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INDIAN SOCIETY FOR BUFFALO DEVELOPMENT
ISBD-IVRI2025
ICAR-IVRI, IZATNAGAR
DECEMBER 15-16, 2025

NATIONAL CONFERENCE

Indian Society for Buffalo Development (ISBD)

Buffalo Production and Health in a Changing Climate: Innovations for a Resilient Future

December 15-16, 2025

Division of Physiology & Climatology
ICAR-INDIAN VETERINARY RESEARCH INSTITUTE
Izatnagar-243 122 (UP) INDIA

National Bank for Agriculture and Rural Development

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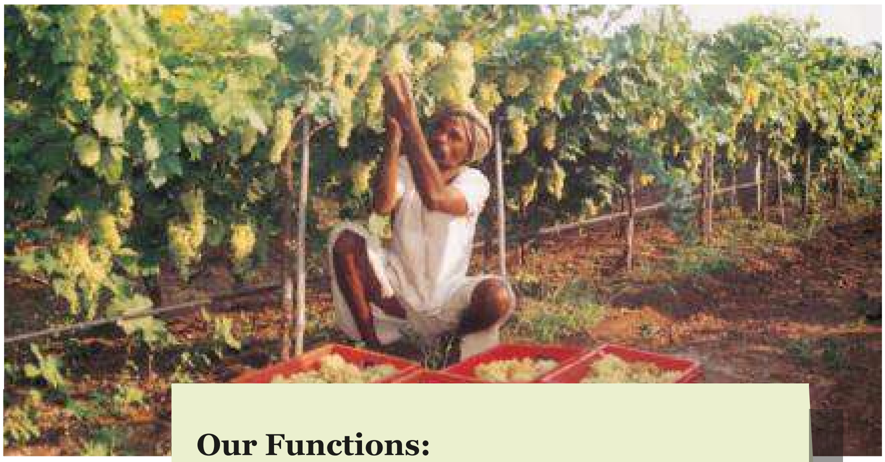

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COMPENDIUM OF ABSTRACTS AND LEAD PAPERS

National Conference of the Indian Society for Buffalo Development (ISBD)

on

Buffalo Production and Health in a Changing Climate: Innovations for a Resilient Future

15-16 December, 2025

Organized by

Division of Physiology and Climatology
ICAR-Indian Veterinary Research Institute
Izatnagar, Uttar Pradesh, India

Under the Aegis of

Indian Society for Buffalo Development (ISBD)

INDIAN SOCIETY FOR BUFFALO DEVELOPMENT
ISBD–IVRI2025
ICAR-IVRI, IZATNAGAR
DECEMBER 15-16, 2025

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Message

Buffaloes have long been the cornerstone of India's dairy sector, providing livelihood, nutrition, and economic stability to millions of farmers in the country. Their unique adaptability, efficient feed utilization, and superior milk quality make them a valuable national asset. However, the rapidly changing climate presents new challenges that demand urgent and innovative scientific responses. Climate resilience in livestock cannot be achieved through isolated efforts. It requires a holistic understanding of animal physiology, genetics, nutrition, management practices and health disciplines combined with advanced tools such as precision livestock farming, molecular diagnostics, and data-driven decision support systems. Interdisciplinary collaboration and integration of traditional wisdom with cutting-edge research will be crucial in ensuring that buffaloes continue to thrive in a changing climate.

In this connection, I appreciate that Indian Society for Buffalo Development (ISBD) is organizing National Conference on the theme **"Buffalo Production and Health in a Changing Climate: Innovations for a Resilient Future"** at ICAR—IVRI, Bareilly. The focus of this conference is therefore both timely and vital in addressing the sustainability of buffalo production systems under evolving climatic scenarios.

The conference will also provide platform to the researchers, academicians, policy makers, and the industry stakeholders to share their ideas for better understanding of buffalo health and production and to formulate farmer-centric strategies for sustainable buffalo husbandry.

I extend my best wished for successful outcome of the conference.

Dated the 09th December, 2025
New Delhi

(M.L. Jat)

सत्यमेव जयते

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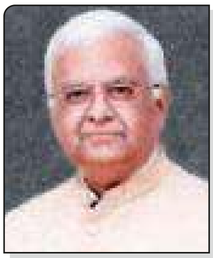

Date: 28/11/2025

Message

I am pleased to extend my warm greetings and best wishes on the occasion of the National Conference of the Indian Society for Buffalo Development (ISBD) on "Buffalo Production and Health in a Changing Climate: Innovations for a Resilient Future" being organized by ICAR-Indian Veterinary Research Institute, Bareilly, on December 15-16, 2025.

The buffalo, aptly called the "Black Gold" of India, is pivotal to our rural economy, dairy production, and nutritional security. Rising climate uncertainties, however, pose serious challenges to its health, productivity, and adaptability. The theme of this conference is therefore timely and forward-looking, underscoring the need for innovative and resilience-oriented approaches in buffalo husbandry and health management.

Future progress will depend not only on scientific advances but also on developing a generation of researchers equipped with cutting-edge biotechnologies, advanced analytical tools, and a strong understanding of climate-livestock interactions. This conference provides an excellent platform to inspire young scientists, encourage interdisciplinary collaboration, and foster transformative ideas for sustainable buffalo development.

I am confident that the deliberations over these two days will meaningfully contribute to strengthening India's roadmap for climate-smart, competitive, and environmentally responsible buffalo production.

I commend the Indian Society for Buffalo Development and the organizing team at ICAR-IVRI, Bareilly, for conceptualizing and hosting this important scientific event, and I wish the conference every success and all participants the very best in their scientific pursuits.

(Sanjay Kumar)

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Dr. Raghavendra Bhatta
Deputy Director General (Animal Science)
M. V. Sc. Ph.D., Postdoctorate (Japan, USA)

No. AS/PS/DDG(AS)/Message/2025
Dated 28th November, 2025

Message

I am delighted to extend my warm greetings to all delegates, organizers, and participants of the National Conference of the Indian Society for Buffalo Development (ISBD) on the theme `Buffalo Production and Health in a Changing Climate: Innovations for a Resilient Future,' being organized by the Division of Physiology and Climatology, ICAR-IVRI, Bareilly, on December 15-16, 2025.

Buffaloes hold a pivotal place in India's livestock-based economy, contributing significantly to milk production, draft power, and rural employment. In the context of global climate change, this vital species now faces a range of challenges - including thermal stress, productivity losses, and shifting disease dynamics. The theme of this conference rightfully underscores innovation and resilience as the two essential pillars for ensuring sustainable buffalo production in an increasingly variable and demanding environment.

Advances across the animal sciences are opening new frontiers in understanding the physiological and molecular mechanisms underlying stress tolerance and adaptive capacity. Coupling these insights with climate-smart management practices, next-generation vaccines and diagnostics, and precision livestock technologies will be crucial for safeguarding animal health, enhancing productivity, and protecting farmer livelihoods. Strengthening interdisciplinary collaboration among scientists, institutions, and stakeholders will further accelerate the development and field-level application of such innovations. This conference arrives at an opportune moment, bringing together eminent experts, young researchers, and policymakers to deliberate on forward-looking strategies and share cutting - edge research outcomes. Such platforms not only facilitate the exchange of ideas but also strengthen professional networks and inspire coordinated efforts toward building climate-resilient livestock systems.

I deeply appreciate the vision and commitment demonstrated by ISBD and the organizing committee in convening this important event. The insights and recommendations emerging from this conference are poised to make a meaningful contribution to advancing resilient, future-ready approaches in buffalo research, production, and health management across India.

(Raghavehdra Bhatta)

भाकृअनुप-भारतीय पशु-चिकित्सा अनुसंधान संस्थान
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डॉ त्रिवेणी दत्त
निदेशक
Dr Triveni Dutt
Director

Message

Buffaloes are the backbone of India's dairy and draught power sectors, contributing significantly to rural livelihoods and national food security. However, accelerating climate change poses serious challenges to their productivity, health, and overall well-being. Rising temperatures, erratic rainfall, increased humidity, and the spread of vector-borne diseases all threaten the efficiency and sustainability of buffalo-based farming systems. A resilient future for buffalo production in India will depend on integrated approaches that combine scientific research, technological innovation, traditional knowledge, and inclusive policy support. By empowering farmers, strengthening veterinary services, and encouraging climate-smart investments, India can safeguard buffalo health, sustain productivity, and ensure that this vital species continues to thrive despite climatic uncertainties. This conference provides a fitting platform to deliberate upon the challenges, share innovative research outcomes, and explore new avenues for climate-smart buffalo management.

The Indian Veterinary Research Institute has long served as a cradle of innovation and excellence in animal research. Over the years, IVRI has contributed significantly to advance knowledge in livestock health and production, which collectively form the foundation of sustainable production. In the face of growing climatic challenges, these scientific strengths have become even more crucial in developing adaptive technologies and policy frameworks that enhance livestock resilience and productivity.

It gives me immense pleasure to welcome all dignitaries, scientists, academicians, and participants to the National Conference of the Indian Society for Buffalo Development (ISBD) on "Buffalo Production and Health in a Changing Climate: Innovations for a Resilient Future," being organized by the Division of Physiology and Climatology, ICAR - Indian Veterinary Research Institute, Bareilly, during December 15-16, 2025. I am particularly pleased that this event brings together a wide network of researchers, scientists, and professionals from across the country. Such scientific interactions foster collaboration, inspire innovation, and generate collective insights that are essential for addressing complex issues like climate resilience and sustainable productivity.

I would like to extend my appreciation to the Indian Society for Buffalo Development and the organising team for their exemplary efforts. The knowledge and outcomes emerging from this conference will be pivotal in driving resilient and innovative path Bo for sustainable buffalo research and production in India.

(Triveni Dutt)

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Message

I am pleased to extend my warm greetings to all delegates of the National Conference of the Indian Society for Buffalo Development (ISBD) on the theme "Buffalo Production and Health in a Changing Climate: Innovations for a Resilient Future, being organized by the Division of Physiology and Climatology, ICAR-IVRI, Bareilly, on December 15-16 2025.

Animal Nutrition and Physiology together form the foundation upon which climate resilience in livestock could be built. Nutritional interventions directly influence thermoregulation, metabolic stability and immune competence, while physiological adaptability determines how efficiently animals can utilize nutrients and maintain performance under environmental stress. This intricate relationship becomes particularly crucial for buffaloes, which, despite their robustness, experience marked physiological strain under rising temperature - humidity conditions.

Advances in adaptive nutrition such as the use of antioxidants, nutraceuticals, and feed additives offer immense potential to mitigate the negative impacts of heat stress. Similarly, research in nutrigenomics, metabolomics, and endocrine regulation is revealing how dietary inputs interact with gene expression to enhance stress tolerance and productivity. Strengthening this interface between nutrition and physiology will be vital for developing innovative feeding and management strategies suited to India's diverse agro-climatic zones.

This conference serves as an excellent platform to deliberate on such scientific innovations and field - level applications. It brings together experts from diverse disciplines to discuss how integrative approaches can safeguard animal health and ensure sustainable productivity in the face of climatic uncertainty.

I applaud the efforts of ISBD and the organizing team of ICAR-IVRI for choosing such a relevant theme and providing a forum for constructive scientific exchange. I am confident that the knowledge shared at this event will guide the redefinition of nutritional and physiological strategies to strengthen the buffalo sector.

(Ashis Kumar Samanta)

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Message

It is my privilege to welcome all delegates to the **ISBD National Conference 2025**, a gathering dedicated to advancing our understanding of buffalo production and health amid the realities of a rapidly changing climate. The theme reflects both the urgency and the optimism with which we approach the future of buffalo science.

Buffaloes are gentle, intelligent, and remarkably adaptable animals—gifted with loyalty, resilience, and a unique ability to thrive where other livestock cannot. The higher fat and solids content of buffalo milk, its superior taste and richness, underscore the exceptional biological value of this species. As was beautifully written decades ago, "The water buffalo is a jewel; we only need to polish it a little more." This belief remains profoundly true today.

Asia, home to 98% of the world's buffalo population, continues to lead global milk and meat production from buffaloes. Yet, the challenges of heat stress, water scarcity, emerging diseases, and ecological variability demand innovative approaches to ensure sustained productivity. The buffalo's admirable hardiness, docility, and capacity to convert low-quality roughages into high-quality animal protein make it vital to climate-resilient agriculture.

This conference creates a platform to explore cutting-edge advancements in genomics, reproductive technologies, climate-smart nutrition, disease mitigation, precision livestock farming, and sustainable management systems. As noted in the classic literature, "Al! those who drink buffalo milk would look graceful and dignified, courageous, self-composed and progressive in attitude," an ode to the cultural and emotional connection people share with this noble species.

I express my sincere appreciation to ICAR–IVRI, Izatnagar, and the Organizing Committee for their dedication and vision in hosting this important national event. I am confident that the interactions during this conference will enrich our collective understanding and strengthen our resolve to build a resilient, sustainable future for buffalo farming and wish it a grand success.

6/12/25

(Inderjeet Singh)

ISBD

Indian Society for Buffalo Development

Sarita Yadav
Secretary ISBD
ISBD National Conference 2025

Message

It is my privilege to welcome all distinguished delegates, scientists, academicians, veterinarians, industry partners and students to the ISBD National Conference 2025 on the theme **"Buffalo Production and Health in a Changing Climate: Innovations for a Resilient Future"** from December 15–16, 2025, at ICAR–Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P.), India.

As highlighted in recent global studies, the water buffalo is among the most resilient livestock species-highly adaptable to diverse and even extreme environments from wetlands and humid tropics to cold climates-making it vital for both rural livelihoods and sustainable food systems.

At ISBD, we strive to create an enabling environment that encourages scientific exchange, promotes innovation and builds strong networks dedicated to the growth of the buffalo sector. This conference provides a unique opportunity for researchers and professionals from across India to engage in forward-looking discussions on emerging technologies, research breakthroughs and sustainable solutions for buffalo production.

I extend my sincere appreciation to the organizing committee for their dedicated efforts in hosting this national event and I warmly welcome all participants whose contributions make this platform meaningful and impactful.

May this conference inspire constructive dialogue, renewed collaborations, and practical innovations that strengthen the buffalo sector-our nation's Black Gold.

Sarita yadav
(Sarita Yadav)

दैहिकी एवं जलवायुकी विभाग
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Gyanendra Singh

Organizing Secretary ISBD
ISBD National Conference 2025

Message

Dear Delegates,

It gives me great pleasure and a profound sense of responsibility to extend a warm welcome to all participants of the National Conference of the Indian Society for Buffalo Development (ISBD) on “Buffalo Production and Health in a Changing Climate: Innovations for a Resilient Future'' being organised by the Division of Physiology & Climatology, ICAR-Indian Veterinary Research Institute, Izatnagar-243 122 (UP) INDIA, during 15th–16th December, 2025.

The theme of this conference reflects the pressing reality of our times. Climate change is no longer a distant threat but a lived experience with profound implications for Buffalo's health, productivity, and welfare. Buffalo rearing, which forms the backbone of rural livelihoods in India, is particularly vulnerable to environmental stress, feed scarcity, and disease emergence. Addressing these challenges requires innovative technologies that not only explain adaptive mechanisms but also provide field-friendly solutions to sustain buffalo production.

This conference is designed as a platform to deliberate on critical areas ranging from Breeding and Omics, physiology and reproduction, Health and disease, Nutrition and management, Production technology and extension practices and potent and novel frontiers in buffalo research. I am confident that this scientific forum will provide an excellent opportunity for eminent scientists, academicians, researchers, and students to exchange ideas, present innovations, and build collaborations that advance the frontiers of research areas, improving buffalo productivity and health. Such interactions are essential for developing climate-smart solutions that safeguard both productivity and animal welfare.

Organizing a national event of this scale has been possible only due to the encouragement of Director ICAR-IVRI and the unwavering supports from the office bearers of the Indian Society of Buffalo Development (ISBD). I extend my heartfelt gratitude to dedicated faculty colleagues, administrative and technical staffs and students whose coordinated efforts across various committees have brought this conference to a reality.

On behalf of the Organizing Committee, I extend my warm welcome to every delegate and participant. Let us together make ISBD IVRI2025, Izatnagar, a milestone event that contributes meaningfully to science, policy, and society. Looking forward for your active participation and valuable contributions in this conference.

(Gyanendra Singh)

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Mandate: *Editing and publication of brochure, banners, certificates, compendium, proceedings etc.*

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Session-I

Keynote Address

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Vice Chancellor BASU and President ISBD

Co-Chairman : **Dr S.S. Dahiya**
Former Director ICAR-CIRB

Rapporteurs : **Dr Hari Abdul Samad** (Senior Scientist, ICAR-IVRI) &
Dr Brijesh Kumar (Scientist S.S), ICAR-IVRI

KN 1. Buffalo Production and Reproduction: Past, Present and Future

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ABSTRACT

The water buffalo (Bubalus bubalis) underpins milk and meat supply for millions of smallholders in Asia and is increasingly important in Mediterranean and other regions. Despite a global population of about 209 million head and superior ability to convert low-quality roughage into high-value milk and meat, buffalo have historically been managed in low-input, multi-purpose systems with modest productivity and poor reproductive efficiency. This review synthesizes the evolution of buffalo production and reproduction into three phases–past (1950–2000): present (2000–2025) and future (2025-2050)–with emphasis on advances from Indian institutions such as ICAR-NDRI and ICAR-CIRB.

In the past, technological foundations were laid through artificial insemination (AI): semen cryopreservation and basic selection, but progress was constrained by species-specific features (seasonality, silent estrus, prolonged postpartum anestrus) and late or invasive pregnancy diagnosis. The present phase has been characterized by rapid gains in milk yield, reduced age at first calving and shorter calving intervals in organized herds, driven by fixed-time AI, structured breeding programs, emerging genomic tools and high-quality nutrition. Landmark contributions include early pregnancy biomarkers such as serum MX2, the field-ready Preg-D urine test, proteomic and metabolomic profiling of serum and milk, buffalo cloning by somatic cell nuclear transfer and climate-smart systems such as the Banni night-grazing model.

Looking ahead, integration of genomics, artificial intelligence, precision feeding and low-methane nutritional strategies offers scope to double genetic gain, lower emission intensity and enhance resilience to heat and water scarcity. Cloning and advanced embryo technologies will remain strategic tools for conserving elite and rare germplasm. However, because 90-95% of buffalo are still kept by smallholders, equitable diffusion of AI/FTAI services, diagnostics and precision tools through cooperatives and digital platforms is essential. The convergence of biological innovation with inclusive institutions can enable buffalo to contribute disproportionately to sustainable, low-emission livestock systems and rural livelihoods in the coming decades.

Keywords: *Buffalo, reproduction, precision, climate*

1. Introduction

The domestic water buffalo (*Bubalus bubalis*) is a primary dairy species in South Asia and a high-value niche dairy animal in Mediterranean countries. Global numbers exceed 200 million, with India alone housing more than half the world population. Traditionally valued for draught power and manure, buffalo are now increasingly managed as specialized dairy and meat animals. Their ability to thrive on crop residues and marginal lands and the high fat and protein content of buffalo milk, make them critical to food and nutrition security and to climate-resilient livestock systems.

The evolution of buffalo production and reproduction can be viewed in three overlapping phases. The past (1950–2000) established technological foundations such as AI and semen cryopreservation. The present (2000–2025) has seen rapid gains through fixed-time AI, biomarkers, metabolomics, cloning, genomics and climate-smart production models. The future (2025–2050) will likely be shaped by genomic selection, AI-enabled decision support, low-methane nutrition and institutional innovations ensuring that smallholders benefit from these advances.

2. Past: From multi-purpose draught animal to dairy resource

Buffalo were historically kept in low-input, multi-purpose smallholder systems. Typical herds comprised 2-5 animals providing draught, manure, meat and, secondarily, milk. Animals grazed extensively on communal and marginal lands, often with minimal supplementation or veterinary care. Under these conditions, indigenous buffalo typically produced 2–3 L of milk per day, with lactation yields of 700–1,000 kg, age at first calving (AFC) of 48–54 months and calving intervals (CI) of 18–24 months or longer.

Research during this era documented buffalo-specific reproductive constraints: smaller ovaries and fewer primordial follicles than cattle, marked seasonality mediated by photoperiod and melatonin, a high incidence of silent estrus and prolonged postpartum anestrus. Non-genetic constraints—including undernutrition, parasitism, infectious disease and heat stress—further depressed fertility and extended calving intervals.

Technological progress nevertheless occurred. AI was first demonstrated in buffalo in the 1940s and was gradually expanded through national programs. Semen cryopreservation methods were adapted from cattle and then modified for buffalo’s greater cryo-sensitivity. However, poor estrus expression, inconsistent semen quality and weak extension limited field conception rates to 15-25% in many programs.

Pregnancy diagnosis relied on per-rectal palpation, transrectal ultrasonography and laboratory-based hormonal assays (progesterone, estrone sulphate, pregnancy-associated glycoproteins). Early pregnancy factor attracted interest but tests were complex and non-specific. None of these methods offered a rapid, inexpensive, cow-side diagnosis within the first three weeks after insemination, when rebreeding open animals would most shorten CI.

3. Present: Technological Acceleration (2000-2025)

3.1. Production systems and genetic improvement

Since 2000, buffalo production has shifted toward specialized dairy systems in many regions. Commercial herds of 80-160 lactating buffaloes are now common in India, Pakistan, Egypt and Italy. Animals are housed in loose housing or paddocks, milked mechanically and fed total mixed rations or unifeed based on maize silage, high-quality hay, concentrates and by-products. In Mediterranean Italian herds, average yields of 2,000–2,300 kg/lactation with ~8% fat and ~4.6% protein are reported; Indian national averages have risen from ~3.4 to ~4.5–4.6 kg/day over two decades. Organized herds often achieve AFC near 30–34 months and CI close to 400–420 days when nutrition and reproduction are well managed.

Structured breeding and performance recording underpin these gains. Italy's Mediterranean program records a large proportion of cows, progeny-tests bulls and selects for mozzarella yield rather than volume alone. In India, the National Project for Cattle and Buffalo Breeding and National Dairy Plan expanded semen production, introduced minimum standards for semen stations and increased AI coverage, improving average conception rates to ~35%.

Genomic tools are now emerging. High-quality reference genomes and SNP panels have been developed for several buffalo breeds. Early genomic selection and GWAS efforts have begun to identify QTL for milk yield, composition, fertility and thermotolerance. Implementation is currently limited by smaller reference populations and less comprehensive recording than in dairy cattle, but the technical framework for genomics-enabled breeding exists.

3.2. Reproductive technologies

Fixed-time AI (FTAI) protocols such as Ovsynch and progesterone device-based regimens have greatly mitigated problems of poor and silent estrus. Properly implemented, these protocols yield conception rates of 40-55% in organized herds and modified double-Ovsynch strategies have achieved even higher rates in acyclic heifers.

Advanced reproductive technologies are now routinely applied in nucleus herds. Ovum pick-up (OPU) combined with in vitro embryo production (IVEP) allows repeated recovery of oocytes from elite females, producing multiple embryos for transfer or cryopreservation. Sexed semen, using flow cytometry to enrich for X-bearing sperm, is being evaluated to accelerate genetic gain and shift sex ratios towards females in strategic herds.

India has taken a global lead in buffalo cloning. ICAR-NDRI produced the first cloned buffalo via somatic cell nuclear transfer (SCNT): followed by multiple clones of elite Murrah animals. These clones have attained puberty, conceived and lactated, demonstrating the feasibility of SCNT in buffalo. ICAR-CIRB has evaluated semen quality and fertility of cloned bulls and integrated them into AI programs. Although high costs and ethical concerns preclude mass deployment, SCNT is strategically important for conserving rare germplasm, multiplying top genomically selected donors and research on gene function.

3.3. Biomarkers, diagnostics and precision tools

A major advance in the present phase has been the development of buffalo-specific biomarkers and field-usable diagnostics, particularly from ICAR-CIRB Hisar. Proteomic profiling of serum and milk identified pregnancy-associated proteins and set the stage for early pregnancy tests. A key breakthrough was the demonstration that the interferon-stimulated gene product MX2 is strongly upregulated at mRNA and protein levels in pregnant buffalo heifers between days 14 and 28 post-insemination, but not in non-pregnant controls. This identified serum MX2 as a practical early pregnancy biomarker and provided a basis for MX2-based ELISA tests.

Complementing this work, the Preg-D urine test was developed and patented as a simple, thermophilic, colorimetric assay for pregnancy detection in buffalo. It is a rapid, farmer-friendly alternative to blood tests, ultrasound or rectal palpation. It is built on metabolomics work showing that specific small molecules in maternal urine change consistently during early gestation. The test uses a thermophilic colour reaction: when the urine of a pregnant animal is added to the reagent on the strip or in the tube, pregnancy-associated metabolites drive the reaction towards an orange to red/purple colour, whereas urine from non-pregnant animals produces only a very pale background colour. As currently configured, Preg-D can usually be applied from about day 18-22 after insemination, with the most reliable results from day 30 onwards. It is designed as a true pen-side test: only urine (no blood): no cold chain, no instrumentation and results in a few minutes. This makes it particularly suitable for smallholders and field veterinarians who need to screen many animals and cannot rely on laboratory infrastructure. Validation data indicate that, when all samples are included—including those from dehydrated animals, animals with urinary tract infections, very cloudy or effervescent urine—the sensitivity already exceeds 90% and overall accuracy is above 80%.

Metabolomics (serum and milk) and "lactomics" have further expanded diagnostic possibilities. Changes in proteins such as synaptojanin-1, apolipoproteins and von Willebrand factor reflect early embryo-maternal interactions; milk metabolite signatures distinguish healthy, subclinical and clinical mastitis and reflect energy balance around calving. These insights support development of on-farm spectroscopic tools for health monitoring, mastitis screening and precision feeding.

3.4. Behaviour, climate adaptation and precision farming

The Banni buffalo production system in the saline grasslands of Kachchh, Gujarat, demonstrates climate-smart management based on animal behaviour. Banni herds graze 8-10 hours at night, led by a dominant "bell" cow, thereby avoiding extreme daytime heat, saving water and exploiting widely scattered forage. Returning at dawn, animals receive water and a concentrate-jaggery mix, reinforcing predictable movement patterns. This model drastically reduces feed and labor costs while maintaining milk yields of 12-20 kg/day under harsh conditions.

Parallel efforts in precision livestock farming have introduced IoT-based sensors for activity and rumination, mobile applications for record-keeping and heat alerts and indices of heat stress tailored to buffalo. These tools are currently most feasible in organized herds but can be down-scaled through cooperatives and service providers.

3.5. Nutrition, methane and metabolic health

Buffalo possess higher crude fiber digestibility and slightly better energy conversion efficiency than cattle, supporting cost-effective use of crop residues and low-quality forages. Modern diets for high-yielding buffaloes typically balance metabolizable energy at ~2.6 MFU for maintenance plus 0.4-0.5 MFU per kg milk and provide 18-22% crude protein in the concentrate portion, with appropriate bypass protein.

Under climate-resilience programs such as NICRA, NAINP and CIRB have evaluated low-methane feeding options: high-digestibility forages, tannin-rich tree leaves, specific oilseed cakes and additives (plant secondary metabolites, ionophores, targeted probiotics) that shift rumen fermentation towards propionate and away from methane. These strategies reduce emissions per unit milk without sacrificing productivity, especially when combined with manure biogas capture.

Management of peripartum metabolic health focuses on limiting negative energy balance, early provision of high-quality colostrum, rapid restoration of feed intake and maintaining body condition scores around 3.0-3.5. Such practices improve fertility, reduce disease and support longer productive lifetimes.

4. Future Directions (2025-2050)

In the coming decades, buffalo production and reproduction will likely be shaped by four interacting trends. First, precision farming and digitalization will expand through wearable sensors, automated heat detection, predictive analytics and blockchain-based product traceability. Robotic milking adapted to buffalo and AI-based decision support for breeding and feeding are realistic medium-term prospects in larger herds.

Second, genomics-enabled breeding will mature as reference populations grow. Genomic estimated breeding values for milk, fertility, health and thermotolerance, integrated with AI, OPU-IVEP and, selectively, cloning, could double annual genetic gain compared to conventional progeny testing.

Third, climate-smart buffalo systems—combining thermotolerant genotypes, low-emission diets, Banni-like grazing models and crop-buffalo-biogas integration—will be central to low-carbon dairy strategies, especially in South Asia and emerging African buffalo regions.

Finally, equity and smallholder inclusion will be decisive. Expansion of village-level AI/FTAI services, dissemination of farmer-friendly diagnostics such as Preg-D, strengthening of cooperative value chains and appropriate climate-finance mechanisms are essential to ensure that technological advances translate into broad-based improvements in productivity, resilience and rural livelihoods.

5. Conclusions

Buffalo production has evolved from low-input, multi-purpose systems with modest productivity to increasingly sophisticated, technology-driven enterprises. Past innovations in AI and semen freezing laid foundations but were constrained by biology and field realities. Present advances in FTAI, biomarkers, metabolomics, cloning, genomics, low-methane nutrition and climate-smart systems have begun to unlock the species' full productive and reproductive potential.

The future will be defined as much by institutions as by technologies. If genomics, AI and precision feeding are combined with inclusive service delivery, cooperative organization and supportive policy, buffalo can contribute disproportionately to sustainable, low-emission livestock systems and to the livelihoods of millions of smallholders across Asia and beyond.

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Session-II

Breeding & Omics

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Dr Sheikh Firdous Ahmed
Scientist ICAR-IVRI, Izatnagar

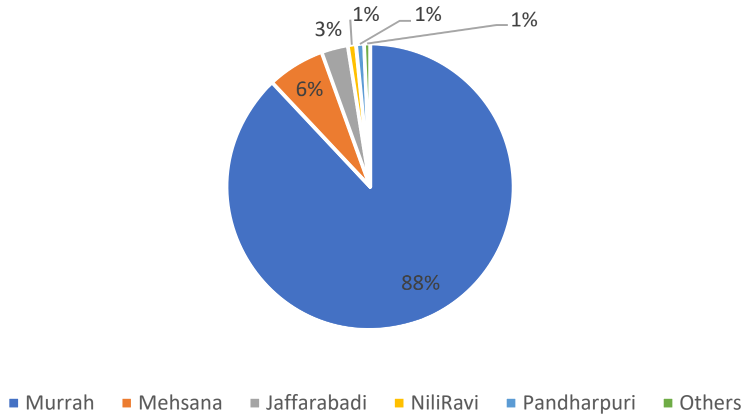
LP 2.1. Development of MAHISHCIP and its use for buffalo development

Nilesh Nayee*, Swapnil Gajjar, Atul Mahajan, Sujit Saha and A. Sudhakar
National Dairy Development Board, Anand

Domestication of buffaloes started later than cattle domestication. Cattle domestication is estimated to be around 10500 years back (Helmer *et al.,* 2005) whereas buffalo domestication dates back to 3000-6000 years ago. Buffaloes are thought to have reached to Southern Asia through Europe but later on their population remained limited mainly to South Asia (Rehman *et al.*, 2021). With ability to withstand harsh hot and humid climate across the Indian subcontinent and ability to convert fibre rich crop residues into high quality milk makes buffalo preferred animal in many parts of India. Owing to its place in economy of India as a readily liquefiable asset, buffaloes are popularly known as "Black Gold" among livestock owners in India. Buffaloes produce almost half o the milk in India and provide nutrition to the Indian population. Considering variation in agro-climatic conditions across India, various buffalo breeds has been developed in the country, each with some unique characteristics to adopt to the local conditions. At present there are 19 registered buffalo breeds in the country (NBAGR: http://14.139.252.116:8080/appangr/openagr.htm).

Considering the socio-economic importance of buffaloes, the country has focussed on research and developmental activities for buffaloes to increase productivity. Artificial Insemination (AI) which was initially recognized as breeding tool only for cattle is now used equally for buffaloes as well. Out of 141 Million bovine Frozen Semen Doses produced in the country, 53 Million FSDs (38%) are produced from buffalo bulls. The breed wise distribution of FSDs produced in the year 2024-25 is provided in the figure below:

Figure 1: Buffalo FSDs in country 2024-25



Considering sizeable amount of FSDs being produced from buffalo bulls, it is essential to select the bulls with high accuracy to ensure genetic progress in the buffalo population under AI. Genomic Selection (Meuwissen *et al.,* 2001) is a promising breeding tool which provides higher accuracy for breeding value estimates compared to pedigree based EBVs. In order to implement genomic selection a large reference population where all the animals have phenotype as well as genotype is required. Based on this reference, prediction of Genomic Enhanced Breeding Value is done for selection candidates that do not have own performance record. It has been proven that the GEBVs are more accurate compared to pedigree based EBVs. This will accelerate genetic progress in the population under selection. Classically, implementation of Genomic Selection requires reference population consisting of proven bulls. But female reference populations are now gaining in popularity, especially for novel traits, such as feed efficiency, methane emissions and detailed reproductive measures, the traits which are expensive to measure and are therefore available for only some animals in the population (Pryce *et al.*, 2012).

Genomic Selection is whole genome-based selection and for achieving this SNP markers placed across the genome are used. This is achieved by identifying markers that are informative for the population under study and then genotyping with the help of DNA microarrays. Performance of commercially available genotyping microarray Axiom Buffalo GenotypingArray (90 K) (Thermo Fisher Scientific, MA) was evaluated by Thakor et.al 2021 and concluded that levels of SNPs variation using this array could be insufficient to differentiate the other local breed except Pandharpuri, Surti and Jaffarabadi (morphologically distinct breeds): so there is a need to develop SNP chip based on SNP markers identified by sequence information of local buffalo breeds.

Total 196 buffalo samples were collected belonging to 8 different buffalo breeds of India. The samples were collected from native breeding tract of these breeds from farmers/institutions that rear animals having physically true to the breed characters. Care was taken to avoid related animals based on pedigree information when available. The breeds were selected based on their popularity in semen industry, milk production potential in the breed or distinction of phenotypic appearance of the animals of the breed.

Breed-wise number of samples sequenced is given in Table 1. Paired end sequencing on Illumina platform using TrueSeq DNA Library preparation protocol was done at a service laboratory to generate data to provide around 20X coverage of the samples. Only paired end reads that aligned to the reference assembly with quality score of q30 were considered. Considering a small number of samples, data from Banni breed was not used for further analysis.

Table 1: Breed-wise samples and data generated

Breed	No. of samples sequenced	Avg. raw data in Gb	Avg. data pasing Q30 Gb
Banni	5	54	48
Bhadawari	13	62	54
Jaffarabadi	16	63	56
Mehsana	26	65	58
Murrah	87	69	61
NiliRavi	16	63	53
Pandharpuri	17	66	59
Surti	15	67	60

Designing BUFFCHIP

The paired end reads with q30 quality were aligned to the UoAmediterrenean buffalo genome assembly (GCF_003121395.1: https://www.ncbi.nlm.nih.gov/ datasets/genome/ GCF_003121395.1/#:~: text=1.,%5B GenBank%5D%206500688%20%5BRefSeq%5D) using Santeion DNASeq variant calling workflow (https://www.goldenhelix.com/media/pdfs/whitepapers/Sentieon_Genomics_Tools.pdf). The reads were processed breed wise. The raw variants were filtered for the quality using GATK Variant Filteration functionality (Poplin *et al.,*2017). Further, only SNP variant that were genotyped for minimum 95% of the individuals and had minor allele frequency more than 0.1 were retained for further selection using PLINK 1.9 software. Flaking reference sequence of 50 base pairs on either side of the SNP was obtained using bcftools (https://samtools.github.io/bcftools/ bcftools.html). An Illumina Array Design Tool–ADT (sapac.support.illumina.com /downloads /designstudio-assay-design-product-documentation.html) input file was prepared for the filtered SNPs and submitted to ADT for obtaining Illumina design scores. Further only SNPs with Illumina design score of >0.8 were retained for processing.

In first phase, 15000 SNPs from Axiome array were selected. The parameters were set to ensure double SNP density at the end of the chromosomes and minimum gap size (inter-SNP distance) of 10000 Base pairs.

In second phase of SNP selection, remaining 45000 SNPs were selected from discovered variants, to keep average inter-marker distance of 50000 base pairs. The SNP array thus selected was designed and manufactured on Illumina HTS platform and the custom product was named as BUFFCHIP1.

384 samples were genotyped using BUFFCHIP1 using standard Illumina HTS protocol (https://support.illumina.com/content/dam/illumina-support/ documents/documentation/chemistry_documentation/infinium_assays/infinium-hts/infinium-hts-assay-reference-guide-15045738-04.pdf). Primary quality control of genotype data generated showed that 14834 SNPs were not being called/had poor call rates (below 80%) or were monomorphic.

The design was updated with removing all 14834 SNPs that were performing poorly and adding 18834 new SNPs that has high design score. The new design was manufactured on Illumina HTS platform as BUFFCHIP2.

Designing MAHISHCHIP

In order to unify various platforms developed in the country the design was further modified to replace 11948 SNPs with the SNPs that performed well in NBAGR High Density buffalo genotyping chip. Further, new 17697 SNPs were included that were discovered based on alignment of sequence date on new reference assembly NDDB_SH_1 (https://www.ncbi.nlm.nih.gov/ datasets/genome/GCF_019923935.1/) and Parentage SNPs recommended by BAIF. The SNP panel was designed on ThermofisherAxiom genotyping platform and the unified version was named as MAHISHCHIP. The SNP locations were updated on

NDDB_SH_1. The array design was also submitted to Illumina Design Studio for scoring SNPs. Only 574 SNPs were having Illumina score below 0.9, showing that the design is also compatible with Illumina platform. Till date 15,360 samples has been genotyped using MAHISHCHIP.

Use of MAHISHCHIP for improvement of buffaloes:

Genomic Selection: Genomic Selection has come as a boon for our condition as it provides higher accuracy (from 50 to 70% depending upon availability of reference population) compared to pedigree-based ranking at birth of calf. Availability of large number of performance records from existing Progeny Testing projects implemented in collaboration with various agencies (refer to Trivedi et.al. 2021) and genotyping platform like MAHISHCHIP and GAUSHIP has provided opportunity to implement Genomic Selection for Indian conditions. Genomic Selection is now employed for bull calf selection for Murrah, Mehsana, Gir, Sahiwal, Kankrej, HFCB and JCB breeds. The validation studies show that Genomic Selection is around 3 times more accurate than traditional dam's yield-based selection and around 80% more accurate than Pedigree based EBVs obtained for young bulls in PT projects. This is expected to provide more accurate ranking of bulls and bull calves and accelerate progress in the daughters of AI bulls.

All the buffalo bulls available for semen collection at semen stations were genotyped using MAHISHCHIP and their GBVs are provided to semen stations so that bulls with poor GBV are prioritized for replacement with better young bulls.Total 2195 Murrah and 572Mehsana bulls have been evaluated for their Genomic Enhanced Breeding Values (GEBV).

The bull calves produced under Progeny Testing projects are now screened for their Genomic Breeding Values and top bull calves are only purchased from farmers after disease testing. The bull calves are distributed among semen stations across the country for FSD production ensuring availability of better genetics in national AI program. Till date 528 Murrah and 35 Mehsana bull calves have been tested for GEBVs. And bulls with higher GBVs were procured from farmers.

Parentage Testing and Discovery: with majority of bulls used for semen collection being genotyped, it is now easy to provide parentage testing services for AI born buffaloes. The SNP genotype data allows testing of parentage with high accuracy. Further, alternate sires are assigned in case of parentage failure using genotype data repository with high accuracy and pedigree records are corrected in the database.

Overall, the MAHISHCHIP is used for genetic improvement of buffaloes by building more robust pedigree structure and selecting young bulls with high accuracy through Genomic Selection process.

Acknowledgement:

We acknowledge efforts done by field officers of Milk Unions, Milk Federations, Livestock Development Board and other agencies who are ensuring collection of quality phenotypic records under Progeny Testing Projects. These records are backbone of genetic improvement programs of the country. We also thank Department of Animal Husbandry and Dairying, Govt. of India for providing funding for the Genomic Selection program under Rashtriya Gokul Mission and have made policy changes to support scientific advancements. We are grateful to Dr. Ben Rosen and Prof. Curt Van Tassel, APIL, ARS, USDA for their guidance in designing BUFFCHIP. We acknowledge support from NDDB management that has always encouraged us to try innovative approaches.

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LP 2.2. Novel Method of Breed Assignment through Machine learning in Buffalo

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Introduction

Accurate breed assignment and ancestry estimation are core requirements in livestock genomics, particularly for conservation programs, breed registration, genomic selection and traceability. Traditionally, population structure tools such as ADMIXTURE, STRUCTURE and fast STRUCTURE have been widely used to estimate breed proportions based on allele frequencies. While these tools have been powerful for exploratory population genetics, the increasing availability of high-dimensional SNP datasets, large reference populations and diverse breeds has highlighted several limitations of classical admixture models.

Machine Learning (ML): especially supervised learning approaches, now provides a significantly more accurate, flexible and scalable alternative for breed probability estimation. ML models such as XGBoost, Random Forest, Logistic Regression, Support Vector Machines and Deep Learning architectures can learn complex genomic patterns and return breed probability scores with higher precision and robustness than ADMIXTURE-based methods.

This document explains why ML-based breed probability estimation is superior to admixture approaches and outlines how modern genomic prediction pipelines achieve high accuracy in multi-breed datasets.

Why Machine Learning?

I. ADMIXTURE is a Population Genetics Model.It estimates ancestral proportions under the assumption of:

- Hardy-Weinberg Equilibrium (HWE)
- Linkage equilibrium across SNPs
- K ancestral populations with multinomial allele frequency distributions

These assumptions rarely hold for real livestock breeds due to:

- Selective sweeps
- Inbreeding
- Admixture across historical timelines
- Uneven effective population sizes
- Complex LD patterns

In contrast, ML does not require any population genetic assumptions. It learns directly from genotype-label relationships.

II. ML Models Handle High-Dimensional Data Better

ADMIXTURE performance drops significantly when datasets include:

- More number of breeds are included breeds
- >100,000 SNPs
- Related individuals
- Uneven sample sizes across breeds

XGBoost and similar ML models:

- Scale efficiently to millions of SNPs
- Automatically learn non-linear relationships
- Detect SNP interactions
- Are robust to unbalanced datasets (using class weights or SMOTE)

III. ML–Faster, More Scalable and More Reproducible

ADMIXTURE runtimes increase rapidly with K and sample size. Multiple replicates are required for stable results.

ML pipelines:

- Train in minutes
- Evaluate in seconds
- Provide reproducible outputs
- Can be deployed as automated pipelines or APIs

IV. ML Outputs True "Breed Probabilities"

ADMIXTURE proportions reflect ancestry components, not actual breed membership probabilities. Machine learning outputs calibrated probabilities for:

- Breed assignment
- Multi-breed mixtures
- Uncertain classifications (useful for admixed or crossbred animals)

This is extremely useful for breed societies, genomic registries and livestock certification programs.

ML Pipeline for Breed Probability Estimation

A standard ML pipeline for breed probability estimation includes:

Step 1: High-quality SNP filtering
Step 2: Selection of informative markers (e.g., chi-square, ANOVA F-test, SHAP values)
Step 3: Dimensionality reduction (optional: PCA)
Step 4: Training ML models (XGBoost, Logistic Regression, Random Forest)
Step 5: Cross-validation (stratified k-fold)
Step 6: Probability prediction for new individuals
Step 7: Model calibration and confidence interval estimation
Step 8: Reporting (confusion matrix, per-breed F1, ROC-AUC)

Applications in Livestock Genomics

- Breed confirmation for registration
- Traceability in value chains (milk, meat)
- Detecting crossbreeding and grading levels
- Classifying unknown or mixed-animal populations
- Conservation prioritization
- Selection of reference populations for genomic evaluations
- Breed authentication for semen, embryos and genomic chips

Conclusion

Machine learning has transformed the ability to classify livestock breeds with high confidence and accuracy. Unlike ADMIXTURE, which infers generic genetic clusters, ML models directly learn discriminative breed-specific SNP patterns. This leads to:

- More reliable breed probabilities
- Higher accuracy across closely related breeds
- Robustness to missing data, LD structure and sample imbalance
- Scalable deployment for large national genomic programs

ML-based breed classification is now the modern, evidence-based standard for livestock genomic prediction pipelines.

LP. 2.3. From Genome to Algorithms: Harnessing Omics and Artificial Intelligence for Precision Buffalo Breeding

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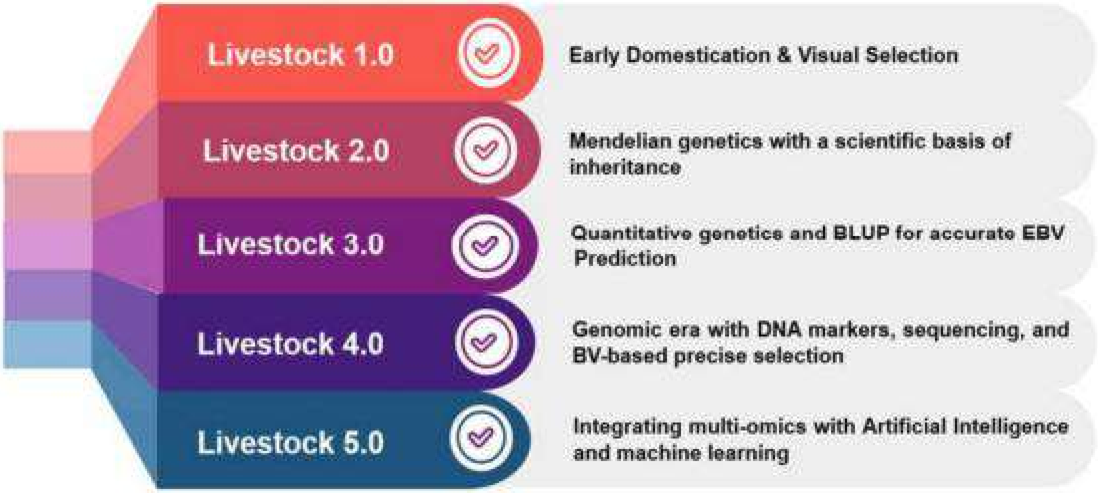
Buffaloes at the Core of India's Dairy Sustainability

Buffaloes (*Bubalus bubalis*) are central to sustainable dairying in India and many tropical regions, contributing substantially to milk production, draught power, rural livelihoods and nutritional security. Their milk, naturally richer in fat, protein and calcium supports premium dairy industries and yields higher returns through value-added products such as *khoa* and ghee. Buffaloes are also farmer-friendly and eco-efficient: they thrive on low-quality roughages, show strong adaptability to tropical stressors, live longer and fit well within smallholder production systems, making them a backbone of dairy economies across South Asia (Michelizzi *et al.*, 2010; Khedkar *et al.,* 2016). Despite these strengths, buffalo production remains underutilized globally due to several biological and management constraints, including poor heat tolerance, delayed puberty, longer calving intervals, lower fertility, high initial purchase cost and regionally variable consumer preference for buffalo milk. These challenges, coupled with the inherently complex polygenic architecture of productivity and functional traits, limit the effectiveness of conventional pedigree and phenotype-based selection programmes.

Livestock 5.0: The New Paradigm



This paradox, high value but slow genetic progress underscores the urgent need for modern, data-driven breeding strategies. Advances in Omics technologies and artificial intelligence (AI) now offer transformative solutions, enabling deeper dissection of the genome, mapping of regulatory and metabolic networks, enhanced prediction accuracies and algorithm-guided selection decisions. Together, these innovations mark a pivotal shift toward precision buffalo breeding capable of unlocking the species' full productivity, resilience and sustainability potential.

Livestock improvement has progressed through a dynamic pathway, reflecting advances in biological understanding, data capture and technological innovation. Livestock 1.0 relied largely on visual appraisal and observable performance, where selection was intuitive and based on traditional knowledge rather than structured genetic principles. With the rediscovery of Mendelian inheritance, Livestock 2.0 introduced the foundation of classical genetics, enabling breeders to recognize patterns of heredity and segregation and to implement simple pedigree-based selection and organized mating strategies. The advent of quantitative genetics in the mid-twentieth century led to Livestock 3.0, where statistical models such as Best Linear Unbiased Prediction (BLUP) allowed the integration of phenotypes, pedigrees and environmental factors to generate reliable Estimated Breeding Values. This greatly improved the accuracy and consistency of selection decisions across diverse livestock populations. The genomic era marked the onset of Livestock 4.0, driven by molecular markers, SNP chips, genome sequencing and genome-wide association studies, which enabled the estimation of genomic breeding values at a much younger age, reducing generation intervals and accelerating genetic gain.

The current paradigm, livestock 5.0, represents the convergence of multi-omics technologies and computational intelligence. This futuristic yet rapidly emerging phase integrates genomics, transcriptomics, proteomics, metabolomics, epigenomics and metagenomics with artificial intelligence, machine learning, digital phenotyping and real-time sensor-based monitoring. livestock 5.0 shifts from single-layer genetic information to a systems-level understanding of how molecular networks shape complex traits such as feed efficiency, fertility and resilience to climate stress. It focuses not only on maximizing productivity but also on ensuring animal welfare, environmental sustainability, biodiversity preservation and robustness under climate change. Through this fusion of Omics and artificial intelligence, livestock 5.0 enables highly precise, algorithm-supported selection strategies that accelerate genetic progress and support the development of superior, high-performing populations.

Positioning Buffalo Breeding Within the Livestock 5.0 Framework

Buffalo breeding is increasingly compatible with the Livestock 5.0 approach owing to recent advancements in species-specific genomic and molecular resources. Understanding how these capabilities integrate into buffalo improvement requires a brief look at the historical structure of breeding in the species. Traditionally, buffalo selection relied on visual appraisal, pedigree records and progeny testing (PT). Although PT remains the most reliable method for evaluating sires, its practical implementation in buffalo has been slow because of long generation intervals, low artificial insemination coverage and limited performance recording. These constraints delay the release of proven bulls by several years and restrict the overall rate of genetic gain. Marker-assisted selection (MAS) provided an early molecular enhancement, but its impact was limited because only a small proportion of genetic variance was captured through the few available markers and the generally weak linkage disequilibrium between markers and causal variants (Boichard *et al.*, 2016).

High-density SNP genotyping, whole-genome sequencing, GWAS outputs, transcriptomic datasets and emerging epigenomic information have substantially improved our understanding of the genetic and regulatory architecture of key buffalo traits, including milk yield, fat synthesis, fertility and other important traits. These resources now support genomic selection that enables early, accurate prediction of breeding values, thereby shortening generation intervals compared with progeny testing. With these molecular datasets now established for buffalo, the species is effectively aligned with the analytical expectations of the Livestock 5.0 paradigm.

Multi-Omics Frameworks for Next-Generation Buffalo Breeding

Omics technologies provide multilayered biological information spanning genomics, epigenomics, transcriptomics, proteomics, metabolomics, lipidomics, phenomics and microbiome profiling (metagenomics/meta-transcriptomics) from a high-throughput workflow. Together, these platforms offer an integrated genome-to-phenome perspective and greatly enhance the ability to dissect productivity, fertility, adaptability and resilience traits in buffaloes.

Genomics

Genomics forms the foundation of precision breeding by enabling genome-wide discovery of SNPs, InDels and structural variants through high-throughput sequencing. To strengthen these efforts, the development of buffalo-specific SNP arrays such as the Axiom® Buffalo Genotyping Array (90K; Iamartino*et al.,* 2017): BUFFCHIP and the Mahish Chip will support accelerated routine genotyping and enable more comprehensive species-wide variant characterization. Building on these enhanced genomic resources, genomic selection (GS) (Meuwissen *et al.*, 2001) leverages thousands of genome-wide markers to estimate breeding values with substantially higher accuracy than traditional pedigree-based methods. The single-step GBLUP (ssGBLUP) framework (Aguilar *et al.,* 2010; Christensen and Lund, 2010) further advances this by integrating pedigree, phenotypic and genotypic information into a unified relationship matrix, enabling robust genomic prediction even when only a subset of animals is genotyped.

Transcriptomics

Transcriptomics adds a functional layer by quantifying RNA expression across tissues and physiological states. RNA sequencing (RNA-seq) (Wang *et al.*, 2009) enables high-resolution profiling of gene expression and regulatory pathways. Integrating transcriptomic signatures with genomic variation improves the prioritisation of functional variants by linking DNA polymorphisms to expression changes. Approaches such as eQTL mapping and transcriptome-wide association studies (TWAS) (Chhottaray*et al.,* 2023) further strengthen gene-trait inference and help identify regulatory mechanisms that remain undetected through GWAS alone.

Epigenomics, Proteomics, Metabolomics and Metagenomics

Other Omics layers refine biological interpretation. Epigenetics examines heritable regulatory changes such as DNA methylation, histone tail modifications, chromatin remodelling and non-coding RNA activity that alter gene expression without modifying the underlying DNA sequence, thereby influencing phenotypic variation and adaptive responses (Skvortsova*et al.,* 2018). Proteomics links gene expression to cellular function through mass spectrometry-based protein profiling. Metabolomics captures biochemical end products, improving genotype-phenotype mapping and enhancing prediction accuracy (Chakraborty *et al.,* 2022). Metagenomics and meta-transcriptomics reveal the composition and active functions of the rumen microbiome, which influences feed efficiency, methane emissions and health (Shabat*et al.*, 2016; Li and Guan, 2017). Integrated through systems genomics (Kadarmideen, 2014): these multilayer datasets strengthen biological resolution and provide a basis for precision buffalo breeding.

Integrating Artificial intelligence with Multi-Omics for Precision Breeding

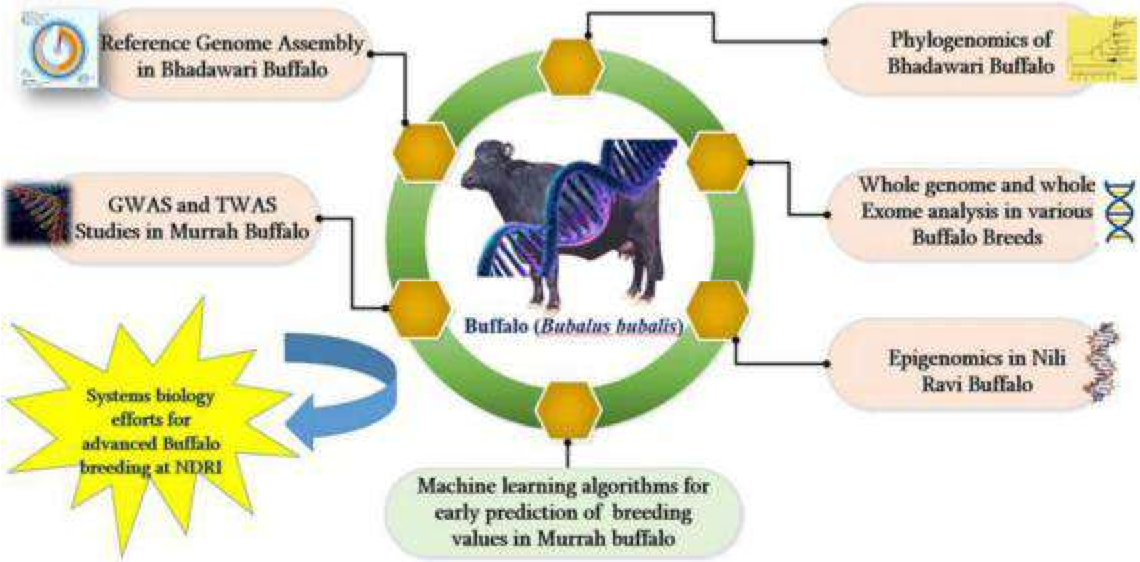
Dense SNP chips and cheaper genotyping made genomic prediction routine in buffalo and other species, typically implemented through GBLUP or Bayesian regression methods. However, as genomic and Omics datasets expanded, new challenges emerged: extreme marker-to-animal ratios ("large p, small n"): heterogeneous and noisy data and the need to model non-additive effects, complex interactions and genotype-by-environment relationships (Nayeri*et al.,* 2019; Chafai*et al.,* 2023). These patterns are often difficult to capture with strictly linear parametric models.Machine learning (ML) and broader artificial intelligence (AI) frameworks offer complementary tools for this new data landscape. Supervised learning algorithms, such as random forests, gradient boosting, kernel methods (e.g. RKHS, SVM) and deep neural networks, can learn nonlinear relationships between high-dimensional genomic (and multi-omics) inputs and complex phenotypes, can be applied to genomic prediction in buffalo. ML has also been used for feature selection (identifying informative SNP subsets or genes): genotype imputation and integration of genomic data with sensor-derived phenotypes and health records. In parallel, advances in bio-sensing, wearable devices and precision livestock farming platforms now generate continuous data streams on activity, health, feeding and environmental exposure, creating an opportunity for AI systems that combine genomics, phenomics and management data into real-time decision tools.

For buffalo breeding, where reference populations are still relatively small and several important traits such as fertility, thermotolerance, disease resistance and methane emissions are difficult or expensive to measure, AI and machine learning offer clear advantages. By integrating information from genomic markers, Omics datasets, longitudinal performance records and, in future, sensor-based phenotypes, these models can improve prediction accuracy even in limited or heterogeneous datasets. They also support early selection and help develop decision-support tools that are suited to the biological and management conditions typical of buffalo production systems. Within the Livestock 5.0 framework, AI therefore acts as the computational engine that converts large, complex buffalo datasets into actionable selection indices and management recommendations, complementing genomic selection rather than replacing it and pushing precision buffalo breeding from data collection toward truly data-driven decisions.

Systems Biology and Data-Driven Genomics Initiatives in Buffalo Improvement at NDRI

At ICAR-NDRI, coordinated efforts have been made to advance buffalo genomics, multi-omics and computational breeding. A major step has been the development of breed-specific reference genomes, including the recent *de novo* assembly of the Bhadawari buffalo, which complements existing Murrah and Jaffarabadi assemblies and provides a valuable foundation for variant discovery, gene annotation and comparative analyses. Targeted and genome-wide sequencing platforms—such as GBS (Genotyping-by-Sequencing): WGS (Whole Genome Sequencing) and WES (Whole Exome Sequencing) have enabled the identification of SNPs, InDels and functional genomic regions associated with milk yield, composition traits, fertility, lactation persistency, thermotolerance and adaptation across breeds including Murrah, Pandharpuri, Chilika, Chhattisgarhi, Gojri and Nili Ravi.

Epigenomic studies using RRBS (Reduced Representation Bisulfite Sequencing) in Niliravi buffalo have revealed differential DNA methylation patterns linked to phenotypes such as pigmentation, underscoring the regulatory significance of epigenetic variation. Transcriptome-level approaches, including RNA-seq and TWAS (Transcriptome-Wide Association Studies): in Murrah Buffaloes have further identified expression signatures and biological pathways associated with fat %, SNF% and other lactation traits, demonstrating the value of integrating expression data into genomic prediction. Additional work on mitochondrial genome assemblies has contributed to resolving phylogenetic relationships among global buffalo populations.



Complementing these molecular datasets, machine-learning frameworks, including Random Forest, Support Vector Machine, Artificial Neural Networks and boosting algorithms such as XGBoost (Extreme Gradient Boosting) have been evaluated for breeding-value prediction in Murrah Buffalo, with models such as XGBoost achieving high predictive accuracy for 305-day milk yield. Together, these genomics, multi-omics and AI-driven initiatives form a unified systems-biology platform that deepens biological understanding, enhances genomic prediction accuracy and strengthens the foundation for future-ready, precision breeding programmes tailored to the genetic architecture and production environments of Indian buffaloes.

Challenges and Future Directions

While Omics technologies offer powerful opportunities for buffalo improvement, several challenges continue to restrict their large-scale use. Multi-omics datasets are inherently high-dimensional, requiring sophisticated statistical and computational frameworks for filtering, imputation, normalization, transformation, quality control and scaling (Li *et al.,* 2022). Technical issues within next-generation sequencing (NGS) workflows, including errors during sample handling, library preparation, PCR enrichment and sequencing can obscure true biological variation and hinder the detection of low-frequency variants (Ma *et al.,* 2019; Satam*et al.,* 2023). Short-read sequencing also performs poorly in repetitive and long-range genomic regions, reducing the contiguity and overall quality of genome assemblies. In India, fragmented herd structures, limited pedigree and phenotypic recording make it difficult to establish large, well-characterized buffalo reference populations, which are essential for accurate genomic prediction (Vohra, 2018). Artificial intelligence (AI) and machine learning (ML) face similar constraints: most models require large, clean and consistently recorded datasets, yet buffalo data remain small, heterogeneous and uneven across production systems. As a result, model generalizability is often poor and the “black-box” nature of deep learning reduces biological interpretability.

Advancing Omics-driven buffalo breeding will require coordinated efforts to build large, well-phenotyped, reference populations and to expand the use of long-read sequencing and pangenome assemblies for more complete variant discovery. In parallel, exploring genome editing tools such as CRISPR for targeted improvements and understanding host-microbiome interactions for feed efficiency and methane mitigation will open new avenues for enhancing buffalo productivity and resilience. Ultimately, embedding these technological innovations within a systems biology framework, supported by improved national data recording and digital infrastructure, will accelerate genetic gain, enhance environmental adaptability and contribute to the long-term sustainability of buffalo production systems.

Summary

Precision buffalo breeding is entering a transformative phase powered by genomics, multi-omics and artificial intelligence, enabling deeper decoding of complex traits and accelerating genetic gain. Breakthroughs in reference genomes, GWAS, TWAS, epigenomics, proteomics, metabolomics and AI-enabled prediction have shifted buffalo from a traditionally data-limited species to one supported by comprehensive molecular and computational resources. Realizing the full value of these innovations, however, requires their integration into structured breeding programs built on larger reference populations, robust and continuous phenotyping and explainable AI systems that ensure biologically credible predictions. With strategic investment in data infrastructure and systems-biology frameworks, India is well positioned to convert these scientific advances into sustained improvements in productivity, fertility, thermotolerance and resilience. Embedding these modern tools into routine breeding pipelines will ultimately allow the Livestock 5.0 vision to materialize, driving faster, smarter and more sustainable genetic progress in this cornerstone species of the dairy sector.

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LP 2.4. The trajectory of buffalo genomics in India: Progress and applications

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Buffaloes belong to the Bovidae family, which is divided into two genera: Bubalus and Syncerus. The genus Syncerus includes the African buffalo (*Synceruscaffer*): found in Sub-Saharan Africa. Conversely, the genus Bubalus comprises four wild species native to tropical Asia: the wild water buffalo (*Bubalus arnee*): the tamaraw (*Bubalus mindorensis*): the mountain anoa (*Bubalus quarlesi*) and the lowland anoa (*Bubalus depressicornis*). The domesticated water buffalo (*Bubalus bubalis*) is believed to have originated in regions such as the Indian subcontinent, China and Southeast Asia. Two primary subspecies of domesticated water buffalo are recognized today: the river buffalo (*Bubalus bubalis bubalis*): predominantly found in the Indian subcontinent and the Mediterranean, primarily valued for milk production and the swamp buffalo (*Bubalus bubalis carabanesis*): prevalent in Southeast Asia, mainly used for meat and draft work (Ahlawat *et al.*, 2024).Water buffaloes are considered a vital livestock resource in tropical and subtropical regions due to their exceptional milk production, adaptability to hot and humid environments and efficient feed conversion. According to the 20th Livestock Census, buffaloes constitute 20.46% of India's total livestock population of 536.76 million. They are integral to rural livelihoods, food security and the agricultural economy, contributing significantly to India's milk production, accounting for 44.81% of the 230.58 million tons of milk produced annually (Annual Report 2022-23, dahd.gov.in). In addition to milk and meat, buffaloes provide essential draught power for agricultural activities and produce dung, which is utilized as fuel and fertilizer. Buffaloes are often preferred over cattle in various parts of India due to their higher milk fat content and greater disease resistance. Their ability to maintain productivity under suboptimal management and feed conditions further enhances their utility.The diversity observed among Indian buffaloes in terms of physical characteristics, physiological traits and economically significant attributes is the result of a long history of local adaptation, artificial selection, mutations and genetic drift. Over the past few decades, extensive research has been conducted to unravel the genetic diversity and explore the genomic potential of buffaloes in India.

Evolution of buffalo genomics research in India

Genomic studies in India began in the 1990s with the use of microsatellite markers to assess genetic diversity in livestock. By the 2000s, single nucleotide polymorphism (SNP) markers gained prominence due to their higher resolution in livestock research. The 2010s saw the advent of genome-wide association studies (GWAS): which enabled the identification of genetic markers linked to desirable traits. The advent of Next Generation Sequencing (NGS) technologies has since transformed genomic research, allowing for comprehensive genome analysis and providing deeper insights into the complexities of buffalo genomics. Recent advances in genomic selection, marker-assisted breeding and conservation genomics are driving precision breeding and biodiversity preservation, ensuring adaptation to environmental challenges. Furthermore, transcriptomic and epigenomic analyses, such as transcriptome and methylome profiling, have provided valuable insights into gene expression and regulatory mechanisms.

Mitochondrial DNA-based studies

Mitochondrial DNA (mtDNA) possesses distinct characteristicsincluding a higher mutation rate than nuclear DNA, absence of introns and recombination and high copy number, that make it a powerful tool for phylogenetic and population genetic studies in livestock. A foundational study in India analyzed mitochondrial D-loop and cytochrome b gene sequences from eight riverine buffalo breeds in comparison with swamp buffaloes (Kumar *et al.*, 2007). This research provided evidence of separate domestication events for riverine and swamp buffaloes. Nagarajan *et al.* (2015) reinforced this conclusion by analyzing mtDNA control region sequences from riverine buffaloes sampled across India, Iran, Egypt and Pakistan. Their findings proposed that river buffaloes were first domesticated in the northwestern region of India before dispersing globally. Furthermore, Singh *et al.* (2020) explored D-loop sequence data from riverine, swamp and hybrid buffaloes in India, pinpointing northeastern India near the Indo-China border as the likely origin of swamp buffaloes.Subsequently, Singh *et al.* (2022) genetically characterized the Diara buffalo, a population adapted to wet paddy agriculture on the marshy banks of the Ganga River in Bihar, India. Mitochondrial D-loop sequencing of Diara buffaloesrevealed high haplotype and nucleotide diversity. The Diara population exhibited greater genetic diversity than the Murrah breed. The findings highlighted the Diara buffalo as a unique and genetically diverse local ecotype, underscoring its value for conservation and adaptation to specific agro-ecological niches.Although previous studies have primarily focused on specific mitochondrial regions, a recent investigation by Ahlawat *et al.* (2024) employed whole mitochondrial genome analysis to provide a comprehensive view of maternal diversity in buffaloes from the Indian subcontinent. Phylogenetic analysis revealed that Indian swamp buffaloes primarily belong to the SA haplogroup, with the novel identification of haplogroup SB2b in swamp buffaloes. For riverine buffaloes, the findings aligned with the known RB1, RB2 and RB3 sub-haplogroups, further supporting northwestern India as a key domestication center. Hierarchical AMOVA analysis revealed that 92.42% of total genetic variation was attributable to differences between the two subspecies. Pairwise F_{ST} analysis indicated substantial genetic differentiation between riverine and swamp buffaloes (F_{ST}=0.944). Whole mitogenome-based phylogenetic trees provided a clear demarcation of their evolutionary paths.

Microsatellite-based genetic diversity analysis

The genetic polymorphism and diversity of registered Indian buffalo breeds have been extensively studied over the past two decades using microsatellite markers, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs). Due to their neutrality, co-dominant inheritance and high polymorphic information content, microsatellite markers have become the preferred tool for assessing genetic diversity. Almost all registered buffalo breeds have been characterized using these markers, with most studies focusing on single populations to evaluate parameters such as allelic richness, gene flow and population bottlenecks. These investigations have generated substantial molecular data on buffalo characterization. For instance, SSR-based genetic analysis of Dharwadi buffaloes highlighted sufficient genetic variability within this buffalo population thereby generating valuable information for its recognition as a distinct breed (Sharma *et al.*, 2019). Some studies have also compared multiple populations to assess diversity patterns. For example, analysis of genetic diversity and population structure among three North Indian buffalo breeds: Murrah, Nili-Ravi and Gojri using STR markers showed average genetic differentiation (F_{ST}) between the breeds. STRUCTURE analysis assigned the populations into three clusters, revealing genetic admixture between Murrah and Nili-Ravi breeds while identifying Gojri as a distinct germplasm. This indicated a possible shared origin for Murrah and Nili-Ravi buffaloes (Vohra *et al.*, 2021a).Another notable study integrated genetic and geospatial data to analyze the genetic structure of buffalo populations. Using a panel of 100 microsatellite markers, 1055 samples from 12 breeds/populations across India were genotyped (Vijh *et al.* 2018). The study found a higher overall genetic differentiation (F_{ST}=10%) among the breeds than previously reported. STRUCTURE analysis supported the classification of Indian buffalo populations into four primary groups, providing evidence of genetic connectivity between Murrah, Nili-Ravi and several other breeds, highlighting their role in breed improvement programs. The use of a large number of microsatellite markers enhanced genome coverage, leading to precise and unbiased insights into genetic relationships and greater differentiation among buffalo populations.

Genome-wide association studies (GWAS)

Genome-wide association studies (GWAS) have become indispensable for deciphering the genetic underpinnings of key economic traits in water buffalo. Owing to rapid progress in genomic technologies, researchers in India have generated important insights into markers linked with production, reproductive efficiency and disease resistance. One notable advance involved a comparative analysis of water buffalo and cattle genomes to detect signatures of selection. By sequencing 79 buffaloes representing seven breeds and contrasting them with 294 cattle genomes, investigators uncovered common selective regions associated with body size, suggesting parallel domestication pressures in both species (Dutta *et al.*, 2020). These cross-species comparisons have deepened our understanding of the genetic determinants of domestication and adaptive traits in buffalo.The Murrah breed, renowned for its rich milk characterized by high fat and solids-not-fat (SNF) content, has been central to several GWAS initiatives in India. Using double-digest restriction-associated DNA (ddRAD) sequencing, one study identified more than 38,000 SNPs associated with milk yield, lactation persistence and fertility-related traits. Specific genomic intervals linked to test-day milk production, fat percentage and SNF were mapped, laying the groundwork for genomic prediction and early selection of superior animals. Additionally, several SNPs exhibited pleiotropic effects across multiple production traits, underscoring the value of GWAS in enhancing breeding programs for Murrah buffaloes (Vohra *et al.*, 2021b). Jaglan *et al* .(2023) identified seven SNPs in genes such as NCBP1 and FOXN3 associated with mastitis susceptibility, revealing roles in immunogenetics and metabolism. Complementarily, Chhotaray *et al.* (2023) used transcriptome-wide association studies (TWAS) to pinpoint key regulatory genes (ADCY5, PRL, GHR) and pathways (Relaxin, AMPK, JAK-STAT) governing lactation efficiency. Additionally, efforts to understand the genetic basis of growth, production and reproduction traits in Murrah buffaloes have also yielded significant results using weighted single-step GWAS (WssGWAS): identifying genomic regions explaining over 0.5% of additive genetic variance (George *et al.*, 2024).

Transcriptomic studies

Transcriptome studies in buffaloes have focused on identifying gene expression patterns related to lactation, immunity, host-parasite interactions, fertility etc. To understand the interplay of various genes and pathways, the milk transcriptome from three lactation stages of Murrah buffalo has been analyzed by RNA sequencing. Distinct patterns of gene expression have been delineated across early, mid and late lactation stages. Key genes such as CSN2, CSN1S1, CSN3, LALBA, SPP1,and TPT1 showed consistent high expression throughout lactation (Arora *et al.*, 2019). Early lactation was dominated by genes driving key metabolic processes, while mid lactation reflected a combination of metabolic and immune-related functions. In contrast, late lactation was primarily associated with heightened immune activity. Genes central to milk synthesis showed peak expression during the initial phase and progressively decreased as lactation advanced. Fourteen genes displaying significant differential expression were identified as promising candidates for future investigations into the molecular regulation of lactation. Overall, these insights provide a clearer picture of how gene expression shifts in coordination with milk production and immune function throughout the lactation period.Further, an in-depth comparison of the milk somatic cell transcriptomes in Sahiwal cattle and Murrah buffaloes highlighted notable immune response distinctions between the two species. Buffaloes exhibited an enrichment in innate and adaptive immune-related genes and higher expression of host defense peptides (Ahlawat *et al.*, 2021). These findings suggested that buffaloes possess a more robust immune mechanism, likely contributing to their lower susceptibility to mammary infections compared to cattle.

In another study, a functional genome profile of Murrah buffaloes analyzed RNA-seq data from high- and low-yield multiparous animals to investigate milk production traits. Differential gene expression revealed 142 upregulated and 83 downregulated genes associated with TNF-alpha, NF-kappa B and MAPK PI3K-AKT signaling pathways. These genes were linked to cell signaling, proliferation, differentiation, RNA splicing, fat metabolism and inflammasome activity, emphasizing the role of post-transcriptional and post-translational modifications in lactogenesis(Sikka *et al.*, 2023).A separate investigation compared sperm functionality and gene expression patterns in buffalo bulls with contrasting fertility levels. Bulls classified as low fertile exhibited significantly reduced sperm plasma membrane and acrosomal integrity ($P<0.05$): along with elevated protamine deficiency and lipid peroxidation ($P<0.05$): relative to their high-fertility counterparts. Using an mRNA microarray platform, the study identified 709 transcripts that differed markedly between the two groups. Most of these fertility-associated transcripts were linked to molecular binding functions, gene regulation, protein synthesis and key metabolic activities, predominantly localized in the nucleus, nucleoplasm and cytosol. Additionally, spermatozoa from low-fertile bulls showed disruption in several critical pathways, including MAPK signaling, ribosomal function and oxidative phosphorylation (Paul *et al.*, 2021).Transcriptome analysis has also been attempted for understanding host response to various diseases in buffaloes. For example, gene expression changes in buffalo milk affected by clinical mastitis compared to healthy controls have been analysed (Ahlawat *et al.*, 2022). The study identified a substantial transcriptional shift in mastitic milk, with 1,014 genes showing increased expression and 999 exhibiting reduced expression. Functional enrichment analysis indicated that many of these genes were associated with immune function and inflammatory processes. The pronounced upregulation of defense-related molecules, including granulysin, perforin, various granzymes, β-defensins, cathelicidins and peptidoglycan recognition protein 1, pointed to an intensified immune response within the affected mammary gland. In addition, several major regulatory factors such as **E2F4, E2F1, RBL2, FOXM1, IRF1 and MYB** emerged as important contributors, underscoring their involvement in controlling key cellular and metabolic pathways.

Development and utilization of SNP Chips in buffaloes

Advances in whole-genome single-nucleotide polymorphism (SNP) analysis have transformed the study of animal genetics by enabling detailed characterization of genetic variation within livestock populations. The creation of SNP chips has become achievable due to well-annotated reference genomes, sophisticated bioinformatics and advanced high-throughput technologies for data processing. SNP genotyping arrays serve as efficient tools, offering a cost-effective and less complex alternative to whole-genome sequencing, allowing breeders to obtain comprehensive genomic profiles for selective breeding. This technology enhances the accuracy and efficiency of predicting an animal’s breeding value through the genotyping of numerous polymorphic markers. Researchers in India have employed the Axiom® Buffalo Genotyping Array to examine genetic variability and population structure across different buffalo breeds from diverse geographical regions (Thakor *et al.*, 2021). However, an evaluation of the Illumina® BovineHDBeadChip for genotyping Indian riverine buffalo breeds produced less promising results, underscoring the necessity for a customized SNP marker panel specifically designed for Indian riverine buffaloes (Shah *et al.*, 2022). In a significant recent advancement, a "Unified Genomic Chip" known as the

Mahish Chip has been developed in India through collaborative efforts of many research institutes. This specialized SNP chip is expected to facilitate the direct application of DNA-based technologies to improve the genetic potential of buffalo populations in the country.This innovation will enable early identification of genetically superior bulls that can significantly enhance livestock productivity and breed improvement efforts.

Conclusion and future perspectives

Indian buffalo genomics has made remarkable progress, using advancements in molecular biology and next-generation sequencing technologies to address challenges and exploit opportunities in buffalo breeding and conservation. These efforts have significantly enhanced the understanding of buffalo genetics, from deciphering population structure using microsatellites to employing GWAS for pinpointing markers linked to economically important traits. Mitochondrial DNA studies have unraveled the maternal genetic diversity of Indian buffaloes, tracing domestication events and highlighting genetic distinctions between riverine and swamp buffalo subspecies. The advent of transcriptomic analyses has provided new insights into gene expression and regulatory mechanisms underlying traits like lactation, disease resistance and reproduction. Innovations such as the development of customized SNP chips will revolutionize precision breeding by enabling early selection of superior buffaloes and enhancing genetic gains.As research progresses, expanding datasets and incorporating advanced bioinformatics tools will further refine our understanding of buffalo genetics. Future efforts should focus on functional validation of candidate genes, integration of multi-omics data and the implementation of large-scale genomic selection programs. These steps are crucial for accelerating genetic gain, enhancing climate resilience and ensuring the sustainable conservation of valuable buffalo genetic resources, solidifying India's leadership in buffalo genomics.

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ORAL PRESENTATION

OP-2.1. Decoding Early Pregnancy in Buffalo Through mRNA-lncRNA-circRNA-miRNA Network Integration

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Early pregnancy in buffalo involves highly coordinated molecular events, yet the regulatory roles of non-coding RNAs (ncRNAs) remain insufficiently understood. ncRNAs–lncRNAs, circRNAs and miRNAs– modulate gene expression through competitive endogenous RNA (ceRNA) interactions and may influence early embryonic development. This study integrated mRNA-lncRNA-circRNA-miRNA expression profiles to unravel regulatory networks associated with early pregnancy.Whole-transcriptome sequencing of cotyledonary tissue from early (0-40 days; n=4) and mid-pregnancy (41-80 days; n=3) using RiboZero depletion generated ~75 million clean reads per sample (80-95% alignment to NDDB_SH_1). circRNAs were identified using CIRI2 and SkeeCRIT, lncRNAs using CLEAR and differential expression was assessed via DESeq2 ($|\log_2 FC| \geq 2$; padj< 0.05 for mRNAs/lncRNAs; p<0.05 for circRNAs). Eight qPCR-validated pregnancy-associated miRNAs were used for target prediction (miRanda, miRWalk). Pearson correlation ($r \geq 0.9$) combined with miRNA-target pairing enabled ceRNA network construction. Functional enrichment (STRING, g: Profiler, ClueGO): clustering (MCODE) and hub-gene analysis (CytoHubba) were performed. A total of 375 mRNAs, 115 lncRNAs and 175 circRNAs were significantly expressed. Several circRNA-associated genes ($\log_2$FC>2) were linked to immune modulation, extracellular matrix organization, epithelial differentiation, nutrient sensing and early embryonic development. Seven major functional clusters highlighted pathways essential for implantation. Key regulators identified across cluster and hub analyses–including buf-miR-27, circRNA40, EPB42, lncRNA16, lncRNA2, lncRNA22, lncRNA23, LOC102405260, LOC123330880, OSMR, TSPAN7 and WT1–were specific to early pregnancy and may play crucial roles in implantation biology. These findings provide new insight into ncRNA-mediated regulation during early pregnancy in buffalo and identify potential biomarkers for early pregnancy detection.

OP-2.2. Comparative Transcriptome Profiling Unveils the Genetic Basis of Salinity Tolerance in Chilika Buffalo

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The Chilika buffalo, an indigenous breed from India's eastern coastline, is well adapted to the brackish and saline conditions of the Chilika Lake ecosystem. This study investigates transcriptome-wide differences between Chilika and Murrah buffalo to unveil the molecular mechanisms underlying the Chilika breed's adaptation to its high-salinity habitat. To identify differential gene expression associated with salinity tolerance, transcriptome data were generated from eight skin tissue samples of Chilika and Murrah buffalo. Following quality assessment and filtering, the high-quality reads were mapped to the buffalo reference genome (*Bubalus bubalis NDDB_SH_1*) using the HISAT2 alignment tool.Read quantification was carried out using the feature Counts tool and Differentially Expressed Genes (DEGs) were identified through the edgeR package in R.A total of 10,034 DEGs (777 upregulated and 9257 downregulated expression) were found to be significantly expressed in skin tissue (p-value<0.05 and log2fold change>2).Further, a total of eleven DEGs (*KRT1, KRT5, KRT6A, KRT10, DSG1, PRR9, ELOVL3, S100A8, SFN, ASPRV1* and *TCHH*) were identified as being related to salinity. Pathwayenrichment analysis indicated that several key pathways, including neuroactive ligand-receptor interaction, PPAR signaling, fat digestion and absorption, complement and coagulation cascades, GABAergic and glutamatergic synapses, steroid biosynthesis, FOXO signaling, Hippo signaling, biosynthesis of unsaturated fatty acids and fatty acid elongation, play crucial roles in salinity adaptation. These pathways collectively contribute to maintaining skin integrity and resilience, enhancing the synthesis of protective skin proteins and supporting homeostasis, osmotic regulation and water-electrolyte balance essential for survival in saline environments. In conclusion, this study uncovers the genetic basis underlying the adaptive function of the Chilika buffalo and deepens our understanding of salinity-driven genomic evolution. These insights can support the strategic and sustainable utilization of this unique germplasm within its native Chilika ecosystem.

OP-2.3. Mitogenome and CNV profiling of 450 genomes reveals domestication history and adaptive divergence of global buffaloes

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Domestic buffaloes are dispersed across all five continents and serve as a key livestock species in tropical and subtropical ecosystems, providing milk, meat and draught power for rice cultivation. River and swamp buffaloes are widely accepted to share a common origin from the wild Asian buffalo (*Bubalus arnee*); however, the demographic history and domestication processes of these lineages remain insufficiently explored. Mitochondrial DNA (mtDNA) is considered a robust marker for reconstructing domestication pathways and resolving the phylogenetic structure within species. Similarly, copy number variations (CNVs) also play a major role in livestock adaptation, as they encompass larger genomic regions than SNPs and therefore exert stronger phenotypic effects. By analysing both mitochondrial and nuclear genomic variation, it is possible to reconstruct the timing, geographic centres and genetic consequences of buffalo domestication. In this study, complete mitochondrial genomes and CNV profiles were analysed from 450 whole-genome resequencing (WGS) datasets representing 57 global populations across 17 nations. The dataset included 13 wild, 289 riverine and 145 swamp buffaloes, with 93 samples belonging to seven Indian breeds. MtDNA analysis identified 1,428 variable sites with 742 parsimony-informative sites in riverine buffaloes, yielding a high haplotype diversity (0.997). Within Indian populations alone, 866 variable sites were detected. Both riverine (Tajima's D: -2.44; Fu and Li's D: -6.32) and Indian (Tajima's D: -2.22; Fu and Li's D: -3.82) groups exhibited significant negative neutrality statistics, indicating signatures of population expansion and directional selection in the mitochondrial genome. Phylogenetic reconstruction confirmed sub-haplogroups RB1, RB2 and RB3 in Indian breeds, reinforcing the role of northwestern India as a principal domestication centre for riverine buffalo. A high-resolution CNV map was constructed for Buffalo global breeds using WGS. Severalgenes within overlapping CNVRs, including *ZEB2*, *IL10* and *SDC1*, were found to be shared among breeds and were implicated in growth factor signalling, homeostasis and milk production. The CNVRs offered key insights into the evolutionary history of these breeds and highlighted genomic regions likely subjected to selection within each population. Overall, the study enhances the global understanding of buffalo mitochondrial and nuclear diversity, offering key insights into domestication, evolutionary history and breed adaptation.

Keywords: *Global buffaloes, Domestication, MtDNA, CNVs, WGS*

OP-2.4. Big Data and Multi-Omics Network Analysis for Identifying Sex-Dependent Reproductive Biomarkers in Farm Animals

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Multi-Omics approach is one of the advanced techniques in the biomarker discovery. The role of Multi-Omics approach in drug discovery and cancer studies by usingan integrated analysis of mRNA, lncRNA, miRNA and proteomics have lead to the identification of specific molecules in terms of diseased. In case, of reproductive biology and biotechnology Multi-Omics studies revealed a prominent information helped in deciphering infertility and helped in embryo production success. The main objective is to develop a network analysis between X and Yspermatozoa of cattle by integrating miRNA, mRNA, lncRNA and proteomics data, for identifying sex-specific pathways and biomarkers for a better reproductive success in cattle reproduction. Data related to X and Y spermatozoa of cattle such asmRNA, miRNA from NCBI; proteomics data (proteome X change) retrieved and analyzed to identify the DEPs, Differential miRNA, DEPs and DElncRNA done by using command line by using miRDeep2, salmon, bowtie2, cuff compare and finally the DESeq2 RStudio package for DE analysis. Further integration was done to find out the co-differential expression patterns of all data types, genetarget prediction and functional enrichment analysis by using RStudio packages like dply, BiomaRt, limma and Genomic Ranges. In the end a comprehensive ceRNA Network Construction was done to know the effects on each other molecules in terms of fertilization and embryo development. In differential analysis a total of 81 genes are significantly down and 16 genes are significantly up in mRNA; in lncRNA analysis 31 are significantly down regulated and 18aresignificantly up regulated; in miRNA 13are significantly down and 8 are significantly up. By using Target Scan prediction we found out the miRNA targets and further integration for co-expression analysis showed major reproductive pathways regulation in terms of motility and fertilization. The ceRNA network analysis reveled the major interactions and enrichment analysis helped to find sex-specific reproductive molecular Interactomes in cattle. In conclusion, Interactome and interwork analysis by using Bioinformatics approach helped in finding major sex-specific regulatory mechanisms either in X/Y spermatozoa of cattle.

Keywords: *Multi-Omics,miRNA,DEPs,Interactome,Sex-sortedsemen,Sorting*

OP 2.5 Construction of the Indian Buffalo Pangenome Using an Iterative Mapping Approach

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Buffaloes are regarded as “Black Gold” due to their significant contribution to global food security and the economy through the production of milk, meat, skins, draught power and other essential resources. High-quality reference genomes are fundamental in modern genomics, enabling advancements in animal productivity, health and genetic improvement. A pangenome represents a more comprehensive genomic framework capable of capturing population-specific variation, unlike a single reference genome that is typically assembled from one or a few individuals and therefore fails to encompass the full genetic diversity of a species. Constructing a pangenome from a large number of individuals helps overcome reference bias associated with linear genomes and supports more accurate downstream analyses. In this study, we constructed the first Indian buffalo pangenome using Whole Genome Sequencing (WGS) data from 264 animals generated by the National Dairy Development Board (NDDB, Gujarat) through Illumina paired-end sequencing. These samples represent ten buffalo breeds, including nine riverine breeds (Murrah, Mehsana, Jaffarabadi, Bhadawari, Banni, Nili-Ravi, Surti, Pandharpuri and Toda) and one swamp breed (Luit). Using the PanGenome Analysis Pipeline, we identified non-reference novel sequences (NRNS) absent from the current riverine buffalo reference genome (NDDB_SH_1). The major steps included quality filtering of raw reads, alignment to NDDB_SH_1, extraction of unaligned reads, de novo assembly, contamination removal and redundancy filtering.

This workflow yielded 87 Mb of high-confidence NRNS from the 264 individuals. These sequences were then integrated with the NDDB_SH_1 reference genome to generate the first-ever Indian buffalo pangenome, offering an expanded and more accurate representation of buffalo genomic diversity.

Keywords: *Pangenome, buffalo, WGS buffalo, Non Reference Sequences*

OP-2.6. Comprehensive CNV Landscape and Trait-Linked CNVRs in Murrah Buffalo

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Worldwide, riverine buffaloes-particularly the Murrah breed-are valued for their great economic value in dairy and meat production systems, their remarkable ability to adapt to difficult environmental conditions and their effective use of roughages of low quality. Copy number variants (CNVs) and other structural genomic variations are still poorly understood in this species, despite their significance. In the present study, we conducted a comprehensive genome-wide assessment of CNVs in 237 Murrah buffaloes utilizing the Axiom® Buffalo 90K Genotyping Array (Affymetrix, Santa Clara, CA, USA). Using the univariate method of the Copy Number Analysis Module (CNAM): CNV detection was carried out on every autosome. A total of 7,834 CNVs with an average size of 102.04 kb and lengths ranging from 7.3 kb to 3.47 Mb were found in 201 animals. The conserved character of CNV landscapes among ruminants was highlighted by the fact that these structural variants accounted for roughly 9.24% of the buffalo genome, a percentage that is in line with CNV reports in similar livestock species including cattle, sheep and goats. 1,322 CNV regions (CNVRs) were found when overlapping CNVs were combined to refine the dataset. 436 genes were found inside 146 CNVRs that were present in at least 12 individuals, according to gene annotation, suggesting areas of possible biological and evolutionary significance. Notably, 45 genes previously linked to 63 economically significant traits-such as immunity, growth, reproduction, milk production and adaptability-were found in 32 CNVRs. These trait-associated CNVRs highlight the functional significance of structural changes in influencing phenotypic variablity within the Murrah breed. This study offers the most comprehensive CNV landscape for Murrah buffalo to date, providing insights into the structural genetic architecture. In order to increase productivity, resilience and overall genetic merit in buffalo herds, functionally relevant CNVRs and the genes that are linked to them offer interesting targets for next genomic selection, functional validation and breeding initiatives.

OP-2.7. Polymorphism in STAR gene known to affect silent heat in buffaloes

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Silent estrus significantly reduces reproductive performance in buffaloes due to ineffective estrous expression. The present study was aimed to ascertain the polymorphism in the *Steroidogenic Acute Regulatory* (*STAR*) gene in murrah buffaloes. Genomic DNA (30 normal/control and 30 exhibiting silent estrus/test) was isolated from blood samples obtained from the animals housed at the Cattle and Buffalo farm of ICAR-IVRI, Izatnagar and processed for genotyping using the PCR-RFLP technique using *BSu*RI restriction enzyme. The amplification of the *STAR* gene locus (fragment of 203 bp) was performed, followed by restriction enzyme digestion, to identify the genotypes of the animals. In the normal / control group, the genotypic frequency observed for AA, AB and BB genotypes was 50, 23 and 27% respectively. The gene frequency of A and B alleles were 62 and 38% respectively. Similarly, for silent heat/test group, the genotypic frequency observed for AA, AB and BB genotypes was 23, 20 and 57% respectively. The gene frequency of A and B alleles were 33 and 67% respectively.

Keywords: *Buffalo, Steroidogenic Acute Regulatory (STAR): silent estrus, PCR-RFLP, BSuRI*

OP-2.8. Proteome-Driven Insights into Thermotolerance: A Roadmap for Climate-Smart Buffalo Breeding

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Buffalo production systems face growing vulnerability under accelerating climate change, with heat stress emerging as a critical constraint to reproductive efficiency. Buffalo bulls, being inherently thermosensitive, experience sharp declines in seminal quality during thermal load, ultimately threatening fertility, genetic progress and national dairy productivity. The present study employed an integrative reproductive and proteomic approach to decipher the biological mechanisms underpinning thermotolerance in Murrah breeding bulls. Fifteen bulls were monitored across contrasting environmental conditions and categorized into heat-stress affected, heat-stress resilient and comfort-season groups. Classical semen evaluations revealed a pronounced seasonal depression in motility and acrosome integrity in heat-affected bulls. To map the molecular response to heat, high-resolution LC-MS/MS-based seminal plasma proteomics was conducted, identifying 1,222 proteins involved in cellular protection, energy metabolism, oxidative defense, membrane stabilization and fertility regulation. Distinct proteomic signatures differentiated thermoresilient from thermosusceptible bulls. Heat-affected bulls overexpressed canonical stress proteins, including HSP70, HSPA1L and Serum Albumin, indicative of activated protein-repair and cytoprotective pathways attempting to mitigate thermal and oxidative insults. Conversely, heat-resilient bulls showed elevated levels of fertility-enhancing and immunoprotective proteins such as DEFB122A, PRDX5 and ACRV1 markers linked to antioxidative capacity, membrane stability and sperm longevity. Notably, several unique proteins identified exclusively in heat-affected bulls (e.g., SCRN1, GALE, B4GALT3, PRXL2B, GSTP1, TSTD1, SELENBP1, SLC1A1, HS6ST1, HYAL2) emerged as strong biomarker candidates for large-scale semen quality screening and early detection of thermosensitive sires. This integrative analysis provides the first comprehensive proteomic evidence of how thermal stress reshapes buffalo reproductive biology at both cellular and molecular levels. More importantly, it identifies novel protein biomarkers with direct utility in breeding programs aimed at selecting climate-resilient, high-fertility bulls. Incorporating these omics-driven indicators into genetic improvement frameworks can reduce insemination failures, safeguard reproductive efficiency and build a thermotolerant buffalo population capable of sustaining milk production under future climate scenarios. The study demonstrates how innovative reproductive omics can accelerate climate-smart buffalo breeding, ensuring sustainable production, enhanced animal health and food security in a warming world.

Keywords: *Heat stress, Seminal plasma, Biomarkers, Climate resilience*

Poster Presentation

PP-2.1. GWAS with PCA Identifies Genes with Comprehensive Effects on Buffalo Milk Traits

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90 DNA samples 0f five riverine Indian Buffalo Breeds Bhadawari (15): Mehsana (15): Murrah (30): Pandharpuri (15) & Surti(15) were sequenced via genotyping-by-sequencing employing the double-digestion restriction-associated DNA (ddRAD) tag. Alignment was done against *Bubalus bubalis* (water Buffalo) NDDB_SH_1 reference genome. Total 2,31,483 high quality SNPs were identified. Distinct clusters were revealed between animals of five breeds using principal component analysis with genomic data. This study is done as a proof of concept of using principal components (PCs) as dependent variables for a genome-wide association study (GWAS). In this study, principal component analysis (PCA) on 9 typical traits related to milk, growth and reproductiontraits (Average milk yield, Age at first calving, Calving Interval, Animal Height, Body Weight and Heart girth, Milk fat percentage, Milk protein percentage and SNF percentage) revealed that the first principal component (PC1): provided the most information on traits that determine milk traits. A genome-wide association study (GWAS) using PC1 as a dependent variable was used to find out the genes (present on Chr 2) that are comprehensively controlling the milk traits. STAT1, STAT4, EPHA2 and INSIG2 are important genes related with milk yield, mammary gland development and fat metabolism in milk; Gene MYLK3I (Chr18) related to Calciumsignalling pathway, muscle system development are some important findings of the study. Identification of such genomic regions shall help in carrying out an early selection of high-yielding persistent buffaloes and, in the long run, would be helpful in shaping their future genetic improvement programs.

Keywords: *Buffalo, GWAS, PCA*

PP-2.2. Evaluation of the Indian Buffalo Pangenome as an Enhanced Reference for Read Mapping and Variant Discovery

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Traditional single linear reference genomes often fail to capture the full extent of genomic diversity present within livestock species because it was built from one or few individuals, leading to reference bias and reduced accuracy in downstream analyses. To address this limitation, we recently constructed the Indian buffalo pangenome by integrating the NDDB_SH_1 reference assembly with 25,403 non-reference novel sequences (NRNSs) totaling ~87 Mb. In the present study, we evaluated the effectiveness of this pangenome as a reference for read alignment and variant discovery. Whole-genome sequencing data from 23 buffalo individuals were aligned to both the NDDB_SH_1 reference and the newly constructed pangenome. Mapping statistics were assessed using Samtools v1.11. For SNP and indel discovery, duplicate reads were marked using Picard Tools v3.1, followed by variant calling with GATK v4.6.2.0 using best-practice workflows. High-confidence variants were filtered based on QUAL>30, QD>2 and MQ>20. Across all samples, the pangenome demonstrated consistently higher alignment rate. The average mapping rate

increased from 92.58% (NDDB_SH_1) to 94.10%, while the average properly paired read percentage improved from 77.24% to 78.81%. Several genetically diverse individuals showed improvements of up to 8-10%, indicating that the added NRNSs effectively resolve previously unmapped genomic regions. Although detailed variant counts varied across samples, the pangenome consistently yielded a higher number of high-quality SNPs compared to the single reference, reflecting improved sensitivity and reduced reference bias. Overall, our findings demonstrate that the Indian buffalo pangenome provides superior read mappability and more accurate variant detection, validating its utility as an enhanced reference for population-scale buffalo genomics.

Keywords: *Pangenome, buffalo, WGS buffalo, Non Reference Sequences, genome mapping*

PP-2.3. Inter-breed genomic scans uncovered adaptive signals underlying salinity tolerance in Chilika buffalo

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Chilika buffalo, indigenous to the eastern coastal region of India, are primarily distributed around the brackish waters of the Chilika Lake in Odisha. This breed exhibits a remarkable ability to thrivein saline lake environments, reflecting a high level of ecological adaptation to salinity stress. Despite this unique resilience, their population has declined in the last decade, largely due to the increasing preference for Murrah buffalo to enhance milk productivity in the region. However, Murrah animals and their crossbreds may not be equally suited to withstand the harsh, high-salinity conditions characteristic of the Chilika ecosystem. The present study investigated the genomic mechanisms underlying the Chilika buffalo's adaptation to a harsh saline environment by conducting aninter-breed genomic analysis with the Murrah buffalo. Whole-genome resequencing (WGS) was performed on DNA samples from 18 Chilika and 10 Murrah buffaloes. Following stringent quality control and removal of low-quality reads, the high-quality sequences were aligned to the buffalo (*Bubalus bubalis*) reference genome (NDDB_SH_1) using BWA.A total of 22,177,610 SNPs in Chilika and 17,472,799 SNPs in Murrah buffalo were identified and subsequently used for downstream analyses. Inter-breed genomic contrast analyses were performed to detect regions exhibiting disproportionate variability and differentiation between Chilika and Murrah buffalo using the VCF tool. Gene annotation was done using GeneXtract, an in-house Python-based GUI tool. A total of 54genes were identified in common within the selective sweep region. Furthermore, genes such as *CAMKMT, COMMD1, EXOC6B, PRDM8, B3GNT2, MYLK3* and *NCKAP5*were identified as being related to salinity. Pathwayenrichment analysis indicated that several key pathways, including Glycosaminoglycan biosynthesis–keratan sulfate, Glycosphingolipid biosynthesis–lacto and neolacto series and Lysine degradation, play crucial roles in salinity adaptation. In conclusion, this study elucidates the genetic underpinnings of local adaptation in the Chilika buffalo and enhances our understanding of salinity-driven genomic evolution. These insights will contribute to the sustainable utilization of this unique germplasm within its native Chilika habitat.

PP-2.4. A Novel Surfaceome-Guided Approach to Next-Generation Sex-Specific Sperm Sorting

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The surfaceome comprises plasma membrane proteins with extracellular domains that mediate communication between the intracellular and extracellular environment. In spermatozoa, the surfaceome consists of receptors, transporters, ion channels, adhesion molecules and membrane-associated enzymes, providing an accessible pool of candidate biomarkers for distinguishing X- and Y-bearing sperm. This study aims to identify surface proteins differentially expressed between X and Y sperm to support next-generation, non-invasive sex-sorting strategies. LC-MS/MS proteomics datasets from Proteome X change were analyzed to identify DEPs, functionally annotated using DAVID and g: Profiler and corresponding FASTA sequences were used to predict differential and sperm-type-specific surface proteins. From the analysis, we have found

that 36 and 40 proteins were up-regulated in X and Y sperm, respectively. Furthermore, 151 and 88 proteins are specifically upregulated in X and Y spermatozoa and bioinformatics analysis for the plasma membrane fraction with surface-exposed proteins revealed a total of 38 proteins with transmembrane domains by using deepTMHMM and TMHMM online servers. Sub-localization tools like BUSCA, deeploc 2.0 and WolfPsort predicted the localization of the proteins in the organelles. Finally, using Venny 2.0, we have filtered further into a very few proteins for Surfaceome analysis. In conclusion, this analysis has predicted the potential surfaceome targets for sorting of X and Y sperm by proposing a next-generation sorting technique.

Keywords: *Sperm Surfaceome, Differential Proteomics, X- and Y-Bearing Sperm, Sex-Sorting Biomarkers, Bioinformatics Analysis*

PP-2.5. Genetic Characterization of Exon 11 of the LHR Gene and its Association with Reproductive and Production Traits in Surti Buffaloes

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The objective of the present investigation was to characterize genetic variation within exon 11 of the Luteinizing Hormone Receptor (LHR) gene and to assess its association with key production and reproductive traits in Surti buffaloes. The study involved 50 Surti buffaloes maintained at the Livestock Research Station, Kamdhenu University, Navsari. Blood samples were collected from all animals and genomic DNA was extracted following standard protocols. A 303 bp fragment corresponding to exon 11 of the LHR gene was amplified using polymerase chain reaction (PCR). Genotyping of the amplified product was performed through PCR-RFLP analysis employing the *HhaI* restriction enzyme to detect polymorphic sites. Restriction digestion revealed two genotypes, TT and TC, indicating the presence of a single nucleotide polymorphism within this exon of the LHR gene. Analysis of genotype and allele frequencies confirmed that the population is under Hardy-Weinberg Equilibrium.To determine the potential functional relevance of the observed polymorphism, association analyses were conducted for key reproductive traits i.e.age at first calving, service period and calving intervalas well as production traits including total milk yield and lactation length. Statistical evaluation indicated that the LHR exon 11 genotypes exerted no significant influence on any of the examined traits. These findings suggest that the identified polymorphism in exon 11 of the LHR gene does not significantly affect reproductive performance or milk production in Surti buffaloes. Although the LHR gene plays a central role in reproductive hormone signaling, the specific genomic region examined in this study appears to contribute minimally to phenotypic variation in the traits assessed and hence showing that any genotype would perform almost equally as far as these traits are concerned. Future research incorporating larger sample sizes, additional genomic regions and functional validation studies is recommended to enhance understanding of the genetic determinants influencing fertility and productivity in Surti buffaloes.

Keywords: *LHR Gene, Surti buffalo, Polymorphism*

PP-2.6. Genomic Signatures of Selection Reveal Adaptive and Economically Important Traits in Murrah Buffalo

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Riverine buffaloes, particularly the Murrah breed, are significant worldwide for their ability to thrive in challenging environments and efficiently convert low-quality feed into high-value milk. Comparative genomics shows that, after diverging from cattle, buffalo evolved expansions and contractions in gene families related to protein degradation, olfaction, detoxification and immune regulation. In this study, we analyzed genomic signatures of selection in 333 Murrah buffaloes genotyped with the Axiom® Buffalo Genotyping Array 90K. We employed three complementary statistical methods–Tajima's D, Composite Likelihood Ratio (CLR) and Integrated Haplotype Score (iHS)–to identify regions of the genome under selective pressure. Several candidate genes associated with milk production and dairy traits exhibited strong selection signals. These included genes involved in milk protein synthesis (CSN1S1, CSN3): fat metabolism (LPL, DGAT1): as well as significant QTL-linked genes related to milk yield and composition (ANKRD17, RASSF6). We also identified loci associated with heat tolerance (HSF1, HSF5) and coat color variation

(MC1R, KITLG): reflecting adaptation to tropical production systems. Additionally, we identifiedunique genomic regions under selection for horn patterning. Strong selection signals were identified near HOXD1 and ZEB2 on chromosome 2, TWIST1 on chromosome 8 and RXFP2 on chromosome 13–genes critical for craniofacial development and horn bud formation. Collectively, the genes and QTLs identified provide valuable insights into the selective forces shaping the Murrah genome for milk production, horn morphology and overall performance. This study serves as a crucial genomic resource to support future association studies and improved breeding strategies for Indian buffalo populations.

PP-2.7. Variability in OXT gene in Indian water buffaloes known to affect quiet ovulation

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Quiet estrus or silent estrus, is one of the key phenomena that is known to limit the reproductive efficiency and herd management in the dairy industry. The present study was aimed to investigate the polymorphism in the *Oxytocin*(*OXT*) gene in Indian water buffaloes of murrah breed. Genomic DNA (30 normal/control and 30 exhibiting silent estrus/test) was isolated from blood samples obtained from the animals housed at the Cattle and Buffalo farm of ICAR-IVRI, Izatnagar and processed for genotyping using the PCR-RFLP technique using *Eco*47I restriction enzyme. The amplification of the *OXT* gene locus (fragment of 214 bp) was performed, followed by restriction enzyme digestion, to identify the genotypes of the animals. In the normal / control group, the genotypic frequency observed for AA, AB and BB genotypes was 20, 27 and 53% respectively. The gene frequency of A and B alleles were 33 and 67% respectively. Similarly, for silent heat/test group, the genotypic frequency observed for AA, AB and BB genotypes was 50, 23 and 27% respectively. The gene frequency of A and B alleles were 62 and 38% respectively.

Keywords: *Buffalo, Oxytocin gene (OXT): silent estrus, PCR-RFLP, Eco47I*

PP-2.8. High-Resolution Copy Number Variation Landscapes of Indian Buffalo Breeds Using a Read-Depth Framework on Whole-Genome Resequencing Data

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Structural variants, particularly copy number variations (CNVs): play a critical role in shaping genomic diversity and influencing key physiological and pathological traits. The present study provides a high-resolution CNV landscape for seven Indian buffalo breeds using whole-genome resequencing data. Following stringent quality control, reads were aligned to the latest *Bubalus bubalis* draft genome and CNVs were identified using the read-depth-based tool CNVnator across multiple bin sizes. Adjacent CNVs were subsequently merged to define copy number variation regions (CNVRs): enabling comparative assessment of their genomic distribution, size and frequency across breeds. The analyses demonstrated that higher sequencing depth substantially improved CNV detection resolution, as reflected by the reduced average CNVR size at 30X (0.201 Mb) compared with 10X (0.013 Mb): with Banni buffaloes presenting the smallest and most refined CNVRs. Deletion and duplication events dominated the CNV spectrum, whereas mixed CNVs occurred far less frequently in all breeds. The number of CNVs varied widely across populations: Murrah exhibited the highest count at 10X (2,627 CNVs): while Pandharpuri showed the greatest number at 30X (25,688 CNVs). In contrast, Surti consistently displayed the lowest CNV counts across both sequencing depths (2,092 and 17,373 CNVs). When aggregated into CNVRs, Jaffarabadi presented the highest number of regions (833 at 10× and 10,179 at 30×): whereas Surti again showed the lowest (783 and 7,553, respectively). Across all breeds and coverage levels, deletions were more prevalent than duplications, suggesting shared structural variant patterns within buffalo genomes. Functional annotation of genes overlapping common and large CNVRs revealed signatures of selection and offered insights into the evolutionary divergence of these geographically distinct breeds. Overall, this work represents the first comprehensive, high-resolution CNV map across major buffalo populations and provides a valuable resource for understanding their evolutionary history and the genomic determinants underlying breed-specific traits.

Session-III

Physiology and Reproduction

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LP-3.1 Buffalo Production under Impending Climate Change: Opportunities and Challenges

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ABSTRACT

The effectiveness and sustainability of the buffalo production systems of our nation are seriously threatened by impending climate change. The physiology of buffalo, feed availability, reproductive efficiency and total productivity are all negatively impacted by rising temperatures, higher humidity, protracted heat waves, irregular rainfall and frequent droughts. The reduced feed intake, lower milk yield, impaired rumen function, suppressed immunity and lower conception rates are all consequences of heat stress, which are already noticeable in hot, humid and hot dry regions of the nation. These difficulties worsen the livestock production system due to a lack of water and deteriorating natural feed resources. Our country has special prospects despite these limitations. Native buffalo breeds are well-suited to climate-smart livestock systems due to their innate flexibility, resilience and disease tolerance. Climate impacts can be considerably reduced by implementing better housing designs, cooling techniques (misting, shading and wallowing), drought-resilient fodder cultivation, balanced ration formulation and mineral supplementation. Additional avenues for adaptation include promoting integrated farming practices, using ICT-based early warning systems and selecting for thermotolerance. In the face of future climate uncertainty, buffalo production in India may remain robust and sustainable with targeted research, policy support and farmer training.

1. Introduction

The ecology encompassing the human, agricultural and animal farming sectors is seen as seriously threatened by global warming. The genuine and urgent problem of climate change seriously threatens the survival and sustainability of bovine species worldwide. Abrupt and significant climate changes have increased the frequency of variations in environmental variables, especially ambient temperature, humidity, drought and floods, wind speed and solar radiation. The severity and duration of these extreme weather events may increase in the years to come, which could harm all life on Earth, including humans, agriculture and the animal farming system. The demand for livestock products is expected to increase by the middle of the twenty-first century and it has been established that climate change will affect livestock production through competition for natural resources, changes in the quantity and quality of feed, effects on livestock health, heat stress and biodiversity loss. Climate change is another significant and detrimental environmental stressor for livestock, impacting their well-being and productivity, decreasing reproduction and impairing immunity and health. According to future projections, the severity of the heat stress issue will increase as climate change is linked to increased global warming concerns (Gupta *et al.*, 2022).

The Intergovernmental Panel on Climate Change estimates that by 2100, the average global surface temperature will increase by 1.4°C to 5.8°C (IPCC, 2007). The global temperature has been rising by 0.2°C every ten years. Heat stress in buffaloes causes increased respiration rate, pulse rate, rectal temperature and water intake, along with decreased feed intake (Das *et al.*, 2016). Physical activity, feeding and rumination time and standing and lying behaviors, all are change simultaneously. When sensible (conduction, convection, radiation) and insensible (evaporative cooling) heat loss methods are inadequate, heat load and core body temperature rise.

Changes in energy metabolism, cellular processes, and molecular pathways result from the activation of the neurological and endocrine systems, which promote adaptation during both acute and chronic stress periods. Cellular adaptation is achieved through the production of heat shock proteins (HSPs), leading to dynamic changes in DNA and RNA functions as well as molecular and enzymatic pathways. Recent DNA research indicates that buffalo breeds traditionally raised in hot environments may have developed greater heat tolerance. The ruminant sector is believed to be one of the largest sources of greenhouse gases (GHGs). However, there are strategies to reduce climate change by lowering GHG emissions from ruminants.

Ruminants emit methane (CH4) and nitrous oxide (N2O) through processes like denitrification and nitrification in manure, as well as the anaerobic digestion of organic matter in the rumen and manure. Quantifying these emissions using experimental methods is difficult and time-consuming, and analyzing the results to develop adaptation and mitigation strategies is complex. Computer simulation models offer significant potential for predicting GHG emissions to help address these challenges. These models often consider all farm activities while accurately estimating GHG emissions from both direct and indirect sources. They are quick and efficient at forecasting emissions and provide valuable data for implementing effective GHG mitigation techniques on farms. Furthermore, these models assist in assessing the success of various mitigation strategies aimed at reducing GHG emissions.

Climate change presents several new challenges to buffalo production, especially in tropical regions like India. Variations in rainfall patterns and frequent droughts affect the availability and quality of green fodder, leading to nutritional deficiencies and lower productivity. Herd health is also at risk due to shifts in parasite loads and disease vectors driven by climate change. Water scarcity increases stress and endangers buffalo welfare, as water is essential for wallowing and thermoregulation. Collectively, these factors threaten the long-term sustainability and profitability of buffalo farming, highlighting the need for rapid adaptation strategies such as improved housing, efficient water management, climate-resistant nutrition, and genetic selection for heat tolerance.

Challenges:

Feed, water and forage dynamics

Climatic changes affect the amount, quality and seasonal availability of fodder. Prolonged droughts diminish pasture production and enhanced reliance on agricultural wastes and commercial feeds, hence escalating expenses. Increased CO_2 and temperature can alter the nutritional composition of forages, frequently diminishing protein levels, which may lead to decreased intake and milk production. Water scarcity both for livestock hydration and cooling applications further constrains management alternatives. Climate change induces increased unpredictability and severity in weather patterns, necessitating the creation of feed sources that are tolerant to drought and elevated temperatures while maintaining nutritional quality (Thornton *et al.*, 2021). The focus is given on developing drought and heat-resistant forage species capable of maintaining biomass production and nutritional quality in these challenging conditions (Thornton *et al.*, 2022). Examples comprise certain cultivars of sorghum, millet, and drought-resistant legumes, which exhibit superior water usage efficiency compared to conventional winter season grasses (Plaizier *et al.*, 2018). The research emphasizes the utilization and processing of agricultural wastes and residues (e.g., treated straw, crop stover) to enhance their digestibility and palatability, providing a dependable feed buffer during extreme climatic stress. Advanced feed preservation techniques, including enhanced ensiling processes, are essential for sustaining nutritional quality throughout extended storage durations.

Ensuring sufficient water consumption is the main dietary approach, since water serves as the principal channel for thermoregulation via sweating and panting. Water controls body temperature, eliminates waste through urine, feces and respiration, facilitates nutrition delivery, sustains electrolyte equilibrium and offers a fluid medium for fetal development. Saliva production needs water for effective swallowing and mastication. Furthermore, lactating buffaloes require water for milk synthesis. Livestock must have unrestricted access to water, as insufficient hydration can lead to stress or dehydration in animals (Squires *et al.*, 2016). Animals require continuous, unimpeded access to adequate water resources (Gaughan *et al.*, 2019). Water sources must be situated close to feeding and resting locations to minimize the physical distance and effort needed for drinking (Kadzere *et al.*, 2002). The trough space allocated each animal should be augmented to mitigate competition that obstructs access for subordinate animals. Troughs require regular cleaning to avert the accumulation of contaminants and pathogens, as substandard water diminishes palatability and consumption. Furthermore, supplying colder water serves as a direct heat sink, compelling the body to expend energy to warm it, thereby efficiently dissipating heat from the core (Gaughan *et al.*, 2019).

Milk production and quality

Animals continuously monitor and assimilate internal and exterior signals for homeostatic control (Nakamura *et al.*, 2008). An equilibrium internal environment is crucial for optimal growth, reproduction and productivity, despite variations in these regulating mechanisms among and within species (Collier *et al.*, 2019). Homeostatic and homeorhetic processes may be compromised by exposure to elevated ambient temperatures, high humidity, bright sunshine, or combinations thereof, frequently indicated by the temperature–humidity index (THI). Ultimately, these environmental pressures diminish physiological efficiency and productive performance by modifying metabolic, endocrine and thermoregulatory responses.

The thermoregulation of dairy buffaloes is sustained within a limited thermoneutral range of 15–25 °C. Any elevation above this range elevates core body temperature and activates thermoregulatory responses, including increased breathing, gasping, sweating, and vasodilation. Heat is predominantly dissipated by radiation, conduction, convection, and evaporation; yet, the efficacy of each mechanism is contingent upon variables such as air circulation, coat characteristics, and ambient humidity (West, 2003).

Evaporative cooling is diminished in efficacy during hot and humid conditions. Buffalo depend more on panting and peripheral vasodilation than horses due to possessing fewer sweat glands. Blood flow to the skin rises, resulting in diminished nutrient transfer to the udder and gastrointestinal organs due to decreased mammary and splanchnic circulation (Collier *et al.*, 2019).

Prolonged hyperthermia (> 39 °C) results in electrolyte imbalance, dehydration, and alterations in acid-base equilibrium. The temperature-humidity index (THI) correlates favorably with rectal temperature, respiration rate, and heart rate, although THI values between 72 and 74 result in a significant decline in milk production (Bernabucci *et al.*, 2009). The nonspecific physiological response of body is to high ambient temperatures and relative humidity is known as heat stress, and it causes a number of bodily alterations, as well as an imbalance in the internal thermal milieu, that compromise various animal production performances. Heat stress is known to reduce average daily gain in response to a purposeful reduction in feed intake. Due to their poor capacity to sweat and effectively regulate body temperature, especially buffalo, those with black coats or high metabolic rates, are more susceptible. To reduce the negative effects of heat stress and ensure animal welfare and productivity, effective management strategies are essential. These include providing shade, adequate ventilation, clean drinking water, and cooling equipment (Sogoe and Minca, 2024).

Heat stress poses a considerable problem to the global dairy business due to its detrimental impact on production and profitability. Milk production is among the most thermally sensitive physiological characteristics in dairy animals. Exposure to elevated ambient temperatures, particularly when coupled with high humidity, generates a thermal burden that surpasses the animal's capacity for homeostasis, resulting in a significant decline in production. Heat stress in dairy calves arises when their core body temperature increases, inhibiting fat production in the mammary gland. This leads to reduced milk production and dry matter consumption, along with alterations in milk composition, such as decreased amounts of fat, protein, lactose, and solids-not-fat, among other constituents (Dunn *et al.*, 2014). The Temperature Humidity Index (THI), which integrates ambient temperature and relative humidity, is commonly employed as a measure of thermal stress in cattle. With the escalation of climate change leading to more frequent and prolonged heatwaves, it is imperative to comprehend the relationship between Temperature-Humidity Index (THI) and milk production, as well as the related seasonal, regional, and economic ramifications (Thornton *et al.*, 2021).

Empirical research indicates that the dairy industry has the most significant economic losses due to heat stress relative to other livestock sectors (St-Pierre *et al.*, 2003). Under heat stress, dairy buffaloes reduce their dry matter intake, contributing around 35% to the reduction in milk production (Rhoads *et al.*, 2009). Moreover, high-yielding dairy breeds are particularly susceptible due to their greater body size and elevated metabolic rate, which generate increased internal heat, hence enhancing their vulnerability to heat stress (Rojas-Downing *et al.*, 2017). Consequently, milk production declines when environmental and physiological heat stress escalates (Kadzere *et al.*, 2002). Heat stress further influences milk composition. Numerous studies indicate that lactating buffaloes begin to suffer heat discomfort at a THI of 72, after which both milk protein and fat concentrations progressively decline. Comparable decreases in milk constituents under elevated THI circumstances have been shown in goats (Salama *et al.*, 2020) and buffaloes (Seerapu *et al.*, 2015). Heat stress induces alterations in the metabolic profile of the mammary gland in lactating dairy animals, affecting pathways including glycolysis, lactose production, ketone metabolism, the tricarboxylic acid (TCA) cycle, and amino acid and nucleotide metabolism. Heat stress diminishes milk production and affects milk composition and somatic cell count (Liu *et al.*, 2019). These disturbances impede the availability of crucial substrates required for milk production in the mammary tissue of lactating animals. Consequently, heat stress modifies both milk production and composition by affecting the metabolic processes in mammary gland tissues (Fan *et al.*, 2018).

Reproduction and fertility

Buffalo reproduction is adversely affected by elevated temperatures: estrus manifestation diminishes, conception rates decline, and embryonic losses increase during warm times. Climatic factors may modify seasonal breeding patterns, hence undermining conventional management and artificial insemination programs. Decreased fertility results in economic costs stemming from extended calving intervals and diminished lifetime production. Recent veterinary study verifies alterations in serum hormone levels and reproductive indices in buffaloes subjected to temperature stress.

Disease and parasite dynamics

Elevated temperatures and humidity, together with modified seasonal patterns, frequently extend the distributions and life cycles of parasites, vectors, and diseases. Alterations in disease pressures, such as tick-borne infections and mastitis pathogens, can elevate morbidity, mortality, and veterinary expenses. Heat-stressed animal exhibit diminished immunological responses, hence exacerbating illness effects.

Climate change may affect animal health in four principal ways: heat-related illnesses and stress, extreme weather events, the adaptation of livestock production to altered environments, and the emergence or resurgence of infectious diseases, particularly those transmitted by vectors that are significantly influenced by environmental and climatic factors. Prolonged climatic alterations may undermine several societal facets, either directly or indirectly. For instance, elevated average temperatures may increase the incidence of illnesses and raise air ventilation costs, as seen with Lyme disease. Severe weather phenomena also pose a danger to society. Severe heat can elevate morbidity and mortality rates, particularly among at-risk groups, and adversely affect some crops. The following are the immediate effects of heat stress on animals. Indirect impacts include acclimatization, a phenotypic response that animals acquire in response to certain environmental stimuli. Thermoregulatory reactions that mitigate heat load are crucial to the acclimatization of animals to elevated temperatures. Immediate reactions encompass reduced feed intake, elevated respiration rate, increased water consumption, and hormonal alterations that influence the susceptibility of target tissues to external stimuli (Collier and Zimbelman, 2007). Buffaloes subjected to heat stress frequently experience substantial weight loss and deterioration of bodily condition due to diminished energy intake resulting from decreased feed consumption, culminating in a negative energy balance (Lacetera *et al.*, 1996). Numerous studies have shown alterations in glucose, protein, and lipid metabolism, along with changes in liver function in animals subjected to heat stress (Moore *et al.*, 2005). California epidemiology research indicated that calves born in July exhibited higher death rates. It has been proposed that heat stress may diminish the protective efficiency of colostrum in cows (Nardone *et al.*, 1997) and may influence passive immunoglobulin transfer in newborn calves (Lacetera, 1998).

Opportunities:

Buffaloes have distinct advantages such as efficient fiber digestion, fat-rich milk, robust performance on low-quality forages, and tolerance to wetland and humid environments. Particular swamp and smallholder-adapted breeds have superior tolerance to local stresses compared to exotic cattle. Nonetheless, high-yielding riverine breeds selected for milk production may exhibit greater heat sensitivity due to their elevated metabolic rates, which generate excess internal heat. The total susceptibility depends on breed, production level, management practices, and local climatic conditions. Understanding this paradox is essential for formulating adaptive methods.

Adaptation strategies

Housing management: shade, water and microclimate

Offering shade, access to wallows or ponds, sprinklers and enhanced ventilation markedly alleviates heat stress in buffaloes. Minor microclimate modifications such as orienting shelters, employing reflective roofing and planting trees can reduce peak temperatures in animal resting areas. Observational studies indicate that these methods can partially reinstate feed intake and milk production during elevated temperatures. Nevertheless, water-intensive cooling techniques, such as sprinklers and ponds, require sustainable water management in areas with constrained water supplies.

In building cattle shelters, particularly in light of climate change, the susceptibility of dairy sector is paramount. Deficient modeling and suboptimal material selection in conventional housing have evidently resulted in declines in milk production across several climatic regions. Dairy housing systems require immediate, scientifically proven design modifications to enhance animal comfort and production in response to shifting climatic trends. A crucial element of animal housing that directly influences health and well-being is the flooring. Cement concrete is frequently used for dairy animals due to its durability, strength and thermal properties; however, it has significant disadvantages. The challenging, frequently slick terrain diminishes animal comfort and may lead to deformities and hoof injuries, hence elevating the risk of laminitis (Vanegas *et al.* 2006). Research indicates that animals maintained on concrete surfaces are more prone to developing or exacerbating heel erosion and have an elevated risk of lameness compared with those on rubber flooring (Vanegas *et al.* 2006). Effective flooring design and management are crucial for regulating productivity, promoting bovine health and longevity and ensuring comfort. Studies regularly demonstrate that dairy buffaloes prefer soft, elastic, dry and slip-resistant flooring.

The design of the roof structure and the selection of suitable materials are essential for effective environmental management in animal housing, a need common to all such facilities. The roof must provide adequate shade to prevent direct sunlight from entering the building. It must also enhance natural ventilation, which is crucial for interior cooling and promoting gas exchange. A study in Kerala, examined the roofing materials employed in local buffalo shelters. The results revealed that tiled roofs were the most prevalent kind, accounting for 32% of the structures. Additional materials identified were tin sheets (22%), asbestos (19%), traditional coconut palm leaf thatch (12%), aluminum sheets (7%), and concrete (3%), as documented by Prasad *et al.* (2013). Roofing materials for animal shelters must have superior insulating properties. If conductive materials like metals are used, their outer surfaces must reflect sunlight. Aluminum sheets are optimal for this use, effectively reflecting solar radiation and offering significant endurance. In tropical environments, especially for small-scale enterprises, indigenous materials that are more cost-effective and readily available such as dried grasses are often preferred (Eva Van laera *et al.*, 2014).

Nutritional interventions

Modifying ration composition in hot seasons by augmenting calorie density, improving rumen-protected nutrients and adding electrolytes and antioxidants can counteract reduced intake and oxidative stress. The strategic application of bypass fats, rumen-protected amino acids and feed additives (vitamin E and selenium) may enhance production and reproduction during heat stress. The availability of local feedstuffs should inform formulation to ensure adaptation remains practical and economical.

Heat stress reduces dry matter intake (DMI) and nutritional efficiency, thereby reducing production (Rhoads *et al.*, 2009). Administering high-concentration diet under elevated temperatures enhances consumption and reduces internal heat generation, as acetate metabolism produces more heat than propionate metabolism. Administering low-roughage diets before calving maintains dry matter intake, reduces body fat mobilization and mitigates the effects of heat stress. Research indicates that cows fed diets containing 12% NDF, rather than 18%, have increased DMI and milk production during heat stress (Adin *et al.*, 2008). Maintaining sufficient forage levels is essential to avert acidosis and providing high-quality forages can reduce heat-related fermentation losses compared with inferior forages. Incorporating fat into ruminant diets is an additional method to maintain nutritional intake, as lipids yield a lower increase in heat (Moallem *et al.*, 2010). Buffaloes experiencing heat stress that are provided with fat-supplemented diets exhibit enhanced milk yield, increased milk fat content and reduced respiratory rates during the early morning (Linn *et al.*, 2004). While augmenting concentrates and reducing fiber improve yield under heat stress, the effects of dietary protein amount and quality remain ambiguous (Arieli *et al.*, 2003). Many studies demonstrate that a crude protein concentration of 15.3% facilitates milk production in heat-stressed buffaloes, contingent upon suitable ratios of rumen-degradable organic matter to degradable protein and effective evaporative cooling conditions (Arieli *et al.*, 2003). Excessive fat inclusion may hinder fiber digestion, reduce milk fat, and diminish dry matter intake (Bauman *et al.*, 2008).

Supplemental additives and exogenous fibrolytic enzymes can improve digestibility under heat stress. Yeast supplementation stabilizes rumen function, promotes fiber degradation, increases microbial protein synthesis and elevates both feed intake and milk yield (Ayad *et al.*, 2013). Yeasts appear to confer greater benefits under stress conditions; however, results differ depending on dietary composition, lactation phase and stress intensity (Brunoa *et al.*, 2009). Plant-derived feed additives, particularly phenolic compounds and isoflavones like daidzein, might mitigate oxidative stress and enhance heat tolerance and performance (Megahed *et al.*, 2008). Furthermore, antioxidants such as vitamins C and E efficiently mitigate heat-induced effects.

Niacin, particularly in rumen-protected form, helps animals manage heat stress by enhancing evaporative heat loss and marginally reducing body temperature; however, its impact on milk supply depends on the stage of lactation and the intensity of stress (Zimbelman *et al.*, 2010). Enhancing the dietary cation–anion difference (DCAD) with sodium bicarbonate and potassium carbonate augments milk production during thermal stress, while excessive sulfate and chloride must be avoided. In these species, developing diets according to the optimal protein concept guarantees balanced amino acid profiles, minimizes excess nitrogen excretion, and lowers heat production from amino acid oxidation. Modifying feeding schedules by providing extra meals or increased amounts at cooler times, such as early morning or late evening, can help sustain feed intake during elevated temperatures (Metzler-Zebeli *et al.*, 2008).

Reproductive management

The hypothalamic-pituitary-gonadal (HPG) axis is disrupted. Reduced gonadotropin-releasing hormone (GnRH) pulses result in less follicle growth and smaller surges of luteinizing hormone (LH). Elevated temperatures hinder fertilization potential by altering meiotic progression, inducing mitochondrial dysfunction and increasing reactive oxygen species production. Temperature substantially impacts spermatogenic cells in men; oxidative damage to lipid membranes and DNA diminishes sperm survival and motility (García-Ispierto *et al.*, 2007).

Embryo implantation is hindered when progesterone levels decline owing to dysfunctional luteal activity. Alterations in the microenvironment caused by uterine warmth and prostaglandin production increase the incidence of early embryonic loss. Consequently, reproductive failure indirectly leads to diminished lactation persistence.

Health Management

Improved disease surveillance, targeted parasite management and immunization timing aligned with fluctuating seasonal risks are essential. The integration of vector control strategies, including environmental management and judicious acaricide use, mitigates disease loads exacerbated by climate change.

Genetic and breeding approaches

Genetic selection for thermotolerance provides a sustained form of adaptation, but at the cost of associated trade-offs. Prioritizing high milk production often diminishes heat resistance, a comprehensive breeding strategy that incorporates physiological and fitness traits (such as reduced resting body temperature, heat-shock protein indicators, and fertility during heat stress) can improve long-term resilience. Crossbreeding with indigenous, thermotolerant ecotypes can yield short- to medium-term benefits, but necessitates meticulous planning to safeguard milk quality and local breed resources. Genomic technologies and marker-assisted selection are becoming accessible and can expedite the discovery of thermotolerant lineages.

Technological and Genetic Advancements:

Genomic Selection**:** Research into the buffalo genome is identifying genes linked to heat tolerance, disease resistance, and higher productivity (milk and meat yield).

Climate Services: Utilizing weather-based advisories and climate information can help farmers to better plan and adapt their operations to anticipated changes.

Contribution to Food Security and Livelihoods: With the global demand for animal protein increasing, buffalo production can play a vital role in ensuring food security, particularly in developing countries where buffalo farming is a major livelihood source for smallholder farmers.

Conclusion

Buffalo production faces a multifaceted array of climate-induced challenges such as thermal stress, altered feed and water availability, and evolving disease dynamics that will diminish productivity and jeopardize farmer’s livelihoods if left unmitigated. The species inherent flexibility, along with effective on-farm practices, precise instruments, breeding methodologies, and favorable legislation, offers many avenues for enhancing resilience. Interventions must be tailored to the local environment, economically viable, and attuned to the circumstances of smallholders. Integrating short-term management strategies such as shading, cooling, and enhanced feeding can optimize the success of buffalo systems in the face of impending climate change.

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LP-3.2 Buffalo Reproduction: Emerging challenges and future technologies in Climate Change

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Climate change has emerged as a major challenge to livestock production systems, and water buffalo—an important species for milk, meat, and draught power in tropical and subtropical regions—is increasingly vulnerable to rising temperatures. Although buffaloes possess certain adaptive traits for coping with heat, the rapid increase in global warming and frequent heatwaves are surpassing their thermoregulatory limits, resulting in significant declines in productivity, health, and particularly reproductive performance. Climate change–induced heat stress alters behavioural, physiological, and metabolic responses in buffaloes, ultimately reducing fertility (Napolitano *et al.*, 2023). Elevated Temperature–Humidity Index (THI) disrupts endocrine balance, suppresses estrus expression, impairs follicular development, and lowers conception and pregnancy rates, while heat stress further compromises oocyte competence, embryo viability, and male fertility through reduced semen quality and increased sperm DNA damage. Heat stress–mediated oxidative imbalance and cellular dysfunction severely affect oocyte maturation, sperm function, and early embryonic development, thereby diminishing reproductive success in buffaloes and other livestock (Khan *et al.*, 2023). As buffalo-based dairy systems are concentrated in climate-vulnerable regions such as South Asia, understanding the multifactorial effects of heat stress on reproductive physiology and developing sustainable mitigation strategies is crucial for safeguarding reproductive efficiency and overall productivity.

Emerging Challenges

The primary challenge is increased heat stress, which negatively impacts all aspects of reproduction in both male and female buffaloes.

Climatic factors affecting livestock

Temperature, humidity and solar radiation are the major environmental factors resulting heat stress to the animals. Solar radiation is again influenced by photoperiod and ozone layer depletion. Whereas wind speed and rainfall help to reduce these effects (Bohmanova *et al.*, 2007). Temperature and relative humidity are generally considered the most substantial causative agents of heat stress and are often combined together in a thermal heat index (THI) (Dikmen *et al.*, 2009). The climatic factors affecting livestock are as follows.

Extreme temperature and heat stress:

Temperature fluctuations outside the optimal range for livestock can severely affect their physiological functions. Animals are homeotherms and they maintain a stable internal body temperature. Exposure to extreme temperatures, especially high heat, disturbs the animals' physiology and affects both production and reproduction. One of the rising concerns for gradual temperature increase is global warming. It has been discussed separately in the next section of this article.

Humidity:

Combined effect of high temperature and humidity increases the stress level increases. Animals struggle to combat heat stress through evaporative cooling mechanism as humidity hampers sweating and panting. That's why the temperature and humidity are considered together to measure the stress level in animals.

Drought and nutritional deficiencies:

Drought and dietary deficiencies caused by climate change profoundly impact livesstock by reducing water availability and limiting forage growth. Water scarcity leads to dehydration, heat stress, and reduced animal productivity, while diminished pastureland and forces farmers to rely on expensive or low-quality feed alternatives. Poor nutrition weakens animals' immune systems, reduces reproductive performance, and slows down growth rates. Additionally, long-term drought can damage land, reducing its future grazing potential and leading to overgrazing.

Environmental pollution:

Environmental pollution exacerbated by climate change significantly impacts livestock by degrading essential resources critical for their health and productivity. Pollution from agricultural runoff, industrial waste, and atmospheric contaminants leads to toxic exposure, respiratory issues, and various animal diseases.

This contamination also diminishes the nutritional quality of forage, exposing livestock to harmful chemicals that can accumulate in their bodies. As a result, animals experience weakened immune systems, lower reproductive efficiency, and increased disease susceptibility (Nkuruma, 2023).

Global warming and heat wave:

Many of the changes observed in the climate are slow and may take hundreds or thousands of years to show effects, while many are already set in motion, such as global warming (IPCC, 2024). It is well known that the emission of greenhouse gases due to human activities is the major reason behind this increase in temperature. Interestingly, the effects are not the same in every place globally, and the warming over land is greater. The effects of global warming can be measured and witnessed grossly, such as a rise in sea level, decreased snow and ice surface coverage and increased frequency of hot days

Reproductive physiology affected by climate change:

Heat stress occurs when the animals are exposed to hot and humid environments and they are unable to adapt effectively. The physiological responses to regulate the body temperature are overwhelmed, resulting in decreased feed intake, milk production etc. The effect of heat stress on reproduction is more profound and persisting as it affects the hypothalamic-hypophyseal-gonadal (HPG) axis (Roth, 2017). Hence, the fertility of the animals may be affected for several weeks or months after the onset of stress. The overall effects of heat stress on the reproductive system of male and female animals are depicted in Fig. 1.

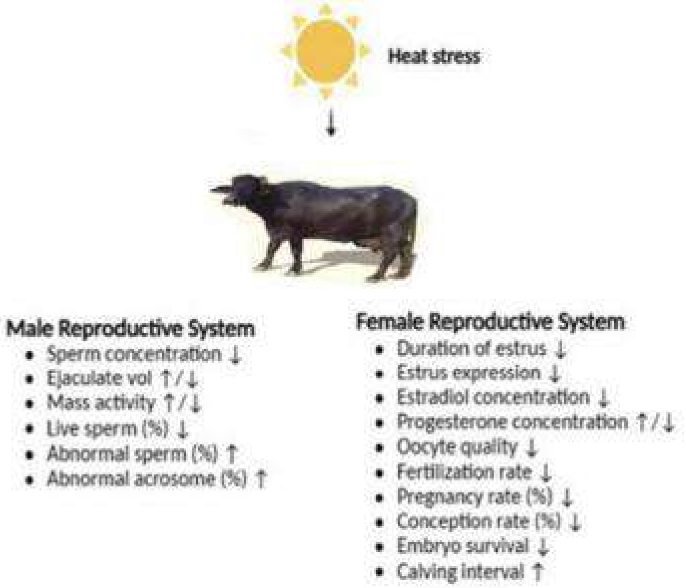

Fig. 1. Depiction of the overall effects of heat stress on reproductive system of male and female animals

In the case of females, heat stress reduces the degree of dominance of the selected follicle, and there is also reduced steroidogenic activity of its theca and granulosa cells, resulting in a fall in blood estradiol level. The metabolic state of the animal and the nature of heat stress (acute/chronic) can increase or decrease the plasma progesterone levels (Khodaei-Motlagh *et al.*, 2011). These endocrine changes diminish follicular activity and modify the ovulatory mechanism, leading to a decline in oocyte and embryo quality. The uterine environment is also altered, reducing the chance of embryo implantation. At birth, the bovine ovary contains about 150,000 primordial follicles, each with an oocyte arrested at prophase I. Throughout the female's life, these follicles are recruited into a growing pool, which leads to the development of the preovulatory follicle. The development of primordial follicles in the preovulatory stage involves primary, secondary, early antral, and antral follicles. The primary and secondary follicles are gonadotropin-independent, and the recruitment is triggered by paracrine ovarian factors such as stem cell factor, epidermal growth factor, basic fibroblast growth factor, leukemia inhibitory factor, nerve growth factor, etc. (Roth, 2017). Interestingly, the different follicular phases respond differently to heat stress. The primordial, primary and secondary follicles are heat resistant, whereas the developing antral follicles, including the dominant and preovulatory follicles are sensitive to heat exposure (Roth, 2017). The stage-dependent pattern of resistance/sensitivity of the ovarian pool of follicles to heat stress has been depicted in Fig. 2.

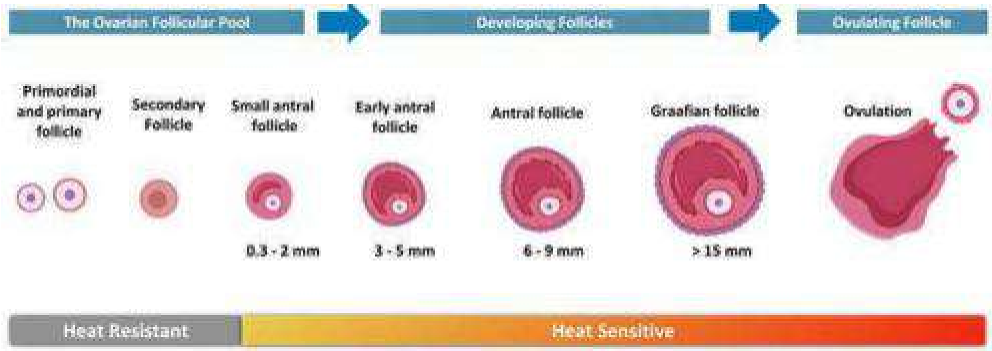

Fig. 2.: A stage-dependent pattern of resistance/sensitivity of the ovarian pool of follicles to heat stress. The primordial, primary, and secondary follicles are heat resistant, whereas the developing antral follicles, including the dominant and pre-ovulatory follicles, are sensitive to heat exposure (Roth, 2017)

Impaired Estrus and Ovulation: High temperatures increase the incidence of "silent heat" (poor or unobservable estrus signs), shorten the duration of estrus, and disrupt normal ovarian function and hormone levels, making natural breeding or accurate timing for artificial insemination difficult.

Reduced Fertility and Embryo Survival: Heat stress before and immediately after insemination significantly lowers conception and pregnancy rates and increases early embryonic mortality. The development and quality of oocytes and embryos are compromised by hyperthermia.

Male Reproductive Issues: Elevated testicular temperature due to heat stress can damage sperm quality, motility, and concentration, leading to male infertility problems.

Nutritional Stress: Climate change alters the quantity and quality of available feed and forage due to droughts and changing weather patterns, impacting the overall health and reproductive capacity of buffaloes.

Disease Dynamics: Changes in climate patterns can alter the prevalence and spread of vector-borne diseases, further impacting animal health and reproductive efficiency.

Future Technologies and Strategies

Future approaches combine advanced biotechnologies and improved management practices to mitigate the adverse effects of climate change.

Assisted Reproductive Technologies (ARTs):

Fixed-Time Artificial Insemination (FTAI): Hormonal protocols (like Ovsynch and CIDR-Synch) are used to synchronize ovulation, allowing for insemination without the need for heat detection, which is particularly difficult during hot periods.

Modified Ovsynch: An Estrus Synchronization and Fixed Time AI (FTAI) Technology to Enhance Fertility in Postpartum Cows and Buffalos under Tropical Field Conditions

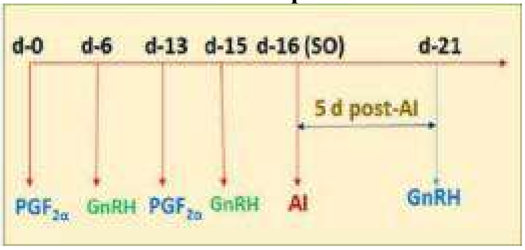

Fig. 3: Modified Ovsynch Protocol

In Vitro Embryo Production (IVEP) and Transfer (ET): Oocytes can be collected via ovum pick-up (OPU) from genetically superior or heat-stressed donors and fertilized in a controlled laboratory environment. Embryos are then transferred to recipient animals, bypassing sensitive early developmental stages in the heat-stressed animal's body.

Sex-Sorted Semen and Embryos: Technologies to predetermine the sex of offspring help farmers manage herd composition, focusing on valuable females for milk production or males for meat, thereby accelerating genetic gain and potentially reducing overall herd numbers and associated greenhouse gas emissions.

Cryopreservation and Gene Banks: Improved vitrification techniques for oocytes and embryos allow for the long-term preservation of valuable genetics, safeguarding against climate-related losses and facilitating genetic exchange.

Genetic Improvement:

Genomic Selection: Identifying and breeding buffaloes with genes associated with heat tolerance, disease resistance, and high reproductive efficiency to develop climate-resilient breeds.

Environmental and Nutritional Management:

Improved Housing and Cooling Systems: Providing effective cooling systems, such as fans, sprinklers, foggers, and access to natural water bodies (wallowing areas) can significantly reduce heat stress and improve fertility rates.

Nutritional Adjustments: Modifying diets with energy concentrates, specific mineral mixtures (including nano-minerals for better absorption), and antioxidants helps maintain metabolic balance and reduce the negative impacts of heat stress.

Data and Automation:

Precision Livestock Farming: Utilizing automation, sensors, and data analytics (including infrared thermography) to monitor animal health, detect heat stress early, and inform management decisions for more efficient and sustainable farming practices.

Conclusion:

It is evident that heat stress affects both the male and female reproductive systems of domestic animals to a great extent. Additionally, climate change particularly the global warming is worsening the situation. This results in loss of production and economic damage to farmers. Though animals try to adapt the condition to some extent, mitigation strategies must be taken to reduce the adverse effects of thermal stress. It includes housing management, nutritional strategies, hormonal interventions, assisted reproductive technologies, selection of heat-tolerant breeds and reduction of livestock greenhouse gas emissions. These will minimize the stress and improve the reproductive health of the animals resulting a better economic condition for the producers. The future of reproductive biotechnologies in water buffalo in holds significant promise for enhancing genetic quality and productivity. Fixed-time artificial insemination remains the commonly used technology, with advances in assisted reproductive technologies (ART) such as in vitro embryo production (IVEP), embryo transfer (ET), and the use of sex-sorted sperm increasingly adopted to improve breeding efficiency. These technologies overcome traditional breeding limitations, such as low reproductive rates, genetic diversity constraints, and the production of sex-predetermined offspring. The application of multiple ovulation and embryo transfer (MOET) is constrained by poor embryo recovery in this livestock species. Somatic cell nuclear transfer (SCNT) offers great potential for producing sex-predetermined and genetically superior buffalo but requires further research to increase efficiency. Cryopreservation of buffalo genetics is bolstered by the establishment of Gene Banks. Challenges such as high costs, the need for skilled personnel, and infrastructure development remain constraints. Integration of genomic selection, automation, and expansion of ET programs are clear directions. Strengthening research and collaboration among Southeast Asian countries is essential to fully realize the benefits of these biotechnologies and ensure sustainable and profitable buffalo farming.

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LP-3.3 Prospects of genome editing tool in the buffalo improvement programme

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1. Background:

Buffaloes play a vital role in the Indian livestock sector in maintaining nutritional food security, contributing more than 49.2% of total milk production with 20.45% of the livestock population (https://static.pib.gov.in). India is home to the world's largest buffalo population, with over 109 million buffaloes as per the 20th livestock census. According to DAHD 2013, Murrah buffalo (Riverine, *Bubalus bubalis*) is one of the superior Indian buffalo breeds, which comprises around 44% of the total buffalo population, and is the major source of milk, meat, and draft power in India, and is well known for its high-quality milk fat and protein along with its adaptation to wide environmental conditions and feed conversion efficiency (DAHD 2017-2018). Moreover, the reproductive performance in buffalo remained much lower than in cattle, and with the population on decline in certain countries. Thus, FAO has termed buffalo as "an asset undervalued" (Nanda and Nakao, 2003). India is home to 21 unique buffalo breeds and is well characterised by NBAGR, Karnal (https://nbagr.res.in/buffalo-breed). The first 10 identified buffalo breeds are Murrah, Surti, Mehsana, Jaffarabadi, Bhadwarai, Nili Ravi, Nagpuri, Pandharpuri, Marathawadi, and Toda breeds. In addition, recently, 11 breeds of buffalo have been recognised as breed characteristics by NBAGR, Karnal (https://nbagr.res.in/buffalo-breed).

Buffalo has an inherent capacity to efficiently convert the feed with poor nutritional value and is highly adapted to various environmental conditions with diverse topography, climate, and vegetation (Li *et al.*, 2020). However, upon comparing other livestock species, water buffaloes have an inefficient evaporative cooling system due to their dark coat color, small hair follicles, and inadequately dispersed sweat glands (Marai and Haeeb, 2010). That results in impairment of growth, production, and reproduction performance. Intensive research on genetics, genomics, nutrition, advanced biotechnological, and management practices could unlock further productivity gains. Technology advances, such as the inclusion of genetic selection to enhance production traits, precision livestock farming to optimise the management practices, an automated feeding system to optimize nutritional delivery, artificial intelligence in improving the food supply chain by considering buyer demands, predicting future demands of milk and meat, and the harnessing the power of a genome editing tool in enhancing buffalo health and productivity.

2. National Breeding Programs in Buffalo Genetic Improvement

In 1970-71, the National Project on Buffalo Improvement via All India Coordinated Research Project was initiated to produce proven bulls for enhancing milk production through semen conservation, dissemination, and a field progeny testing programme. In 1987, the 1st Dairy Herd Improvement Program was initiated by the National Dairy Development Board in India, focusing on milk recording and selective breeding to improve buffalo genetics in various districts of Gujarat (Trivedi, n.d.). Network project on Buffalo Improvement was started in 1993 at the Central Institute for Research in Buffalo at Hissar to produce improved germplasm on a larger scale for the buffalo improvement program. The main goal is to produce a larger number of daughters per bull for accurately evaluating breeding bulls, the method commonly known as progeny testing. In 2014, Rastriya Gokul Mission was implemented for the development and conservation of the indigenous bovine breeds. The National Livestock Mission was started to enhance farm food-producing animal productivity.

3. Advanced Assisted Reproductive Tools in Buffalo Genetic Improvement

The reproductive efficiency in buffalo is lower than cattle, and it is confronted with huge challenges due to both intrinsic and extrinsic factors. This includes an inherently low number of follicles in their ovaries, poor estrous manifestation, silent heat, marked seasonal infertility, delayed puberty, postpartum anoestrous, long calving interval, high incidences of atresia, and apoptosis (Srirattana *et al.*, 2022). Assisted reproductive technologies are major interventions for the efficient utilization of follicle reserve in buffaloes to enhance their reproduction and production capacity. For enhancing the productivity of the buffalo species, various reproductive biotechnological tools have been applied. These include artificial insemination, multiple ovulation embryo transfer (MOET), ovum pick up, *in vitro* maturation, *in vitro* fertilisation, embryo transfer technology, intracytoplasmic sperm injection, cryopreservation of germ cells, sex sorted semen, and reproductive cloning (Hufana-Duran *et al.*, 2025). At NDRI Karnal, the in vitro fertilization (IVF) technique was successfully used to produce the world's first IVF calf in buffalo named "PRATHAM." (Sarkar *et al.*, 2021). Reproductive cloning includes somatic cell nuclear transfer. The embryo Biotechnology laboratory at NDRI Karnal and CIRB Hissar established the hand-guided cloning technology and produced cloned buffalo offspring using various somatic cells as a nuclear donor (Panda *et al.*, 2012, 2011; Saini *et al.*, 2018; Selokar, --). These reproductive biotechnology tools help in boosting milk production, improving reproduction, faster growth of elite animals, controlling and preventing infectious, zoonotic diseases (https://buffalopedianew.cirb.res.in/modern-technologies/).

4. Genetic Selection in Buffalo Genetic Improvement

Over the last 20 years, the reproductive physiology of Indian buffaloes has changed rapidly due to the physiological adaptation for acquiring the high milk production that leads to reproductive decline. It is due to the selective breeding focusing on milk production traits to achieve excellent milk quality and quantity, rather than the reproductive traits in buffalo (Shao *et al.*, 2021). Inclusion of female reproductive traits in the breeding goals is one of the alternatives to reduce the fertility costs, and it has been adopted by several countries. Up to now, selective breeding and progeny testing have been practiced in many institutional dairy farms to enhance milk production. The growing importance of buffaloes requires that they undergo an accelerated rate of genetic gain for efficiency of production, product quality, and sustainability. Integrating reproduction, growth, and production traits in breeding programs is required for genetic improvement. However, due to the polygenic nature of fertility traits, many of the studies are insignificant. Great genetic associations between traits are produced by linkage disequilibrium, and the pleiotropic effects of genes provide better results in genetic improvement studies (George *et al.*, 2023). The Open Nucleus Breeding System (ONBS) is a livestock breeding strategy that enhances genetic improvement through intensive selection and bidirectional gene flow between elite herds and base herds, employing MOET (https://www.nddb.coop/services/animalbreeding/opennucleus). Research indicates that ONBS can lead to a 10-15% increase in annual response to selection compared to traditional breeding methods.

5. Genome Editing in Buffalo Genetic Improvement

The FAO estimates demand for animal-based food products will increase by 70% to feed the projected 9.7 billion people on the globe (Alexandratos, n.d.). Meeting these future demands for animal-based products in a way that has minimal impact on the environment by keeping the high standard of animal welfare practices requires the implementation of advanced reproductive biotechnology and cutting-edge genome editing (GE). Notably, the GE tool allows for introducing polymorphisms seamlessly or even creating novel changes predicted to result in improved gain, which is not possible in traditional livestock breeding that is restricted by genetic linkage and the available genetic variation (Laible *et al.*, 2015).

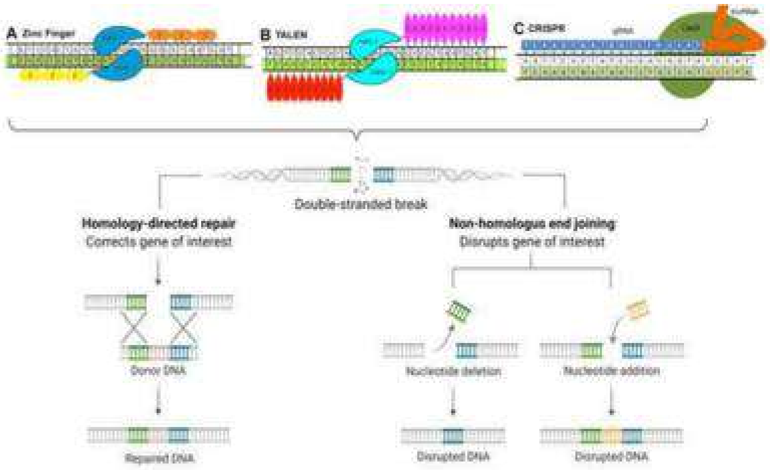

Fig. 1. Schematic of ZFN, TALEN, and CRISPR/Cas9 workflow in gene editing

Gene editing is a groundbreaking biotechnology tool that presents significant opportunities and challenges in buffalo breeding. The genome editing toolbox comes with mainly three different categories, such as ZFNS, TALENs, and CRISPR/Cas9, and has been successfully implemented in livestock species such as cattle, pigs, sheep, goats, and chickens (Tait-Burkard *et al.*, 2019). ZFN and TALEN are artificial tools based on protein-DNA interaction, while CRISPR has evolved from a bacterial defence mechanism based on RNA-DNA interaction. Though the modalities of these genome editors are different, the mechanism of action will be similar in all three categories, i.e., causing double-strand break (DSB) at user-defined, specified location of the genome and subsequent endogenous cellular DNA repair pathways (Panda *et al.*, 2013; Wefers *et al.*, 2013) (Figure 1). DSBs are repaired most frequently by error-prone non-homologous end-joining pathways to create indels that cause loss of function or/and rare homologous end-joining pathway results in the gain of function. The CRISPR/Cas9 system can achieve precise DNA cleavage by guiding the Cas9 protein with a single guide RNA (sgRNA). Due to the simplicity, efficiency, and cost-effectiveness standpoints, the CRISPR/Cas9 tool stands distinct and is foreseeable that a significant number of genome-edited livestock will be produced over the next decade.

Further expanding the genetic toolbox, base-editors and nickase systems, which can drive editing, are now popular in this field as they perform the genetic modification without the need for introducing DSBs (Pickar-Oliver and Gersbach, 2019). BEs are classified into cytosine base editors (CBEs) and adenine base editors (ABEs) based on the type of base-modifying enzyme used. CBEs can convert C-G to T-A, while ABEs can convert A-T to G-C (reference). In addition, prime editing has been developed by deploying reverse transcriptase and peg RNA to cause point mutations and achieve precise insertions and deletions without requiring DSBs or donor DNA templates.

6. Methods to deliver the genome editors into the Buffalo genome:

Two conventional techniques, such as Somatic cell nuclear transfer (SCNT) and zygote microinjection, are commonly used in generating genome-edited animals (Figure 2). However, both techniques are technically challenging, expensive, labor-intensive, and require expert skills to need sophisticated instruments. Only a few laboratories in the world are used. The emergence of cutting-edge reproductive technologies tools such as zygote electroporation, zygote transduction with recombinant adeno-associated virus, and surrogate sire technology, will provide animal breeders with a new toolkit for delivering the genomic editors to reproductive germ cells (McFarlane *et al.*, 2019).

Zygotic microinjection: Electroporation leads to pores in the zygotic membrane, without the need for zona pellucida dissolution, allowing the genome editors to pass through it to the nucleus, where genome editing happens (Miao *et al.*, 2019). It has two limitations: it only takes < 1 kb of homology-directed repair template for generating a knock-in animal and causes mosaicism in genome-edited offspring.

Somatic Cell Nuclear Transfer: A groundbreaking discovery made by Wilmut and Campbell for showing transdifferentiation of somatic cells in the enucleated oocyte environment into the pluripotent embryonic state, which leads to a full-term pregnancy and birth of live offspring (Campbell *et al.*, 1996; Wilmut *et al.*, 1997). A simplified version of SCNT evolved that does not require of expensive micromanipulator, known as the Hand Made cloning technique (Panda *et al.*, 2011; Vajta *et al.*, 2001). Cloning technology is a key technology for generating gene-edited livestock that bypasses the problematic mosaicism of zygotic microinjection and facilitates producing a homozygous live-born offspring within one generation (Ledesma and Van Eenennaam, 2024).

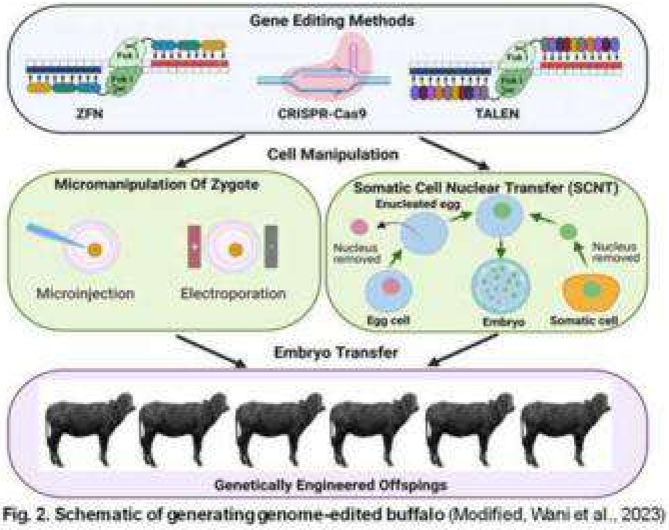

Fig. 2. Schematic of generating genome-edited buffalo (Modified, Wani et al., 2023)

Surrogate Sire Technology: This technology describes the creation of male animals (Cattle, Sheep, and Goats) lacking endogenous germline stem cells by knocking out the *NANOS2* gene. Spermatogonial stem cells (SSCs) from donor males can be transplanted in the above males, providing a source of self-propagating stem cells that produce mature sperm from the donor (Ciccarelli *et al.*, 2020).

7. The application of gene editing technology in buffalo breeding

The advent of the genome editing tool provides genetic resistance to pathogenic infections, offering new frontiers for the prevention and control of infectious diseases in livestock. In recent years, genomic editing tools have been efficiently utilised to remarkably reduce breeding costs and boost the efficiency of developing disease-resistant livestock. Such examples of targeting monogenic traits to minimise disease infestation include targeting *PRNP* for prion disease, *SP110* and *NRAMP1* for tuberculosis resistance, and LYZ for mastitis (reviewed in (Xu *et al.*, 2025)). Other frontiers of application of genome editing in improving productivity by targeting MSTN and IGF2, improving milk quality by targeting *LALBA*, *LYZ*, *BLZ*, and sex skewed to produce only females by targeting the *SRY* gene (reviewed in (Xu *et al.*, 2025)). Technology has also been used in animal welfare projects, such as creating hornless dairy cattle (Carlson *et al.*, 2016) and heat-tolerant cows (Onasanya *et al.*, 2020).

8. Regulatory approval

Though the gene editing technology is at the doorstep for any laboratory scientist who aspires to utilise it, there is a risk associated with unintended or off-target editing that leads to long-term consequences. That needs careful design of the experiment to manipulate the mammalian genome. Though the technology has an unprecedented opportunity to improve animal welfare and performance while reducing the environmental footprint of livestock production, it remains unclear in many countries like India, the regulatory pathway to bring genome-edited animal-derived foods to market (Zhao *et al.*, 2019). Currently, regulations differ substantially between geopolitical regions around the globe. Secondly, technological awareness is needed for the public acceptance and careful consideration by healthy dialogue among all stakeholders, of the consumption of GE livestock, by establishing an appropriate regulatory framework. Within years, it is imperative to allow livestock farmers to access genome editing tools to enhance livestock health, productivity, and global food security sustainably.

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Oral Presentation

OP-3.1. CRISPR/Cas9-Mediated Disruption of the β-Lactoglobulin Gene in Buffalo Mammary Epithelial Cells

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β-Lactoglobulin (BLG) is the predominant whey protein in buffalo milk and a major allergen associated with milk-related hypersensitivity in humans. Targeted reduction or elimination of BLG through genome editing offers a promising strategy for producing hypoallergenic dairy products. This study aimed to optimize a CRISPR/Cas9-based approach to disrupt the BLG gene in buffalo mammary epithelial cells. Single guide RNAs (sgRNAs) targeting exon 1 were complexed with Cas9 and delivered into mammary epithelial cells via electroporation, a method that demonstrated high reliability and efficiency for ribonucleoprotein (RNP) delivery. Optimization of the sgRNA-to-Cas9 ratio revealed that 250:500 ng consistently achieved maximal editing efficiency, resulting in 74.2% disruption of the BLG gene. Genomic editing was confirmed by T7 endonuclease I assay and relative mRNA expression analysis, while Western blotting demonstrated complete loss of BLG protein expression. These findings establish that CRISPR/Cas9 can effectively target and disrupt the BLG gene in milk-producing cells. The optimized protocol provides a robust foundation for generating BLG-free buffalo and improving the allergenic profile of buffalo milk, representing a significant step toward the development of hypoallergenic dairy products.

OP-3.2 *In vitro* Development of Embryonic Stem Cells from Preimplantation Buffalo Embryos

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The objective of the present study was to develop *in vitro* fertilized buffalo embryos of different stages, culture embryos on feeder free culture system (fibronectin), formation of embryonic stem cell colony and characterization of embryonic stem cell colonies by the use of immunocytochemistry. Buffalo cumulus-oocyte complexes (COC's) obtained from slaughterhouse ovaries were matured and fertilized *in vitro*. The fertilized oocytes (400) were then cultured in modified synthetic oviductal fluid (mSOF) medium containing bovine serum albumin (BSA) and fetal bovine serum (FBS) and evaluated for the developmental stages of preimplantation early stage embryos. The overall embryos developed at the end of *IVC* were 43.98±2.11%. Bovine ES cells were attached to fibronectin that supported the attachment and proliferation of primary colonies. The ES colonies were dome shape. Embryonic stem cells express high levels of alkaline phosphatase staining. The expression of a panel of pluripotency related surface antigens, which included the SSEA-1, SSEA-4, TRA-1-60, TRA-1-81and the transcription markers like Oct4 and Nanog, was examined by indirect immunofluorescence in preimplantation *in vitro* produced different stages of embryos up to compact morula stage and in ES cells derived from such embryos and were confirmed in present study. It can be concluded that embryonic stem cells showed intense alkaline phosphatase staining as a proof of stemmness. Positive fluorescent staining of panel of surface antigens, that included SSEA-1, SSEA-4, TRA-1-60 and TRA-1-81, indicates the pluripotency in ES cells. The transcription markers like Oct4 and Nanog were also confirmed.

Keywords: *Embryonic stem cells (ESC's), Immunocytochemistry, Early stage embryos, Fibronectin, transcription markers*

OP-3.3. Exploring the Impact of Similar Range of Temperature Humidity Index on Testicular Thermal Profiles and Semen Quality Parameters of Murrah Bulls under Heat Stress

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The present study was aimed to explore the impact of similar range of Temperature Humidity Index (THI) on skin surface temperature at different anatomical sites, testicular thermal profiles and semen quality of Murrah buffalo bulls. The study was conducted at Artificial Breeding Research Centre of ICAR-National Dairy Research Institute, Karnal (Haryana) on eight adult healthy Murrah buffalo bulls. Data was collected during hot dry, hot humid, autumn and winter season. Environmental parameters in terms of dry bulb and wet bulb temperature, relative humidity were recorded throughout the study to calculate THI. Skin surface temperature (SST) at various anatomical sites (ear, eye, muzzle, forehead, flank and rump) along with testicular surface temperature (TST) at distal, middle and proximal pole was recorded with the help of Infrared thermography. Semen ejaculates were collected at weekly interval using an Artificial Vagina. The SST of eye and forehead (afternoon hrs) was significantly ($p<0.05$) higher during hot humid compared to hot dry season. However, the values of SST were significantly ($p<0.05$) higher during summer (hot dry and hot humid) season compared to autumn and winter seasons. Proximal and distal pole surface temperature was significantly ($p<0.05$) higher during the hot humid compared to the hot dry season in the afternoon and morning hours, respectively. Whereas, testicular temperature gradient (°C) was significantly ($p<0.05$) lower during hot humid compared to hot dry season during morning. The mean values of fresh as well as frozen sperm abnormalities (head, mid piece, tail and total abnormality, %) were significantly ($p<0.05$) higher during hot humid compared to hot dry season. The present study demonstrates that comparable THI ranges exert differential impacts on testicular thermal profiles and semen quality of Murrah bulls under heat-stress conditions. Hot-humid season imposed greater thermal load than hot-dry season, indicating compromised scrotal thermoregulation and resulting in elevated sperm abnormalities. These results emphasize the need for season-specific heat-ameliorative strategies, particularly during the hot-humid period, to maintain reproductive efficiency in Murrah buffalo breeding programs.

Keywords: *Murrah bulls, Infrared Thermography, Scrotal gradient, Semen, Thermal Stress.*

OP-3.4. Luteal insufficiency and its impact on conceptus development in buffalo

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Luteal insufficiency (LI) in buffaloes is characterized by inadequate function of the corpus luteum (CL), leading to low progesterone (P4) concentrations in the circulation. This hormonal deficiency results in embryonic mortality and reduced fertility rates. The present study aimed to identify LI through P4 profiling and assessment of CL dynamics in buffaloes. Additionally, we investigated the expression profiles of ISGs and conceptus development in LI-affected buffaloes. Based on P4 profiles, LI was identified in 67% of buffaloes with a history of conception failure. These buffaloes (n=27) were classified into supra-basal P4 (n=5), delayed P4 rise (n=5), early fall of P4 (n=8) and normal P4 (n=9). The basal P4 concentration in the supra-basal group was 1.11±0.05 ng/mL. On day five, the lowest P4 concentrations were observed in the delayed rise group (1.57±0.17 ng/mL). A marked drop in P4 concentration was observed in the early fall group on day 18 (0.81±0.12 ng/mL). The development of the CL was delayed in the slow P4 rise group compared to the control, and a significant reduction in CL diameter was noted on day 18 in the early fall group compared to other groups. Color Doppler ultrasonography revealed a marked reduction in CL vascularity at the early luteal stage in the slow P4 rise group, with a further reduction in the early fall group compared to the remaining groups. Cut-off serum P4 concentrations of ≥0.95 ng/mL on day 0, ≤1.86 ng/mL on day 5, and ≤1.98 ng/mL on day 18 of the estrous cycle can be used to predict LI. The conception rate was lower in LI buffaloes (16.7%) compared to those with normal P4 (66.7%). The relative expression of ISG15 remained at basal level in non-pregnant buffaloes. In pregnant groups, ISG15, MX2, and OAS1 expression was significantly upregulated (37-68 fold) on day 18. In LI buffaloes associated with embryonic mortality, MX2 expression remained at basal levels, while ISG15 showed moderate upregulation on days 18 and 27 (2.7-3.5 fold), and OAS1 was significantly upregulated (9.41 fold) on day 27 ($P<0.05$). In conclusion, the marked upregulation of ISGs on day 18 in pregnant buffaloes and their persistent basal level in non-pregnant buffaloes suggest their potential use in monitoring conceptus development. Establishing the true pattern of luteal function would facilitate targeted therapy, either with a luteotrophic or antiluteolytic approach, to address the low conception rate associated with LI in buffaloes.

Keywords: *Buffalo, corpus luteum, progesterone, pregnancy, embryonic mortality*

OP-3.5. A New role of NNC 55-0396: Reduces Capacitation-Like Changes Through Inhibition of Cholesterol Efflux, Calcium Influx and Protein Tyrosine Phosphorylation During Sperm Cryopreservation in Buffalo

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Cryopreservation induces premature capacitation-like changes (cryocapacitation) in buffalo (Bubalus bubalis) spermatozoa, primarily through excessive calcium influx via CatSper channels, compromising post-thaw fertility. This study investigated NNC 55-0396 (NNC), a selective T-type voltage-gated Ca^{2+} channels (CatSper channel) inhibitor, for its ability to mitigate cryocapacitation when added to Tris-egg yolk-glycerol extender. Semen from four Murrah buffalo bulls was cryopreserved with 0, 2, 5, or 10 μM NNC. Post-thaw evaluations included progressive motility and various sperm kinetic parameters, plasma membrane integrity (PMI), acrosome integrity, capacitation status via chlortetracycline (CTC) assay, intracellular calcium ($[Ca^{2+}]$ i) by flow cytometry, protein tyrosine phosphorylated proteins (TPP) by Western blotting, and in vitro zona pellucida binding ability. At 5 μM, NNC significantly ($P<0.05$) improved total sperm motility (TM; 55.00±4.7 vs. 41.46±3.57 control), progressive motility (PM; 37.62±3.44 vs. 27.39±2.91 control) and rapid motility (RM; 49.72±4.46 vs. 36.62±3.38 control). However, various sperm kinetics parameters like VAP (μm/s), VSL (μm/s), VCL (μm/s), ALH (μm), BCF (Hz), STR (%), LIN (%), EL (%) and head size (μ) were similar ($P>0.05$) in NNC treated and non-treated (control) sperm samples. NNC did not affect sperm plasma membrane integrity and also did not affect the sperm motility over the period of time (120 minutes). Interestingly, at 5 μM and 10 μM it prevented the loss of cholesterol from the sperm cells (5 μM; 32.92±0.67 and 10 μM; 31.87±0.10 vs. 23.45±0.34 μg/100 million control). The percent of live sperm with low calcium were higher ($P < 0.05$) in sperm treated with NNC compared to control which indicated that NNC reduced massive calcium influx during sperm cryopreservation as indicated by reduced $[Ca^{2+}]$ _i in live sperm (high Fluo-3 AM: 55.85±4.94% 2 μM; 52.82±2.76% 5 μM; 54.32±3.90% 10 μM vs. 37.91±1.53 %; $P < 0.05$). NNC also inhibit ($P<0.05$) the expression of TPP (Mol. Wt.; 32, 45, 75, 85 and 120 kDa) in dose dependent manner which are the final hallmark of cryocapacitation. Further, The CTC assay indicated reduced capacitated sperm (Pattern B: 38.00±5.85% 2 μM; 25.92±4.04% 5 μM; 24.49±5.18% 10 μM vs. 54.96±6.53% control; $P < 0.01$) and increased uncapacitated sperm (Pattern F: 54.37±5.38% 2 μM; 69.25±3.68% 5 μM; 68.92±4.58% 10 μM vs. 33.69±5.23% control; $P < 0.05$). In *in vitro* capacitation media, NNC 55-0396's effects were reversed, allowing normal capacitation. At 5 μM NNC, sperm showed enhanced zona binding after *in vitro* capacitation 354.75±33.75 vs. 231±24.56 control. These findings suggest NNC 55-0396 minimizes cryocapacitation by inhibiting calcium influx and TPP, enhancing buffalo sperm cryotolerance and fertilizing potential.

Keywords: *Buffalo spermatozoa; Cryocapacitation; NNC 55-0396; CatSper; Calcium influx; Protein tyrosine phosphorylation; Zona pellucida binding*

OP-3.6. Heat Stress–Induced Impairment of Insulin Signaling in Bubaline Skeletal Muscle and Its Modulation by Alpha-Lipoic Acid Supplementation

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Heat stress disrupts metabolic homeostasis in ruminants, yet molecular insights into insulin signaling dysfunction in buffalo skeletal muscle remain limited. This study delineated the downstream insulin receptor signaling alterations induced by chronic heat load and evaluated the modulatory efficacy of alpha-lipoic acid (ALA), a redox-active insulin-sensitizer. Eighteen male Murrah buffaloes (293.61 ± 4.66 kg) were randomly allocated to non-heat-stressed control (NHSC), heat-stressed (HS), and heat-stressed + ALA (HSLA; 32 mg/kg BW) groups. HS and HSLA animals were exposed to controlled heat stress (40 °C; 6 h/day) for 21 days. Skeletal muscle biopsies collected on day 21 were analyzed for mRNA (IR, IRS1, AKT,GLUT4) and protein abundance (IRS1, AKT, pAKT, GLUT4). Heat stress markedly upregulated IR and IRS1 transcripts ($P<0.05$) but failed to activate the downstream AKT–GLUT4 axis, evidenced by a 59.2% reduction in AKT expression (HS vs HSLA) and no significant increase in GLUT4. Protein abundance mirrored transcript patterns: IRS1 increased in HS, whereas AKT and GLUT4 remained unchanged from NHSC. Although pAKT was elevated in HS, the response was insufficient to support GLUT4 translocation, indicating an early "unplugging" of the insulin signaling cascade.ALA supplementation substantially restored insulin signaling, enhancing AKT (56.7% vs NHSC; 30.91% vs HS), GLUT4 (23% vs NHSC), and pAKT (13.48% vs HS), suggesting improved PI3K–AKT-mediated glucose uptake and protection against heat-induced oxidative inhibition of IRS1/AKT signaling. Collectively, prolonged heat stress initiated a potential "unplugging" of the insulin signaling axis at the IRS1–AKT–GLUT4 node, suggesting a beginning of impaired insulin sensitivity in bubaline skeletal muscle and ALA effectively mitigates this disruption. ALA thus emerges as a promising nutritional strategy to strengthen metabolic resilience and productivity under climate-induced heat load in Bubaline Species.

Keywords: *Heat Stress***,** *Insulin Signaling***,** *Alpha-Lipoic Acid (ALA)***,** *Skeletal Muscle***,** *AKT–GLUT4 Pathway***,** *Buffalo (Bubalus bubalis)*

OP-3.7. Development of ELISA for the detection of interferon stimulated genes coded protein in the buffalo plasma for the early detection of pregnancy

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Buffalo the black gold of the tropics despite superior production traits remains a poor breeder. Embryonic mortality (65%) occurring early during pregnancy mostly between 16 and 18 days being the major constraint. An early and accurate diagnosis of pregnancy can thus improve herd economics by reducing time gap between subsequent inseminations. Interferon tau (IFNT) was considered, which acts as first pregnancy recognition signal in ruminants and stimulates expression of various Interferon stimulated genes (ISGs) in the peripheral blood mononuclear cells (PBMC's), concomitant with IFNT signaling occurring around maternal recognition of pregnancy (MRP). Present study was planned to demonstrate the expression dynamics of ISGs (OAS1, MX1, MX2 and ISG15) in PBMCs during peri-implantation period in buffalo and subsequent molecular cloning and expression of suitable ISG coded protein(s) in suitable prokaryotic host expression system, with an aim to raise antibodies for development of field assay based on ELISA. Blood samples were taken from 10 healthy multiparous pregnant/inseminated buffaloes at days 14, 16, 18, 20, 22 post AI, against 10 control/non pregnant buffalo and expression dynamics of ISG's was studied in PBMC's using qPCR analysis. Expression profile of most ISGs was observed to increase through day 14 to day 20 post AI and declined thereafter. Based on differential expression OAS1 and MX2 were identified as suitable ISG candidate biomarkers for accurate pregnancy diagnosis within 18 days post AI. Molecular cloning and expression of selected ISGs in a suitable prokaryotic expression vector was performed. Bulk expression of the recombinant proteins was done and purified by affinity chromatography and confirmed by Western blot using Mouse Monoclonal His-probe antibodies. Purified protein was used to raise hyperimmune sera in laboratory animals. Reactivity of hyperimmune sera against recombinant ISG coded proteins was checked by western blot and indirect ELISA. Standardization of indirect enzyme linked immunosorbent assay (ELISA) for AS1 and MX2 genes was done using recombinant protein and raised antibodies. Standard

curves for ELISA were thus obtained at different dilutions and optimal concentrations of antigen and antibodies were ascertained. The standardized ELISA values could further be used to develop a field level diagnostic kit based on lateral flow assay, for the detection of early pregnancy in buffaloes.

OP-3.8. Assessment of Semen Quality Traits of Murrah Breeding Bulls

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The present study was conducted on 65 Murrah breeding bulls to evaluate semen quality parameters. Key traits analysed included ejaculate volume (ml), sperm concentration (million/ml), initial motility (%), and post thaw motility (%). A total of 1,280 ejaculates were collected and analysed using standard laboratory procedures. The average ejaculate volume recorded was 5.48 ± 1.36 ml, ranging from 3.0 to 9.31 ml, while the mean sperm concentration was 1360.25 ± 230.42 million/ml, with individual bulls showing variations between 1022.66 to 2376 million/ml. The average sperm motility was 75.42 ± 0.77%, and progressive motility averaged 56.13 ± 1.01%, indicating good viability across the population. Month-wise analysis showed slight variations in semen quality parameters, with peak motility and concentration recorded in the second month, suggesting seasonal and management influences on semen characteristics. Correlation analysis indicated a moderate positive association between ejaculate volume and sperm motility, while concentration and motility showed a weak negative relationship. These results highlight individual variability among Murrah bulls, emphasizing the importance of regular semen evaluation for selection and breeding programs. The findings provide essential baseline data for improving artificial insemination (AI) efficiency in Murrah buffaloes. Regular monitoring of semen quality traits can enhance fertility outcomes, aid in identifying superior bulls for breeding, and contribute to the genetic improvement of the herd. This study underscores the relevance of systematic semen evaluation in breeding centers to optimize reproductive performance and support the national Murrah buffalo improvement program.

Keywords: *Murrah buffalo, semen quality, ejaculate volume, sperm concentration, motility, artificial insemination.*

OP-3.9. Protective Effects of Melatonin and Betaine Against Heat Stress-Induced Apoptosis in Buffalo Granulosa Cells Using a 3D Spheroid Model

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Buffaloes are polyestrous, yet their reproductive activity follows a marked seasonal rhythm, with significantly higher fertility in winter than in summer. Prolonged photoperiods, elevated ambient temperatures, endocrine disruptions, and increased oxidative stress during the summer months collectively impair reproductive cyclicity, contributing to a high incidence of summer anestrus (36.6–59.6%) in buffaloes. Heat stress (HS) negatively affects granulosa cell proliferation, steroid hormone production, and follicular growth, ultimately compromising ovarian function and the fertility of buffaloes raised in tropical climates. The present study investigated the protective role of melatonin and betaine on buffalo GCs function under induced HS conditions using a 3D spheroid culture model. The MTT assay revealed that melatonin and betaine promoted GC proliferation, with maximal responses at 300 ng/ml and 0.78 mg/ml, respectively. Under control conditions, GCs formed compact spheroids that grew progressively. In contrast, exposure of differentiated follicular (DF-like) GCs to HS (40.5°C, 2–24 hr) caused time-dependent spheroid disintegration, reduced *PCNA, CYP19, FSHR,* and *RUNX1* expression, and elevated *HSP70* and *BAX* levels. Both melatonin and betaine mitigated these changes, maintaining spheroid structure, GC-specific gene expression (CYP19, FSHR, RUNX1), and reducing heat stress-induced expression of *BAX* and *HSP70* by increasing the basal expression of antioxidant key genes (*CAT* and *SOD2*) while attenuating heat stress-induced apoptosis with a significant improvement in the estradiol production under heat stress conditions. The findings demonstrate that HS exposure impairs the proliferation and function of GCs, and that melatonin and betaine confer thermo-protective effects by sustaining proliferation, steroidogenesis, and antioxidant defense while attenuating heat stress-induced apoptosis.

Keywords: *Heat stress, granulosa cells, melatonin, betaine, steroidogenesis, apoptosis, antioxidant defense*

Poster Presentation

PP-3.1. Late-Gestation Heat Stress Alters Placental Morphology and Efficiency in the Murrah Buffaloes

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The placenta plays a central role in foetal development and subsequent lactation performance by supporting nutrient transfer, heat dissipation, gas exchange, and the secretion of key hormones required for mammary gland development. Heat stress during late gestation can disrupt these functions and compromise both prenatal and postnatal outcomes. This study investigated the impact of late-gestation heat stress on placental morphology and efficiency in Murrah buffaloes. The work was conducted during the summer months (May- June; THI 82.03) of 2025 at the Livestock Farm, ICAR–IVRI, Bareilly, Uttar Pradesh. Multiparous pregnant buffaloes (N=12; average age of 6.25±0.65 years) were dried off 55±5 days before their expected calving date and exposed to ambient heat stress during late gestation. Gestation length and calf birth weight were recorded. Placentas were collected from buffaloes within 3.00±0.24 hours of calving, and total placental weight, number of cotyledons, cotyledon weight, and surface area were evaluated within 1 hour of expulsion. Foetal cotyledons were morphologically convex and non-pedunculated. Heat-stressed dams had gestation lengths of 308±1.78 days, and their calves weighed 35.5±1.17 kg at birth. The birth sex ratio was (male: female) 0.50. Placentas from heat-stressed dams weighed 4.12±0.09 kg and contained 106.41±5.12 cotyledons, with a placental efficiency of 8.64±0.32%. These findings indicate that ambient heat stress at late gestation may induce thermoregulatory pressure and gross morphological adaptations in the placenta. Effective management of heat stress during late gestation is therefore crucial for optimising foetal growth, improving postnatal outcomes, and enhancing the resilience and productivity of dairy buffaloes in a warming climate.

Keywords: *Heat stress, Placental morphology, Late gestation, Birth weight, Placental efficiency*

PP-3.2. Thermo-Protective Effects of Curcumin on Bubaline Granulosa Cells *In Vitro*

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Heat stress severely compromises ovarian granulosa cell (GC) viability and steroidogenic functions, contributing to reduced reproductive efficiency in buffaloes. Curcumin, a bioactive polyphenol, is recognized for its cytoprotective potential. The present study investigated whether curcumin supplementation can mitigate heat stress induced granulosa cell dysfunction *in vitro*. Bubaline GCs were cultured and exposed to 38.5°C, 39.5°C, and 40.5°C for 24 and 48 hours, and assigned into control (untreated) and curcumin treated (5µM) groups. Enzyme linked immunosorbent assay (ELISA) analysis revealed significantly improved progesterone and estradiol secretion in curcumin supplemented cells compared with control, indicating enhanced steroidogenic competence under elevated temperature exposure. Gene expression profiling demonstrated upregulation of Steroidogenic Acute Regulatory Protein (StAR), Proliferating Cell Nuclear Antigen (PCNA), and B-cell lymphoma-2 (Bcl-2), alongside down regulation of apoptotic markers Bcl-2-associated X protein (Bax) and Cysteine-aspartic protease-3(Caspase-3) and the Heat Shock Protein 70(HSP70). These responses reflect improved cellular survival, sustained hormone synthesis, and reduced heat induced cellular injury. The study concludes that curcumin supplementation supports granulosa cell viability and steroidogenesis under thermal stress, suggesting its potential as a nutraceutical intervention to improve reproductive performance in buffaloes facing heat stress related challenges.

Keywords: *Curcumin, heat stress, bubaline granulosa cells, steroidogenesis, apoptosis*

PP-3.3. Hot Fomentation as an Adjunctive Technique for Accelerating Cervical Dilation in Buffaloes with Incomplete Cervical Dilation

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Incomplete cervical dilation (ICD) is a notable cause of dystocia in buffaloes, largely due to their fewer cervical annular rings and slower cervical tissue remodelling during labor. Standard cervical relaxation protocols employing PGF2α, dexamethasone, estrogen, and valethamate bromide are routinely practiced, yet the progression to full dilation often remains prolonged. Local heat application has a physiological basis for enhancing cervical pliability through improved vascular perfusion and collagen softening, though controlled field data in buffaloes are scarce. To assess the effectiveness of hot fomentation with cotton gauze, when used alongside standard therapy, in reducing cervical dilation time and improving cervical dilation scores in buffaloes with ICD. Sixteen buffaloes from first to fifth parity diagnosed with ICD at RVP-OPD-1, ICAR-IVRI, Izatnagar, were randomly allocated into two groups (n=8 per group). The control group received the routine cervical relaxation regimen comprising PGF2α (cloprostenol), dexamethasone, estrogen, valethamate bromide, and intravenous fluids. The treatment group received the same protocol supplemented with manual cervical massage and hot fomentation using sterile cotton gauze soaked in 45–50°C CMC solution, applied intermittently for 20–25 minutes. Recorded parameters included the time from initiation of therapy to complete cervical dilation and cervical dilation scores (0–5 scale) at 0, 2, and 4 hours. Fetal viability at birth was also monitored. Statistical analysis was carried out using an independent t-test in GraphPad Prism, with significance set at $P<0.05$. Buffaloes treated with hot fomentation showed significantly improved cervical progression. The mean time to complete cervical dilation in the hot fomentation group was 19.2±1.3 hours, whereas the control group required 29.4±1.7 hours ($P<0.01$). Cervical dilation scores at 4 hours were higher in the treatment group (3.8±0.4) compared with controls (2.4±0.5; $P<0.05$). Fetal viability did not differ between groups. The accelerated cervical response in the treatment group aligns with the expected physiological effects of localized heat, which enhances blood flow, relaxes collagen fibres, and facilitates more efficient cervical softening an advantage of clinical relevance in field obstetrics. Hot fomentation with cotton gauze is an effective, inexpensive, and field-friendly adjunct to the standard therapeutic protocol for ICD in buffaloes. Its use markedly reduces cervical dilation time and improves cervical dilation scores without compromising maternal or fetal outcomes.

Keywords: *Buffalo, cervical dilation score, Hot fomentation, Incomplete cervical dilation.*

PP-3.4. Management of Luteal Cyst–Associated Anestrus in a Buffalo Using Prostaglandin and GPG Protocol: A Clinical Case Report

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A six-year-old pluriparous (second-parity) buffalo weighing approximately 450 kg was presented with a clinical history of persistent anestrus for the preceding four months. Per-rectal examination identified a right ovarian luteal cyst, consistent with luteal-type ovarian dysfunction and considered the primary etiological factor for the prolonged anestrus. An initial therapeutic attempt involved intramuscular administration of cloprostenol sodium (Vetmate®, 2 ml), a synthetic $PGF_2\alpha$ analogue indicated for luteolysis of functional corpora lutea and luteal cysts. The treatment successfully induced behavioral estrus, after which artificial insemination was performed. However, the animal subsequently failed to conceive, suggesting incomplete cyst resolution, suboptimal ovulatory response, or compromised follicular dynamics. Following a two-month interval, a controlled breeding intervention utilizing the GPG (Ovsynch) protocol was implemented to re-establish synchronized follicular development and ovulation. On Day 0, intramuscular administration of GnRH was performed to induce ovulation or luteinization of dominant follicles and initiate a new follicular wave. This was followed on Day 7 by an intramuscular injection of $PGF_2\alpha$ to induce luteolysis. A second GnRH dose was administered on Day 9 to synchronize ovulation. The buffalo was subsequently exposed to natural mating. Pregnancy diagnosis conducted two months later via per-rectal palpation confirmed conception. This case underscores the therapeutic effectiveness of the GPG protocol in correcting luteal cyst–associated anestrus and achieving successful pregnancy in buffaloes unresponsive to initial prostaglandin-based management.

Keywords: *Pluriparous Buffalo, Anestrus, Ovarian luteal cyst, GPG protocol, Synchronization*

PP-3.5. Dystocia Due to Fetal Muscular Hypertrophy in Murrah Buffaloes: A Case Series

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Muscular hypertrophy, or double muscling, is a recessive lethal genetic trait typically observed in European beef cattle and is characterized by hyperplasia and hypertrophy of muscle fibers without true duplication. Although rare in buffaloes, excessive fetal muscular development, particularly in the cervical and shoulder regions, can lead to severe feto-maternal disproportion and result in dystocia. This case series describes (n=5) full-term first to fourth parity Murrah buffaloes presented to the Referral Veterinary Polyclinic (RVP), ICAR-IVRI, Izatnagar, following unsuccessful delivery attempts by field veterinarians and prolonged straining. Detailed clinical and per-vaginal examinations confirmed muscular hypertrophy as the primary cause of dystocia. Appropriate obstetrical interventions, including either fetotomy or caesarean section based on case assessment, were performed to relieve dystocia, resulting in successful maternal recovery without postoperative complications. This report highlights muscular hypertrophy-induced dystocia as an uncommon yet significant clinical condition in buffaloes, emphasizing the need for early referral, accurate diagnosis, and timely obstetrical management to optimize outcomes.

Keywords: *Dystocia, Double muscling, Fetotomy, Muscular hypertrophy.*

PP-3.6. Testicular temperature gradient and vascularity scores as indicators of semen quality in summer-stressed Murrah bulls

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High summer temperatures compromise reproductive efficiency in Murrah buffalo bulls by altering testicular heat regulation and limiting blood perfusion. The present study investigated how the testicular temperature gradient (TG) and testicular vascular flow relate to semen quality in twelve adult Murrah bulls exposed to intense summer stress (average THI 80.6). Scrotal surface temperatures at the proximal and distal poles were recorded every two weeks using infrared thermography, and TG was determined from these values. Testicular blood flow was evaluated monthly through Colour Doppler ultrasonography using subjective vascularity scores (1-5) and the proportion of area showing Doppler signals. Semen was collected fortnightly with an artificial vagina and examined for concentration, motility traits, viability, acrosome status, HOST response, and morphological abnormalities. Based on TG, bulls were categorized into two groups (>3 °C and <3 °C). Bulls maintaining a TG above 3 °C exhibited markedly superior perfusion (52.7±1.6% vs. 40.6±2.1%) and higher vascularity scores (3.3±0.1 vs. 2.5±0.1). They also showed better semen attributes, including higher progressive motility (79.3±1.6% vs. 71.1±1.2%), greater viability (82.9±0.9% vs. 76.4±1.7%), improved acrosome integrity (86.6±0.2% vs. 84.0±0.3%), stronger HOST response (68.0±0.7% vs. 63.0±0.5%), and fewer abnormal spermatozoa (10.8±0.2% vs. 13.3±0.3%). Correlation and regression analyses demonstrated clear positive relationships between TG, vascular indices, and semen quality, while

higher THI values were linked with reduced testicular efficiency. Overall, thermography and Doppler-based assessments provide dependable, non-invasive indicators of heat-related reproductive decline and can help guide management interventions during periods of severe heat stress.

Keywords: *Testicular thermoregulation; Temperature gradient; Infrared thermography; Colour Doppler ultrasonography; Testicular perfusion; Heat stress; Semen quality*

PP-3.7. Successful obstetrical management of dystocia caused by hydroallantois in a graded Murrah buffalo: A case report

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A six-year-old full-term pregnant Murrah buffalo was presented to the Referral Veterinary Polyclinic (RVP-VGO) with a history of prolonged gestation, anorexia, and progressive abdominal distension. Clinical and ultrasonographic examinations revealed a closed cervix, accumulation of allantoic fluid, and non-functional placentomes, confirming the diagnosis of hydroallantois. Parturition was induced with cloprostenol, dexamethasone, and oestradiol benzoate, while valethamate bromide and misoprostol were administered to facilitate cervical dilation. Transcervical allantocentesis with a Foley catheter was performed for gradual evacuation of allantoic fluid. Continuous intravenous fluid therapy and supportive treatment was provided to prevent hypovolemic shock and secondary infections. A dead male foetus with mandibular malformation (brachygnathism) was delivered by gentle traction, and the dam recovered uneventfully without any complications. Necropsy was done on the calf revealed no remarkable abnormalities. The present case report highlights the successful management of hydroallantois in a buffalo through combined medical and obstetrical interventions.

Keywords: *Hydroallantois, Misoprostol, Transcervical allantocentesis, and Brachygnathism*

PP-3.8. Melatonin administration as a strategy to augment OPU–IVF based embryo production in buffalo during summer season

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Heat stress during summer season impairs ovarian function and reduces the efficiency of *in vitro* embryo production (IVEP) in buffaloes. Melatonin, known for its antioxidant and endocrine-modulatory properties, was evaluated as an adjunct strategy to improve OPU–IVF outcomes under heat-stress conditions. A total of 18 parous buffaloes were divided into two equal groups (n=9). The melatonin group received melatonin at 18 mg/50 Kg body weight subcutaneously, dissolved in corn oil, whereas the control group received corn oil alone. Each animal underwent two OPU sessions: the first OPU on Day 10 after treatment and the second OPU on Day 20. Follicular population, oocyte yield and quality (Grades A–D), and IVF parameters were assessed. The melatonin-treated buffaloes exhibited a higher total follicular count compared with the control group. Notably, the number of small and medium follicles was higher ($p<0.05$) in the melatonin group, suggesting improved follicular recruitment and enhanced ovarian activity during summer heat stress. In the first OPU, melatonin-treated animals showed higher total oocyte recovery (5.44±0.73) compared with control (3.00±0.38), accompanied by an increased proportion of Grade B and C oocytes, indicating improved developmental competence. In the second OPU also, the melatonin group exhibited a higher total oocyte yield (6.67±1.38 vs. 3.11±0.37) than control group. The number of Grade A (1.11±0.24 vs. 0.33±0.15, $p=0.036$), Grade B (1.22±0.32 vs. 0.44±0.18, $p=0.036$), and Grade C oocytes (1.56±0.32 vs. 0.67±0.22, $p=0.005$) was also significantly greater in the melatonin group. Similarly, cleavage and blastocyst production rate were also significantly higher in melatonin group ($p<0.05$) across first and second OPU. In conclusion, melatonin administration during summer season enhances follicular growth, oocyte quality, and oocyte developmental competence across sequential OPU sessions. Therefore, melatonin may serve as a practical and effective treatment for improving OPU–IVF efficiency in buffaloes during the summer season.

Keywords: *Buffalo, OPU-IVF, Melatonin, Summer season*

PP-3.9. POC: Urine-based paper strip tests for pregnancy detection in buffaloes

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Early and accurate pregnancy diagnosis in buffaloes is essential for efficient reproductive management, timely rebreeding decisions, and maintaining optimal milk production. Traditional techniques such as rectal palpation and ultrasonography, although dependable, require trained professionals and can cause discomfort or stress to the animals. In comparison, urine-based testing offers a fast, non-invasive, and affordable option. In the conventional Preg-D assay, urine samples were used for pregnancy detection, and the procedure involved a mandatory heating step, requiring approximately 30 minutes to obtain results. The upgraded Preg-D 2.0 platform utilizes urine along with a set of specific reagents that interact with pregnancy-associated metabolites. These interactions produce a distinct and measurable colour change, which can subsequently be quantified using an optical density (OD) meter. In this work, we further advance this concept by developing a urine-based dipstick designed for rapid and user-friendly pregnancy diagnosis. The dipstick functions by incorporating reagents that react selectively with analytes found in the urine of pregnant animals. This reaction generates a visible colour change that can be interpreted directly with the naked eye, eliminating the need for heating steps or external instrumentation. To ensure stable performance, the dipstick incorporates with stabilizing and inert components to enhance shelf life, and colour development, resulting in a more robust, rapid, and user-friendly pregnancy diagnostic tool.

Keywords: *Metabolite, paper Strips, pregnancy diagnosis, dipstick*

PP-3.10. Epsilon-Polylysine as a Sustainable Antibiotic Free Additive for Enhancing Buffalo Semen Cryopreservation

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Ensuring high reproductive performance in buffalo breeding programs requires maintaining semen free from excessive microbial contamination, as bacteria can disrupt sperm viability and fertilizing capacity during processing and storage. In most artificial insemination (AI) centers, antibiotics are routinely added to semen extenders to control bacterial growth, but the rising concern over antimicrobial resistance has created a demand for alternative strategies. The present study examined the suitability of ε-polylysine (ε-PL), a naturally occurring antimicrobial compound, as a potential replacement for conventional antibiotics in buffalo semen preservation. This study evaluated the efficacy of epsilon-polylysine (ε-PL), a naturally occurring antimicrobial peptide, as a substitute for antibiotics in buffalo semen extenders, focusing on its effects on bacterial load, sperm quality, and functional competence during cryopreservation. Semen from Murrah buffalo bulls was extended with Tris–egg yolk–glycerol-based extenders supplemented with either penicillin-streptomycin or ε-PL (0.64 g/L and 1.28 g/L). Extended samples were cryopreserved in 0.25 mL straws and evaluated post-thaw for microbial load (plate count), sperm motility and kinetics (CASA), acrosome integrity, mitochondrial membrane potential, and mitochondrial superoxide production (flow cytometry). Extenders containing ε-PL significantly reduced bacterial growth, showing effects comparable to antibiotics ($P>0.05$). Post-thaw motility parameters remained acceptable in all groups. Semen extended with 0.64 g/L ε-PL exhibited superior acrosome integrity and mitochondrial membrane potential compared to antibiotic controls, along with reduced mitochondrial superoxide generation ($P<0.05$). ε-PL effectively limited microbial contamination while supporting sperm structural and functional integrity. Its use in semen extenders offers a promising antibiotic-free strategy, reducing reliance on conventional antimicrobials and improving the sustainability of buffalo breeding program.

Keywords: *Antibiotic free extender, buffalo semen, cryopreservation, epsilon- polylysine*

PP-3.11. Distribution, Morphometric Evaluation and Steroidogenic Profile of Cyclic and Acyclic Ovarian Follicles in Buffalo

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The water buffalo (*Bubalus bubalis*) is an economically important livestock species in tropical and subtropical regions, contributing substantially to milk, meat, and hide production. However, reproductive inefficiency remains a major constraint, with acyclicity representing one of the most prevalent disorders affecting fertility and productivity. Acyclicity, defined as the absence of ovarian cyclic activity and failure to form a functional corpus luteum (CL), commonly occurs during the postpartum period or under conditions of nutritional, environmental, or metabolic stress. Prolonged anestrus ultimately increases the number of days open, veterinary interventions, and culling rates. Understanding the distribution pattern of follicles and their functional status based on steroidogenic characteristics during different stages of cyclic and acyclic states is therefore essential for developing targeted reproductive interventions. In this study, paired ovaries were collected from healthy adult buffaloes (n=75) at slaughter. Ovaries were classified as cyclic or acyclic based on the presence of a CL. Surface follicles were enumerated and categorized into small (<5 mm), medium (5–10 mm), and large (>10 mm). Follicular fluid from large follicles was analyzed for estradiol (E_2), progesterone (P_4), and androstenedione (A_4), and follicles were retrospectively classified as active (P_4:E_2 <1), intermediate (P_4:E_2 1–10), or atretic (P_4:E_2 >10). Acyclic buffaloes exhibited a greater proportion of ovaries with only small follicles (36.36%), compared with cyclic animals (9.43%), whereas only large follicles (>10 mm) occurred predominantly in cyclic buffaloes (26.42% vs. 4.55%). Cyclic buffalo ovaries also showed stage-dependent shifts in follicle distribution with only large follicles observed in 26.1% at Stage 3 and 57.1% at Stage 4. Despite these morphological differences, large follicles from cyclic and acyclic buffaloes displayed comparable steroidogenic patterns, with progressive increases in P_4 and decreases in E_2 from active to atretic categories. Further, the E_2:A_4 ratio was also significantly greater in active follicle than the atretic follicle. Thus, evaluation of the follicles based on steroidogenic profile provided biologically meaningful insights for follicular atresia than classification based solely on size and stages of cyclicity and would be useful to develop hormonal intervention for augmenting reproductive efficiency.

PP-3.12. Recombinant human FSH as a viable alternative to porcine FSH for ovarian stimulation in Buffalo: Effects on Follicular response, Oocyte quality and Embryo development

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Ovarian stimulation is crucial for maximizing oocyte recovery in buffaloes undergoing *in-vitro* embryo production (IVEP) using ovum pick up (OPU). Conventional protocols predominantly rely on porcine follicle-stimulating hormone (pFSH), but its high cost, labour-intensive administration, and limited availability restrict large-scale application. The present study was designed to evaluate recombinant human FSH (rh-FSH) as a potential alternative to pFSH for ovarian stimulation in buffaloes. Thirty cyclic Murrah buffaloes were randomly allocated into three groups (n=10). The control group underwent OPU on Day 4 post-estrus without any stimulation. The pFSH group received 200 mg pFSH intramuscularly in a tapering regimen for three consecutive days starting on Day 1 post-estrus, followed by a 40-hour coasting phase prior to OPU. In rh-FSH group, rh-FSH was administered intramuscularly at 150 IU on Day 1 and 75 IU on Day 2 (total dose 225 IU), with 40-hour coasting period before OPU. Parameters including follicular response, oocyte yield and quality, and IVF outcomes (fertilization, cleavage, and blastocyst rates) were assessed across all groups. The number of medium-sized follicles differed significantly among the groups ($p<0.0001$), with the highest counts observed in the stimulated groups, whereas large follicles showed no significant variation ($p=0.119$). Total follicular count differed significantly ($p=0.029$), being highest in the pFSH group (12.20±1.82), followed by rh-FSH (11.20±0.61), and control (9.20±0.39). Oocyte recovery also varied significantly (p=0.018), with mean values of 6.20±0.80 (pFSH), 5.60±0.60 (rh-FSH), and 3.20±0.33 (control). Besides, number of grades B (p=0.049) and grade C (p=0.027) oocytes showed marked improvement in the stimulated groups. The blastocyst rate was also highest in pFSH (32.80±4.83), followed by rh-FSH (29.78±4.02), and lowest in control (18.18±3.63). Cost assessment revealed that hormonal stimulation with pFSH required approximately ₹5,000 per animal, while rh-FSH required only about ₹1,500. In conclusion, both pFSH and rh-FSH enhanced follicular growth, oocyte yield, and embryo production. However, rh-FSH offers a more user-friendly, cost-effective, and animal welfare–oriented option, making it suitable for routine buffalo IVEP, particularly in resource-limited settings.

Keywords: *Buffalo, In-vitro embryo production, Ovarian stimulation, Recombinant human FSH, Porcine FSH, Ovum pick-up*

PP-3.13. Studies on Seasonal Variation in Buffalo spermatozoa quality in relation to the changing temperature-humidity index

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Buffaloes are particularly vulnerable to the heat stress due to various intrinsic constraints, such as lesser number of sweat glands and skin colour. Buffalo bull breeding capacity is limited as compared with cattle bulls, due to its small testicular size, low spermatozoa output rate and low epididymal reserve. Buffalo has a lower cholesterol to phospholipid ratio than other species, which increases the risk of damage to sperm plasma membrane. Buffalo is contemplated as a seasonal breeder with more efficient breeding activity in winter and autumn season as compared to other seasons. Environmental elements like temperature, humidity, atmospheric pressure, and day-duration have an impact on sperm quality. Therefore, the current study was proposed with the objectives, to investigate the seasonal variation in fresh spermatozoa quality in relation to the changing temperature-humidity index. Semen samples were collected (6 x 4 ejaculates x 3 seasons) each in summer (Hot dry: April-May), rainy (Humid: July-Oct) and winter season (Cold Dry: Nov-Jan) at a regular interval of 10 days. THI values were categorized in accordance with season into 6 groups Group I (THI 78-80) Group II (THI 81-83), Group III (THI 84-86), Group IV (average THI of Group II and III) for hot dry, Group V (THI 80-82) for humid and Group VI (THI<77) for cold dry. the semen samples were initially assessed for various parameters, including mass activity, individual progressive motility, concentration, viability, and the presence of sperm morphological abnormalities. To ensure consistency throughout the experiment, only ejaculates meeting certain criteria were chosen for assessing semen quality and quantity parameters. These criteria included ejaculate volume greater than 1.5 mL, sperm concentration exceeding 500 million/mL, mass activity higher than 3+, and individual progressive motility exceeding 70%. Group III (THI 84.16) had the greater mean total morphological abnormalities in comparison with other groups. The intact plasma membrane integrity and acrosome integrity percentage were increased (P<0.05) in low THI (58.2). The Group VI exhibited the maximum viability compared to other groups. The findings suggest that Group VI (with the mean THI 58.2) exhibited improvement in DNA integrity compared to other groups. In this current research, the average proportion of DNA integrity was measured at 94.88±0.29%, 94.75±0.45%, 94.83±0.24%, 94.96±0.14% and 95.58±0.26% for Group II to VI, respectively. Seasonal variation had significant influence on the sperm quality parameters, which was higher in winter followed by humid and rainy. With the rise in THI buffalo spermatozoa show an increasing likelihood of experiencing deterioration of spermatozoa quality. In the local Agro-climatic conditions, a THI less than 77 is considered optimal for producing high quality buffalo semen with good semen quality parameters for the semen collected during the summer months, ejaculates of ‘low THI days’ may be preferred over other days for semen processing.

Keywords: *Temperature humidity index, Oxidative stress, Spermatozoa*

PP-3.14. Studies on Cysteine as an additive to Semen Extender to Mitigate Summer Stress in Buffalo Spermatozoa

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Buffalo bulls are highly vulnerable to thermal stress due to limited sweat glands, dark skin and low heat dissipation capacity. Elevated environmental temperature and humidity during summer months cause significant oxidative stress, leading to deterioration in sperm quality and cryosurvival. This oxidative damage arises primarily from excessive reactive oxygen species (ROS) and inadequate antioxidant defense in buffalo spermatozoa. Among antioxidant additives, L-cysteine, a sulphur-containing amino acid with a free thiol (-SH) group, has been proposed as a potential and cost-effective additive in semen extender to counteract oxidative stress. The present study aimed to optimize the concentration of cysteine in semen extender for mitigating summer-induced oxidative damage in buffalo spermatozoa during cryopreservation. To optimize the dose of cysteine in alleviating post thaw oxidative stress of spermatozoa during summer months. The experiment was conducted during the summer season at the Germ Plasm Centre, Division of Animal Reproduction, ICAR–IVRI, Izatnagar (UP). A total of 24 ejaculates were collected from four healthy Murrah buffalo bulls (6 ejaculates per bull). Only ejaculates meeting quality thresholds (volume ≥1.5 mL, mass activity ≥3+, progressive motility ≥70%, and concentration ≥500 million/mL) were selected. Semen samples were divided into four groups: Group I (Control, no cysteine), Group II (2 mmol cysteine), Group III (4 mmol cysteine), and Group IV (6 mmol cysteine). All samples were diluted with Tris–egg yolk–glycerol (TYG) extender to achieve a final concentration of 80 million sperm/mL, equilibrated at 4°C for 4 hours, frozen in liquid nitrogen, and later evaluated for post-thaw quality parameters, including viability, acrosome integrity, plasma membrane integrity, sperm abnormalities, DNA integrity, malondialdehyde (MDA) concentration, intracellular ROS, and total antioxidant capacity (TAC). The supplementation of cysteine produced a dose-dependent effect on post-thaw sperm quality. The inclusion of 4 mmol cysteine (Group III) significantly ($p<0.05$) improved all evaluated parameters compared to the control and other treatment groups. Group III exhibited the highest sperm viability (77.0±0.42%), acrosome integrity (71.65±0.63%), and plasma membrane integrity (65.21±0.86%), alongside the lowest proportion of sperm abnormalities (13.79±0.22%). DNA integrity was highest in Group III (95.63±0.17%) compared to Control (93.50±0.24%) and higher-dose Group IV (91.71±0.37%). Biochemical analyses supported these findings: Group III recorded the lowest MDA concentration (0.47±0.01 µmolmL^{-1}), lowest intracellular ROS levels (26.42±0.99%), and highest total antioxidant capacity (1041.79±18.24 nmolmL^{-1}). In contrast, a higher concentration of cysteine (6 mmol) resulted in diminished sperm quality and elevated ROS, indicating potential pro-oxidant or osmotic effects at excessive doses. These findings demonstrate that 4 mmol cysteine is the optimum concentration for inclusion in buffalo semen extender during summer, effectively reducing oxidative stress and enhancing post-thaw sperm viability and integrity. The antioxidant action of cysteine likely stems from its thiol-mediated ROS scavenging and reinforcement of the sperm plasma membrane against lipid peroxidation. Supplementing semen extenders with 4 mmol cysteine represents a cost-effective and physiologically compatible strategy to mitigate heat-induced oxidative damage, thereby improving cryopreservation outcomes under tropical climatic conditions.

PP-3.15. Humanin supplementation protects buffalo spermatozoa from cryostress by modulating oxidative stress associated gene expression

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Cryopreservation of buffalo semen is widely used in animal breeding, but the freezing and thawing process often damages sperm cells due to oxidative stress. This study was conducted on the role of humanin in transcript expression to establish a relationship between humanin supplementation and its

ameliorative effect on cryostress-altered parameters through the expression of genes. Semen from three Murrah buffalo bulls was divided into two groups: one extended with a standard semen dilutor and the other with the same dilutor plus 5 µM humanin. Samples were tested at different stages like fresh, pre-freeze, and post-thaw for sperm quality, oxidative stress levels, and expression of specific genes related to antioxidants and cell survival. The results showed that semen treated with humanin had better post-thaw sperm motility, viability, acrosome integrity, and membrane integrity than the control group. Markers of oxidative stress were lower, and total antioxidant levels were higher in the humanin supplemented group. Gene analysis revealed that humanin boosted the expression of antioxidant-related genes (NRF2 and SOD2) while reducing the activity of genes linked to oxidative stress (ROMO1). Computer-assisted semen analysis further confirmed improved sperm movement patterns in the treated group. These findings suggest that humanin helps protect sperm by reducing oxidative damage and cell death during cryopreservation. This study elucidates the impact of cryopreservation on the expression of antioxidant-associated genes in buffalo spermatozoa and investigates the role of humanin in modulating transcript expression, aiming to establish a relationship between humanin supplementation and its potential ameliorative effects on cryostress-induced molecular alterations.

Keywords: *Antioxidant genes, Apoptosis, Buffalo Spermatozoa, Cryoprservation, Humanin, Oxidative Stress*

PP-3.16. Improving Buffalo Productivity and Thermoregulation under Heat Stress through Dietary Strategies in Tropical Climates

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Climate change has caused rising temperatures and altered weather patterns, significantly affecting livestock productivity, especially in tropical and subtropical areas. Heat stress is a significant challenge for livestock, impacting growth, milk production, reproductive success, and overall health. Buffaloes, a key source of milk, are particularly vulnerable to high temperatures and humidity. Understanding how heat stress affects their physiological, biochemical, and molecular responses is essential for developing management strategies that support their productivity and welfare amid climate change. This study aimed to examine the physiological and molecular adaptations of lactating Murrah buffaloes and heifers exposed to heat stress under Hot-Dry and Hot-Humid conditions. The experiment lasted six months across two seasons: hot-dry and hot-humid, at Kathura Village, Sonipat, Haryana. A total of 48 animals were included, consisting of 24 heifers (6-9 months old) and 24 lactating buffaloes (1-3 parities). They were divided into four groups: Control (traditional diet), T1 (traditional diet + cumin), T2 (traditional diet + molasses), and T3 (traditional diet + both cumin and molasses). Physiological measures such as respiratory rate (RR) and rectal temperature (RT), along with production data such as body weight and milk yield, and molecular responses, including gene expression of hsp70 and hsp110, were assessed during the study. Results revealed that both hsp70 and hsp110 expression significantly increased ($p<0.05$) under heat stress, with the highest levels in the Control group. Growth measurements indicated that T3 animals had higher body weights (233.83 kg and 246.17 kg in the Hot-Dry and Hot-Humid seasons, respectively) than controls. Milk yield was also greater in the experimental groups, with T3 producing the most milk (12 kg in Hot-Dry and 12.78 kg in Hot-Humid). Physiological responses such as RR and RT were notably lower in T3, indicating improved thermoregulation. These findings demonstrate that dietary supplementation with cumin and molasses can enhance growth, milk production, and heat tolerance in buffaloes.

Keywords: *Murrah buffalo; Thermal stress; Physiological response; Gene expression; Molecular response*

PP-3.17. Impact of FSH Stimulation and Seasonal Variation on Oocyte Quality and In Vitro Embryo Production in Buffaloes

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Buffalo reproduction is highly vulnerable to environmental stressors, particularly high ambient temperatures, which negatively impact follicular growth, oocyte developmental competence, and overall in vitro embryo production (IVEP) efficiency. Hormonal pretreatment with follicle-stimulating hormone (FSH) has been proposed as a strategy to enhance ovum pick-up (OPU) outcomes and subsequent embryo yield, especially during thermally stressful periods. The present study aimed to evaluate the influence of season on follicular response, oocyte quality, and IVEP performance in buffaloes subjected to FSH-stimulated and non-stimulated OPU protocols. A total of 48 pluriparous buffaloes (n=24 in each season), subdivided into stimulated (n=12) and non-stimulated (n=12) groups for both summer and winter, were assessed. Animals in the treatment group received FSH before OPU, whereas the control group underwent OPU without hormonal intervention. Retrieved cumulus–oocyte complexes (COCs) were graded, and viable oocytes were processed for maturation, fertilization, and embryo culture. Seasonal differences were evident, with winter supporting superior follicular output and oocyte competence. In FSH-treated buffaloes, winter yielded more aspirated follicles (216 vs. 183), higher oocyte recovery (47.2% vs. 38.2%), and a greater proportion of quality Grade A and B oocytes than summer. These improvements contributed to enhanced viability (77.4% vs. 62.8%), cleavage (51.13% vs. 42.75%), and blastocyst development (28.41% vs. 25%). Even in non-stimulated animals, winter favored better oocyte grades and embryonic development. Across both seasons, FSH stimulation consistently improved follicular recruitment, oocyte yield, and IVEP efficiency, with its benefits being particularly notable during summer heat stress. Overall, the findings highlight that winter conditions favor reproductive performance and that FSH stimulation serves as a valuable intervention to counteract seasonal constraints and improve biotechnology-driven embryo production in buffaloes.

Keywords: buffalo reproduction, FSH stimulation, oocyte quality, ovum pick-up, *in vitro* embryo production.

Session-IV

Health & Diseases

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		Dr Ajay Kumar Yadav Scientist ICAR-IVRI Izatnagar

LP-4.1. Discovery of M-cells and P-cells in buffalo tonsils: Assessing their morphology and superiority

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Introduction

The nasopharyngeal tonsil is lined by pseudostratified columnar ciliated epithelium with goblet cells, along with a distinct follicle-associated epithelium (FAE). Embedded within the FAE are microfold (M) cells, which have been identified in several species, including horses, pigs and buffaloes (Kumar *et al.*, 2001; Ranjit *et al.*, 2011; Girgiri *et al.*, 2019). These specialized epithelial cells are central to mucosal immunity, as they transport luminal antigens to the underlying lymphoid tissue to initiate immune responses. M-cells are characterized by a flattened or dome-shaped apical surface, sparse microvilli and their close association with intraepithelial lymphocytes (Kumar *et al.*, 2001). Additional distinguishing features include elevated apical cholesterol (Madara *et al.*, 1984) and variable alkaline phosphatase and esterase activity (Smith *et al.*, 1987; Owen and Bhalla, 1983). In horses, vimentin expression and binding of the lectin GS1B4 have also been suggested as supplementary markers of M-cells (Kumar *et al.*, 2001). Detailed descriptions of the histoarchitecture of the nasopharyngeal tonsil in horses and buffaloes provide useful comparative insights (Kumar *et al.*, 2001; Girgiri *et al.*, 2019).

Lectins are non-immune glycoproteins that selectively bind terminal carbohydrate residues and serve as valuable histochemical tools for identifying cellular and tissue-specific glycoconjugates (Ibrahim *et al.*, 2013). Their selective affinity allows differentiation of cell populations and facilitates the study of host-microbe interactions. In this context, lectin histochemistry is particularly useful for characterizing potential M-cell markers and examining early events in pathogen recognition at mucosal interfaces. Because glycoconjugates on the apical membranes of the FAE especially those of M-cells serve as primary docking sites for lectins and microbial adhesins (Clark *et al.*, 1993): their identification is important for understanding mucosal defense mechanisms and developing targeted vaccine delivery systems (Giannasca *et al.*, 1994). Notably, the distribution of these glycoconjugates varies across species, with marked differences observed between mice and humans (Sharma *et al.*, 1996).

Given the unique glycocalyx of M-cells, the present study examines lectin-binding patterns in the nasopharyngeal tonsil of adult buffaloes. The objectives are to map glycohistochemical epitopes within the respiratory epithelium, FAE and propria-submucosa and to assess their functional significance. These findings may improve our understanding of disease-related carbohydrate alterations and provide insights into the roles of M- and P-cells in antigen uptake and targeted mucosal immunization.

Materials and Methods

Six clinically healthy adult buffaloes (Bubalus bubalis): 5-6 years of age and of a local mixed breed, irrespective of sex, were used for the study. Heads were obtained from a registered slaughterhouse, where Institutional Animal Ethics Committee approval was not required. A sagittal section of each head was made using an electric saw and tissues from the nasopharyngeal tonsil were collected and immediately preserved in liquid nitrogen. Small fragments of the frozen tonsillar tissue were embedded in Poly Freeze tissue freezing medium (Sigma) and used to prepare cryoblocks. Sections 6-8 µm thick were cut using a cryostat and mounted on glass slides coated with 2% 3-aminopropyltriethoxysilane. The slides were stored at -20 °C until staining. A panel of 21 lectins, grouped according to terminal sugar specificity, was used in the analysis (Table 1). Before staining, frozen slides were brought to room temperature, thawed and air-dried for 30 minutes. Sections were fixed in acetone for 20 minutes, air-dried again and rehydrated in phosphate-buffered saline (PBS, pH 7.4) for 10 minutes. A PAP pen (Sigma) was used to outline the sections. Endogenous peroxidase activity was quenched by incubating the slides in 0.3% H_2O_2 in PBS for 30 minutes. After rinsing in PBS, non-specific binding was blocked with 1% bovine serum albumin (BSA) in PBS for 45 minutes.

Individual sections were then incubated with biotinylated lectins (Table 1) at 10 µg/ml in 0.2% gelatin-PBS for 1 hour in a humid chamber at room temperature. Following three PBS washes (5 minutes each): the slides were incubated with streptavidin Alexa Fluor™ 488 (2 µg/ml in PBS) for 30 minutes and washed twice with distilled water (5 minutes each). Sections were mounted with Mowiol containing 2.5% 1,4-diazabicyclo[2.2.2]octane (DABCO). Control sections were processed simultaneously without biotinylated lectins to evaluate non-specific staining. All stained sections were examined under a fluorescent microscope (Olympus CX-41 Magnus) and images were recorded using a Lumenera Infinity 1 CMOS digital camera.

Table 1: Lectins used for the present study with specific sugar moieties

Lectin	**Common Name**	**Acronym**	**Major sugar moieties/ specificity**
N-acetylglucosamine group			
Triticum vulgaris	Wheat germ	WGA	N-acetyl-D-glucosamine and Sialic acid (sia) (β-GlcNAc)
Succinylated *Triticum vulgaris*	Wheat germ, succinylated	s-WGA	N-acetylglucosamine (β-GlcNAc)
Lycopersicon esculentum	Tomato lectin	LEL	[GlcNAc]1-3, N-Acetylglucosamine
Datura stramonium	Datura	DSL	[GlcNAc]1-3, N-Acetylglucosamine
Solanum tuberosum	Potato lectin	STL	N-Acetylglucosamine (β-GlcNAc)
Griffonia (Bandeiraea) *simplicifolia* lectin II	African shrub	GSL II	α- or β-linked N-Acetylglucosamine
N-acetylgalactosamine group			
Glycine max	Soybean	SBA	α-linked N-acetylgalactosamine
Dolichos biflorus	Horse gram	DBA	α-linked N-acetylgalactosamine (α-GalNAc)
Ricinus communis	Castor bean	RCA	N-acetylgalactosamine
Vicia villosa agglutinin	Hairy vetch	VVL	α- or β-linked terminal N-acetylgalactosamine
Griffonia simplicifolia lectin I	African legume	GSL I	α-linked N-acetylgalactosamine
Galactose group			
Griffonia simplicifolia isolectin B4	Africa shrub legume	GS1B4	Terminal (1-3)-linked galactose epitopes
Arachis hypogaea	Peanut agglutinin	PNA	Galactose-β(1-3) N-acetyl galactosamine Gal-β(1-3)-GalNAc
Artocarpus integrifolia	Jackfruit	Jacalin	Galactose, β(1,3) N-aetylgalactosamine
Erythrina crisagalli	Coral tree	ECL	Galactose, N-acetylgalactosamine, Lactose β -Gal>β-GalNAc
Phaseolus vulgaris Erythroagglutinin	Red kidney beans	PHA-E	Galactose, Complex structures
Phaseolus vulgaris Leucoagglutinin	Red kidney beans	PHA-L	Galactose, Complex structures
Glucose/Mannose group			
Canavalia ensiformis Concanavalin A	Jack bean	Con A	Terminal α-D-mannosyl and α-D-glucosyl groups *α-Man>α-Glc*
Lens culinaris agglutinin	Common lentil	LCA	α-linked Mannose, Glucose specific for *α-Man>α-Glc*
Pisum sativum agglutinin	Pea	PSA	α-linked mannose, glucose *specific for α-Man>α-Glc*
Fucose			
Ulex europaeus agglutinin	Furze gorse	UEA	α-linked fucose

Results and Discussion

The study provides the first comprehensive glycohistochemical characterization of the buffalo nasopharyngeal tonsil using a diverse panel of 21 lectins specific for N-acetylglucosamine, N-acetylgalactosamine, galactose, glucose/mannose, or fucose residues. Comparison of lectins within each carbohydrate group revealed consistent binding trends, with only minor variations among lectins recognizing the same terminal sugars. These predictable patterns, together with the clear structural affinities observed across epithelial and subepithelial components, underscore the reliability of lectin carbohydrate interactions and validate the specificity demonstrated by the negative controls.

Within the pseudostratified columnar ciliated epithelium, goblet cells showed weak reactivity to N-acetylglucosamine-specific lectins, binding only to s-WGA. This suggests a limited presence of β-GlcNAc and sialylated N-acetylglucosamine residues in goblet cell secretions. In contrast, mucous acini exhibited moderate to strong affinity for these lectins, indicating marked compositional differences between glandular acinar mucus and goblet cell mucus. DSL, despite recognizing similar carbohydrate residues, produced weaker staining; the basis of this divergence remains unclear. Robust s-WGA reactivity, particularly along the ciliated surface, highlights the contribution of sialic acid to lectin binding. The strong sialylation of the luminal border is likely attributable to the spread of acinar secretions toward the epithelium. Elevated sialic acid content plays an important defensive role in the respiratory tract by inhibiting viral attachment and neutralizing toxins (Murray *et al.*, 1989). Additionally, lectin binding at the epithelial surface may facilitate mucosal immunity by capturing particulate antigens and directing them toward adjacent M-cells and the recently described P-cells. Comparable staining patterns in sheep have likewise been linked both to mucous coatings and to sparse expression of the corresponding residues on cilia or microvilli (Ibrahim *et al.*, 2014; Scocco *et al.*, 2014).

Most lectins distinctly outlined basal cells, while moderate reactivity in the FAE, M-cells and P-cells indicates that glucosamine and sialic acid residues form important components of these specialized epithelial domains. The close similarity in lectin-binding profiles between M-cells and P-cells suggests that P-cells may share functional attributes with M-cells-a hypothesis further supported by analogous lectin affinities in adjacent lymphoid regions. A clear negative correlation was observed between goblet cell staining and lymphoid tissue reactivity for s-WGA, reinforcing the unique biochemical composition of goblet cells. The consistently strong acetylglucosamine reactivity in mucous acini further emphasizes their biochemical divergence from goblet cells, whereas the inconsistent DSL response highlights an area requiring additional study.

The presence of acetylglucosamine residues in basal cells suggests a role in maintaining epithelial structure and turnover. Apart from GSL II, several of the lectins examined also demonstrated strong endothelial binding, indicating their potential utility as vascular markers in future anatomical and pathological investigations.

Lectins specific for N-acetylgalactosamine showed strong binding to the secretions of both goblet cells and mucous acini within the propria-submucosa, indicating that these secretory cell types share a similar carbohydrate composition. Such mucus plays an essential role in maintaining epithelial integrity by providing lubrication, forming a physical barrier and neutralizing pathogens and toxins (Forstner, 1978). Because mucous secretions can mimic epithelial receptor structures, they also contribute to the entrapment and clearance of microorganisms from the respiratory tract (Abraham and Beachey, 1985). Comparable lectin-binding profiles have been documented in the respiratory epithelium of sheep (Ibrahim *et al.*, 2014) and in humans, DBA and VVL likewise demonstrate stronger labeling of villous goblet cells than those associated with the follicle-associated epithelium (FAE) (Sharma *et al.*, 1996).

Beyond the goblet cells and mucous acini, these terminal sugars were largely absent from other epithelial cell types, the FAE and the underlying lymphoid tissue. Nevertheless, DBA and GSL I produced moderate labeling in the FAE, M-cells and P-cells. Lymphoid regions showed minimal binding overall, with only GSL I and RCA exhibiting detectable affinity. Similar to lectins in the glucosamine-binding category, these N-acetylgalactosamine-specific lectins appear useful for examining endothelial glycocalyx architecture, as the tunica intima displayed clear reactivity. Among all tissues examined, mucous acini reacted consistently and strongly across the lectin panel. These findings mirror those reported in sheep (Ibrahim *et al.*, 2014) and human studies likewise note more robust DBA and VVL binding in villous goblet cells than in those of the FAE (Sharma *et al.*, 1996).

Lectins recognizing galactose residues produced staining patterns broadly comparable to those of the glucosamine-binding lectins, albeit with generally reduced intensity. Basal cells were distinctly outlined by GS1B4 and PHA-E, whereas Jacalin generated weaker staining and was the only member of this group to label goblet cells. Binding detected along the ciliated epithelial surface likely reflects the spread of mucous secretions originating from the glandular acini. Both PHA-E and PHA-L displayed moderate reactivity in the FAE, M-cells and P-cells. The α(1-3)-linked galactose residues recognized by these lectins have previously been employed to distinguish mature M-cells in the pharyngeal tonsils of hamsters (Giannasca *et al.*, 1997) and horses (Kumar *et al.*, 2001). Such distinct lectin-binding signatures have facilitated targeted antigen-delivery strategies in gut-associated lymphoid tissues (Giannasca *et al.*, 1994; Clark *et al.*, 1993) and within the nasopharyngeal lymphoid system of hamsters (Giannasca *et al.*, 1997). Similar staining profiles have been reported in mice using GS1B4 (Sharma *et al.*, 1996).

Within the lymphoid compartments, lymphocytes in the subepithelial and interfollicular zones showed stronger reactivity than those in germinal centers, suggesting a higher affinity of these lectins for T-lymphocytes than for B-lymphocytes. The epithelial lining was also labeled by these lectins, though with lower intensity compared with glucosamine-binding lectins. With the exception of Jacalin, the overall staining distribution of this lectin group closely paralleled that observed in the respiratory epithelium and submucosa of sheep (Ibrahim *et al.*, 2014). Species-dependent similarities have likewise been reported in humans and mice, where macrophages in germinal centers and paracortical areas stain strongly with mannose- and GalNAc-specific lectins (Sharma *et al.*, 1996).

Lectins targeting glucose and mannose residues, such as Con A, demonstrated moderate reactivity in several epithelial cell types with the exception of goblet cells and strong labeling within the FAE, although staining in M-cells was comparatively weaker. Within the lymphoid tissue, the staining pattern again highlighted the greater affinity of these lectins for T-lymphocytes than for B-lymphocytes.

UEA, a fucose-specific lectin, exhibited a distinct and selective binding pattern in the nasopharyngeal tonsil. It labelled goblet cell secretions, the free ciliated border, selected cells within the FAE and mucous acini. Its localization in goblet cell granules and the periciliary layer has been interpreted, particularly in rat studies, as evidence of α-L-fucose incorporation into glycoproteins during the later stages of epithelial maturation (Castells *et al.*, 1991). Outside these regions, UEA showed no affinity for other epithelial elements, including the vascular endothelium, a pattern consistent with findings in the Peyer's patches of rats and rabbits. In contrast, UEA-I and WBA selectively labelled M-cells in the Peyer's patches of mice, underscoring species-specific differences in the cytoplasmic and membrane glycoconjugate architecture of M-cells (Clark *et al.*, 1993; Sharma *et al.*, 1996). Human tissues also show notable divergence: in the nasopharynx, Con A and PNA stain goblet cells and mucous glands similarly, whereas UEA-I, Jacalin and s-WGA do not (Paulsen *et al.*, 2001).

The respiratory epithelium supports innate immunity not only through mucus production but also by synthesizing lysozyme, lactoferrin, α-defensins, antimicrobial peptides and various proinflammatory mediators in response to inhaled particles. Variations in the distribution of defensins along different epithelial surfaces may help shape the density and diversity of resident microbiota (Schroder, 1999). The differential expression of receptors for N-acetylglucosamine, galactose, glucose and mannose on goblet cell and mucous gland secretions may therefore represent a dual-layer defense system designed to limit microbial adherence and invasion.

Vimentin, a type III intermediate filament characteristic of mesenchymal cells, has been proposed as an M-cell marker in rabbits, pigs, cattle and horses due to its selective accumulation near the apical regions of these cells (Gebert *et al.*, 1992; 1996; Lelouard *et al.*, 1999; Kumar *et al.*, 2001). However, subsequent investigations particularly in the rabbit ileum revealed that many vimentin-positive epithelial cells initially identified as M-cells were actually cup cells (Ramirez and Andreas, 2003). In the present study, only a small subset of M-cells and P-cells displayed vimentin immunoreactivity, indicating that vimentin alone is not a reliable marker for these specialized cells in the buffalo nasopharyngeal tonsil. Because vimentin is widely expressed in mesenchymal-derived cells, its diagnostic value in epithelial tissues must be interpreted with caution. Studies across multiple species demonstrate that combining vimentin with additional markers such as specific lectins or defined cell-surface antigens improves the accuracy of M-cell identification (Gebert *et al.*, 1992; Lelouard *et al.*, 1999). A multimarker approach is therefore recommended for precise characterization of M-cells across various animal models.

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LP-4.2. Applications of Immunoinformatics in Development of Multi-Epitopic Vaccines for Animal Diseases

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Introduction

The emergence and re-emergence of infectious diseases in animals present major challenges to global livestock production, food security, trade and public health. Modern veterinary medicine increasingly relies on molecular-level insights to combat animal diseases. Traditional vaccines, although successful against many pathogens, often suffer from limitations such as high production cost, biosafety challenges, poor cross-protection against diverse strains, issues with antigenic drift/shift, incomplete stimulation of cellular immunity, necessity for cold-chain storage and difficulties in culturing fastidious organisms. These challenges have driven vaccinology toward more rational, targeted and computationally guided strategies, which has accelerated the development of genomics-guided, computationally- designed, multi-epitopic vaccines. It has caused a revolutionary shift from empirical vaccinology to rational, precision-based vaccinology. Immunoinformatics, a discipline combining immunology with computational sciences, enables the prediction, mapping and modeling of antigenic determinants to design safer and more effective vaccines. One of its major applications is the creation of multi-epitopic vaccines, which incorporate multiple B-cell, T-cell and helper T-cell epitopes into a single construct to generate broad-spectrum immunity.

Immunoinformatics plays a significant role in the development of multi-epitopic vaccine as it reduces laboratory workload, biosafety risks and time in moving from pathogen sequencing to candidate vaccine. It allows

- Rapid identification of B-cell, T-cell, conformational, linear and promiscuous epitopes.
- Rational vaccine design without pathogen culture.
- Pan-epitope and pan-serotype vaccine development.
- Prediction of immunogenicity, allergenicity, toxicity, solubility and stability prior to laboratory evaluation.

Overview of Immunoinformatics

Immunoinformatics refers to the application of computational algorithms, databases, machine learning tools and structural modeling methodologies to analyze immune responses and predict immunogenic components of pathogens. It integrates genome analysis, epitope mapping, antigen modeling, immune simulation and vaccine design into a cohesive, systematic approach.

Importance in Veterinary Sciences

Veterinary pathogens, including viruses, bacteria and parasites, often exhibit high genetic diversity and complex host-pathogen interactions. Many are difficult to culture or require high biocontainment facilities, have multi-species infection patterns and limited availability of host genetic information. Immunoinformatics helps overcome these limitations by:

- Enabling in-silico screening of pathogens
- Targeting conserved and immunodominant epitopes
- Combining epitopes from multiple proteins and stages of pathogenesis
- Designing non-live, non-infectious vaccines that do not require handling live pathogens.
- Customizing vaccines to specific breeds or species (e.g., bovine BoLA alleles)

Machine Learning and AI in Immunoinformatics

Machine learning and artificial intelligence have become integral to modern immunoinformatics, significantly improving the prediction and design of vaccine components. Deep neural networks are extensively used for predicting MHC-binding motifs with high accuracy, allowing rapid identification of T-cell epitopes across diverse species. Transformer-based models have further advanced this field by enabling more precise protein structure prediction and epitope recognition, leveraging their ability to capture long-range dependencies within amino acid sequences. Evolutionary AI approaches contribute to forecasting immune escape variants by simulating viral or microbial evolution under host immune pressure, thereby aiding in the development of more resilient vaccines. Additionally, generative AI methods are now being used to design synthetic epitopes and novel immunogens, offering unprecedented possibilities for creating highly specific and potent multi-epitopic vaccines.

Multi-Epitopic Vaccines: Concept and Significance

Rationale Behind Multi-Epitopic Vaccines

Multi-epitopic vaccines combine several immune-stimulatory epitopes—B-cell, cytotoxic T-lymphocyte (CTL) and helper T-cell (HTL) epitopes—within a single recombinant construct. This strategy enables:

- Broader immunity across diverse pathogen strains
- Improved activation of both humoral and cell-mediated responses (Th1, Th2, CTL, humoral)
- Targeted immunogenicity without exposure to whole pathogen avoiding the risks associated with whole-pathogen vaccines
- Enhanced safety, stability and ease of production
- DIVA (Differentiating Infected from Vaccinated Animals) capability

Types of Epitopes Included

B-Cell Epitopes: These epitopes induce antibody-mediated immunity and include both linear and conformational epitopes. They are critical for neutralizing antibodies and opsonization.

Cytotoxic T-Lymphocyte (CTL) Epitopes: These MHC-I restricted epitopes activate CD8+ T-cells capable of killing infected cells. They are vital for immunity against intracellular pathogens. These must possess proteasomal cleavage sites.

Helper T-Cell (HTL) Epitopes: These MHC-II restricted epitopes activate CD4+ T-cells, which stimulate cytokine release and enhance both humoral and cellular immunity.

Immunoinformatics Tools and Databases

Immunological Databases: The foundation of immunoinformatics lies in rich biological databases that catalog protein sequences, 3D structures, antigenic profiles and immune epitopes. The Immune Epitope Database (IEDB)provides experimentally validated and predicted epitopes. UniProt, NCBI GenBank and PDB store annotated protein sequences and structures. Livestock-specific MHC allele data are available through specialized databases such as BoLA (Bovine Leukocyte Antigen) for cattle, SLA for pigs and BLA for birds. These resources facilitate genome-wide screening of candidate antigens for epitope prediction.

4.2 Computational Tools

Important tools include:

Sequence-based Tools

- BLAST, PSI-BLAST
- TMHMM (transmembrane prediction)
- SignalP (signal peptides)

Epitope Prediction Tools

- **B-cell**: BepiPred, LBtope, ABCPred
- **T-cell (MHC-I)**: NetMHCpan, NetCTL
- **T-cell (MHC-II)**: NetMHCIIpan

Structural modelling Tools

- AlphaFold2, SwissModel, MODELLER,PyMOL

Immune Simulation Tools

- C-ImmSim, iMODS, DynaMut, Janus Immunogenicity Simulator

Machine Learning Approaches

- ANN, SVM, Random Forest, Hidden Markov Models
- Transformer models for epitope prediction (e.g., EpiBERTope)

These tools collectively streamline predictive vaccine design from sequence to structural validation.

Workflow of Multi-Epitopic Vaccine Design

Pathogen Analysis and Antigen Selection: Computational vaccine design begins with accessing complete genome or proteome sequences of the pathogen. This is followed by pan-genome analysis for conserved regions, functional annotations, screening for immunodominant, surface-exposed, membrane-bound, secretory and virulence-associated proteins, as they are more likely to interact with the host immune system. Conservation analysis across multiple strains helps prioritize proteins capable of generating cross-protective immunity. Prioritization of the selected epitopes is essential based on their surface exposure, antigenicity, low homology with host proteins and conservation across strains.

Antigenicity, Allergenicity and Toxicity Prediction: Candidate proteins undergo antigenicity prediction using VaxiJen, ANTIGENpro or similar algorithms to ensure strong immune-stimulatory potential. Vaccines require high antigenicity scores (>0.5 threshold in VaxiJen). Allergenicity score of the epitopes is predicted using AllerTOP, AllegenFP or AlgPred and only non-allergenic sequences are retailed for further analysis. Toxicity predictors (ToxiPred, ToxinPred2 further exclude toxic elements and ensures biological safety. Only proteins that score high in antigenicity and low in allergenicity and toxicity are chosen for epitope screening.

Epitope Prediction

B-Cell Epitope Mapping: Tools like BepiPred and ABCPred analyze physicochemical properties such as hydrophilicity and surface accessibility to identify linear B-cell epitopes. Structural tools such as ElliPro help identify conformational epitopes. IEDB facilitate prediction of surface accessibility, flexibility, hydrophilicity, antigenic index and beta turn propensitiy.

CTL and HTL Epitope Prediction: For CTL epitopes, algorithms assess peptide binding to host MHC-I molecules, proteasomal cleavage, TAP transport and promiscuity across multiple alleles. HTL epitope prediction includes assessing binding affinity to MHC-II molecules, potential for cytokine induction (e.g., IFN-γ, IL-12, IL-4) and potential for helper T-cell activation. Host species-specific MHC alleles (BoLA in cattle, SLA in pigs, GALA in goats) must be considered, since epitope binding strength varies across cattle, goats, sheep, pigs and poultry. This ensures accuracy of predictions and suitability of epitopes for specific animal species.

Epitope Screening and Selection: Predicted epitopes are screened for antigenicity, immunogenicity score, conservancy across strains, cluster analysis to avoid redundancy, lack of similarity with host proteins, absence of cross reactivity, exclusion of self-antigen, ability to stimulate IFN-γ, IL-2 and IL-4 responses and promiscuous binding to multiple MHC alleles. Only epitopes meeting these criteria proceed to construct assembly.

Designing the Multi-Epitopic Construct

Linkers: Linkers are used to maintain structural flexibility and proper processing of the epitopes inside antigen processing cells, Linkers such as AAY (for CTL epitopes): GPGPG (for HTL epitopes): KK (for B-cell epitopes) and EAAAK (structural separation) ensure proper separation and processing of epitopes.

Incorporation of Adjuvants: To enhance immunogenicity, molecular adjuvants such as β-defensin, TLR agonist peptides (e.g., RS09): or LTB (heat-labile toxin B-subunit) or flagellin fragments for TLR5 activation are added at the N-terminal region of the construct. These adjuvants improve antigen uptake and immune activation.

Structural Modeling and Validation

Secondary and tertiary structures of the designed vaccine are predicted using tools such as PSIPRED, SOPMA, AlphaFold2, Robetta or Modeller. The best structure is selected and subjected to structural refinement to improve the folding accuracy using GalaxyRefine, ModRefiner or 3Drefine. Structural validations is done using Ramachandran plots, ProSA Z-scores and ERRAT analysis (for non-bonded interactions) ensure structural reliability and stability.

Molecular Docking and Molecular Dynamics Simulation

Docking studies evaluate the binding interactions of the vaccine with immune receptors such as TLR2, TLR4, TLR5, MHC (I/II) molecules or B-cell Ig domains using tools like HADDOCK, ClusPro, AutoDock Vina and PatchDock-FireDock. For successful docking, binding energy, hydrogen bonding, interface residues and conformational stability are analyzed. Molecular dynamics simulations assess structural flexibility, binding stability, compactness, interaction energies, RMSD, RMSF, Radius of gyration and conformational changes under physiological conditions, ensuring that the vaccine construct is dynamically stable.

Immune Simulation Studies

Immune simulation studies of the construct is carried out using C-ImmSim, iMODS or DynaMut. Immune simulations facilitate prediction of immunological outcomes such as antibody production (IgG, IgA, IgM): cytokine profiles (Th1/Th2 response, IFNg, IL-2, IL-4 and IL-12): memory cell generation and antigen clearance curve. These simulations help forecast potential in vivo performance before laboratory evaluation.

Codon Optimization and In Silico Cloning

To enhance the possibility of translational efficiency of the construct the nucleotides need to be optimized as per the selected expression system such as *E.coli, Pichia pastoris* or Baculovirus. JCat, SnapGene, GenScript rare Codon Analysis or Benchling can simulate cloning of the optimized gene into expression vectors to confirm correct insertion and expression potential. Optimizing the CAI (>0.9) and GC content (50-60%) ensures efficient expression of the construct.

Applications of Multi-Epitopic Vaccines in Major Animal Diseases

Viral Diseases

FMD: Multi-epitopic vaccines combining VP1, VP2 and VP3 epitopes have demonstrated potential in addressing cross-serotype variability and DIVA compatibility. Computational design helps target conserved antigenic regions critical for long-lived immunity.

PPR: Constructs integrating F, H and N protein epitopes show strong Th1 and CTL responses, offering promising alternatives to conventional live attenuated vaccines.

Avian Influenza and Newcastle Disease: HA, NA, F and HN protein epitopes enable wide-spectrum protection in poultry, reducing mutation-linked vaccine failure.

Bacterial Diseases

***Mycoplasma bovis* and CCPP:** Surface lipoproteins (MIP, MIB) and adhesins form suitable epitope sources, enabling the creation of safe alternatives to bacterins and overcomes their failure.

Brucella abortus / melitensis: Immunoinformatics-designed epitopes from BCSP31, Omp25 and Omp31 proteins enhance Th1-biased immunity, while avoiding biosafety concerns associated with live attenuated strains such as S19 and Rev1. It also has the potential to be used as DIVA.

Pasteurella multocida: Immunodominant epitopes from the PLP, OmpA and OmpH proteins have been used for designing constructs with enhanced neutralizing capacity and increased survival post-challenge.

Parasitic Diseases

Theileriosis: Epitopes selected from the p67 and Tams-1 genes have shown strong potential in multi-epitope constructs targeting schizont and sporozoite stages.

Babesiosis: Antigenic epitopes of RAP-1 and MSP-1 genes have been identified as promising candidates for stimulating CTL responses against intraerythrocytic parasites.

Coccidiosis: Immunogenic epitopes of ROPs, AP2 and AMA-1 have been demonstrated to have vaccine potential and to provide cross-species protection.

Advantages of Immunoinformatics-Based Multi-Epitopic Vaccines

- Rational design, high specificity and precision
- Targeted immune activation
- Ability to address antigenic variability
- Avoidance of whole-pathogen handling so no risk of the live pathogens
- Non-infectious and there is no chance of reversion
- Rapid and cost-effective development
- Strong multi-arm immune activation
- Cross-protective capability
- Facilitates DIVA strategies
- Highly scalable for industrial production

Challenges and Limitations

Despite immense advantages, challenges remain ass the prediction algorithms vary in accuracy, especially for non-model species with limited MHC allele information for the animals. Wet lab validation remains essential to confirm immunogenicity. Regulatory frameworks for synthetic vaccines in animals are still evolving. Additionally, computational pipelines require interdisciplinary expertise and high computational resources.

Future Prospects

The integration of AI-driven epitope prediction, deep learning-based antigen modeling and mRNA/DNA-based multi-epitope vaccine platforms will shape the future of veterinary vaccinology. Synthetic biology approaches, including CRISPR-based antigen design, designer immunogens and nanoparticle epitope display systems (ferritin, lumazine synthase and self-assembling peptide nanoparticles): will further enhance precision and immunogenicity. Reverse vaccinology 3.0 will continue to reduce the time from genome sequencing to vaccine deployment, making rapid-response vaccines feasible for emerging zoonoses. Multi-epitope vaccines and mRNA will further facilitate the species customization of the vaccine constructs.

Conclusion

Immunoinformatics provides an unprecedented opportunity to revolutionize the development of multi-epitopic vaccines for animal diseases by enabling computational prediction, structural modeling, immune simulation, rapid and rational design of immunogens. These vaccines have the potential to provide safe, stable and effective alternatives to traditional approaches, especially for pathogens with high variability or biosafety risks. From pathogen genome mining to immune simulation, every step can now be computationally optimized before laboratory validation, reducing time, cost and biosafety risks. As computational tools advance, immunoinformatics-guided multi-epitopic vaccines will become indispensable in protecting livestock health, enhancing productivity and safeguarding food security.

LP-4.3. Foot-and-Mouth Disease in Buffaloes: Immunological Insights and Their Relevance to National FMD Eradication Strategies

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Foot-and-mouth disease (FMD) remains a persistent threat to India's livestock sector, especially in buffalo-dense regions. Buffaloes, though susceptible to FMD virus (FMDV): exhibit distinct immunological features, including milder clinical signs, lower non-structural protein (NSP) seroconversion and efficient viral clearance, which influence surveillance and control outcomes. This paper synthesises current understanding of buffalo-specific epidemiology, immune responses and DIVA-based serological findings, with emphasis on evidence from Haryana. Buffalo populations consistently showed low NSP reactivity (<10%) and good post-vaccination antibody titres. Management-related risks such as communal grazing, market congregations and migration highlight the need for targeted vaccination, movement regulation and improved biosecurity. Haryana's implementation of biannual FMD+HS vaccination, high serotype-specific immunity and effective DIVA and probang-based surveillance demonstrates a scalable model for eradication. Integrating buffalo-centric immunological insights into the National Animal Disease Control Programme can strengthen early detection, optimize vaccination strategies and accelerate India's progress toward FMD-free status. A coordinated, buffalo-focused approach is essential for sustainable FMD eradication and livestock sector resilience.

Keywords: *Buffalo, DIVA, Foot-and-mouth disease, FMD+HS combined vaccination, Non-structural protein, ELISA, Haryana*

1. Introduction

Foot-and-Mouth Disease (FMD) remains one of the most feared infectious diseases affecting livestock worldwide. As a World Organization for Animal Health (WOAH)-listed disease due to its high transmissibility and socio-economic impact, FMD affects all cloven-hoofed animals, including cattle, buffaloes, sheep, goats, pigs and various wildlife species. India, with one of the world's largest cattle and buffalo populations, continues to battle endemic FMD despite decades of control interventions (Pattnaik *et al.*, 2012; Subramaniam *et al.*, 2022). Buffaloes hold a critical position in this context, contributing substantially to national milk production, serving as draft animals in certain regions and supporting rural livelihoods (Krishnamoorthy *et al.*, 2022). Understanding FMD epidemiology and immunology in buffaloes is therefore essential for achieving long-term national eradication goals. FMD virus (FMDV) is a non-enveloped RNA virus (~8.4 kb) belonging to the genus *Aphthovirus* (family *Picornaviridae*) and comprises seven serotypes (O, A, C, Asia-1, SAT1-3) (Grubman and Baxt, 2004). In India, serotypes O, A and Asia-1 are currently in circulation, with serotype O being the most prevalent (Subramanian *et al.*, 2022; Dalal *et al.*, 2025); serotype C has not been detected since 1995. Economically, FMD causes significant losses due to reduced milk production, treatment costs, mortality in young animals, decreased market value and trade restrictions (Dahiya *et al.*, 2022). During 2013-14, losses in cattle and buffaloes alone were estimated at INR 208.97 billion nationally, including INR 350.4 million in Haryana (Govindaraj *et al.*, 2020).

As India advances under the National Animal Disease Control Programme/Livestock Health and Disease Control Programme (NADCP/LHDCP): run by the Government of India, to control FMD, it is crucial to interpret buffalo-specific immunology, susceptibility, carrier status and sero-surveillance data. This lecture summarises buffalo immune responses to FMDV, highlights DIVA-based insights and discusses how buffalo-focused understanding can strengthen national FMD eradication strategies.

2. FMD in Buffaloes: Unique Epidemiological and Pathobiological Features

2.1 Susceptibility and clinical presentation

Buffaloes are susceptible to FMDV but often show comparatively milder clinical signs and lower reactivity to FMDV non-structural protein (NSP) than cattle under similar exposure (Bora *et al.*, 2014; Dahiya *et al.*, 2025). Clinical signs include pyrexia, vesicles on the tongue, dental pad and interdigital space, lameness, salivation, reduced milk yield, anorexia and lesions on teats.

2.2 Carrier state in buffaloes

Buffaloes can harbour FMDV in the oropharynx for 3-6 months post-infection, similar to cattle (Alexandersen *et al.,* 2003; Pannu *et al.*, 2025). This carrier state may contribute to silent viral persistence in buffalo-dense regions. Active surveillance of animals without apparent clinical signs of FMD along with molecular epidemiological studies is needed to understand FMDV transmission and circulation within distinct geographic regions (Stenfeldt *et al.*, 2013).

2.3 Buffalo management practices and associated risks

Several Indian buffalo husbandry practices facilitate FMDV transmission, including communal water sources, mixed-species grazing, interstate livestock transport, large livestock markets and migratory buffalo herds that interface with wildlife and other domestic animals (Bora *et al.*, 2014). Recognising these risks helps refine targeted vaccination and surveillance.

3. Immunological Insights: How buffaloes respond to FMDV

3.1 Innate immune response

Buffaloes exhibit strong innate immune responses characterised by robust interferon activity, efficient macrophage and dendritic cell function and rapid viral clearance (Vats *et al.*, 2020). They may partly account for the low NSP antibody levels, subject to further validation.

3.2 Humoral immunity

Buffaloes mount a protective humoral response to structural proteins (SPs) following FMDV vaccination. They generally show slightly lower FMDV post-vaccination antibody titres than those of cattle, but consistently exhibit lower NSP seroconversion. This pattern suggests milder infection, efficient viral clearance or inherent resistance traits.

3.3 Cell-mediated immunity (CMI)

Emerging data indicate that buffaloes may possess a sustained T-cell response to FMDV. While humoral immunity neutralises acute infection, CMI plays a key role in limiting viral persistence in oropharyngeal tissues (Maddur *et al.*, 2009).

4. DIVA-based serological insights from buffaloes in Haryana

Detection of antibodies against FMDV NSPs in ELISA is used in population serosurveys as an indicator of exposure to and virus circulation and to differentiate between infected and vaccinated animals (DIVA) (Mohapatra *et al.*, 2011). using recombinant 3AB3 NSP ELISA, conducted through ICAR-NIFMD and LUVAS between 2020-2024, provides important insights.

4.1 Low NSP reactivity

Buffaloes showed consistently low NSP positivity—often below 10%—across multiple districts in Haryana (Dahiya *et al.*, 2025). This suggests minimal viral circulation, effective vaccination and favourable immune responses in buffalo populations. This suggests buffaloes may contribute less to active viral transmission, although short-term carrier status remains possible.

4.2 Age-stratified findings

Buffaloes aged 6-18 months also showed low NSP reactivity, indicating minimal new infections among young animals with waning maternal immunity. In India, the Indian Council of Agricultural Research-National Institute on FMD (ICAR-NIFMD) and ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) have adopted a sampling plan for FMDV sero-surveillance wherein young animals aged 6-18 months are surveyed to detect antibodies against FMDV NSPs to resolve the issue of false positives arising from repeated vaccinations with vaccines containing residual amounts of NSPs.

5. Relevance of buffalo immunology to national FMD eradication strategies

5.1 **Identifying low-transmission zones:** Demonstration of NSP antibody-free status in the population is a prerequisite for declaring disease-free zones and progressively regaining FMD-free status (Crowther, 2007). Buffalo herds, due to their low NSP reactivity and lower clinical incidence, can serve as sentinel populations for detecting silent circulation and for guiding creation of disease-free zones (DFZs). Their demonstrated suitability for combined FMD+HS vaccination further supports streamlined immunization strategies.

5.2 **Mapping buffalo movement and transmission risk:** Seasonal migration for breeding, interstate transport of buffalo bulls to semen stations or meat markets and congregation at livestock markets highlight the need for targeted vaccination and enhanced biosecurity in high-risk movement networks (Subramaniam *et al.*, 2022).

6. Policy and field implications for FMD control in buffalo-dominant regions

6.1 **Strengthening vaccination and awareness:** Challenges include delayed vaccine supply, logistical gaps, limited cold-chain capacity and farmer unawareness. Solutions involve micro-level vaccination calendars, seasonal vaccination aligned with buffalo movement, incentives for compliance, farmer training on symptom reporting and biosecurity, the use of artificial intelligence tools in the reporting system and the digitalization of data at the national level. Dairy cooperatives can play a significant role in community-led disease control.

6.2 **Movement regulation and market biosecurity:** Buffalo movement significantly contributes to the spread. Measures such as solid phase competitive ELISA (SPCE)-based screening at checkpoints, certification for transport, enforcing pre-movement vaccination and improving livestock market hygiene are essential.

7. Haryana as a model for buffalo-based FMD control

The state of Haryana has a total geographical area of 44,212 square km and a livestock population of up to 7.11 million, with cattle and buffalo populations of up to 1.93 and 4.37 million, respectively (Handbook on 20th Livestock Census, 2020). After the commencement of the FMD Control Programme (CP) in 2003-04 through mass vaccination of cattle and buffaloes, there was a remarkable decline in FMD outbreaks in Haryana. The economic cost evaluation revealed the impact of FMDV vaccination under the FMD-CP in India, with a progressive decline in disease prevalence. A subsequent decrease in economic burden was observed in states that implemented the program compared with those that did not (Govindaraj *et al.*, 2021). After the launch of the NADCP in 2019, the cattle and buffaloes of Haryana are being vaccinated biannually against FMD + Haemorrhagic septicaemia (HS) combined oil adjuvanted vaccine (Dahiya *et al.*, 2022).

Haryana has emerged as a national example through high post-vaccination titres (>70% for serotypes O, A and Asia-1): low NSP prevalence (<10%): successful implementation of DIVA and probang sampling and adoption of combined FMD+HS vaccination (Pannu *et al.*, 2025). These achievements demonstrate the potential of buffalo-rich regions to lead the development of DFZs, advance India's Progressive Control Pathway stage and enhance prospects for international livestock trade. This model could be applied to other buffalo-dense states such as Punjab, Uttar Pradesh, Rajasthan and Maharashtra.

8. Conclusion

FMD in buffaloes presents a complex yet manageable challenge. Buffaloes' unique immunological characteristics—lower NSP reactivity, stronger humoral response, subclinical infections and potential resistance—provide a valuable opportunity to shape India's eradication strategies. Evidence from Haryana clearly demonstrates how buffalo-based surveillance and vaccination data can accelerate the formation of disease-free zones, strengthen early detection and support India's transition towards FMD-free status.

As India progresses under the NADCP/LHDCP framework, integrating buffalo-centric insights into national policies will be essential. A coordinated approach involving scientists, field veterinarians, policymakers and farmers—grounded in robust immunological and epidemiological knowledge—will pave the way for sustainable disease elimination. A buffalo-centric roadmap for India's FMD eradication requires strengthened surveillance that integrates DIVA, SPCE and probang sampling while focusing on markets, transport corridors, border districts and incorporating wildlife and small ruminants into routine monitoring. Vaccination strategies should adopt potent, thermostable vaccines, use region-specific booster intervals tailored for buffalo herds and expand the use of combined FMD+HS vaccines in suitable zones. Advancements in diagnostics—such as nanobody-based ELISAs, pen-side rapid antigen tests and GIS-enabled real-time outbreak tracking—must be promoted for faster field responses. Biosecurity policies should prioritize regular market disinfection, regulated animal movement and community-led disease reporting systems to minimize transmission risks.

Finally, international collaboration through GFRA, FAO and WOAH training, improved strain characterization, vaccine matching and alignment with global DFZ standards will further support India's progression toward sustainable FMD control and eventual eradication. The future of India's livestock economy, especially its globally significant buffalo sector, depends on how effectively we leverage these immunological insights to construct a resilient, science-driven FMD eradication strategy.

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Oral Presentation

OP-4.1. Functional role of Ca^{2+} permeable TRPC channels in mediating PGF2α- induced myogenic contraction of oviduct in water buffaloes (*Bubalus bubalis*)

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Prostaglandins are the endogenous mediators for oviductal contraction, however, the underlying Ca^{2+} signalling pathway(s) responsible for PGF2α-induced oviductal contraction in buffaloes is not completely understood. In the present study, the myosalpinx strips from both ampulla and isthmus regions of buffaloes during oestrous stage was isolated and the spontaneous and PGF2α-induced contraction was recorded in the absence and presence of different pharmacological modulators related to Ca^{2+} signalling. Ampulla was found to be more sensitive to contractile effect produced by PGF2α than isthmus, which was found to be largely dependent on extracellular Ca^{2+} as evidenced by the reduction in the contractile effect following removal of Ca^{2+} from extracellular fluid. To unravel the role of ion channels in mediating this spasmogen-induced contraction, the immunolocalization of TRPC3 in the smooth muscle layer of both isthmus and ampulla was detected. Additionally, in the presence of SKF96365 (a nonselective blocker of TRPC) and Pyr3 (a selective blocker of TRPC3): the PGF2α-induced myogenic contraction was significantly ($P<0.05$) inhibited in both ampulla and isthmus. Taken together, TRPC3 ion channels are the major regulator that mediate PGF2α- induced contraction in buffalo oviduct, thus regulating its expression and activity during different reproductive disorders may provide a new insight into therapeutic interventions in long run.

Keywords: *TRPC3, Oviduct, Buffalo, PGF2α*

OP-4.2. Diagnostic utility of Echocardiographic Indices and Acute Phase Protein Response in Buffaloes with Foreign Body Syndrome

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This study evaluated acute phase protein (APP) responses and echocardiographic alterations in 51 buffaloes, including 10 healthy animals (Group I) and 41 diagnosed with foreign body syndrome (FBS) without (Group II) or with (Group III) reticular wall penetration. Diseased animals were assessed at four time points: before surgery (T0): immediately after surgery (T1) and at 12 h (T12) and 24 h (T24) postoperatively. Significant elevations in major APPs—TNF-α, fibrinogen, haptoglobin, serum amyloid A (SAA) and C-reactive protein (CRP)—were observed in both affected groups compared with healthy controls. TNF-α levels were higher in Group II than Group III. Comparing within the groups, TNF-α, haptoglobin and CRP increased significantly at T12 and T24. Fibrinogen and SAA remained elevated but did not vary significantly across time intervals. Echocardiographic evaluation revealed a decline in stroke volume and cardiac output from healthy to affected animals, with the lowest values recorded in Group III. In contrast, ejection fraction and fractional shortening increased progressively from Group I to Group III. Left ventricular Internal diameters, End systolic volume and End diastolic volume also decreased with disease severity.

OP-4.3. Full-length P1 region sequence-based molecular characterization of foot-and-mouth disease virus serotype O from tongue epithelium of affected buffalo in Haryana, India

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Foot-and-mouth disease (FMD) remains a major transboundary animal disease affecting cloven-hoofed animals, necessitating continuous molecular surveillance to track viral evolution and assess vaccine relevance. The causative agent, FMD virus (FMDV): exhibits antigenic diversity, with serotype O being most prevalent in the world as well as in India. In the present study, a clinical sample (tongue epithelium) collected from an affected buffalo in village Khedar, district Hisar (Haryana) and confirmed as FMDV serotype O by RT- multiplex PCR, was subjected to FMDV full-length P1 region amplification. RT-PCR using reported L-P1/2A primers yielded the expected ~3000 bp amplicon, which was gel-purified and sequenced using the Illumina NovaSeq6000 platform. Sequencing generated 3,073 nt, which were aligned and trimmed to obtain a 2,208 nt full-length P1 region. BLASTn analysis showed 87.7% nucleotide identity with the Indian vaccine strain FMDV O/IND/R2/1975, indicating substantial genetic divergence. Multiple sequence alignment (MEGA X) using representative global FMDV serotype O sequences demonstrated that the current Haryana sample clustered closely with isolates from Pakistan, Jordan, Saudi Arabia, Bhutan, Nepal, UAE, Mauritius, Myanmar, China and Bangladesh, suggesting regional circulation links. VP1-based phylogenetic analysis classified this sample within the ME-SA topotype and O/ME-SA/Ind2001e lineage. Amino acid comparison of the P1 region showed a limited number of substitutions in VP4, VP2, VP3 and VP1 structural proteins in comparison with the vaccine strain. Amino acid positions critical for VP1 antigenic sites were found conserved except for an I144V replacement which has been consistently observed across all Indian serotype O isolates, regardless of lineage. The findings highlight the genetic diversity of circulating FMDV serotype O in Haryana and the need for continued genomic monitoring, along with antigenic relationship and neutralization studies, to evaluate and refine vaccine strategies.

Keywords: *Foot-and-mouth disease virus serotype O, P1 region, Phylogenetic analysis, Haryana, Buffalo*

OP-4.4. Glycoconjugate Distribution in the Buffalo Palatine Tonsil: A Lectin Histochemical Study

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The present study provides the most comprehensive account to date of lectin-binding sites in the various structural components of the palatine tonsil of six healthy adult buffaloes. It enabled the identification of specific binding affinities for 21 lectins, including those of the N-acetylglucosamine group (Triticum vulgaris WGA, succinylated WGA, LEL, DSL, STL); N-acetylgalactosamine group (SBA, DBA, RCA, VVL); galactose group (GS1B4, PNA, Jacalin, ECL); and glucose/mannose group (Con A, LCA, PSA) across the structures of the palatine tonsil. The stratum spinosum of both the stratified squamous epithelium and the modified reticular epithelium showed strong demarcation with lectins of the N-acetylglucosamine group. Additionally, VVL, Jacalin and Con A also exhibited strong reactivity in this layer. RCA, PNA, Con A and PSA served as effective markers for the collagen fibers of the subepithelial connective tissue. Lymphoid cells in the interfollicular and parafollicular regions demonstrated receptors for ECL, WGA and Con A, whereas; germinal center B cells reacted exclusively with lectins belonging to the glucose/mannose group. The mucosal secretions and the vascular endothelium were predominantly composed of glucosamine, sialic acid and galactosamine-containing glycoconjugates. The distinct localization patterns of these lectins indicate the presence of specific receptor sites that could have important implications for investigating early disease pathogenesis and for the development of oral vaccines. Furthermore, the findings offer a valuable reference database for comparing histochemical alterations under various disease conditions.

OP-4.5. Effects of Antioxidant Multimineral and Vitamin Supplementations to Transitional Buffaloes on Energy Status, Udder and Calf Health

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The concentrations of various trace elements and vitamins in circulation are diminished in periparturient dairy animals due to the lower feed intake and the massive demand for production. Nutrition and energy have been recognized as the important factors of mammary defense in post parturient period. Adequate availability of vitamins and trace elements are the fundamental requirements in improving energy balance and udder health in periparturient buffaloes. The present study was conducted to evaluate the effects of repeated administrations of antioxidant vitamins and multiminerals in transition buffaloes on udder defense mechanism, energy status and occurrence of intramammary infection (IMI) in early lactation period. Forty-five days prior expected date of calving, forty clinically healthy pregnant buffaloes were randomly divided into five different supplementation groups (n=8): control (basal diet): VES (vitamin E and selenium): MM (multiminerals): AA (ascorbic acid) and Cr (chromium picolinate) in basal diet. The udder health status was evaluated by measuring phagocytic activity (PA): myeloperoxidase (MPO) and nitric oxide (NO) productions in milk leukocytes, energy status was assessed by measuring non-esterified fatty acid (NEFA) and occurrence of intramammary infection (IMI) was evaluated by milk somatic cell count (SCC): bacterial count in milk and visible clinical signs of udder until day 28 post-calving. The results showed that the VES and MM supplementations exhibited significantly higher PA, MPO and NO productions of milk leukocytes till first week of lactation whereas, significantly lower NEFA concentration were noted on day -7 in VES and MM groups, day 1 and 7 in MM group and day 14 in VES, MM, VC and Cr groups as compared to the control group. No significant difference in occurrences of subclinical or clinical IMI was noted across the groups until four weeks of lactation. The mean body weights of calves of VES and MM groups at day 30 of birth were significantly higher than control group. No significant association was observed between supplementations and the incidences of calf diarrhoea, pneumonia and mortality of calves until day 30 of birth. From the findings of the present study, it is concluded that repeated administrations of VES and MM to transition buffaloes could be an effective strategy to maintain good udder health by augmenting milk leukocyte functions, mitigating negative energy balance and preventing incidence of IMI in early lactation.

Keywords: *Buffalo, udder health, energy, antioxidant*

OP-4.6 Molecular Detection of Rotavirus A and Bovine Coronavirus in Calf Diarrhea and Design of Multiple Antigenic Constructs for Improved Diagnostics

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Rotavirus A (RVA) and bovine coronavirus (BCoV) are among the most important viral pathogens associated with neonatal calf diarrhea, a condition that continues to impose substantial economic losses on the livestock industry worldwide. These viruses may act as primary enteric pathogens or occur in synergistic association with a variety of other viral, bacterial, or protozoal agents, thereby exacerbating disease severity. Understanding their prevalence and improving diagnostic tools remain essential for effective surveillance, early detection and timely intervention. In the present investigation, we aimed not only to detect the occurrence of RVA and BCoV in diarrheic buffalo and cattle calves, but also to design and evaluate multiple antigenic constructs (MACs) that may serve as improved diagnostic antigens for serological assays. A total of 304 fecal samples were collected from diarrheic buffalo calves (n=43) and cattle calves (n=261) across organized and unorganized farming systems spanning multiple geographical regions of India, thereby ensuring broad epidemiological representation. All samples were screened using quantitative PCR (qPCR) for precise and sensitive detection. The analysis revealed that 11.62% (5/43) of buffalo calves and 24.13% (63/261) of cattle calves were positive for RVA. In contrast, BCoV was detected in 4.65% (2/43) of buffalo calves and 3.83% (10/261) of cattle calves, highlighting the continued circulation of both pathogens in Indian livestock populations and underscoring the need for improved diagnostic and monitoring tools. To strengthen serological diagnostics, two MACs—one each for RVA and BCoV—were

developed using advanced immunoinformatics approaches. These constructs were designed by selecting immunodominant, antigenic, non-allergenic and non-toxic linear B-cell epitopes, ensuring their suitability as safe and broad-spectrum immunodiagnostic candidates. The commercially synthesized gene constructs were cloned into the pET32a expression vector and transformed into suitable prokaryotic expression hosts (Top10 and BL21 cells). Expression resulted in chimeric recombinant proteins of approximately 111 kDa for RVA and 90 kDa for BCoV. Protein expression and purity were confirmed through SDS-PAGE analysis, followed by western blotting using an anti-His tag antibody to validate identity and specificity. The purified recombinant proteins were subsequently used to generate hyperimmune sera in three laboratory animal models—rabbit, rat and chicken—to assess immunogenicity and cross-species antibody response profiles. Furthermore, the chimeric proteins were evaluated for their potential application as diagnostic antigens in antibody detection assays, with the goal of optimizing and strengthening ELISA-based diagnostics for RVA and BCoV infections. The findings of this study provide valuable epidemiological insights and demonstrate the promise of immunoinformatics-derived MACs as next-generation diagnostic reagents for improved surveillance of enteric viral infections in calves.

Keywords: Bovine coronavirus, calf diarrhea, immunoinformatics, multiple antigenic construct, rotavirus

OP-4.7. *In vitro* lymphocytes response and inducible nitric oxide synthase gene expression in postpartum metritic Murrah buffaloes (*Bubalus bubalis*) lymphocytes

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The present study was done to investigate the immune response and expression of iNOS gene in postpartum metritic murrah buffaloes. Eight metritis buffaloes and another eight normal postpartum buffaloes as control of day 0, day +14, day +21 were selected from dairy cattle yard. Heamatological parameters (TLC, DLC) and plasma nitric oxide (NO) are estimated. I*n vitro* lymphocyte functions with and without mitogen (PHA-P, 5µg/ml) stimulation and lymphocytes expression of iNOS expression gene studies were done. Metritic buffaloes showed significantly higher (P=0.001) total leucocytic count and significantly altered (P<0.001) differential leucocytic count as an increase in neutrophil, decrease in lymphocyte, no change in monocytes percent's as compared to control values. The cellular immunity was significantly altered in postpartum metritic buffalo. The lymphocyte proliferation response (0.96±0.04 vs 1.22±0.05) was found to decrease significantly (P=0.001), nitrite secretion by stimulated (4.85±1.63 vs 2.94±0.73µM/L) and unstimulated (6.95±0.79 vs 1.09±0.41µM/L) lymphocytes was found highly significant (P<0.001) in metritic vs control buffalo lymphocytes functions. Interleukin -6 (IL-6) secretion by PHA-P mitogen stimulated and unstimulated lymphocytes was not significantly different. No difference was observed between metritic and control buffaloes. Metritic Buffalo lymphocytes expressed successfully an appreciable amount of iNOS mRNA gene (250bp) and were found to be similar to iNOS gene expressed by bovine leucocytes. Thus, in conclusion murrah buffalo lymphocytes were capable to expresses iNOS gene and increased production of nitric oxide by *in vitro* buffalo lymphocytes in postpartum metritis murrah buffaloes.

Keywords: buffaloes, in-vitro lymphocytes, iNOS, IL-6, nitric oxide, postpartum metritis

OP-4.8. Efficient Fetotomy-Based Management of Conjoined Fetal Monstrosities in Buffalo as a Case Series

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Conjoined fetal monstrosities in buffaloes are major causes of dystocia due to extensive fusion, shared organs and excessive fetal mass that blocks normal delivery. These abnormalities increase the risk of uterine trauma, septic complications and impaired future fertility. Fetotomy is a practical option for non-viable monster foetuses when supported by adequate lubrication, durable instruments and skilled obstetrical handling. The procedure demands high technical precision and reliable fetotomy wire and cases with deep or restricted fusion may require specially designed knives or partial mechanisation to achieve safe reduction. To evaluate fetotomy techniques used to manage conjoined fetal monstrosities in buffaloes. Eighteen (*n=18*) buffaloes presented to the Referral Veterinary Polyclinic, Izatnagar between January 2021 and October 2025 were included. Assessment involved per-vaginal and ultrasonographic examination where feasible. After confirming fetal non-viability and ruling out vaginal delivery, fetotomy was performed under epidural anaesthesia with ample lubrication. Several malformed twin foetuses showed varying degrees of duplication with shared organs, requiring individualized reduction plans. High-tensile stainless-steel wire was used in all cases and most procedures were completed using three to five planned cuts to minimise trauma and allow staged mass reduction. Modified knives or partial mechanisation were used selectively when access was limited. Postoperative care included antibiotics, anti-inflammatories, fluid therapy and routine genital tract monitoring. The cases included in this series were managed to completion without requiring caesarean intervention. No cases of uterine rupture or severe haemorrhage occurred. Minor cervical abrasions noted in a few animals resolved uneventfully with standard treatment. Fourteen buffaloes returned to normal estrous cyclicity within 55-90 days and ten conceived in the subsequent breeding season, demonstrating favourable reproductive recovery despite severe obstetrical compromise. This four-year case series shows that fetotomy, when supported by accurate anatomical assessment, suitable cutting instruments and selective use of modified or semi-mechanised tools, is a safe and economical option for managing dystocia from conjoined fetal monstrosities in buffaloes. Skilled operators and well-planned reduction cuts are essential for achieving good maternal outcomes.

Keywords: *Buffalo; conjoined twins; fetotomy; fetal monstrosity.*

OP-4.9. Precision Management of Cystic Ovarian Degeneration in Buffaloes Using a Novel Cyst Ablation Device

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Cystic ovarian degeneration (COD) in buffaloes is a functional disorder in which one or more large, fluid-filled follicles fail to ovulate and persist on the ovary for 10 days or more. These persistent anovulatory cysts arise from a disruption in the normal LH surge mechanism, leading to failure of follicular rupture. As a result, the ovary becomes hormonally inactive or erratic, causing nymphomania or anoestrus in case of luteal cyst. COD prevents normal follicular turnover and interferes with cyclic ovarian activity, making it an important cause of infertility and delayed conception in buffaloes. To evaluate the effectiveness of a novel Cyst Ablation Device (CAD) in restoring normal ovarian cyclicity and improving reproductive outcomes in buffaloes affected by cystic ovarian degeneration. Thirteen ($n=13$) buffaloes diagnosed with COD based on per-rectal examination, ultrasonography and reproductive history were selected. Animals were restrained in a chute, the rectum evacuated and the device assembled under aseptic conditions. Local infiltration of 3-4 ml of 2% lignocaine was administered at the sacrococcygeal site. Per-rectal guidance was used to palpate and retract the ovary near the fornix. The cyst was stabilized between the thumb, index and middle finger and the CAD was introduced transvaginally for aspiration followed by controlled ablation of the cystic wall. The ablation procedure was successfully completed in all 13 buffaloes, with complete cyst collapse confirmed ultrasonographically within 7-10 days. A return to normal ovarian cyclicity was observed in 11 buffaloes (84.61%): while two buffaloes (15.38%) required an additional follow-up hormonal treatment due to delayed follicular response. Nine buffaloes (69.23%) exhibited estrus and conceived in the subsequent breeding cycle. No complications such as haemorrhage, adhesions, or ovarian trauma were recorded. The results indicate that the cyst ablation device is a simple, minimally invasive and effective tool for managing cystic ovarian degeneration in buffaloes. Its precision-based mechanism enables rapid cyst resolution, restoration of normal follicular dynamics and encouraging conception rates, making it a practical and field-friendly alternative to repeated hormonal therapies.

Keywords: *Buffalo, cyst ablation, cystic ovarian disease, ultrasonography.*

Poster Prresentation

PP-4.1. Successful Management of Extreme Lateral Head Deviation Through Fetotomy in Uterine Torsion Corrected Buffalo

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Dystocia in buffaloes is often complicated by uterine torsion and when accompanied by fetal maldisposition, management becomes clinically challenging. A 3rd-parity buffalo was presented to the Referral Polyclinic, ICAR-IVRI, Izatnagar, with a history of prolonged unproductive straining. Per-vaginal and per-rectal examination confirmed right-sided post-cervical uterine torsion exceeding 180°. After successful detorsion using the modified Schaffer's method, per-vaginal delivery remained unsuccessful due to severe fetal malposture characterized by extreme lateral head deviation with forelimbs extended. Manipulative correction was attempted but the dead fetus could not be brought into normal presentation because of rigidity and limited intrauterine space. Thus, fetotomy was performed with careful removal of the deviated head and anterior part using a fetotome and obstetrical wire, followed by extraction of the remaining fetal body in a controlled manner. Adequate lubrication, epidural anaesthesia and fluid therapy were provided to minimize maternal trauma and stress. The dam recovered uneventfully with postoperative administration of antibiotics, NSAIDs, uterotonic agents and supportive care. This case highlights the importance of timely diagnosis, strategic obstetrical decision-making and the role of fetotomy as a life-saving alternative when fetomaternal disproportion and head maldisposition persist even after torsion correction. Efficient clinical handling can significantly enhance dam survival and reproductive future in high-value buffaloes.

Keywords: *Buffalo, dystocia, fetotomy, lateral head deviation, uterine torsion.*

PP-4.2. Microbial Clearance Following Intrauterine Administration of Immunomodulators Buffaloes Suffering from Subclinical Endometritis

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The present study evaluated the therapeutic efficacy of three intrauterine treatment regimens in eighteen repeat breeder buffaloes diagnosed with subclinical endometritis. Animals were randomly allocated into three equal groups (n=6/group). Treatment Group I received intrauterine infusion of lysozyme (4 mg dissolved in 40 ml normal saline, pH 7.4) for two consecutive days. Treatment Group II was administered a single intrauterine infusion of 5 percent N-acetylcysteine (60 ml normal saline). Treatment Group III received a crude proteolytic enzyme formulation consisting of trypsin (8 mg): chymotrypsin (8 mg): papain (4 mg): α-tocopherol acetate (120 mg) and retinyl palmitate (58.83 mg) dissolved in 10 ml distilled water as a single intrauterine infusion. Uterine samples were collected pre- and post-treatment at the subsequent oestrus to assess microbial load and treatment response. Pre-treatment results revealed that all 18 samples (100 percent) were positive for bacterial isolates, with 61.11 percent presenting mixed infections. A total of 29 isolates were identified, with *Staphylococcus* species (51.72 percent) being the most prevalent, followed by *Streptococcus* species (20.69 percent): *E. coli* (17.24 percent) and *Bacillus* species (10.34 percent). Post-treatment, only 7 of 18 samples (38.89 percent) remained positive, all yielding single isolates. Among these, *Staphylococcus* species (57.14 percent) remained predominant. Comparative assessment indicated that lysozyme treatment achieved the highest reduction in bacterial load, followed by N-acetylcysteine and the

proteolytic enzyme formulation. The marked decline in bacterial positivity and the shift from mixed to single infections demonstrate enhanced uterine clearance. Overall, lysozyme exhibited superior therapeutic efficacy in mitigating subclinical endometritis in repeat breeder buffaloes.

Keywords: *Subclinical endometritis, Repeat breeder buffaloes, Immunomodulators*

PP-4.3. Diagnosis and Management of Traumatic Reticulo Pericarditis in a Buffalo- A case Report

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A six year old eight months pregnant Buffalo was presented to the Referral Veterinary Polyclinic and Teaching Veterinary Clinical Complex, ICAR- IVRI, Bareilly with the history of anorexia, fever, tachypnea since five days. Clinical examination revealed brisket edema, jugular pulsation, recurrent bloat, tachycardia and reduced rumen motility. Heart auscultation revealed muffled heart sound, deep palpation of the abdominal wall caudal to the xiphoid and pinching of the wither elicited grunt and hematology revealed leukocytosis. Echocardiographic examination was done in the third, fourth and fifth intercostal spaces in right and left side of the animal in standing position. Echocardiography revealed pericardial effusion with anechoic fluid around pericardial sac and inflammatory fibrinous changes. Abdominal ultrasound showed reticular wall thickening and reduced motility. The case was diagnosed as traumatic reticulo pericarditis from anamnesis, physical examination and confirmed by ultrasonographic examination. Animal was treated medically with broad spectrum antibiotics, anti inflammatory drugs and supportive fluid therapy.

Keywords: *Buffalo, Traumatic reticulo pericarditis, Ultrasonography, Brisket edema*

PP-4.4 Surgical Correction of Teat Laceration in Two Buffaloes

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Teat injuries in buffaloes could be caused by various factors including trauma, infection, husbandry practices and environmental causes. Teat injury becomes important due to its negative impact on dairy economy. Two buffaloes were presented to the RVP, IVRI, Izzatnagar with the complaint of self-inflicted stamping causing injury to the teat. Teat laceration occurs in buffaloes with high milk yield having well developed, physiological and distended mammary gland, which explains the cause of the teat laceration in presented two cases. Surgical correction was done in both the animals under local infiltration ring block anesthesia using Inj. 2% lignocaine. Lacerated wound was debrided, irrigated with normal saline and cleaned with 5% povidone iodine. Teat canal patency was maintained using sterile infant feeding tube of size FG-8. Three layered suturing technique was done where, first layer mucosa and second layer all inner and outer fibro-muscular layer using simple continuous pattern was sutured with Vicryl No. 2-0 and skin was sutured with interrupted pattern using Nylon No.0. To maintain the teat canal patency the infant feeding tube was sutured to the skin of teat in figure of eight manner using Nylon No. 0 for 5 days. Post-operatively animal was administered with Inj. Intacef Tazo- 4.5 gram intravenously for seven days and Inj. Melonex- 15ml intramuscularly for three days. Sutures were removed after 12 days and teat laceration healed uneventfully in both the animals with no untoward complications.

Keywords: *Buffalo, Surgery, Teat laceration, Teat repair, Ring block*

PP-4.5. Surgical Management of Umbilical Hernia in Buffalo Calf with Decellularized Allogenic Graft

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A six-month-old female buffalo calf was presented to the RVP, IVRI with a history of progressive abdominal bulging at the umbilicus, reduced weight gain and intermittent discomfort during activity.

Physical examination revealed a soft, fluctuant swelling with a clearly palpable hernial ring, suggestive of an umbilical hernia. To confirm the diagnosis and assess the viability of herniated structures, ultrasonography was performed, which identified omentum and a loop of small intestine within the hernial sac, with no signs of vascular compromise. Surgical intervention was planned both to restore normal abdominal integrity and to safeguard future production potential. Under aseptic conditions, the hernial sac was opened, herniated omentum and intestine were inspected and the contents gently reduced into the abdominal cavity. The hernial ring was closed using a vest-over-pants suture pattern to provide strong apposition and minimize tension. To reinforce the repair and reduce recurrence particularly important in young, growing animals exposed to environmental stress, a decellularized allogenic buffalo dermal graft was applied as an onlay biological scaffold. The graft contributed to improved structural stability and facilitated host tissue incorporation, representing a locally available, climate-resilient biomaterial that supports emerging trends in sustainable veterinary innovation. Postoperative care included antibiotics, analgesics, controlled exercise and nutritional support. The calf recovered uneventfully, with complete healing noted on follow up and resumption of normal growth parameters. This case highlights the importance of early surgical correction of hernias to prevent production losses, especially as climate variability, heat stress and nutritional fluctuations can exacerbate healing challenges in young livestock. The successful use of a decellularized allogenic dermal graft underscores how bio-engineered, regionally available materials can enhance surgical outcomes and contribute to resilient buffalo health systems in a changing climate.

Keywords: *Allogenic graft, Buffalo calf, Decellularized, Umbilical hernia*

PP-4.6. Clinico-Therapeutic Approach for Management of Sugarcane Roughage-associated Acute Ruminal Acidosis in Buffalo

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Ruminal acidosis is a metabolic disorder characterized by a drop in ruminal pH resulting from excessive fermentation of carbohydrates or rapidly soluble sugars, as recent findings have shown. In acute form, rumen pH falls below 5.5 and microflora mainly comprises lactic acid-producing bacteria. In northern India, sugarcane (*Saccharum officinarum*) harvest coincides with the cold feed scarcity season and bovine farmers often use sugarcane tops, which are high in sugars and low in crude protein (~6%): to supplement the dietary requirements of dairy animals; a risk factor predisposing to ruminal acidosis. Two lactating buffalo cows (aged 6 and 7.5 years) were presented to the Referral Veterinary Polyclinics (RVP): IVRI, Bareilly, for chief complaints pertaining to anorexia and hypogalactosis. The animal had a history of feeding on excessive sugarcane tops acquired from the owner's farm. On clinical observation, the animal showed visible signs of tympany and ruminal stasis; acidic ruminal fluid; hemogram indicated neutrophilic leukocytosis and lymphocytopenia (<17%). The animals were treated with appropriate fluid therapy along with Sodium bicarbonate (8.4%) infusion, Flunixin meglumine (1.1mg/kg): Strepto-penicillin (5g/day): Vitamin B complex, Simethicone, along with probiotics and recovered uneventfully. After a few weeks, another case of ruminal acidosis in buffalo associated with sugarcane roughage in the diet was reported, it was treated and the owner was also properly educated; this calls for widespread awareness, an in-depth survey and a comparative analysis between cattle and buffalo.

Keywords: *Acute ruminal acidosis, Saccharum officinarum, Clinico-therapeutics, Awareness, Buffalo*

PP-4.7. Ultrasonographical Assessment of Traumatic Pericarditis in Four Buffaloes

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Traumatic pericarditis being the most common disorder in buffaloes following diaphragmatic hernia, in developing countries associated with high economical loss. Main predisposing factor was the indiscriminate eating habits of the bovines. The most common clinical signs were anorexia, dyspnea, tachycardia, distention of the jugular veins, muffled heart sounds and submandibular, brisket and ventral oedema. Ultrasound plays an important role in early diagnosis of traumatic pericarditis in bovines. Among four buffaloes, one buffalo had sign of open mouth breathing, two buffaloes showed jugular pulsation, three buffaloes had brisket as well as ventral oedema, all the four were anorectic and had tachycardia. Upon thoracic ultrasonographic examination, cardiomegaly in two buffaloes was evident and thickened cardiac wall with deformity in the characteristic recognisable cardiac shape was noticed in all four animals. Accumulation of hypoechoic fluids (usually pus) was interspersed with echogenic deposits of fibrin within the pericardium causing tamponade. In one buffalo, an extensive pleural effusion was also evident causing caudal displacement of the heart from its normal position. Thickening in the myocardium and deposition of echogenic fibrinous aggregations with hypoechoic fluids on the ventricles, atria and valves were also detected. In conclusion, ultrasonography, with the aids of other diagnostic tools such as clinical findings, laboratory analysis and radiography, has a high efficacy in evaluation of buffaloes with traumatic pericarditis.

Keywords: *Traumatic Pericarditis, Ultrasonography, Buffalo.*

PP-4.8. A Rare Case of Hermaphroditism in a Buffalo Calf: Clinical Management and Implications for Resilient Buffalo Production

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A rare case of hermaphroditism was presented in a three-month-old buffalo calf at the RVP, IVRI, illustrating how congenital reproductive anomalies can influence future buffalo production in the context of a changing climate and evolving management systems. The calf was brought with a history of abnormal external genitalia. Clinical examination revealed an enlarged clitoris-like structure, absence of perineal opening and palpable testis-like structures in the inguinal region. Ultrasonography confirmed the presence of both ovarian and testicular tissues, consistent with true hermaphroditism. This condition, though uncommon, has growing clinical relevance as climate stress, endocrine-disrupting environmental exposures and nutritional imbalances may contribute to reproductive developmental disorders in livestock. Heat stress and altered feed quality a rising challenge in many buffalo-rearing regions—are suspected to influence hormonal signaling pathways during fetal development, potentially increasing the risk of intersex conditions. Poor growth and compromised urinary passage observed in this calf further indicate secondary health challenges that can affect long-term productivity. Surgical management involved corrective reconstruction to establish a functional urinary outlet and removal of dysplastic gonadal tissues to prevent future complications such as infertility, tumor formation, or urinary retention. Postoperative care included antibiotics, analgesics, controlled nutrition and growth monitoring. Successful recovery highlighted the importance of early diagnosis and timely surgical intervention. From a herd-health perspective, awareness among farmers, improved prenatal nutrition, climate-adaptive housing and surveillance for congenital anomalies can strengthen resilience in buffalo production systems. This case underscores that veterinary innovation through advanced diagnostics, surgical expertise and preventive reproductive health strategies plays a vital role in ensuring sustainable buffalo productivity in an era of climatic and environmental change.

Keywords: *Congenital, Endocrine, Hermaphroditism, Infertility, Ultrasonography*

PP-4.9. Progressive Hydramnios Resulting from Congenital Cranium Bifidum in a Murrah Buffalo and Its Successful Obstetrical Resolution

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Hydramnios, the dropsical accumulation of excessive amniotic fluid, is an uncommon gestational disorder in buffaloes and is primarily linked to fetal anomalies that impair the normal swallowing mechanism. The condition progresses gradually and may culminate in maternal discomfort, dystocia, or obstetrical complications. Although several fetal defects such as cleft palate, oesophageal atresia and skeletal deformities are associated with hydramnios, its occurrence with Catlin mark (cranium bifidum) in buffaloes is rare. To document the clinical presentation, obstetrical management, fetal abnormalities and therapeutic

outcome of a hydramnios case in a Murrah buffalo associated with Catlin mark and to emphasize the clinical relevance of early diagnosis and timely intervention. A 7-year-old Murrah buffalo in third parity was presented with progressive abdominal distension and no straining. Clinical assessment included per-rectal and per-vaginal examinations followed by transrectal ultrasonography. Supportive stabilization (dextrose, Ringer's lactate, vitamin supplementation) preceded induction of parturition using dexamethasone, cloprostenol, estradiol benzoate and valethamate bromide. Cervical massage with warm sodium carboxymethyl cellulose gel facilitated dilatation. Obstetrical manipulation after full cervical dilation enabled correction of left lateral head deviation and controlled traction for fetal delivery. Post-partum management included antimicrobial, anti-inflammatory, antihistaminic and ecbolic therapy. Ultrasonography revealed an anechoic, fluid-filled amniotic sac consistent with hydramnios. Parturition was successfully induced and a dead male calf was delivered vaginally after ~12 hours. Gross examination showed a distinct cranial fissure due to defective frontal bone ossification, confirming Catlin mark. This defect plausibly contributed to impaired fetal deglutition and excessive amniotic fluid accumulation. The dam responded favourably to post-partum therapy and recovered uneventfully with normal lactation. The case reinforces the known pathophysiological association between fetal CNS/cranial defects and hydramnios and aligns with rare previously reported buffalo cases involving congenital anomalies. Timely diagnosis combined with controlled induction of parturition and careful obstetrical manipulation enabled successful resolution of hydramnios without requiring caesarean section. Early ultrasonographic screening for fetal anomalies and appropriate reproductive management strategies may mitigate risks and improve prognostic outcomes in similar cases.

Keywords: *Catlin mark, fetal anomaly, hydramnios, Murrah buffalo, obstetrical management.*

PP-4.10. Partial P1 region-based detection and molecular typing of foot-and-mouth disease virus serotype O from clinically suspected cases in Haryana, India

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Foot-and-mouth disease (FMD) continues to pose a significant threat to cloven- hoofed livestock across India due to its rapid spread and economic consequences. The disease is caused by the foot-and-mouth disease virus (FMDV): which displays extensive antigenic and genetic variability. FMDV serotype O predominates in the country. In Haryana, mass vaccination of all cattle and buffaloes against FMD is carried out by the Government, supported by the Livestock Health and Disease Control Programme (LHDCP): to control the disease. Despite this, due to a dense livestock population, occasional FMD incidents are observed, though on a very mild scale. In the present investigation, clinical samples from FMD-suspected animals in Haryana in 2024 were analyzed to detect and type FMDV based on the partial P1 region. FMDV serotype O was confirmed in 13 of 14 samples from buffaloes and goats across three districts (Bhiwani, Hisar and Jind) using RT-multiplex PCR. The 1D/2B gene-specific reported primers confirmed a 249 bp PCR product specific for FMDV serotype O. This was followed by partial P1 region amplification (covering partial 1C, full-length 1D and 1A and partial 1B genes) using 1C/2B gene-specific reported primers, which yielded a 1165 bp RT-PCR product. These findings highlight the continued circulation of FMDV serotype O in Haryana and underscore the importance of routine molecular surveillance. The FMDV P1 region-based analysis further supports timely detection and guides effective control and vaccination strategies.

Keywords: Foot-and-mouth disease virus serotype O, P1 region, RT-PCR, Buffalo, Goat, India

PP-4.11. Successful Surgical Management and Clinical Evaluation of Open Tibial Fractures in Buffaloes

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Open fractures in large animals pose a challenging situation for field veterinarians. Six buffaloes (200-300 kg body weight) were presented with open tibial fractures to the RVP, IVRI, Izatnagar. The fracture repair was done with circular external skeletal fixation (ESF) system. The treated animals were evaluated postoperatively for the functional limb outcomes. The healing progress of the animals was also evaluated

based on different clinical and radiographic examinations. The level of fracture reduction, alignment and fixation ranged between satisfactory to adequate in all the animals. The fixators were well tolerated and maintained satisfactorily by all the animals till the complete repair of the fracture. Adequate fracture healing with satisfactory functional recovery was observed in all the animals in about 3 to 4 months. The system of circular-ESF was found to provide adequate stability in open tibial fractures in animals weighing 200-300 kg. Therefore, the open long bone fractures, especially tibial fractures, can be effectively managed in buffaloes using circular-ESF system.

Keywords: *Buffaloes, circular ESF, Fractures, Long bones, Surgery*

PP-4.12. Successful Surgical Management of Diaphragmatic Hernia in a Pregnant Murrah Buffalo

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A seven-year-old pregnant Murrah buffalo (8.5 months gestation) was presented with a five-day history of recurrent tympany, anorexia and dyspnea. Clinical examination revealed congested mucous membranes, brisket edema, dehydration (>8%) and muffled heart sounds. Radiographic evaluation confirmed diaphragmatic hernia with a hernial ring of approximately 11.5 cm. Haematological findings indicated neutrophilia and lymphopenia suggestive of acute inflammation. The animal was unresponsive to medical therapy, necessitating surgical intervention. Exploratory laparo-rumenotomy was performed under paravertebral anesthesia to decompress and evacuate ruminal contents, followed by diaphragmatic herniorrhaphy through a ventral midline approach caudal to the xiphoid cartilage. Adhesions between the reticulum and diaphragm were bluntly dissected and the hernial ring was closed with No. 3 silk in a continuous lockstitch pattern. Post-operative therapy included intravenous fluids, antibiotics, anti-inflammatory agents and vitamin supplementation. Despite satisfactory initial recovery, the animal aborted on the fourth post-operative day, likely due to surgical stress, followed by retention of placenta and purulent discharge from both surgical sites. Appropriate medical and local wound management resolved complications and the buffalo later returned to normal estrous cycles with normal milk production. This case emphasizes the importance of timely diagnosis and meticulous surgical management of diaphragmatic hernia in pregnant buffaloes to prevent life-threatening complications and ensure post-operative recovery. The report highlights radiography as a vital diagnostic tool and supports single-stage herniorrhaphy as an effective treatment for diaphragmatic hernia in advanced pregnancy.

Keywords: *Buffalo, diaphragmatic hernia (DH): pregnancy, surgery*

PP-4.13. Nasal Schistosomiasis in a She-Buffalo: A Case Report

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Nasal schistosomiasis, also known as bovine nasal granuloma or *snoring disease*, is a chronic parasitic condition caused by *Schistosoma nasale*, primarily affecting cattle and buffaloes in tropical and subtropical regions. It causes considerable economic losses in livestock-dependent regions of Asia and Africa, primarily due to reduced productivity, chronic illness and treatment costs. A 5-year-old she-buffalo was presented to the Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram, with a history of inappetence, reduced milk yield, bilateral nasal discharge and respiratory distress. There was no prior history of deworming or vaccination. Clinical examination revealed mild fever, pale mucous membranes suggestive of anaemia and crackles upon auscultation of the lungs. Haematological analysis confirmed anaemia and neutrophilia. Faecal examination showed amphistome eggs. Nasal scrapings revealed characteristic Napoleon hat-shaped eggs along with motile miracidium, confirming the diagnosis of nasal schistosomiasis. Therapeutic management included antibiotics, antihistamines, anthiomaline and haematinics. The animal exhibited gradual improvement and achieved uneventful clinical recovery.

Keywords: *Anthiomaline, Buffalo, Nasal Schistosomiasis, Schistosoma nasale*

PP-4.14. Acute Bovine Enzootic Haematuria in Four Buffaloes: A Clinical Case Report

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Bracken fern (*Pteridium aquilinum*) poisoning remains a significant toxicological concern in buffaloes, particularly in areas with unrestricted grazing. This report describes an acute outbreak of bracken fern toxicity involving four buffaloes from a smallholder herd. According to the owner's history, three animals died within 48 hours after showing signs of acute haemorrhagic syndrome after coming from grazing. The fourth animal was presented to the Veterinary Clinical Complex, ICAR-IVRI, Izatnagar, with a one-day history of scleral bleeding, conjunctival petechiae, bleeding in faeces and reddish-brown urine. Clinical examination revealed pale and congested mucous membranes, scleral haemorrhages, haematuria, melena, tachycardia and signs of systemic shock. Haematological findings indicated severe thrombocytopenia, leukopenia, lymphopenia and anaemia, suggestive of bone marrow suppression and haemorrhagic diathesis. Biochemical analysis revealed elevated serum urea and creatinine, hypoproteinaemia and prolonged clotting time, supporting a diagnosis of bracken fern toxicosis. Supportive treatment included intravenous fluids, haematinics, vitamin K_1, antihistamines, corticosteroids and broad-spectrum antibiotics to control secondary infections. Activated charcoal suspension was administered to reduce toxin absorption. The animal showed gradual clinical improvement over a period of supportive management. This case underscores the highly fatal nature of acute bracken fern poisoning and highlights the importance of early clinical recognition, aggressive supportive management and prevention through grazing control. It further emphasizes the need for timely referral to veterinary centres for better outcomes in acute bovine enzootic haematuria.

Keywords: Bovine Enzootic Haematuria, Bracken Fern, Buffalo, Haemorrhagic diathesis

PP-4.15. Development and Clinical Evaluation of an Ultrasound-Guided Keyhole Tube Cystotomy in Buffalo Calves

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The present study was conducted to evaluate and compare the efficacy and postoperative complications of USG guided keyhole tube cystostomy (KHTC) and open surgical tube cystostomy (OSTC) for the management of obstructive urolithiasis in buffalo calves with an intact urinary bladder. Eighty-four calves presented between January 2023 and January 2025 were randomly divided into two groups: KHTC (n=42) and OSTC (n=42). Diagnosis was based on clinical signs, radiography and ultrasonography. The majority of cases occurred in intact male calves aged 3-5 months, predominantly during winter and were associated with feeding wheat bran, rice bran and rice straw. Reduced water intake and high dietary phosphorus were identified as major predisposing factors. All surgeries were performed under 2% lignocaine epidural anesthesia. The mean surgical time was significantly shorter in KHTC (11.22±3 min) compared to OSTC (25.90±4 min). Postoperative complications were lower in KHTC, including catheter blockage (9.7%): wound abscess (4.8%): suture dehiscence (2.4%) and catheter dislodgement (7.3%): than in OSTC (19.5%, 19.5%, 17.1% and 12.2%, respectively). Return to normal urination occurred earlier in KHTC (7-10 days) than OSTC (10-14 days). It is concluded that USG guided KHTC is a simple, cost-effective and reliable technique with shorter operative time, fewer complications and faster recovery.

Keywords: *Buffalo calves, Complications, Keyhole tube cystostomy, Urolithiasis, USG*

PP-4.16. Systemic Pathological Changes in a Buffalo with Femur Dislocation Leading to Septicemia

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A necropsy was conducted on a non-descript six-year-old female buffalo submitted to the ICAR-Indian Veterinary Research Institute, Izzatnagar, Bareilly (Uttar Pradesh). Postmortem evaluation revealed a slipped and dislocated femur, which resulted in complete recumbency for four days prior to death despite therapeutic intervention. Externally, the carcass was in good condition; however, pale mucous membranes, hind limb rigor mortis, hemorrhagic areas, purulent discharge and blackened musculature were evident. Thoracic examination showed frothy exudation within the airways, tracheal and pulmonary hemorrhages, emphysematous changes in the cranial lung lobes, consolidation in the caudal lobes and fibrinous pleural involvement. The heart exhibited petechial hemorrhages on the epicardial and endocardial surfaces, with coagulated blood present in the ventricles. The liver displayed rounded margins, darkened patchy zones and mottled necrotic areas on sectioning. The spleen and kidneys showed petechiae and congestion at the cortico-medullary junction. The intestines exhibited marked serosal and mucosal congestion. Histopathologically, the lungs demonstrated mild subacute bronchointerstitial pneumonia, while the liver showed vacuolar degeneration accompanied by mononuclear cell infiltration around the central vein. The kidneys revealed dense interstitial infiltration of mononuclear cells. Collectively, the pathological findings support trauma-associated septicemia secondary to femoral displacement as the cause of death.

Keywords: *Necropsy, Femoral dislocation, Trauma, Bronchointerstitial pneumonia, Systemic pathology*

PP-4.17. Foetal Maceration in a Buffalo and its Successful per Vaginal Management

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Foetal maceration refers to putrefaction and autolysis of the foetus after ossification at any stage of gestation. This report describes a case of foetal maceration in a 6-year-old non-descript buffalo in its second parity that had completed 340 days of gestation without exhibiting any signs of parturition. The animal showed no relaxation of the Sacro-sciatic ligament, no mammary gland development and a foul-smelling vaginal discharge. Clinical examination revealed that the animal was active and alert with normal physiological parameters. Per rectal examination identified a contracted uterus located near the pelvic brim along with a thickened uterine wall and palpable bony prominences corresponding to a hard foetal mass. Placentomes and fremitus were absent. Per vaginal examination showed dilated cervix with fetid discharge. Based on history and gynaeco-clinical findings the case was diagnosed as foetal maceration. Under epidural anaesthesia using 5 ml of 2% lignocaine hydrochloride, the foetal bones protruding through the cervix were removed manually with adequate lubrication. The expelled mass consisted of macerated foetal tissues with partial autolysis. The uterus was flushed with three litres of warm saline followed by intrauterine administration of oxytetracycline bolus and metronidazole. Supportive treatment included antibiotics, antihistamines, anti-inflammatory drugs, intravenous fluids, multivitamins and herbal ecbolic for five days. The owner was advised to avoid breeding the animal for at least two estrous cycles. Delayed cases of foetal maceration may cause extensive endometrial damage and adhesion making removal difficult without hysterectomy.

Keywords: *Buffalo, foetal maceration, autolysis, per vaginal extraction, uterine lavage*

PP-4.18. Bioengineered SVF Seeded Amnion Grafts for Managing Corneal Affections: Eco-Adaptive Regenerative Therapy for Corneal Regeneration in Buffaloes

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Corneal affections in buffaloes pose significant challenges, leading to ocular morbidity that adversely affects vision, productivity and overall welfare. With climate change predisposing livestock to exposure to environmental stressors increased dust, heightened ultraviolet radiation, emerging infectious agents, the incidence and severity of corneal lesions are expected to rise. This necessitates innovative, sustainable and species-specific therapeutic strategies that strengthen climate resilience in buffalo health management. The present study investigates the therapeutic potential of a stromal vascular fraction (SVF)-seeded decellularised buffalo amniotic membrane (dBAM) graft as a bioactive scaffold for corneal repair. The dBAM, derived from buffalo placenta, demonstrated excellent transparency, mechanical stability and immunocompatibility following decellularization. Five clinical buffalo cases with diverse corneal affections were managed using SVF-seeded dBAM grafting after standard ophthalmic examination and surgical preparation. Grafts were secured using simple interrupted sutures. Healing progression was assessed through serial ophthalmic evaluations, fluorescein staining, vascularisation pattern and epithelialisation rate, pain assessment and restoration of corneal clarity. Across all cases, the SVF-dBAM construct promoted rapid epithelial regeneration, reduced corneal edema, minimized stromal scarring and effectively preserved globe integrity. The bioactive scaffold provided temporary protection while facilitating native stromal regeneration, resulting in shorter recovery periods compared to conventional medical management alone. This study underscores the value of an indigenous, buffalo-specific, cell-based regenerative graft as a cost-effective and climate-resilient therapeutic innovation. Integration of such biomaterials into routine ocular health care and adoption of such indigenous regenerative approaches can significantly enhance buffalo health, productivity and welfare, serving as a robust model for sustainable veterinary ophthalmic care in a changing climate.

Keywords: *Buffalo, Corneal affections, Decellularized amnion, Regenerative therapy, Ophthalmic surgery*

PP-4.19. Obstetrical intervention for fetal hydrocephalus: successful management of four dystocia cases in buffaloes

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Fetal hydrocephalus is a congenital anomaly characterised by pathological accumulation of cerebrospinal fluid within the cranial vault, causing pronounced enlargement of the fetal head and predisposing large animals to obstructive dystocia. Although the condition is widely recognised in Indian cattle, its occurrence in buffaloes is comparatively infrequent; however, it remains a clinically significant contributor to difficult parturition that often requires timely obstetrical or surgical intervention. Over a three-year period, the Referral Veterinary Polyclinic, ICAR-IVRI, Bareilly (Uttar Pradesh) recorded 168 dystocia cases in cattle and buffaloes of which 114 buffalo cases, of which four were definitively attributed to fetal hydrocephalus. All four cases were managed via controlled per-vaginal delivery. Under epidural anaesthesia, an adapter-shielded Buhner's needle (conceal) was used to create a precise stab incision in the distended fetal cranium, permitting evacuation of accumulated cerebrospinal fluid. Subsequent reduction in head size facilitated the application of gentle obstetrical traction, enabling successful delivery without undue trauma to the dam. The consistent success of this approach highlights the clinical utility of decompressive cranial puncture as a practical, field-applicable technique for resolving hydrocephalus-associated dystocia in bovines. These observations reinforce the value of prompt obstetrical decision-making, meticulous supportive care and sustained emphasis on maternal nutrition and gestational management to mitigate the occurrence of congenital anomalies such as hydrocephalus and to optimise peripartum outcomes.

Keywords: *Buffalo, Dystocia, Fetal Hydrocephalus, Obstetrics, Parturition*

PP-4.20. Fetal Monstrosity in Buffalo: Study of Different Cases

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Fetal anomalies are rare in nature and mostly associated with the difficulty in birth or other complication during the pregnancy or parturition. Per-vaginal deliveries of these fetuses are very difficult. In present study total 04 types of cases are discussed which was presented to handled with almost full gestation or prolong gestation. The first case discussed here, was Chiral Fetus that arises due to prolong gestation, dam was presented with the history of one month prolongation of gestation than normal. In this case delivery was done per vaginally with manual interventions. Fetus was born with long hairs on its all over body with increased weight. Second case was presented with the history complete parturition after per-vaginal extraction there was conjoint fetuses with its thorax and having the 8 limbs. Third case was presented with difficulty in birth after complete gestation and a dicephalic fetus was delivered per vaginally with assistance. Fourth case was presented with full term without successful delivery of fetus. An ankylosed fetus was delivered per-vaginally with manual assistance. All these cases were of buffalo. Per-vaginal examination of monster fetus and assessment of percentage of per-vaginal delivery is at most important to save the precious animals and to avoid the cesarean section which is unnecessary burden to the owners. Besides these we also handled the cases of hydrocephalus, penta-limbic conjoint fetus, polydectila, double tailed, twin fetus with single anus, etc.

Keywords: Buffalo, Dystocia, Monster, Dicephalic, Conjoint fetus, prolong gestation, Chiral fetus.

PP-4.21. Operative management of dystocia in buffalo through cervico-episiotomy approach: An efficient mean to prevaginal delivery of fetus

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Incomplete cervical dilatation (ICD) is the most frequent obstetrical abnormality contributing to dystocia in buffaloes and is encountered in both heifers and multiparous animals. Factors such as suboptimal nutritional management, a history of recurrent vaginal prolapse, delayed presentation beyond 72 hours and concurrent uterine torsion can predispose the cervix to ischemia, thereby restricting its physiological dilatation. Under such compromised conditions, cervicotomy serves as an effective alternative to immediate caesarean section, while marked vulval stenosis in selected animals necessitates episiotomy to enable safe fetal extraction. This report documents seven ICD cases presented to the Referral Veterinary Polyclinic (RVP): ICAR-IVRI, all of which were successfully managed through cervicotomy, episiotomy, or a combined approach. Each buffalo had completed a full gestation and exhibited persistent, forceful straining at the time of arrival. Vulval stenosis was identified in four animals, whereas inadequate cervical dilatation without vulvar involvement was observed in the remaining three. Obstetrical intervention consisted of combined cervicotomy and episiotomy in three cases, isolated episiotomy in two cases and isolated cervicotomy in two cases. All procedures were performed under low caudal epidural anaesthesia using 4 mL of 2% lignocaine hydrochloride following thorough lubrication of the birth canal. Episiotomy involved a 9-13 cm dorsolateral vulvar incision made with a scalpel blade and subsequently sutured after fetal delivery. Cervicotomy consisted of 3-5 cm deep incisions placed at two to three sites on the cervical ring; outer ring involvement was noted in two of the seven animals. All foetuses were in anterior presentation and fetal extraction was successfully completed in every case. Postoperative management included routine antimicrobial and anti-inflammatory therapy, with all animals exhibiting uneventful recovery during follow-up.

***Keywords:** Buffalo, Cervicotomy, Dystocia, Episiotomy, Parturition.*

PP-4.22. Lacrimal glands in Buffalo -A Gross, histological and histochemical study

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The present study was conducted on 10 male buffalo calves of non-descript breed and between 1-2 year of age. The objective of investigation was prepared to record the anatomical features of lacrimal gland in buffalo calves. The gross biometrical parameters viz; length, width and thickness of gland were taken by means of digital Vernier callipers. The weight of gland was taken on an electronic weighing balance. The tissue was collected and thin paraffin sections (5 µ) were cut and stained with various stains. The gland was consisted of stroma and parenchyma. The stroma was composed of loose connective tissue capsule which encircled the gland. The thin connective tissue septae were extended from the capsule and divided the gland into lobes and lobules. Some acinar cells were surrounded by basal myoepithelial cell also evident. The intralobular duct was sectretory and was lined by simple cuboidal epithelium. The several excretory ducts were also observed in the capsule and they were lined by pseudostratified epithelium with goblet cells interposed between epithelial cells.

Keywords: *Anatomy, Buffalo, Gland, Lacrimal*

PP-4.23. Maternal dropsy condition in buffaloes as an emerging and life-threatening obstetrical condition: A case series

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Dropsical conditions of pregnancy involving the fetal membranes namely hydramnios, hydro allantois and allantoic oedema are characterized by rapid and abnormal accumulation of fluid. In hydroallantois, the fluid is typically watery and amber-coloured, whereas in hydroamnios, it is more viscous and syrup-like. Hydroallantois is commonly associated with a sudden and excessive rise in allantoic fluid volume around 7-7.5 months of gestation, resulting in marked bilateral distension of the abdomen. In contrast, hydroamnios progresses gradually and is often linked to fetal developmental anomalies. The present communication describes observations from 15 confirmed cases of dropsical conditions in bovines presented to the RVP Clinic, IVRI. The primary complaints reported by owners included progressive abdominal enlargement and respiratory distress. Diagnosis was established based on trans-rectal examination and ultrasonographic evaluation. At presentation, gestational age of the affected animals ranged between 7-7.5 months (7/15) and 9-9.2 months (5/15): with clinical symptoms developing over the preceding 12-18 days. Induction of parturition was attempted in all cases. Cervical dilation to 2-3 finger width was attained within an average of 10-12 hours and complete dilation followed by fetal expulsion required approximately 28-32 hours. Fluid volume removed per case ranged from 150-200 litres in 13 out of 15 animals. The fluid had an average viscosity of 1.015 and a specific gravity of 6.5. Examination of placental tissues revealed hypertrophy of approximately 40-45 placentomes and placental hematomas were noted in 4 animals. Fetal abnormalities were also detected in 4 out of the 15 cases. Body condition score of the dams declined from 3.5-4 at presentation to 2.0-2.5 post-drainage. Maternal survival was compromised in 6 cases. Post-partum monitoring showed that 9 out of 15 animals subsequently developed metritis.

Keywords: *Buffalo, maternal dropsy, hydroallantois, parturition*

PP-4.24. Fetal Ascites in a Buffalo Leading to Obstetrical Emergency: Clinical Management and Outcome

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Fetal ascites is a rare but important cause of dystocia in buffaloes. Excessive peritoneal fluid accumulation enlarges the fetal abdomen, leading to fetal-maternal disproportion and obstructed parturition. Early diagnosis and timely obstetrical intervention are essential to ensure dam survival. This report describes the

diagnosis and successful management of dystocia due to severe fetal ascites in a Murrah buffalo. To document the clinical presentation, obstetrical management including fetal abdominal puncture and postpartum recovery in a buffalo affected with dystocia due to fetal ascites. A 5-year-old Murrah buffalo in second parity at full-term gestation was presented with continuous straining and reduced appetite, urination and defecation. Per-vaginal examination revealed a fully dilated cervix, but delivery was obstructed by a markedly distended fetal abdomen. Adequate lubrication was applied and fetal limb correction attempted. Due to extreme abdominal enlargement, fetal ascitic fluid was evacuated by puncturing the fetal abdomen using a concealed knife, allowing controlled drainage of approximately 60-80 liter of fluid. Following significant reduction in fetal size, controlled forced traction using obstetrical ropes, synchronized with abdominal contractions, successfully delivered a dead fetus. Postpartum therapy included IV fluids (DNS, RL): antibiotic (X-Ceft): NSAID (Flunixin): Oxytocin, calcium, antihistaminic support, M-Block, Belamyl, Involon syrup and tetanus toxoid. The dam showed postpartum weakness but recovered gradually with supportive care. Fetal ascites may arise from congenital anomalies, hepatic or cardiac dysfunction, or intrauterine pathology. Drainage of ascitic fluid is critical when abdominal enlargement prevents fetal extraction. In this case, abdominal puncture followed by controlled traction minimized maternal trauma and ensured successful resolution of dystocia. Fetal ascites should be considered in cases of obstructive dystocia. Fetal abdominal puncture to reduce size, followed by careful traction, is an effective and lifesaving method. Supportive postpartum therapy enhances maternal recovery.

Keywords: *Dystocia, Fetal Ascites, Controlled Traction, Murrah, Fetal Fluid Drainage*

PP-4.25. Histological evaluation of conjunctival and corneal tissue damage associated with prolonged sunlight exposure in grazing buffalo

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Prolonged ultraviolet (UV) and high-intensity sunlight exposure is an emerging welfare concern for grazing buffaloes, particularly under changing climatic conditions. Buffaloes possess darker pigmentation and a semi-aquatic evolutionary background, yet extended exposure to intense solar radiation may predispose them to ocular surface damage, discomfort and reduced visual function. This study aimed to characterize the histopathological alterations in the conjunctiva and cornea of grazing buffaloes chronically exposed to high sunlight intensity and to compare these findings with animals maintained under shaded conditions. A total of 12 adult Murrah buffaloes were examined, including 6 animals continuously exposed to open-grazing sunlight and 6 housed with access to shade. Ocular clinical assessment recorded tearing, photophobia, conjunctival hyperaemia and corneal haze. Tissue samples of bulbar conjunctiva and cornea were collected post-slaughter and processed using routine paraffin embedding. Haematoxylin-eosin staining were performed to assess epithelial integrity, inflammatory infiltration and neovascularization. Buffaloes exposed to prolonged solar radiation showed significant epithelial thinning, loss of microvilli, squamous metaplasia and reduced goblet cell density in conjunctival tissue. Corneal sections demonstrated superficial keratinization, disrupted stromal collagen fibres, focal oedema and increased fibroblast activation. Moderate infiltration of lymphocytes and macrophages was frequently observed. Animals with shaded access exhibited comparatively intact epithelial layers, organized stromal structure and minimal inflammatory changes. The findings indicate that chronic sunlight exposure in grazing buffaloes can induce measurable conjunctival and corneal damage, potentially compromising ocular health and visual comfort. Provision of shade structures, strategic grazing schedules and monitoring of ocular surface health are recommended to mitigate sunlight-associated ocular pathology in buffalo production systems. This study contributes valuable histological evidence supporting welfare-driven management in buffaloes under high-radiation environments.

Keywords: *Sunlight exposure, Ocular histopathology, Conjunctiva, Cornea, UV-induced tissue damage.*

PP-4.26. Clinical Management of True Breech Dystocia in a Multiparous Non-Descript Buffalo Under Field Conditions

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Dystocia in buffaloes represents a critical reproductive emergency in rural dairy systems, often leading to considerable economic loss when timely intervention is unavailable. True breech presentation, although less common than fetal-maternal disproportion, poses a significant obstetrical challenge due to bilateral hind-limb flexion beneath the fetal trunk. This report documents the successful field management of true breech dystocia in a third-parity non-descript buffalo, attended by the Mobile Veterinary Unit (MVU) following an emergency call through the 1962 helpline at Sikosa village, Balod district, Chhattisgarh. The buffalo presented with prolonged straining, restlessness and absence of fetal parts at the vulvar opening. Per-vaginal examination revealed complete cervical dilation with the fetus positioned in true breech posture. Thorough lubrication and precise intra-pelvic manipulation were performed to sequentially extend the hind limbs, followed by controlled traction to safely deliver the fetus per vaginam. Post-partum management included systemic antibiotics, NSAIDs, calcium therapy and uterine ecbolics, resulting in an uneventful recovery of the dam within 48 hours. This case emphasizes the importance of rapid field response via emergency veterinary services, early diagnosis of malpresentation and skilled obstetrical manipulation in resolving breech dystocia. Strengthening field-level obstetric capabilities and emergency service accessibility can substantially improve reproductive health outcomes in buffalo-dominated dairy systems.

Keywords: *Buffalo, breech dystocia, emergency veterinary service, obstetrical correction, field intervention.*

PP 4.27. Comparative microanatomy of the tunics architecture in Buffalo and Cattle.

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The structural organization of the ocular tunics plays a critical role in determining the visual performance and environmental adaptability of domestic ruminants. This study presents a comparative overview of the three principal tunics of the eye—fibrous, vascular, and nervous—in Buffalo (*Bubalus bubalis*) and cow (*Bos taurus*). Notable interspecies differences were observed in the fibrous tunic, where buffalo showed a thicker cornea with flattened curvature and a more melanized sclera, adaptations that enhance protection and optimize vision in high-glare, wetland environments. In the vascular tunic, buffalo exhibited a more heavily pigmented iris, thicker tapetum lucidum, and fewer but robust ciliary processes, reflecting specialization for dim-light and variable light conditions. The nervous tunic revealed a higher rod-to-cone ratio in buffalo, supporting superior night vision but slightly reduced visual acuity compared to cows. Conversely, cows displayed finer ciliary processes, lighter pigmentation, and greater cone density, contributing to better daylight vision and accommodation. These findings highlight functional anatomical adaptations shaped by ecological and behavioural differences, offering valuable insights for veterinary ophthalmology, comparative anatomy, and species-specific clinical diagnosis.

Keywords: *Melanized sclera, Ocular tunics, Pigmented Iris, corneal morphology, Tapetum lucidum adaptation*

Session-V

Nutrition and Management

Chairman : **Dr Pawan Singh**
Head LPM ICAR-NDRI, Karnal

Co-Chairman : **Dr Narayan Dutta**
Head ICAR-IVRI Izatnagar &

Dr L.C. Chaudhary
Former Head ICAR-IVRI Izatnagar

Rapporteurs : **Dr Anju Kala**
Scientist (S.S) ICAR-IVRI Izatnagar

Indu Devi
Scientist (S.S) ICAR-NDRI, Karnal

LP-5.1. Exploring Smartphone and Image Analysis Tools for Colorimetric Methods Used in Buffalo Research

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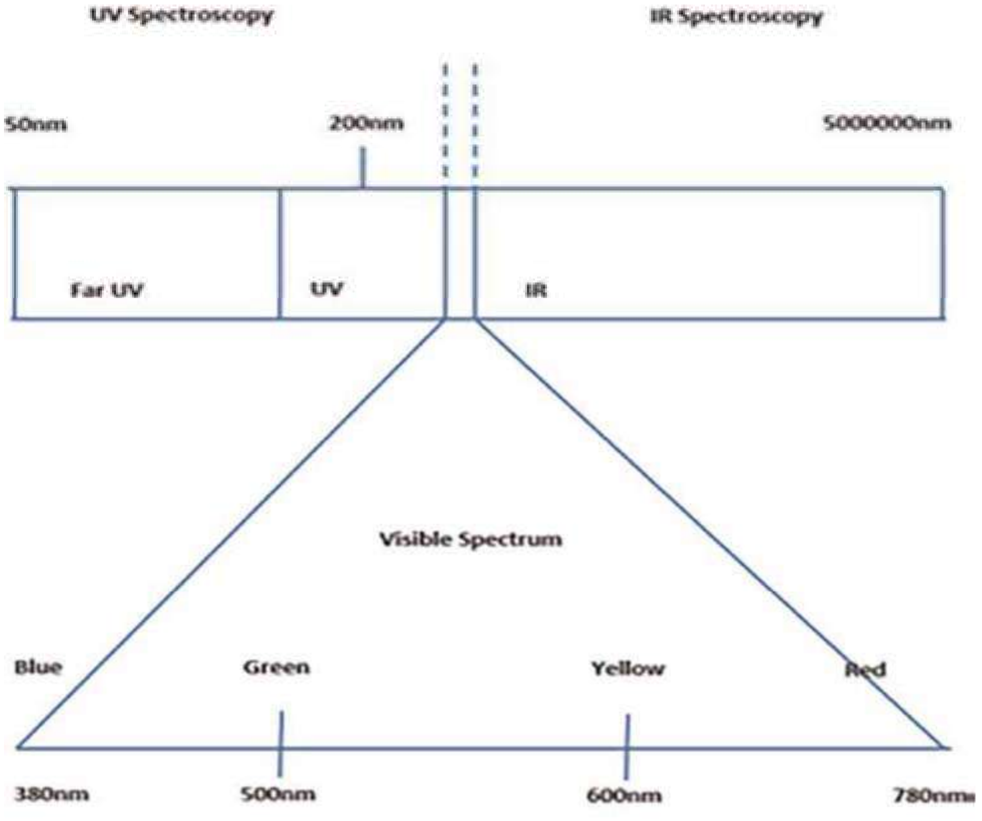
Buffalos are valuable animals that contribute to both economy and animal proteinsources with milk and meat production in India. Environmental conditions or physiological status such as summer, severe winter, pregnancy, lactation, and disease patterns may also be reflected in variations of the serum biochemical parameter values in animals and humans. Buffalo serum biochemical parameters provide a valuable tool for detecting changes in herd health, which could help for taking timely management decisions. Colorimetricassay methods detect serum biochemicallevels in terms of values for protein, vitamins, minerals, hormones etc., through visible colourintensity changes thatcan be perceptible and measured by Spectrophotometers at different wavelengths ranging from 380 to 750 nm.



Colorimetry

Colorimetryis referred if the measured light is in the visible range of the electromagnetic radiation. In this method abeam of light from a light source is allowed to pass via sample holder containing theanalyte in the solution, the intensity of light transmitted will be less than the lightpassing through sample in the cuvette. The absorbed light is in proportion to theconcentration of the analyte. The colour of the sample is an intrinsic characteristic ofthe solution, or it can be evolved by the addition of suitable reagents. The absorption ofthe sample is compared to that of standards from which the concentration of testsample can be calculated.

In modern times, obtaining and sharing analytical data timely on site is becoming increasingly important. Smart phone digital image colorimetry (SDIC) develops into a hot research topic. SDIC refers to a colorimetric analysis method based on digitizing images collected by some image acquisition tools such as mobile phones, digital cameras, webcams, scanners, and so on. The lightness and portability of smartphones and digital cameras make the use of these two digital products in SDIC far exceed that of webcams and scanners. Compared to digital cameras, smartphones are widely used as image acquisition tools in SDIC due to their rapid increase in use, remarkable improvement of camera functions, and the widespread use of mobile applications. In the procedure of SDIC, there are several steps including two necessary ones, image acquisition with smartphone and colour quantification using specific image processing software (Adobe Photoshop, Image J, Matlab, Pantone Studio, etc.) under suitable colour space. Then, the relationship between quantified images data and analyte concentration can be constructed and the results can be read out using customized mobile APPs. Thus, SDIC on smartphone has attracted increasingly greater attention.

Smart phone based digital image colorimetry (SDIC) has emerged as a viable alternative to conventional spectrophotometers. SDIC is a unique technique that takes advantage of smartphones' availability and affordability to transform analytical instrumentation, with enormous advantages in scientific research involved in buffalo experiments. Furthermore, the analytical usage of SDIC has also been improved by the availability of free apps and easy-to-use programs for data processing. Colorimetry is based on the measuring the colour intensity at different wavelengths and its absorbance as depicted below:

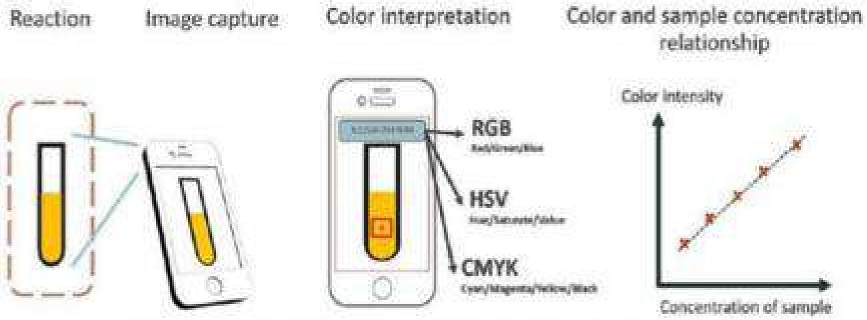
Colour of solution	**Filter**	**Wavelength (nm)**	**Range (nm)**
Blue green	Red	620	650-700
Blue	Yellow	590	570-600
Purple	Green	550	505-555
Red	Blue green	500	495-505
Orange	Green Blue	490	475-495
Yellow	Blue	445	420-475
Yellowish Green	Violet	410	400-420

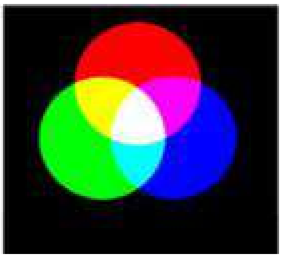
SDIC is based on colorimetric principles, where light is absorbed or transmitted by a sample solution at specific wavelengths to estimate the concentration of analyte present. The concentration of analyte in solutions and their absorbance exhibit a linear relationship. The readings of SDIC, like the traditional UV-vis spectrophotometry, are proportional to absorbance, obeying Beer-Lambert's law. The fundamental law underlying all spectrophotometric estimations is the Beer-Lambert Law. According to Beer-Lambert law, the absorbance (A) of light by a solution is directly proportional to the concentration (c) of the solution and the path length (l) by which the light passes through the solution (that is, the cuvette width).

A or OD (optical density)=-log (I_t/I_0) where A is the absorbance (A or OD) of light by a solution, $I_t$is the intensity of light measured after transmitting the solution and I_0 intensity of light measured prior to enter the solution. Absorbance (A) is inversely proportion to the transmittance of light intensity. According to Beer-Lambert law, the absorbance (A) of light by a solution is directly proportional to the concentration (c) of the solution and the path length (l) by which the light passes through the solution (that is, the cuvette width). This relationship is pivotal for estimating the analyte concentration in solution. The absorbance of one solution (unknown solution) is compared to a set of solutions with known concentration.

All the spectrophotometric methods in measuring different compounds like protein, enzyme inhibition, biochemical estimations/metabolites and drugs etc use this principle based on the measurement of absorbance (A) of selected analyte and its intensity of colour under selected wavelength (nm).

Colorimetric information can be obtained by smartphones under stabilized light conditions and that image can be analysed using free apps available from internet sources. SDIC can be useful as spectrophotometer and microplate reader in visible spectrum. The working principle of SDIC is given below for understanding.





After capture of image, the colour spaces can be quantified using RGB, HSV and CMYK modes. However, RGB (red, green, blue) is regarded as the most commondigitally coded colour model and employs the three primarycolours of red, green, and blue as shown in the image below.

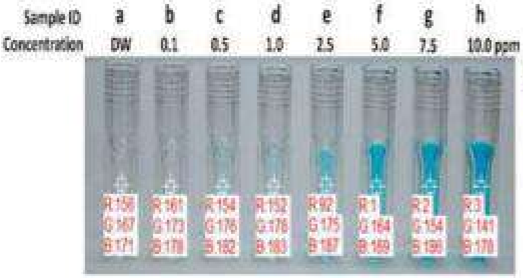
RGB is an additive colour model meaning that the presentedcolours are generated through combinations of thethree primary colours. For example, yellow is representativeof acombination of green and red. With 8 bits of colour depth, colourintensities ranged from 0 to 255(2^8).For example, absolutewhite is a combination of Red, Green and Blue with [255:255:255] whereas Black is with [0:0:0] of Red, Green and blue. Similarly, the different colours have different codes of digital values of Red, Green and blue. Free android apps like Colour Grab and Colour Picker gives RGB digital values for solutions that are exposed to smart phone camera. These values of (R, G and B) for each reading can be summated and the average value (R+G+B/3) of this summation is taken for both blank and standard/test samples.

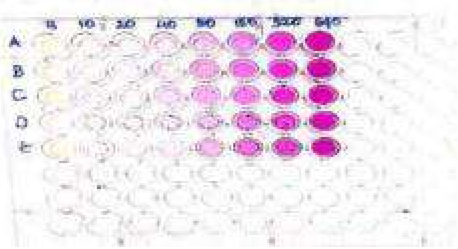
OD or A= -log (average RGB value of Blank/average RGB value of standard/test conc/) follows linear relationship as in Spectrophotometer.

Typical designs for taking images of cuvette/tube solutions or microplate reader with smart phone with stabilized light conditions are given below for variety of applications in the scientific field.

Cuvette model design

Microplate Design





Data conversion method from RGB values obtained to Absorbance can be done as shown below. However, Image J like apps. Calculate R+G+B/3 values also that can be utilized for quantification.

Conc ppm	R	G	B	R+G+B	R+G+B/3	Value
DW (0) (blank)	156	167	171	156+167+171	494/3	164.66
1.0	152	178	183	152+178+183	513/3	171.00
2.5	92	175	187	92+175+187	454/3	151.33
7.5	02	154	186	02+154+186	342/3	114.00

Absorbance= -log (R+G+B/3) blank/(R+G+B/3) sample

For image processing certain web apps like trigit (http://trigit.com.au) (https://ij.imjoy.io/) can also be used. Even Image J app can be downloaded in the desktop/laptops for analysing RGB values obtained for the test/standard samples. These readily available sources can be utilized in buffalo research with limited available resources without compromising quality of research work.

Further Reading

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LP 5.2 Methane Emission from Livestock and its Mitigation: What We Know and What We Need to Know

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Climate change is adversely impacting the livestock farmers across the globe. The challenge for the livestock farmers is multifaceted as they have to increase the overall production to meet the nutritional demand of ever increasing population and at the same time also need to minimize the impact of climate change and environment of the livestock. Intergovernmental Panel on Climate Change defined carbon dioxide, methane, nitrous oxide, hydrofluorocarbons, perfluorocarbons and sulfur hexafluoride as greenhouse gases and among these CO_2, CH_4 and N_2O are naturally occurring GHGs, while the remaining are do not exist in the atmosphere and are added in the small quantities due to the industrial activities.

The Methane Challenge

After carbon dioxide, methane is the second most significant greenhouse gas (Yusuf *et al.*, 2012) prevails in the atmosphere at a concentration of 1890 parts per billion. The atmospheric concentration is annually rising with a rate of 10 parts per billion (Saunois *et al.*, 2016). Methane has accounted for roughly 30% of global warming since pre-industrial times and carries a global warming potential 25-28 times greater than carbon dioxide over a 100-year period. The livestock sector stands as a major contributor to anthropogenic methane emissions. Current estimates suggest that livestock production accounts for 12-19.6% of global greenhouse gas emissions, with considerable variation depending on the methodology and metrics used for calculation. Of particular concern is enteric fermentation—the digestive process in ruminant animals—which alone contributes approximately 2 gigatons of CO_2 equivalents annually. Worldwide, cattle and buffaloes contribute 77 and 13% of the enteric methane emissions, respectively. The annual enteric methane emissions from these two species in India is estimated to be 7.83 teragram, which is approximately 85% of the total enteric methane emissions in the country (Bhatta, 2023). The process of methanogenesis occurring in the rumen results in an energy loss of 8-12%, which the host animal could have utilized for different body functions (Malik *et al.*, 2023). A recent estimate indicated that methane emissions resulting from enteric fermentation in dairy animals are responsible for an annual energy loss of 38x10^8 Giga calories (1 Gcal=10^6 calories). Thus, the methane emission from the livestock represents a double burden of nutrition gaps in the warming world and defined as production inefficient process for the obligatory removal of metabolic hydrogen from the rumen. The relatively low concentration of methane in the atmosphere is usually led to the underestimation of its significance in climatic impacts. A significant decrease in enteric methane is necessary for the optimum utilization of feed energy to bridge the gap between nutrient deficit and availability; to enhance the production efficiency of livestock and reducing the contribution of livestock in global warming.

The urgency of addressing livestock methane is underscored by the Global Methane Pledge, which commits over 100 signatory countries to reducing methane emissions by 30% by 2030. Achieving this ambitious target while meeting growing global food demands presents a complex challenge that requires innovative solutions spanning nutrition, genetics, management and policy.

Sources of Emissions

Enteric Fermentation

The primary source of livestock methane is enteric fermentation, a natural digestive process in ruminant animals including cattle, sheep, goats and buffalo. During this process, microorganisms in the rumen—primarily methanogenic archaea—break down fibrous plant material and produce methane as a metabolic byproduct. Contrary to popular belief, over 90% of enteric methane from cattle is released through eructation (burping) rather than flatulence.

Individual cattle can produce between 70-120 kg of methane per year, with variations depending on diet quality, animal size, productivity level and individual animal characteristics. Globally, the 1.5 billion cattle raised for meat and dairy production emit at least 231 billion pounds of methane annually. Notably, cattle account for approximately two-thirds of livestock-related greenhouse gas emissions, with beef and dairy production being the largest contributors.

Manure Management

The second major source of livestock methane emissions is manure management. When manure is stored in anaerobic conditions—particularly in slurry form—microbial decomposition produces significant methane. In contrast, manure deposited directly on pasture decomposes aerobically and produces minimal methane. In the United States and Wisconsin, the increasing adoption of slurry manure storage systems represents the fastest-growing source of agricultural greenhouse gas emissions, highlighting the importance of manure management strategies.

What We Know: Mitigation Strategies

Feeds and fodders

Fodder quality and type have a significant influence on the intensity of methane emissions. In an animal study, Kurihara *et al.* (1995) recorded less methane production in Italian ryegrass hay-fed cows than those fed on maize silage. Due to higher passage rate, Digestible fodders stay for a short time in the rumen and thereby produce less methane. Methane emission from the animals fed on leguminous forage is less than those feeding on grasses (Ramirez-Restrepo and Barry, 2005). The VFA profile, thereby methane yield, is affected by the type of carbohydrates fermented in the rumen (Bannink *et al.*, 2006). On a concentrate-based diet, fermentation of sugars and starch showed 25 and 15% lesser methane yields, respectively, compared to a roughage diet (Bannink and Dijkstra, 2005). The extent to which high-grain diets lower methane emissions depend on the source of the grain. For example, more significant reductions can be achieved with maize than barley (Beauchemin and McGinn, 2005). McGinn *et al.* (2009) observed 23.9% less methane on the feeding of maize distillers' dried grains compared to barley grain feeding in growing beef cattle. The desirable 15-21% reduction in enteric methane emission may be achieved by feeding more digestible feeds like legumes (Benchaar *et al.*, 2001). Lucerne or red clover feeding decreases methane emission due to the secondary plant metabolites such as tannins (Ramirez-Restropo and Barry, 2005) or saponins (Malik *et al.*, 2009, 2010).

Ration Balancing

Ration balancing with locally available feed resources at the farmer's doorstep is an effective and easiest way to alleviate methane emission without compromising the production or health performance of the animals. Livestock in the developing world is scattered, reared mainly by marginal/landless farmers and exclusively fed on a fibrous diet with negligible or zero concentrate. Feeding of highly lignified forage leads to higher methane emission from enteric fermentation. Due to the lack of awareness among the stakeholders, livestock is generally fed with an imbalanced diet, resulting in more enteric methane emissions with poor nutrient utilization for productive functions. This can be done by developing the minimum methane model by considering locally available feed resources. National Dairy Development Board (NDDB) launched a ration balancing program (RBP) for the small dairy farmers in different agro-climatic regions. In one study, Garg *et al.* (2012) reported about 14% reduction in methane emission in buffaloes by feeding balanced ration formulated from the locally available feed resources at farmers' doorstep. Studies in India established that increasing concentrate proportion in the diet from 25 to 75% decreased methane emission to the tune of 18-20% (Singh, 2001).

Plant secondary metabolites

Plant secondary metabolites (PSM) are now known for their anti-methanogenic action. Animal nutritionists focusing on enteric methane amelioration from livestock explored most available feeds, herbs, leaves and other herbal materials for their PSM content. In the author's laboratory, more than thousands of conventional, unconventional feeds, tree leaves and other phyto materials have been tested for their phenolic (tannin) content under an ICAR ongoing project entitled '*Estimation of enteric methane emission under different feeding systems and development of mitigation strategies'*. All the phyto-materials that possess substantial tannin content were tested for the effect of limited supplementation on *in vitro* and *in vivo* methane emissions.

Harit Dhara

The decade-long exhaustive research under the project yielded two products, namely Tamarin Plus and *Harit Dhara* to ameliorate about 15-20% enteric methane emissions in livestock. Tamarin plus is an anti-methanogenic complete feed block enriched with tanniniferous agricultural waste tamarind seed husk. This product is an exclusive feed formulation and can be offered *ad libitum* to ruminants to meet their maintenance requirement and for methane amelioration. *Harit Dhara is a*n anti-Methanogenic feed supplement prepared using selected phyto-sources containing condensed tannins, hydrolyzable tannins and saponins. This product is very effective in reducing enteric methane emissions by 20% when incorporated in livestock feed. Feeding of *Harit Dhara* in cattle and buffalo costs Rs. 3-5 per day.

Oat brewery waste

Recent studies (Malik *et al.*, 2024; Prajapati *et al.*, 2025 communicated) conducted at the National Institute of Animal Nutrition and Physiology revealed that the oat brewery waste has great potential for decreasing enteric methane emission from livestock while feed fresh. The results from the studies indicated that the concentrate in the diet can be successfully replaced by the oat brewery waste up to 20% level without any adversity on the nutrient utilization and production performance in growing sheep.

3-Nitrooxypropanol (3-NOP): This synthetic compound, marketed commercially as Bovaer, represents the most advanced methane mitigation technology currently available. 3-NOP specifically inhibits methyl-coenzyme M reductase, an enzyme critical for the final step of methane formation. Studies demonstrate consistent methane reductions of approximately 30% in dairy cattle and up to 45% in beef cattle. As of 2024, 3-NOP has received regulatory approval in over 65 countries, including the United States, European Union, Australia, Brazil, Canada and the United Kingdom. The required dose is remarkably small—approximately ¼ to 1 tablespoon per cow daily—making it practical for implementation in intensive feeding systems.

Seaweed: Red seaweed species, particularly *Asparagopsis taxiformis* and *A. armata*, have demonstrated dramatic methane reduction potential. The active compound bromoform inhibits methane-producing enzymes in the rumen. Studies have reported methane reductions ranging from 37.7% to over 90%, depending on dose, feed quality and inclusion rate in the diet. Recent research on grazing beef cattle showed an average 37.7% reduction in methane emissions without adverse effects on animal performance. However, significant challenges remain regarding scalability, potential bromoform accumulation in milk and meat, ozone depletion concerns and the need for sustainable aquaculture production systems. Various studies conducted in India and elsewhere indicated that some of the seaweeds are quite effective in decreasing enteric methane emissions from livestock (Malik *et al.*, 2025; McGurrin *et al.*, 2023). Seaweeds are nowadays are being used in the nutraceutical industries and therefore generating huge waste, which potentially cause the health hazards. Recently, work carried out at the National Institute of Animal Nutrition and Physiology revealed that the seaweed biowaste from the nutraceutical industries can be effectively used to minimize the enteric methane emission (Mohapatra *et al.*, 2024, 2025).

Genetic and Breeding Strategies

Selective breeding represents a particularly attractive mitigation approach because genetic improvements are permanent, cumulative across generations and do not require ongoing intervention once established in the population.

Research has demonstrated that methane emission traits are heritable, with heritability estimates ranging from 0.13 to 0.47 depending on the specific trait definition and stage of lactation. Several methane-related traits are being investigated such as Methane production (MeP): Absolute daily methane output (g/day); Methane yield (MeY): Methane per unit of dry matter intake (g/kg DMI); Methane intensity (MeI): Methane per unit of product (g/kg milk or meat) and Residual methane production: Methane output after accounting for production, body weight and intake

Innovative approaches using rumen microbiome data as biomarkers for methane production offer cost-effective alternatives to expensive direct measurement. Research has shown that rumen microbial gene abundances can predict both feed efficiency and methane emissions, potentially enabling large-scale genetic selection without requiring respiration chambers for every animal.

What We Need to Know: Critical Research Gaps

Measurement and Monitoring

Scalable Measurement Technologies: Current gold-standard methods for measuring individual animal methane emissions—such as respiration chambers and the GreenFeed system—are expensive and impractical for large-scale deployment. While mid-infrared (MIR) spectroscopy of milk shows promise for dairy cattle, accuracy remains limited and the approach is inapplicable to beef cattle or non-lactating animals. Development of low-cost, accurate, real-time measurement technologies suitable for pasture-based systems represents a critical need.

Standardization: Lack of standardized measurement protocols complicates comparison across studies and regions. Establishing internationally accepted methods for methane quantification, including appropriate correction factors for different production systems, is essential for robust global assessments.

Feed Additive Research

Long-term Efficacy and Safety: Most feed additive studies span weeks to months, yet commercial application requires evidence of sustained efficacy over years. Potential adaptation of rumen microbiomes, residue accumulation in animal products, environmental impacts of large-scale use and effects on animal health and welfare all require comprehensive long-term investigation.

Combination Approaches: Limited research examines potential synergies or antagonisms when combining multiple mitigation strategies. For instance, could low-dose bromoform combined with 3-NOP achieve greater reductions than either alone? How do genetic selection and feed additives interact?

Phenotype-Genotype Relationships: While heritability estimates exist, the specific genetic architecture underlying methane production variation remains poorly understood. Identifying causal variants through genome-wide association studies could enable more precise genomic selection. The relationship between host genetics and rumen microbiome composition—and how this interaction influences methane production—requires deeper investigation.

Tradeoffs and Correlations: Concerns exist that selecting for reduced methane might compromise fiber digestion efficiency, a fundamental advantage of ruminant production systems. Comprehensive evaluation of genetic correlations between methane traits and animal health, fertility, longevity, product quality and stress resilience is essential before implementing large-scale breeding programs.

Multi-species Application: Most genetic research focuses on dairy cattle, with limited attention to beef cattle, sheep, goats and buffalo. Given regional and production system variation, species-specific breeding strategies require development.

Microbiome Manipulation

Microbial Ecology: While we know methanogens produce methane, the complex interactions among bacteria, archaea, protozoa and fungi in the rumen remain incompletely understood. How do these interactions influence methane production? Can we promote hydrogen-utilizing bacteria that compete with methanogens? What determines microbial community resilience and stability following intervention?

Probiotic and Prebiotic Approaches: Early-stage research on direct microbial interventions—such as introducing hydrogen-consuming bacteria or bacteriophages targeting methanogens—shows promise but faces substantial challenges. Ensuring colonization persistence, preventing microbial adaptation and maintaining intervention effectiveness across diverse animals and diets all require extensive investigation.

Emerging Technologies

Vaccines: Anti-methanogen vaccines targeting key methanogenic species represent an intriguing possibility but face technical hurdles including mucosal immunity challenges and methanogen diversity.

Gene Editing: CRISPR-based approaches might enable precise modifications to improve feed efficiency or alter rumen microbial communities, though regulatory, ethical and practical considerations require careful navigation.

Artificial Intelligence: Machine learning applications for predicting emissions, optimizing feeding strategies and identifying low-emitting animals show considerable promise but require larger, higher-quality datasets for training and validation.

Conclusions and Future Directions

Significant progress has been made in understanding and mitigating livestock methane emissions. Proven technologies like Harit Dhara, 3-NOP, promising approaches such as Asparagopsis seaweed and advances in genetic selection provide realistic pathways toward meaningful emission reductions. Nutritional interventions alone could potentially achieve 30-60% methane reduction in intensive systems. However, substantial knowledge gaps persist. Scaling solutions for understanding long-term efficacy and safety, developing cost-effective measurement technologies and navigating complex socioeconomic and equity considerations all require urgent research attention. The challenge is particularly acute in low- and middle-income countries where livestock provide essential livelihoods and food security.

The goal should not be methane reduction at any cost, but rather sustainable intensification that meets growing food demands while minimizing environmental impact and supporting producer livelihoods. Achieving the Global Methane Pledge's 30% reduction target by 2030 is technically feasible but will require coordinated action across research, industry, policy and farming communities.

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LP-5.3 Balancing Nutrition for Augmenting Productive and Reproductive Performance in Buffaloes

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Abstract

Balanced nutrition of livestock is a backbone for obtaining optimum productive and reproductive performance. Ration formulated for the livestock should be properly balanced in terms of energy, protein, vitamins and minerals. Low or excess of nutrients adversely affect the performances of livestock. Here in this chapter, optimum nutrients requirement of buffalo has been discussed. The buffalo (Bubalus bubalis) is a cornerstone of dairy and meat production, particularly in Asia. Optimizing their productive and reproductive efficiency is intrinsically linked to a precise and balanced nutritional program. Unlike cattle, buffaloes have unique physiological characteristics, including a lower metabolic rate and differences in rumen morphology, which influence their nutrient requirements and utilization efficiency. This paper reviews the critical nutritional components—energy, protein, minerals and vitamins—highlighting the comparative requirements and the buffalo's unique adaptations.

Introduction

Optimizing reproductive efficiency is crucial for dairy buffalo profitability, as inefficiency leads to significant economic losses like increased calving intervals and decreased milk production. Nutritional manipulation is a key strategy for improving reproductive performance, as nutrient deficiencies or excesses can negatively alter the efficiency. Essential reproductive events requiring nutritional support include reaching puberty, early conception and achieving timely subsequent calving. The ultimate management focus should be on improved reproductive efficiency, as it is the main driver for commercial dairy husbandry.

In many regions of the world, especially Asia, the buffalo (*Bubalus bubalis*) is essential to the production of both meat and milk. A precise and balanced nutritional regimen is directly linked to maximizing their productivity as measured by growth rate, milk yield and reproductive efficiency. Buffaloes, in contrast to cattle, have distinct physiological traits that affect their food needs and utilization efficiency, such as a lower metabolic rate and variations in rumen shape. Buffaloes are superior to cattle in physiological and behavioral adaptation for efficiently utilizing low-quality, high-fiber diets and managing heat. Buffaloes spend more time physically breaking down tough fibers through rumination, retain the feed longer and have a stronger, more specialized rumen microbial population. The nutrient requirement for buffaloes is differed in comparison to cattle because of their unique metabolism, their ability to utilize low-quality roughages (FAO, 2020) and variations in their milk composition (Borghese and Mazzi, 2005). The efficiency of nutrient utilization is more in buffaloes comparison to cattle. They have slower rate of passage of feed in the digestive tract, allowing for a longer time for microbial digestion.

Dry matter requirement

Table 1: Buffalo vs cattle daily feed requirement

Category	Animal Type	DM requirement %	Green fodder (kg/d)	Dry fodder (kg/d)	Concentrate (kg/d)	Key difference
Maintenance	Cattle (indigenous)	2-2.5% of body weight	15-20	4-5	1-1.5	Lower DMI than crossbred
	Cattle (crossbred)	2.5-3.0% of body weight	20-25	4-6	1.5-2.0	Higher basal requirement due to higher metabolic rate
	Buffalo	2.5-3.0% of body weight	15-20	6-8	1.5-2.0	Higher fiber /roughage utilization efficiency, need more fodder for maintenance
Lactation (milk production)	Cattle (indigenous and crossbred)	Up to 3.5% of body weight	25-35	3-5	Maintenance plus 0.4 kg for every kg of milk production	For milk production concentrate requirement is slightly higher in buffalo than cattle due to higher fat content (≥6% fat)
	Buffalo	Up to 3.5 to 4.0% of body weight	25-35	3-5	Maintenance plus 0.5 kg for every kg of milk production	
Late pregnancy (last 2-3 months)	Cattle	2.5% of body weight	15-20	4-5	Maintenance plus 1-1.25 kg	Higher additional concentrate than cows, slightly higher protein requirement in buffaloes than cattle
	Buffalo	2.5-3.0% of body weight	15-20	4-5	Maintenance plus 1.5-1.75 kg	

Energy optimization for production and reproduction

Energy status is the primary driver of optimum reproductive success in dairy buffaloes. Energy is the most critical and often the most limiting nutrient in buffalo diets, dictating both the quantity and quality of production. It is primarily supplied by carbohydrates (fibrous and non-fibrous) and fats. Negative Energy Balance (NEB): common in high-yielding animals during early lactation due to inadequate energy intake and fat mobilization, significantly delays estrus and conception. The mobilization of body fat increases blood levels of Non-Esterified Fatty Acids (NEFA) and ketone bodies. Simultaneously, metabolic hormones like insulin and IGF-1 decrease, while Growth Hormone (GH) increases. These metabolic changes negatively impact fertility by disturbing Luteinizing Hormone (LH) pulse frequency and altering follicular and luteal development. Dietary fat supplementation is critical for maintaining energy balance and improving reproductive efficiency. As fatty acids and cholesterol are substrates for reproductive hormones, supplemental fat may increase hormone levels and improve dominant follicle diameter and embryo quality. The total dietary fat should not exceed 6-7% of the diet, with up to 3-4% coming from supplemented fat. Supplementing buffaloes with bypass fat (100-150 g/day or 2.5% of DMI) has been shown to improve milk yield, milk fat content and reduce the time to uterine involution and the service period. The TDN requirement for buffaloes vs cattle is shown in Table 2.

Table 2: The total digestible nutrient (TDN) requirement for cattle vs buffaloes (Based on ICAR Standard and common nutritional guidelines)

	Animal Type	TDN kg/d	Additional requirement per kg milk (g)	Recommended TDN% in the concentrate diet	Key difference
Maintenance	Cattle (400-500 kg body weight)	3.0-3.5 kg	-	55-60 %	Maintenance requirement is proportional to metabolic body weight ($W^{0.75}$)
	Buffalo (500-600 kg body weight)	3.5-4.0	-	55-60 %	Maintenance requirement is higher due to larger body size
Lactation (milk production)	Cattle (4% Milk fat)	Maintenance	350 g	65-70 %	Higher requirement in buffalo due to 1.5-2.0 times higher fat and total solid content of buffalo milk
	Buffalo (6-7% Milk fat)	Maintenance	400-500 g	70-75 %	
Late pregnancy (Steaming up)	Cattle	Maintenance	1-1.5 kg	60-65 %	Higher requirement in buffalo than cattle due to larger foetus and preparation for high peak yield
	Buffalo	Maintenance	1.3-1.5 kg	65-70 %	

Buffaloes are biologically adapted to utilize a diet containing higher levels of NDF and lower quality forages than high-yielding dairy cows. A diet formulated for a high-producing dairy cow with 26% NDF might cause health issues (like acidosis) if the NDF is not effective, but a high-NDF diet of 33% could significantly reduce the cow's DMI and energy intake. A buffalo can often thrive on a diet with 30–34% NDF and achieve higher DMI and production compared to cattle on the same high-NDF feed, thanks to its more robust rumen function (Table 3).

Table 3: Neutral Detergent Fiber (NDF) Requirement Comparison

Characteristic	**Cattle (High-Yielding Dairy Cow)**	**Buffalo (High-Yielding Milch Buffalo)**	**Difference**
Minimum Total Dietary NDF	25%–30% of diet Dry Matter (DM) is the typical range for peak lactation.	28%–33% of diet Dry Matter (DM) is often considered safe or even optimal.	Buffaloes can utilize higher total NDF levels while maintaining production, due to their superior fiber digestion efficiency.
Minimum Forage NDF (FNDF)	18%–21% of diet DM. This ensures adequate physically effective NDF (peNDF).	20%–23% of diet DM. A slightly higher minimum helps ensure sufficient rumen scratch factor and saliva production.	FNDF is essential for stimulating chewing/rumination and maintaining a healthy rumen pH (preventing acidosis).

Rumen Fiber Digestion	Good/Efficient. Fiber digestion is easily compromised if the rumen pH drops below 6.0 (due to excess starch/Non-Fiber Carbohydrates).	Superior/Highly Efficient. Buffaloes generally exhibit 5%–8% higher fiber digestibility than cattle, particularly with low-quality roughages.	This is attributed to a larger rumen, higher activity of cellulolytic microbes and a longer retention time of feed particles.
Influence of NDF content on feed intake	NDF acts as a stronger "fill" factor, meaning high NDF levels limit Dry Matter Intake (DMI) more quickly in cattle.	The fill effect is less pronounced (or occurs at a higher level) due to their superior fiber degradation, allowing them to eat more volume of high-NDF feed.	
Optimum Rumen pH	Approx 6.0–6.4. Needs more precise management to prevent acidosis when NDF is low and concentrates are high.	Approx 6.2–6.6. Buffaloes show greater buffering capacity and are slightly more resistant to depressed rumen pH, particularly when fed diets high in roughage.	

Optimizing protein for maximizing production and reproduction

Protein is vital for maintenance, reproduction, growth and lactation in animals. However, the level of dietary protein must be carefully balanced, as both deficiency and excess negatively impact performance. Low protein severely affects rumen microbial growth and fermentation, which decreases the capacity to digest organic matter and depresses feed intake. Conversely, excessive protein, especially when accompanied by an energy shortage, increases the energy requirement for the elimination of surplus urea, which can negatively affect health and reproduction. When Rumen Degradable Protein (RDP) is fed in excess, it is converted to urea, increasing Blood Urea Nitrogen (BUN) and Milk Urea Nitrogen (MUN). High dietary Crude Protein (CP) is negatively associated with fertility parameters; feeding 10-15% excess CP may increase services per conception and prolong calving intervals and supplementation of 15-19% excess CP has been reported to lower conception rates. This decreased fertility is likely due to the negative effect of increased urea levels on the uterine environment, which can lower the uterine pH during the luteal phase and impair successful embryo implantation and development, particularly at the cleavage and blastocyst formation stages. Proper energy intake is required alongside protein to maximize efficiency and avoid these negative reproductive outcomes. The optimum RDP and UDP requirement for cattle and buffaloes are shown in Table 4.

Table 4: RDP and UDP requirement cattle vs buffaloes

Feature	**Buffaloes (Bubalus bubalis)**	**Cattle (Bos taurus/indicus)**	**Implication**
RDP Utilization Efficiency	Higher. Buffaloes are reported to be superior to cattle in utilizing Rumen Degradable Protein (RDP) and Non-Protein Nitrogen (NPN) sources.	Lower comparative efficiency in utilizing NPN.	Buffaloes can thrive on lower-quality, high-fiber diets that have a high proportion of RDP/NPN, as they are more effective at converting this into Microbial Protein (MP).
Rumen Fermentation	Exhibit higher ruminal ammonia and Volatile Fatty Acid (VFA) concentrations.	Ammonia and VFA concentrations are generally lower when fed the same diet.	Higher ammonia concentration in the buffalo rumen indicates a more efficient and complete breakdown of RDP, leading to greater Microbial Protein synthesis.
Total Protein Requirement for Milk	Higher. The requirement for Digestible Crude Protein (DCP) needed per kilogram of milk produced is higher (approximately 55-65 g/kg of milk).	Lower. The DCP requirement is typically lower (approximately 45-50 g/kg of milk).	Due to the significantly higher protein (and fat) content of buffalo milk, the total Metabolizable Protein (MP) requirement (supplied by both Microbial Protein and RUP) is higher, necessitating a greater total protein supply in the diet.
RUP (Bypass Protein) Need	Critical for high-producing buffaloes. Despite superior RDP utilization, the sheer volume of protein required for high-yield milk production (due to richer milk) often requires supplemental RUP.	Critical for high-producing cows. RUP is essential to meet the animal's amino acid requirements that cannot be met by microbial protein alone.	In both species, a balanced RDP:RUP ratio is essential. For high-yielding buffaloes, the total Metabolizable Protein need is very high, which must be met by both maximum Microbial Protein from RDP and targeted supplementation of RUP.

Optimizing minerals for efficient production and reproduction

The success of the dairy buffalo economy hinges on optimal reproductive rhythm (Dhaliwal, 2005). Major reproductive disorders include anestrus (Ahmed *et al.*, 2012a) and repeat breeding. These issues are frequently associated with a reported deficiency of specific minerals in field conditions. Correction requires targeted, high-quality and economically feasible mineral supplementation (Butani et al, 2018).

Major minerals, including Calcium (Ca) and Phosphorus (P): are vital for both productive and reproductive efficiency in buffaloes. Ca deficiency is common around parturition and can cause reproductive issues such as delayed first estrus, delayed uterine involution, retained placenta and an increased incidence of dystocia. Maintaining a balanced Ca:P ratio (ideally 1.5:1 to 2.5:1 for lactating animals) is crucial, as Ca excess can impair the absorption of P, Mn, Zn and Cu, negatively affecting reproductive health. P deficiency is linked to reduced production, abnormal sexual behavior, decreased ovarian activity, delayed sexual maturity and low conception rates. Clinical signs of P deficiency include silent/irregular estrus, long inter-calving periods and embryonic death due to poor uterine muscle tone. Both Ca and P play roles in male fertility: Ca is essential for preserving spermatozoa motility and viability, while P is needed for glycolysis and motility and its concentration in seminal plasma correlates positively with semen quality. Additionally, Sodium (Na) and Potassium (K) regulate sperm motility and Magnesium (Mg) is required for sperm capacitation, hyperactivation and the acrosome reaction, with its level positively associated with semen's total antioxidant status. The requirement for phosphorus is often considered higher in buffaloes, especially high-yielding dairy buffaloes, due to their intense utilization in body maintenance and milk production.

Trace minerals are critical micronutrients for buffalo reproductive performance, with both deficiencies and excesses causing problems. Manganese (Mn) deficiency is linked to delayed estrus, reduced conception rates and deformed calves. Copper (Cu) deficiency is associated with a long post-partum return to estrus, anoestrus, silent heat and abortion, while Cu-rich diets enhance spermatozoa motility and may affect LH release. However, both deficient and excess Cu levels can negatively affect spermatogenesis. Conversely, excessive Molybdenum (Mo) intake can decrease libido and cause sterility in males and delay puberty, reduce conception rate and cause anoestrus in females. Cobalt (Co) deficiency impairs fertility and increases early calf mortality. Iron (Fe) is required for normal spermatozoa production and function, with its total content in seminal plasma correlating with sperm motility and viability, although excessive Fe can negatively affect sperm morphology and DNA integrity. Zinc (Zn) is essential for sexual maturity, early puberty attainment and seminal quality. Finally, managing the Dietary Cation-Anion Difference (DCAD) through chloride (Cl) addition is a key nutritional strategy to effectively reduce the incidence and severity of parturient hypocalcemia, which is important for maximizing subsequent reproductive efficiency. Buffaloes generally have a higher requirement or utilization rate for certain trace minerals, particularly Copper (Cu) and Zinc (Zn): which are crucial for immune function and reproduction.

Optimizing vitamins for efficient production and reproduction

Vitamins C, D, E and B-complex are generally either synthesized by rumen microorganisms or the animal body, or are widely available in common feedstuffs, meaning adult buffaloes typically do not require extensive supplementation. The major exception is vitamin A (or its precursor β-carotene): which is often deficient in mature forages and poor-quality roughages. Vitamin A is crucial for embryonic development and its deficiency leads to reproductive issues such as delayed sexual maturity, abortion, birth of weak calves, retained placenta and metritis. A daily intake of 2-3 kg of green fodder usually meets the requirement; otherwise, lactating buffaloes need supplementation of 20,000 to 45,000 IU/day and growing buffaloes need 2,000 to 8,000 IU/day. Dairy animals deficient in β-carotene suffer from delayed uterine involution, first estrus and an increased incidence of cystic ovaries. Additionally, Vitamin E acts as an important antioxidant; supplementation at a dose of 1,000 IU for 30-60 days postpartum has been shown to improve reproductive performance by decreasing the postpartum estrus interval, days open and services per conception in buffaloes. It was reported that supplemental Choline (a B-vitamin) has shown to improve liver function and reducing the severity of fatty liver during NEB, which can indirectly enhance fertility. Folic Acid (B_9) and Vitamin B_{12} are important in one-carbon metabolism and have been shown to potentially improve milk yield and reproductive parameters.

Plant-Derived Bioactive Components: The Future of Augmentation of buffalo performances

The use of plant-derived bioactive components, often referred to as nutraceuticals or phytogenic feed additives (PFAs): represents a novel strategy to enhance buffalo performance naturally and sustainably. These components include essential oils, saponins, tannins (Barman *et al.*, 2006; 2008) and flavonoids. Supplementation of moderate level of tannins (Condensed and Hydrolyzable) protect dietary protein from excessive ruminal degradation (acting as a natural RUP source). This can increase the supply of essential amino acids to the intestine, benefiting milk protein synthesis and reproductive physiology. Essential oils Compounds (thymol, cinnamaldehyde) can selectively inhibit certain rumen microbes, such as methanogens and deaminating bacteria which leads to improve feed efficiency and reduce methane emissions.

Flavonoids and Polyphenols of plant bioactive compounds possess strong antioxidant properties that help counteract the increased oxidative stress associated with the transition period, high milk production and postpartum reproductive events. Reducing oxidative damage to immune cells and reproductive tissues (e.g., uterine tissue, corpus luteum) is key to reducing incidence of diseases like metritis and improving the quality of the egg/embryo.

Conclusion

Optimal production and reproduction in buffaloes hinges on a precisely formulated diet that meets the demands of high lactation while simultaneously supporting the complex hormonal and physiological events of the reproductive cycle. The challenge lies in management of Negative Energy Balance that occurs postpartum. A holistic nutritional strategy must focus on: 1) Maximizing DMI and Energy Density through high-quality forages and protected fats, 2) Balancing RDP and RUP to ensure both efficient microbial protein synthesis and adequate supply of limiting amino acids, 3) Strategic supplementation of Trace Minerals (Se, Zn, Cu) and Vitamins (A, D, E): often via injectable or organic/chelated forms for better bioavailability, especially during the critical transition period (3 weeks pre- to 3 weeks post-calving): 4) Incorporating Plant-Derived Bioactive Components to sustainably enhance rumen function, protein utilization and boost the animal's natural antioxidant and immune defenses.

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LP-5.4 "Opportunities and Challenges in Application of Artificial Intelligence in Livestock Management"

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1. Introduction

Livestock management is undergoing a rapid digital transformation driven by advances in Artificial Intelligence (AI): computer vision, edge computing and Internet of Things (IoT). Across dairy, poultry and companion animals, AI systems now support real-time disease detection, automated monitoring, welfare assessment and decision-support analytics. These technologies enable preventive healthcare, reduce economic losses, optimize feeding and breeding strategies and contribute to sustainable livestock production. This chapter presents the current technological trends, emerging opportunities and persisting challenges in AI-driven livestock management, followed by a detailed summary of the work undertaken under the AI-DISA, PLW and Shria initiatives.

2. Current Trends in AI for Livestock Management

2.1 Vision-Based Health Assessment and Phenotyping

Deep learning models such as YOLO, Faster R-CNN, Mask R-CNN and Transformer-based architectures are now extensively used to detect lesions, assess lameness, estimate body condition and compute live weight from images and videos. Mobile phones and low-cost cameras have made AI-driven livestock phenotyping more accessible and scalable.

2.2 Wearable and Smart Sensor Integration

Livestock are increasingly monitored using smart collars, accelerometers, GPS modules, temperature sensors and rumination monitors. Data from these devices, when processed through machine learning models, enables prediction of estrus, heat stress, sickness behavior, movement abnormalities and environmental stress responses.

2.3 Edge-AI for On-Farm Real-Time Monitoring

With the rise of edge computing, AI models are deployed directly on embedded devices or farm gateways, enabling real-time inference without dependence on continuous internet connectivity. This trend is essential for remote livestock farms.

2.4 Multimodal AI for Behavior and Welfare

AI systems integrating image, audio, thermal, environmental and movement data support robust behavior classification, welfare scoring, stress detection, aggression monitoring and early identification of anomalies such as off-feed behavior.

2.5 Robotics, Automation and Smart Infrastructure

AI-enabled robotics now assist in milking, feeding, barn cleaning, drone-based monitoring and automated weight estimation—reducing labor dependency and enhancing farm precision.

3. Opportunities Enabled by AI in Livestock Systems

3.1 Early Disease Detection

AI enables rapid identification of visual and behavioral abnormalities, enabling earlier treatment and reduced mortality.

3.2 Precision Livestock Farming (PLF)

Individual-level monitoring allows personalized feeding, reproductive management and targeted interventions, enhancing efficiency.

3.3 Enhanced Welfare Monitoring

AI-driven welfare systems track comfort, stress and behavior changes, enabling humane and ethical livestock management.

3.4 Climate-Smart Decision Support

AI can predict heat stress, optimize ventilation, model disease outbreaks and support climate-resilient farming strategies.

3.5 Automation of Routine Activities

Robotic systems powered by AI reduce workload, minimize error and ensure continuous operations.

4. Challenges in AI Adoption

4.1 Data Limitations

High-quality annotated datasets remain limited, reducing model robustness in diverse farm conditions.

4.2 Environmental Variability

Lighting, breed variation, occlusion and background differences make model generalization difficult.

4.3 Infrastructure Gaps

Weak connectivity, unreliable power supply and lack of hardware impede deployment.

4.4 High Initial Costs

Sensors, cameras, edge devices and cloud infrastructure require investment, particularly challenging for smallholders.

4.5 Ethical and Behavioral Concerns

Continuous monitoring raises issues around privacy, data governance and potential behavioral changes due to devices.

4.6 Need for Capacity Building

Widespread adoption requires training veterinarians, paravets and farmers on AI-enabled tools.

5. Work Done: AI-Driven Livestock Disease Detection, Advisory Systems and Image-Based Weight Estimation

Artificial Intelligence (AI) and deep learning are transforming livestock health management by enabling real-time disease detection, automated decision support and precision livestock farming. The suite of applications and models developed under this work demonstrates significant progress toward operationalizing AI for animal health, productivity monitoring and field-ready veterinary advisory systems. The following summarizes the major contributions under the AI-DISA platform (bovine and canine disease detection): the hybrid autoencoder-based classification framework, the Pig Live Weight (PLW) system and the Shria intelligent assistant.

5.1 AI-DISA for Bovine Disease Detection

A robust AI-powered pipeline has been developed to automatically diagnose **Foot and Mouth Disease (FMD): Mastitis and Lumpy Skin Disease (LSD)** using mobile-captured images. The workflow allows users to upload images through the AI-DISA mobile application, after which deep learning models infer the disease type and generate an advisory from expert-curated knowledge bases.

YOLOv5 architectures (n, s, m, l, x variants) were fine-tuned for lesion localization and classification. The dataset incorporated diverse symptom presentations and anatomical regions, with augmentation improving generalization. High mAP@0.5 scores and strong confusion matrix performance validated model reliability in field environments. Deployment on an NVIDIA GPU server enables rapid inference, making the system suitable for veterinarians, dairy farmers and field workers. This establishes a scalable framework for bovine disease surveillance.

5.2 AI-DISA for Canine Disease Detection Using Hybrid Autoencoders

To extend AI-based services beyond cattle, a comprehensive canine disease detection system has been developed using datasets collected via the **NIBLD (National Image-Based Livestock Diagnostics)** mobile app. This platform ensures standardized, high-quality image capture and metadata recording, supporting disease-wise dataset organization and augmentation.

A novel hybrid modeling strategy was developed:

- **Class-wise supervised autoencoders (AEs)** were trained to capture disease-specific latent features.
- **Six AEs** (five disease-wise + one auxiliary) generated latent embeddings.
- Combined embeddings were used to train a downstream classifier.

This design improves discrimination among visually similar diseases and performs well on small, heterogeneous datasets. The architecture provides a strong foundation for expanding to multi-species veterinary diagnostics.

5.3 PLW: Image-Based Live Weight Estimation of Pigs

The **Pig Live Weight (PLW)** application represents a shift toward non-invasive, stress-free livestock monitoring. A CNN model (Keras + TensorFlow) estimates live weight from 2D images uploaded by the user. Experimental results showed **R^2 values of 0.734-0.801** and **82% testing accuracy**.

Key features:

- Digital herd management
- Individual pig profiles with temporal image history
- Day-wise weight tracking
- Automated readiness for sequential weight prediction

This tool reduces the need for manual weighing, lowering stress on animals and improving on-farm decision making.

5.4 Shria: Smart Heuristic Response-Based Intelligent Assistant

Shria functions as the conversational AI layer, guiding users through disease detection workflows, advisory interpretation and application navigation. Integrated with AI-DISA and PLW, Shria enhances accessibility and usability for farmers, veterinarians and field staff.

6. Overall Contribution and Impact

The integrated work across disease detection, advisory systems, hybrid AI modelling and livestock weight estimation demonstrates a major advancement in **AI-enabled veterinary informatics**. Together, these systems:

- Reduce diagnostic turnaround time
- Improve early detection and treatment effectiveness
- Provide automated lesion localization for precise assessment
- Strengthen multi-species surveillance
- Enable digitized herd management
- Support scalable, real-time monitoring solutions

Collectively, this work contributes to a comprehensive ecosystem for smart veterinary diagnostics and precision livestock farming, aligned with national priorities in Digital Agriculture, One Health, animal welfare and sustainable livestock production.

Oal Presentation

OP-5.1. Evaluation of rumen attributes on gradual supplementation of nitrate along with nitrate reducing bacteria in buffalo for reducing enteric methane

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In the present scenario, the problem of climate change is a major global issue and it is mainly due to the increasing greenhouse gases in the atmosphere. The three major greenhouse gases are carbon dioxide, methane and nitrous oxide. Indian buffaloes alone produce fifty percent of the total milk produced by the buffalo population of the entire world. They also account for around 44% of the country's overall milk output (FAO, 2024). The contribution of buffaloes towards global methane production is substantial of about 11.3% and for India; the figures are even higher of about 40%. Reducing methane emissions will produce a positive environmental impact, increase animal production efficiency and will help to achieve goals of net zero carbon emissions by 2070 as per COP26 summit (2021). To reduce the quantity of methane emission from rumen, strategies adopted include developing genetically less methane-producing animals, dietary manipulations such as feed additives, exogenous enzymes, chemicals, antibiotics/Ionophores, defaunation, plant secondary metabolites, vaccination, vegetable oils and bacteriocins. Recently, the use of terminal electron acceptors like nitrate and sulfate has shown the potential to decrease methane and improve rumen performance at the same time. Nitrate serves as a methane inhibitor by competing for hydrogen and inhibiting methanogens and also provides ammonia to rumen microbial population for their growth. However, the supplementation of nitrate also has its own disadvantages.

It is reduced from nitrate to ammonia through nitrite which is a toxic compound to the host animal when absorbed into the blood. Hence, this study was aimed to evaluate the safe adaptation of rumen to nitrate feeding following a gradual increment in nitrate levels in diet. The experimental feeding (45 days) was done to adapt the animals for the nitrate feeding. The 24 male growing Murrah buffaloes, divided into four groups were fed with concentrate and roughage in the ratio of 50:50. The four groups were control, where basal diet was fed, T1 fed with nitrate @ 2% of DMI, T2 fed with nitrate @ 2% DMI and *S. ruminantium* RM 220 on every alternate week and T3 fed with nitrate @ 2% DMI and *S. ruminantium* RM 220 daily. The nitrate feeding was increased gradually @ 0.25% per week for first four weeks followed by 0.50% per week for last two weeks. It was found that during the adaptation period of nitrate, there was no significant difference in DMI among the groups (varied from 5.23 kg to 6.70 kg/ d).

The mean rumen pH values of control, T1, T2 and T3 were 6.12, 6.21, 6.13 and 6.12, respectively and showed no significant difference among the control and nitrate fed groups at different periods (15, 30 and 45 days post feeding). However, the rumen ammonia N increased significantly ($P<0.05$) with increasing the level of nitrate in the diet as compared to control. The rumen nitrite (ranged 0.0011 to 0.0015 mM/dL) did not show any significant difference among the groups and periods. The TVFAs was similar among the groups, but the molar proportion of acetate increased and that of propionate and butyrate decreased in nitrate supplemented groups ($P<0.001$) than the control group. The branched VFAs, isobutyrate and isovalerate showed no difference among the groups and periods, whereas valerate showed a significant decrease with the increasing period ($P<0.001$). The increase in acetate and decrease in propionate resulted in significant increase in acetate to propionate ratio in the treated groups as compared to control.

The population density of total bacteria, *S. ruminantium*, methanogens and protozoa showed no significant difference among the treatments. The activities of rumen enzymes like carboxymethyl cellulase, xylanase, avicelase, urease and protease were not affected due to feeding of nitrate and nitrate along with *S. ruminantium* in buffaloes. Gradual supplementation of nitrate to buffaloes @ 0.25% per week for first four weeks followed by 0.50% per week for last two weeks did not produce any adverse effect on rumen fermentation in buffaloes.

OP-5.2. Effect of maternal programming through nutritional supplementation on behavioural and productive performance in transition Murrah buffaloes

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Maternal programming refers to the influence of the dam's nutritional and metabolic status during late pregnancy on her own health and productivity after calving, as well as on development and future performance of the calf. The transition period around calving is a vulnerable phase for dairy animals, often marked by negative energy balance (NEB): metabolic disorders and suppressed milk production. This study evaluated the impact of targeted supplementation with a polyherbal-multimineral-multivitamin blend on the behavioural patterns, metabolic status and postpartum performance of Murrah buffaloes. In this study, advanced pregnant Murrah buffaloes were screened based on their blood β- hydroxybutyrate (mmol/L): non-esterified fatty acids (μmol/L) and glucose (mg/dl) levels to identify animals at risk of metabolic stress in pre-partum period. A total of 21 advanced pregnant buffaloes were divided into three groups: a healthy control (T0): a NEB-affected unsupplemented group (T**1** or positive control) and a NEB-affected supplemented group (T**2**). Supplementation in T**2** given 30 days before calving and continued until 15 days postpartum. Behavioural parameters such as feeding behaviour, drinking behaviour, rumination and activity patterns were recorded. The T**1** group showed significant declines in feeding and drinking behaviour, increased lying time and significantly lesser standing time, indicating metabolic stress. However, improved behavioural parameters and significantly higher milk yield were observed in the T**2** group as compared to T**1** and exhibited behaviour similar to that of healthy buffaloes (T0). Supplemented animals also exhibited faster metabolic recovery postpartum and produced significantly higher milk than the other two groups. The results suggest that early and strategic nutritional intervention with polyherbal, vitamin and mineral support can mitigate the adverse effects of negative energy balance.

Keywords: *Murrah, Transition, NEB, Behaviour, Polyherbal, Supplementation, Metabolic stress*

OP-5.3. Machine learning-based milk yield prediction of Murrah buffalo using udder morphometric traits

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Udder morphometric traits have been traditionally used to estimate milk yield in dairy animals using classical methods. However, these traditional methods have several drawbacks. This investigation is to develop an ANN-based model for predicting the average daily milk yield (ADMY) of Murrah buffaloes based on udder morphology and compare it with linear and second-order regression models. A total of 72 ADMY records, along with the corresponding udder measurements, were used in the analysis. The presence of a significant positive correlation between ADMY with udder circumference ($r=0.859$, $p<0.01$) and teat circumference ($r=0.812$, $p<0.01$) indicates potential for ANN model development. The BR, GDX and LM algorithms, along with the TANSIG transfer functions, were used to calculate the ANN. The goodness of fit was assessed using the mean square error (MSE): coefficient of determination (R^2): Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). The ANN model with 16 HLNs trained using the LM algorithm emerges as the best-performing model for predicting ADMY in Murrah buffalo, achieving the highest R^2 (0.9285) and the lowest values of MSE (0.0026): AIC (-156.1169) and BIC (-197.6557) among the configurations considered. Comparison between the three models revealed that the ADMY was estimated more accurately by the ANN model than by linear and second-order non-linear models. ANN may therefore be used to predict ADMY with high accuracy and reliability in place of other regression methods.

Keywords: *Artificial neural networks, MATLAB, Udder measurements, Lactation, Correlation*

OP-5.4. A Novel Herbal Feed Additive as a Strategy to Improve Semen Quality and Freezability in Heat-Stressed Buffalo Bulls

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Buffalo bulls are highly susceptible to heat stress during the summer, which adversely affects semen quality, reduces freezability and increases ejaculate rejection rates—posing a major challenge for producing quality frozen semen doses needed to serve the breedable buffalo population under artificial insemination programs. This study aimed to develop an ameliorative strategy through dietary supplementation of a herbal-based feed additive to improve semen quality in buffalo bulls during hot-humid conditions. Eight Murrah buffalo bulls were divided into two groups: control (n=4): receiving only a basal diet as per ICAR (2013) and treatment (n=4): receiving a standardized dose of herbal powder 'X' for 40 days during peak summer (THI 83-86). Scrotal thermography was performed using an infrared thermal camera and semen and blood samples were collected on day 0 and at 15-day intervals up to 60 days. Herbal supplementation significantly improved the testicular temperature gradient from day 30 onward (3.013±0.31 vs. 1.86±0.04). Initial semen quality traits, including mass motility and sperm concentration, improved significantly from day 30 in the supplemented group. Individual motility, plasma membrane integrity (HOST): acrosome integrity and sperm viability showed significant ($P<0.05$) enhancement at fresh, pre-freeze and post-thaw stages. Antioxidant status in seminal plasma improved markedly, with increased TAC, SOD and GPX, accompanied by reduced lipid peroxidation ($P<0.05$). Functional sperm parameters demonstrated reduced capacitation status, lower mitochondrial ROS production, higher mitochondrial membrane potential (JC-1 HMMP%) and increased live acrosome-intact sperm (FITC-PNA) from day 30 onward. Post-thaw CASA analysis revealed significant improvement in total motility, progressive motility, straightness and curvilinear velocity. Overall, the study establishes a novel herbal-based feed additive as an effective package-of-practice to mitigate heat stress-associated reproductive decline and enhance semen quality in breeding buffalo bulls during the summer season.

Keywords: *Murrah Buffalo, Herbal-based Feed Additive, Semen Quality, Summer Season*

OP-5.5. Molecular analysis of methanogenic archaeal diversity in rumen of Murrah buffaloes fed guar

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Methane production in ruminants contributes significantly to greenhouse gas emissions, making the modulation of rumen microbial communities a key strategy for sustainable livestock production. In this study, rumen fluid samples were collected from eight Murrah buffalo calves maintained at the CIRB herd (treatment group, n=6; supplemented with 500 g boiled *Cyamopsis tetragonoloba* (guar) seeds along with basal concentrate mixture and control group, n=2). DNA extracted from rumen fluid was subjected to 16S rRNA V3-V4 metagenome amplicon sequencing using the Illumina Miseq platform. A total of 461 operational taxonomic units (OTUs) were identified, spanning 32 bacterial phyla. Alpha diversity was assessed using multiple indices of richness and diversity. The Shannon index (3.26±0.03) and InvSimpson index (13.88±0.46) were significantly higher in the treatment group, ($p<0.05$) compared with the control group (3.06±0.04, $p=0.04$; and 11.78±0.20, $p=0.006$, respectively): indicating greater bacterial diversity and community evenness in response to treatment. These results indicated that dietary supplementation enhanced the diversity and evenness of the ruminal bacterial community. Beta diversities showed that bacterial communities were distinct between the treatment and the control groups. The control samples clustered closely together, indicating a greater degree of similarity in their bacterial community structures, whereas the treatment group formed separate clusters, suggesting distinct microbial compositions among them. Furthermore, White's non-parametric test identified 66 OTUs as significantly different ($p \leq 0.05$) between control and test groups, supporting the observed differences in microbial community structure. Beta diversity analysis indicated a marked reduction in methanogenic archaeal taxa, including *Methanobacteriales* (*Methanosphaera*, *Methanobrevibacter*) and *Methanosarcinales* (*Methanimicrococcus*) in the treatment group. In qPCR analysis, the treatment reduced archaeal abundance by approximately 10-fold, while

cellulolytic species such as *R. albus* and *F. succinogenes* remained unaffected, indicating inhibition of methanogens without compromising fiber-degrading microbiota. These findings demonstrate the potential of guar seeds as a low-cost anti-methanogenic feed additive for mitigating methane emissions in buffalo production systems while promoting microbial diversity.

OP-5.6. From Linear Models to Machine Learning: Advancing Infrared Thermography-based Core Temperature Prediction in Buffaloes

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Monitoring core body temperature is essential for evaluating physiological status and detecting health deviations in livestock, yet conventional rectal thermometry remains invasive and labor-intensive. Infrared thermography (IRT) offers a non-invasive alternative, but its predictive accuracy depends heavily on the modelling approach used to interpret thermal data. This study compares a simple, interpretable Multiple Linear Regression (MLR) with modern machine-learning (ML) techniques for predicting rectal temperature in buffaloes using thermal images of the eyes. Two datasets comprising 280 and 471 adult female buffaloes were analysed. Statistical tests showed no significant difference between left and right eye temperatures; therefore, the MLR model used the average ocular temperature along with ambient temperature, relative humidity, to predict rectal temperature, yielding a predictive equation with an R^2 of 0.516 and RMSE of 0.272, confirming moderate predictive capability under controlled conditions. In contrast, ML models—Random Forest, XGBoost, Support Vector Machine, k-Nearest Neighbours and Ridge Regression—were integrated into a stacked ensemble. This approach captured non-linear relationships more effectively, achieving a higher R^2 of 0.62 and RMSE of 0.228. Notably, feature-importance analyses revealed that left eye temperature emerged as the strongest predictor, outperforming the right eye despite their statistical similarity under MLR assumptions. This indicates that ML models can detect subtle, asymmetric thermal patterns not captured by traditional linear approaches. Overall, the comparison highlights that while MLR is simple, interpretable and useful as a baseline, advanced ML techniques provide superior accuracy and greater sensitivity to complex thermal-environmental interactions. These findings support the integration of IRT with modern computational methods to enhance non-invasive temperature monitoring in precision livestock systems.

Keywords: *Buffalo; Core body temperature; Infrared thermography; Machine learning; Precision livestock farming*

OP-5.7. Influence of Palm Stearin-Based Diets on Rumen Microbiology in fistulated Buffaloes

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Palm stearin, a highly saturated fat rich in palmitic acid, is increasingly used in ruminant diets to increase energy density. It has a high melting point which allows more fat to bypass the rumen. This study evaluated the effect of palm stearin, with or without sodium bicarbonate, on rumen microbes in fistulated buffaloes. Three rumen-fistulated adult buffaloes were allotted to a 3×3 Latin square design and fed a control diet (T_0), concentrate along with 8% palm stearin (T_1), and concentrate along with 8% palm stearin + 1% sodium bicarbonate (T_2). Rumen liquor samples were collected at 2 and 4 hours after feeding and analysed using real-time PCR to estimate total bacteria, fungi, and methanogens, while protozoa were counted microscopically. Results showed that total bacteria and fungi were not affected by treatments, showing that palm stearin did not alter the rumen microbial population. However, total methanogens were found to be significantly lower at 2 hours in T_1 and T_2 compared to T_0 ($P<0.05$), indicating that palm stearin, especially when combined with sodium bicarbonate, can help reduce methane-producing microbes. Although differences were not significant at 4 hours, the overall trend remained lower in the supplemented groups. Protozoal counts were also similar and remained unaltered among all the treatment groups. Overall, palm stearin feeding, with or without sodium bicarbonate, helped reduce rumen methanogens without affecting other microbial groups, suggesting it may be a useful approach for lowering rumen methane production.

Keywords: Saturated, Buffaloes, Bicarbonate, Treatments, Rumen

OP-5.8 Evaluation of Herbal Feed Additives and Their Combinations by *In vitro* Gas Production Technique

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This study examined the effects of herbal feed additives and their combinations on total gas production, substrate degradation and efficiency of microbial biomass production. The *in vitro* study was performed with 200 mg air-dried substrates following the method of Menke and Steingass (1988). In this study roughage and concentrate (50:50) were used as substrate. *T. cordifolia, W. somnifera* and *A. racemosus* powder were incorporated to the substrate at 0.5, 1.0, 2.0 and 3.0% levels of DM. Further, the mixture of all the three herbs in all possible combinations were incorporated to the substrate at 1.0 and 2.0% levels of DM. The volume of the total gas produced was measured after 24h. The supplementation of *T. cordifolia*, *W. somnifera*, and *A. racemosus* significantly (P<0.01) increased gas production (mL200 mg^{-1}) relative to CON, except for groups supplemented with 0.5% *T. cordifolia*, and *W. somnifera*, however, the gas production was highest at 2 and 3% levels in *W. somnifera* and at 3% in *A. racemosus*. The gas production in *T. cordifolia* was analogous at 1, 2 and 3% levels of inclusion. TDDMR and TDOMR (mg200mg^{-1}; %) were found to be significantly (P<0.01) higher in all the three herbs supplemented groups at all levels as compared to CON. However, TDDMR and TDOMR were highest at 1% in *T. cordifolia*, 2 & 3% in *W. somnifera* and at 3% level in *A. racemosus*. Similarly, MBP (mg200mg^{-1}) and PF (mg, TDOMRml^{-1} gas volume) were also noticeably (P<0.01) higher in all the three herbs supplemented groups at all levels than CON. The MBP and PF were highest at 1% in *T. cordifolia*, Effect of binary combinations of *T. cordifolia, W. somnifera* and *A. racemosus* on substrate degradation was evaluated and found that gas production (mL200mg^{-1}), TDOMR (mg200mg^{-1}; %), TDDDM (mg200mg^{-1}; %), MBP (mg200mg^{-1}) and PF (mg, TDOMR mL^{-1} gas volume) were significantly (P<0.01) higher in all the combination groups as compared to CON. Moreover, gas production was significantly (P<0.01) higher in *T. cordifolia* and *W. somnifera* combination (2:1) group. The TDOMR and TDDDMR were significantly (P<0.01) higher in *T. cordifolia* and *W. somnifera* combination (2:1) group@1% with all the other treatment groups having statistically similar values. MBP (mg 200 mg^{-1}) and PF were significantly (P<0.01) higher in *T. cordifolia* and *W. somnifera* combination (2:1) group @ 1% and *T. cordifolia* and *W. somnifera* combination (1:2) @ 2% than the other combinations. The influence of tertiary herbal blends on total gas production, TDDMR, TDOMR, MBP, and PF was evaluated and the values depict higher TDDMR (mg 200mg^{-1}; %), TDOMR (mg200mg^{-1}; %), PF (mg, TDOMRmL^{-1} gas volume) in all the treatment groups as compared to CON. Moreover, gas volume, TDDDMR and TDOMR, MBP and PF were significantly (P<0.01) highest in *T. cordifolia, W. somnifera* and *A. racemosus* combination (2:1:1) group @1% and lowest in CON, however, other groups have statistical similar values. It can be deduced that the supplementation of *T. cordifolia, W. somnifera* and *A. racemosus* and their combinations have increased gas production relative to CON. Similarly, TDDMR, TDOMR, MBP and PF were higher in the entire herb supplemented group at all levels and combinations as compared to CON.

Keywords: *A. racemosus, W. somnifera, T. cordifolia, and In-vitro gas production*

OP-5.9. Optimizing Semen Quality through Microclimatic Modification and Nutritional Strategies with Insights into Heat-Stress related Gene Expression in Murrah Buffalo Bulls

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Artificial insemination (AI) is recognized as the most efficient and cost-effective breeding technique in livestock, including buffaloes, resulting in an increasing demand for frozen semen from genetically superior bulls. The present study aimed to assess the influence of microclimatic modification and supplementary feeding on physiological responses, blood biochemical profile, and semen quality of Murrah buffalo bulls during different seasons. The experiment was conducted at the Artificial Breeding Research Centre (ABRC), ICAR–National Dairy Research Institute, Karnal, using 18 mature Murrah bulls (4–8 years) divided into three groups based on semen production performance. Bulls in the control group were maintained under conventional housing conditions, while those in the T_1 group were provided with modified microclimatic conditions using foggers and fans during the hot–dry season, fans during the hot–humid season, and curtains with straw bedding during winter. In the T_2 group, similar microclimatic interventions were applied along with supplementary feeding of flaxseed, betaine, β-carotene, and chromium. Physiological parameters were recorded weekly, and blood samples were collected fortnightly during peak seasonal periods for PBMC separation for mRNA expression of heat stress related genes and evaluating biochemical parameters, while semen was collected twice weekly for quality assessment. The Temperature-Humidity Index (THI) during hot–humid, hot–dry, spring, and winter seasons averaged 81.50±0.20, 77.75±0.44, 64.25±0.68, and 57.22±0.49, respectively. The overall mean for rectal temperature was significantly ($p<0.01$) higher in summer season as compared to spring and winter season while T2 group showed lower temperatures as compared to control group in summer season. Similar trend was observed for respiration rate. significantly higher ($p<0.01$) values for sperm concentration, Mass motility, progressive motility, sperm viability (%), HOST positive (%) spermatozoa and acrosome integrity in fresh semen was observed in spring season followed by winter and lowest during hot dry summer season. Bulls of treatment groups showed decrease in relative expression of heat stress related genes (Na+K+ ATPase, HSF1, HSP70, HSP 60 and HSP 90) across all seasons indicates role of microclimatic modification and supplementary feeding in improving semen quality mainly during higher oxidative stress period.

Poster Presentation

PP-5.1. Production and Reproduction Performance of Mehsana Buffalo at Organized Farm

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The present study pertaining to lactation records of 301 Mehsana buffaloes, spread over a period of 30 years (1991-2020): collected from the records maintained at LRS, SDAU, SK Nagar, Gujarat; to evaluate the production and reproduction performance of Mehsana buffaloes. The data pertaining to performance traits was collected from the records of the farm and analysed to know the effects of non-genetic factors like- period of calving, season of calving, parity and age at first calving group on these performance traits. The overall LSM of 305-DMY, TMY and LL was observed 1714.75±27.30 lit (N=1171): 1765.55±28.61 lit (N=1186) and 273.28±3.66 days (N=1164): respectively. The effects of period ($P \leq 0.05$): season ($P \leq 0.01$) and parity ($P \leq 0.01$) was significant on 305-DMY, but TMY was affected by period, season and AFC group ($P \leq 0.01$). Similarly, LL was significantly ($P \leq 0.05$) influenced by the period and AFC group, but season and parity had highly significant ($P \leq 0.01$) effect on LL. The LSM of AFC were observed 1295.54±11.80 days (N=301): but it was significantly ($P \leq 0.01$) affected by the period of birth. Similarly, LSM for SP was calculated 145.72±4.98 days (N=1038) and it was significantly ($P \leq 0.01$) affected by period, season and parity, but AFC group did not have any effect. The LSM of DP was estimated as 213.69± 9.17 days (N=869): while it was significantly ($P \leq 0.05$) affected by period, but effect of season and parity was highly significant ($P \leq 0.01$). Similarly, LSM of CI was observed 451.97±4.97 days (N=1054): which was significantly ($P \leq 0.01$) affected by the period, season and parity. Therefore, based on these observations it was concluded that significant effect of non-genetic factors play an important role to improve the production and reproduction performance of the Mehsana buffalo herd, therefore emphasis must be given on nutrition, management and health cover practices to improve the performance of the indigenous breed on the farm.

PP-5.2. Assessment of Calving Interval Patterns in Major Indian Buffalo Breeds under Small holder Production Systems

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Calving interval (CI) is a key fertility indicator influencing lifetime productivity and economic returns in buffalo production systems. This study analyzed CI across major Indian buffalo breeds using multi-parity data (Lactation 1–5) from eight breeds: Banni, Chilika, Jaffarabadi, Mehsana, Murrah, Nili Ravi, Non-descriptive buffaloes, and Surti. The dataset comprised 9,124 distinct animals and 12,344 total lactation records. Breed-wise and parity-wise summaries were generated to understand differences in reproductive performance under field conditions. Across all breeds, CI showed substantial variability, with overall mean values ranging from 434 to 507 days. Murrah buffaloes exhibited the highest representation (4,402 distinct animals), followed by Jaffarabadi (1,539) and Non-descriptive buffaloes (1,586). Average CI across lactations was shortest in Chilika (434 days) and Jaffarabadi (438 days), whereas non-descriptive buffaloes consistently showed longer intervals (507 days), indicating management or environmental challenges among non-standardized genetic groups. Parity trends revealed that CI was longest in first lactation across most breeds and generally declined or stabilized in later parities, reflecting improved postpartum recovery in mature buffaloes. Murrah, the dominant breed in semi-organized and smallholder systems, maintained CI between 466–495 days across parities, aligning with known physiological patterns under typical field management. Surti and Nili Ravi buffaloes showed moderate CI values (482 and 459 days overall), indicating relatively efficient fertility considering their smaller population size in the dataset. The mismatch between distinct animal count (9,124) and total records (12,344) highlights repeat calving information, yet preliminary variance analysis from the larger project suggests low repeatability and negligible genetic influence on CI, indicating strong environmental domination of fertility outcomes. Overall, the results underscore significant breed differences and large environmental variability affecting CI in Indian buffaloes. The findings emphasize the need for improved oestrus detection, nutritional management, AI timing, and herd-level reproductive governance to shorten CI and enhance reproductive efficiency across diverse production systems. This dataset provides an important baseline for future genetic and genomic fertility studies in buffalo.

Keywords: Calving Interval, Buffalo Breed, Lactation number.

PP-5.3. Effect of Delignified Paddy Straw Feeding on Nutrient Utilisation and Water Consumption in Buffalo Bull Calves.

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The present experiment was conducted to evaluate the effects of replacing normal paddy straw (NPS) with delignified paddy straw (DPS) on nutrient utilization and soundness of digestion in buffalo bull calves. Eighteen buffalo bull calves in the age range of 153-277 days with an initial body weight ranges from 85 to 140 kg were allocated into three dietary treatments having six animals in each group. The three dietary treatments comprised 1) NPS fed group as control or T_1 group, 2) Mixture of NPS and DPS in 1:1 ratio as T_2 group and 3) DPS fed group or T_3 group. The digestion trial included 14 days adaptation period and 7 days collection period. Besides nutrient digestion, the other observed parameters included water consumption and dung score. The dry matter intake(kg/day) of the T_2 group was significantly ($P<0.05$) better than the control group and differences among other two treatments were non-significant. Digestibility values of OM, CF, NDF, cellulose and hemicellulose were significantly ($P<0.01$) higher in DPS, while the digestibility of CP, EE and ADF were statistically similar in all treatment groups. Buffalo calves receiving delignified paddy straw (DPS) showed increased water consumption. The dung score reduced significantly ($P<0.01$) with increased level of inclusion of DPS, with optimum score of 2.50 in T_2 group which became further poorer significantly ($P<0.01$) in T_3. With these findings, it was concluded that DPS is better digested than NPS. A mixture DPS and NPS in 1:1 ratio is advised for optimal feeding.

Keywords: *delignified paddy straw, buffalo bull calves, dung score, nutrient digestibility.*

PP-5.4. Murrah Buffalo Exhibit Superior Nitrogen Metabolism, Growth Efficiency and Rumen Attributes Over Vrindavani and Tharparkar Cattle

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Present study has been undertaken to study nitrogen metabolism, growth and rumen fermentation parameters in three large bovines on a similar diet comprised of concentrate and roughage in the ratio of 70:30. Buffalo outperformed with respect to majority of parameters than the cattle groups. Growth, nitrogen, rumen fermentation, ruminants. Although both cattle and buffaloes have similarities in terms of utilization of coarse fibrous feed stuffs, non-protein nitrogen as protein source, there exists some differences in physiology (rumen, nutrient metabolism). It has been reported that buffalo are superior in terms of body weight gain and rumen fermentation than the cattle. However, few studies found contrasting results. Thus, it is still not known that how these breeds differ in terms of nutritional physiology. With this background, the present experiment has been undertaken. Male growing Murrah buffalo (Average BW 255.5 kg): Vrindavani (BW 273.5 kg) and Tharparkar (BW 205 kg): 6 each were taken and fed as per ICAR (2013) for 120 days. A metabolism trial was conducted and nitrogen contents of samples were estimated by Kjeldahl method. Rumen liquor was collected at 30th day of trial to analyse metabolites and protozoa. The statistical analyses were performed using SPSS, 2010, Version 20, SPSS Inc., Illinois, USA.

PP-5.5. Effects of Mulberry Leaves Supplements on Growth, Physiological Responses and Hormonal Parameters of Growing Buffalo Calves

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The internal defense system of buffalo calves are not well developed and they are prone have various stress during their various growing phases of life which intern drastacally affects their productive potential. In such situation the supplement of Mulberry leaves (*MorusAlba*) may improve their growth, physiological responses, hormonal profile and immunity. The purpose of the current study was to assesse the effects of Mulberry leaves supplementation on the growth, physiological responses and hormonal profile of growing buffalo growing calves in tropical region of India. The study was carried out on 12 male buffalo calves which were apparent healthy and disease free. They were radomly divided into two equal groups 6 each. Group I (n=6) animal were used as the control while Group II (n=6) were supplemented with *Morus alba* (Mulberry) leaf powder at the rate of 30 g per day per calves. The thermal heat index (THI) was calculated. The solar radiation of microclimate was also taken during the experimet. During the experiment. The body weight of calves were recorded at fortnightly interval. The physiological responses viz. respiration rate RR (breath / min): pulse rate PR (Beat/min) and rectal temperature RT (forenheight) were recorded at 15-day interval. The metabolic hormone (T3, T4) and stress hormone (cortisol) were also estimated at fortnighly interval. The THI was in the range of 82-84 and solar radiation was recrded as 14.7- 16.8 during the experimental period. A significant ($p<0.05$) increase in the body weight of treated calves were observed as compared to control calves. The RR, PR and RT of treated and control calves were with in the physiological limit. The T3, T4 and cortisol levels in the treated calves were at higher side but did not reaches to the level of significance.

Keyword: *Mulberry leaves; Heat stress, Buffalo calves*

PP-5.6. Effect of Dietary Palm Stearin Supplementation on Rumen Fermentation Characteristics in Growing Murrah Buffaloes

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This study evaluated the effects of dietary palm stearin (PS) supplementation on rumen fermentation dynamics in growing Murrah buffaloes. Eighteen calves (<18 months) were randomly assigned to three dietary treatments: a control diet (T0) and concentrate mixtures supplemented with 3% (T1) and 6% (T2) PS. The feeding trial was conducted for 165 days and on the last day of the feeding trial rumen liquor was collected to assess pH, ammonia nitrogen (NH_3-N): total volatile fatty acids (TVFA) and the molar proportions of acetate, propionate and butyrate. Supplementation of PS had no significant effect ($P>0.05$) on any rumen fermentation parameter. Rumen pH and NH_3-N concentrations remained similar across the groups and neither TVFA concentration nor the profile of individual volatile fatty acids, including the acetate-to-propionate ratio, differed among the treatments. These findings indicate that dietary inclusion of palm stearin at 3% or 6% in the concentrate mixture does not alter or modify rumen fermentation processes in growing Murrah buffaloes, demonstrating its suitability as a supplementary fat source without impairing rumen function.

Keywords: *Palm stearin (PS): Murrah buffaloes, rumen fermentation, pH, ammonia nitrogen (NH_3-N): Volatile fatty acids (VFA)*

PP-5.7. Evaluation of Dietary Palm Stearin Supplementation on Haematological Indices and Blood Biochemical Parameters Related to Energy, Protein, Lipid Metabolism and Liver Function in Growing Murrah Buffaloes

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The present study evaluated the effects of dietary palm stearin (PS) supplementation on haematological and blood biochemical parameters in growing Murrah buffaloes. Eighteen calves (<18 months) were assigned to three dietary treatments: a control group (T0) receiving a basal ration and two treatment groups

supplemented with 3% (T1) and 6% (T2) PS in the concentrate mixture. The feeding trial lasted 165 days and blood sampling was conducted on days 0th, 80th and 160th to assess indicators of energy, protein and lipid metabolism and liver function. Haemoglobin concentration, packed cell volume, glucose, triglycerides, total protein, albumin, globulin and the liver enzymes AST and ALT did not differ significantly ($P>0.05$) among the dietary groups. Although HDL and LDL cholesterol levels increased numerically with the inclusion of PS, these changes were not statistically significant. In contrast, total cholesterol concentration (mg/ dl) was significantly higher ($P<0.001$) in the PS-supplemented groups compared to the control. All measured parameters remained within normal physiological ranges throughout the study. The findings indicate that dietary PS at 3% and 6% of the concentrate mixture modulates certain lipid components, particularly total cholesterol, without adversely affecting haematological indices, liver function, or overall metabolic status in growing Murrah buffalo.

Keywords: *Palm Stearin, Murrah Buffaloes, Haematological Indices, Blood Biochemical Parameters*

PP-5.8. Effect of Palm Stearin Supplementation on Performance of Lactating Buffaloes

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The dairy sector in India is undergoing rapid expansion and contributes significantly to the national economy. Enhancing milk production in high-yielding buffaloes requires the provision of energy-dense diets that minimize the risk of metabolic disorders, particularly those associated with high-starch feeding. The present study is conducted at ICAR-IVRI, Izatnagar. This study evaluated the potential of palm stearin (PS) as an alternative energy source in the diets of dairy buffaloes through a two-phase research approach. In the first phase, the nutritional characteristics of concentrate mixtures containing varying levels of PS (0%, 3%, 6% and 9%) from two sources (PS1 and PS2) were assessed using the in vitro gas production technique (IVGPT). Net gas production (ml/200 mg) significantly decreased with PS inclusion compared to the control, while methane production also declined, though without statistical significance. No differences were observed among treatments for in vitro true digestibility (IVTD): in vitro true digestible organic matter (IVTDOM): ammonia nitrogen (NH_3-N): or partition factor. The second phase involved 12 early postpartum buffaloes randomly assigned to a control group or a treatment group receiving a diet supplemented with 3% PS. Both groups received standardized concentrate mixtures along with green Napier grass and wheat straw. Although total milk yield and daily milk yield did not differ significantly, the treatment group demonstrated a statistically significant increase in 6% fat-corrected milk (FCM) and energy-corrected milk (ECM) yields. Milk from PS-fed buffaloes showed increased MUFA content and reduced TSFA, TUFA and PUFA levels. Reproductive performance, particularly conception rate, improved in the treatment group. Overall, inclusion of 3% palm stearin positively influenced milk fat yield, milk fatty acid profile.

Keywords: *Murrah, palm stearin, milk yield, milk fat, methane*

PP-5.9. Impact of Deoiled Soyabean Meal Replacement with Dogs on Milk Composition, Haemato-biochemical Parameter and Economics in Murrah Buffalo

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Although India holds the dominant position in global milk production, its dairy industry continues to encounter significant challenges, particularly the insufficient availability of high-quality, energy- and protein-dense feed resources. Feed constitutes nearly 60-70% of total milk production expenditure. Therefore exploring economical and nutritionally viable alternatives is imperative. The present study is conducted at ICAR-IVRI, Izatnagar. The study examined the potential of dried distillers' grains with solubles (DDGS) as a substitute for de-oiled soybean meal in concentrate mixtures for lactating Murrah buffaloes. Animals were assigned to three dietary treatments: Control (0% DDGS): T1 (25% DDGS) and T2 (50% DDGS). Parameters evaluated included dry matter intake, body weight changes, milk yield and composition, as well as serum biochemical profiles. Treatment groups exhibited a modest increase in average daily milk yield (T1: 6.31±0.38 kg; T2: 6.25±0.25 kg) relative to the control group (5.81±0.28 kg). Notably, milk fat content increased significantly in DDGS- supplemented diets (T1: 7.99±0.08%; T2: 7.92±0.06%) compared to the control (7.39±0.03%). Enhanced serum glucose and triglyceride levels indicated improved energetic status in the DDGS-fed buffaloes. Economic analysis further revealed a reduction in feed costs and milk production cost per kilogram. These findings collectively demonstrate that DDGS is an effective alternative feed ingredient, capable of improving milk fat, total solids and overall profitability

Keywords: *DDGS, milk yield, milk fat, metabolic energy, economics*

PP-5.10. Behavioural and Maternal Responses of Periparturient Murrah Buffalo Heifers to Human-Animal Interaction

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The aim of this study was to investigate the influence of human-animal relationship on behavioural reactivity and maternal behaviour of periparturient advanced pregnant Murrah buffalo heifers. For the trial we have selected 12 animals from the Buffalo herd of ICAR-National Dairy Research Institute, Karnal one month prior to the date of calving and divided them into two groups i.e., control (C) group (n=6) and treatment (T) group (n=6). For C no additional human animal interaction was given to animals except for basic handling and for T additional human-animal interaction was given to animals in the form of visual presence, calling them with their name, patting, stroking the ventral neck followed by brushing for 5 min/day to each animal beginning from the day of start of trial till the day of calving (average duration=150 min of HAI). Both the groups were housed separately but in the same shed. Parameters recorded were avoidance distances score and pre-partum ease of handling score (day 1st,3rd, 7th,14th, 21st and 28th day of start of trial): cortisol (weekly) maternal behaviour and temperament score of the heifers (1st 5 days from the day of calving). Statistical analysis was done using repeated measures general linear model was applied to evaluate the effects of treatment (Group): time (Day/Week) and their interaction (Group × Day/Week) on the outcome variable. Results showed that mean avoidance distance was ($P<0.01$) lower on 21st and 28th day of HAI. Significantly ($P<0.01$) lower prepartum ease of handling score was obtained for T from 14th day however, the consistent decrease was seen up to the 28th day of human-animal interaction indicating that animals being less fearful when handled by the handler. For cortisol the result was found to be non-significant between both the groups. The overall licking of calf by dam (duration and frequency) was higher in T with significant ($P<0.05$) and highly significant ($P<0.01$) on day 1 and day 2 of calving. Higher ($P<0.01$) nursing by the dam (duration and frequency) was in T group on day 1 and 2 of calving. The overall temperament score was lower ($P<0.01$) in T (1.03±0.120) as compared to control group (1.76±0.120; $P<0.01$) on day 1 and 3 of calving. It can be concluded that heifers habituated to prepartum human-animal interaction performed better in terms of lower avoidance distance, pre-partum ease of handling score and maternal behaviour. Therefore, it can be recommended that human-animal interaction one month prior to calving may improve heifers' behaviour with familiar handler and maternal behaviour in periparturient Murrah buffalo heifers.

Keywords: *Behaviour, Maternal, Heifers, Buffalo, Avoidance*

PP-5.11. Effect of supplementing sulphur on *in vitro* and *in vivo* feed fermentation in buffaloes

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Rumen microbes require minerals for their optimum functioning. Sulphur is one of the important mineral and is beneficial in two ways first fulfill the mineral requirement of rumen microbes second it act as an alternate electron acceptor in the rumen as it diverts hydrogen towards the production of hydrogen sulphide thus reducing the availability of substrate for methanogenesis. Sulphur is also stimulatory for the growth of rumen microbes especially rumen fungi and, therefore, helps in improving fibre digestibility. Lakhani *et. al.,* (2019) observed methane suppression using sulphate with additives. Gupta *et al.* (2017) also reported

improved IVTD associated with decreased methane production by inclusion of sulphur along with a feed additive. Therefore, the present study was aimed to observe the effect supplementation of sulphur at graded levels on *in vitro and in vivo* feed fermentation. For this study, four fistulated Murrah male buffaloes (average 550±50 kg) were divided in four groups in 4X4 switch over design. The treatments were, T1 fed with basal diet; T2, T3 and T4 fed basal diet supplemented with sulphur @ 0.25, 0.30 and 0.35% of DMI. The basal diet comprised of wheat straw and concentrate in 60:40 ratio (ICAR, 2013) for maintenance. Each phase lasted for 21 days including last two consecutive two days for rumen liquor collection. The rumen liquor was analysed for VFA, CMCase, avicelase, xylanase and protozoa population. The rumen liquor was also used as inocula for *in vitro* feed fermentation (Menke and Steingass, 1988). After 24 hr incubation, gas and methane production, TVFA and *in vitro* true digestibility (IVTD %) were assessed. Supplementation of sulphur had no impact on TVFA, acetate, propionate, butyrate and acetate to propionate ratio in the rumen liquor indicating that there was no change in the fermentation pattern. On an average rumen liquor pH 6.74±0.16, remained similar to the normal rumen environment. The specific activity of carboxymethyl cellulose, amylase were similar to control, whereas, avicelase was higher ($p<0.05$) in treatment groups indicating favourable for fiber digestion as this is one of the key enzymes for fibre degradation. There was no difference in the *in vitro* gas and methane production. The mean value for *in vitro* gas and methane were 140.43 and 23.9 ml/g DM. The average IVTD was 60.5% and there was no difference in any of the in vitro parameters.

Conclusion

The results of the present study demonstrate that the inclusion of sulphur in the diet did not have any effect on both *in vitro* and *in vivo* the feed fermentation, however, activity of avicelase, a key enzyme of fibre degradation, increased indicating a favourable change for fibre degradation.

Table 1: Effect of sulphur supplementation on rumen microbial enzyme activities

Attributes	T1	T2	T3	T4	P value
DMI (kg/d)	9.04±0.21	8.98±0.31	9.07±0.23	8.99±0.22	0.99
CMC	17.68±1.09	16.42±0.36	16.07±0.95	16.70±1.37	0.716
Amylase	40.11±4.27	38.43±4.55	37.15±3.70	32.48±3.59	0.587
Xylanase	57.09±5.85	55.93±3.78	50.13±2.53	50.20±2.28	0.333
Avicelase	6.61^{a}±0.72	9.17 ^{ab}±0.70	9.82^{ab}±0.71	10.53^{b}±1.45	0.039

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PP-5.12. Image processing and deep learning driven determination of body condition score in buffaloes

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Precision in determination of body condition score (BCS) is critical for managing health, reproduction and productivity in high-yielding Murrah buffaloes. Conventional visual scoring which is widely used by veterinary professionals and researchers is highly subjective and prone to inter-observer variation. This subjectivity can delay the detection of under- or over-conditioned animals, particularly in field conditions where experienced clinicians may not be available. An automated, image-based system can therefore rapidly assess BCS, enabling farmers and para-veterinary workers with minimal training to monitor nutritional status and early signs of metabolic or welfare problems using simple photographs. In this work, a deep learning-based approach for automated BCS classification of Murrah buffaloes into three categories (BCS 3, 4 and 5). A dataset of more than 500 rear view images was acquired under farm conditions and divided into training and validation subsets. The rear area including the tail head, pin bone and hook bone was annotated using Roboflow software. The resulting images were pre-processed (224 × 224 pixels, rescaled) and augmented (rotation, translation, shear, zoom, brightness and horizontal flip) to improve generalization. A pretrained MobileNetV2 network, initialized with ImageNet weights, using a frozen feature extractor, was integrated into a transfer-learning pipeline comprising global average pooling, a 512-neuron ReLU dense layer, batch normalization, dropout (0.5) and a final softmax layer for three-class prediction was used in Python. The model was trained for 50 epochs using the AdamW optimizer and categorical cross-entropy loss, achieving ~98% training and ~94% validation accuracy on the validation set with a training and validation loss of <0.2. The proposed framework demonstrates the potential of lightweight convolutional neural networks to deliver rapid, consistent and user-friendly BCS assessment and for the development of mobile application enabling precision livestock management in buffalo dairy systems.

Keywords: *Convolutional neural network, roboflow, precision livestock, animal health*

PP-5.13. Rumen Fermentation Response to Palm Stearin Supplementation with and without Sodium Bicarbonate in Fistulated Buffaloes

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Palm stearin, a highly saturated fat rich in palmitic acid, is increasingly used in ruminant diets to enhance energy density without depressing fibre digestion. Its high melting point also supports greater rumen bypass, making it a suitable fat source for producing animals. The present study evaluated the effects of palm stearin supplementation on rumen fermentation and volatile fatty acid (VFA) profile of fistulated buffaloes. Three adult rumen-fistulated buffaloes were allotted to three dietary treatments in a 3×3 Latin square design: T1–control diet, T2–concentrate containing 8% palm stearin and T3–concentrate containing 8% palm stearin + 1% sodium bicarbonate. At the end of the feeding period, rumen fluid samples were collected and analysed for key fermentation parameters including pH, ammonia nitrogen (NH_3-N, mg/dL): total volatile fatty acids (mM/DL). Results indicated that palm stearin supplementation had no significant (p>0.05) effect on any of the rumen fermentation parameters across the groups. Rumen pH, ammonia-nitrogen and lactic acid concentrations remained statistically similar (P>0.05) among treatments, indicating that palm stearin did not adversely affect fermentation patterns. Major VFAs (acetate, propionate, butyrate) also remained unchanged by dietary supplementation of palm stearin. Overall, supplementation of 8% palm stearin, with or without 1% sodium bicarbonate, did not impair rumen fermentation or microbial ecology in ruminants, indicating its safe inclusion in concentrate mixtures of producing animals.

Keywords: *Palm stearin, fermentation, ammonia, rumen, ruminants*

PP-5.14. Evaluation of baseline circadian behaviors and daily time budget of loose housed lactating herd of buffaloes

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Evaluation of baseline normal circadian behaviors round-the-year under man-made husbandry conditions serves as references points and provides valuable insights for designing husbandry practices according to animal’s behavioral needs. Furthermore, any deviation from normal behavioral repertoire helps in finding deficiency in management protocols and also helps in estrus and disease detection. The aim of this study was to investigate the circadian behaviours and daily time budgets of loose housed lactating herd of buffaloes maintained at Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal, India. For this purpose feeding, rumination, water drinking and resting behaviors of an average 79 lactating Murrah breed of buffaloes per month (range: 69-117) loose housed in a single group were measured at fortnightly intervals throughout the year using Close-Circuit Television Cameras. Significance of difference in means of experimental variables were analysed using one-way ANOVA using SPSS. Overall mean daytime feeding time and feeding frequency were 399.03±8.11 min and 10.03±0.832 which was decreased (P<0.01) in summer months (May-October: 358.97±5.63 to 340.52±9.77 minutes/day and July-October: 6.35±0.42 to 5.22±1.27 bouts/day). Mean daytime manger visits were 11.08±0.75 which increased (P<0.01) from November (10.79±0.72) to April (13.06±0.64). Mean daytime and night feeding time (minutes/day) was 160.75±6.74 (2.68 hours/day) and 238.27±6.98 (3.97 hours/day) which varied (P<0.05) in summer and winter. Mean rumination time (minutes/day) was 435.43±9.39 (7.25 hours/day) of which 74.21±0.87% was performed in lying and 25.79±6.86% in standing position. Mean water drinking time and frequency were 14.04±4.38 min and 11.52±0.78 which increased (P<0.05) during summer months (23.28±8.14 min and 19.58±1.95). Overall mean daily resting behaviors including standing time, lying time, loafing time, idle

standing and idle lying time were 789.91±9.24, 650.08±10.41, 57.88±4.56,206.38±6.97 and 327.23±6.91 min respectively. The study indicated that resting and rumination was lower than the normal during summers highlighting the need for improving protection from heat stress and comfortable flooring. More than half of feeding was done during nights during summers indicating the need for increasing night feeding of lactating buffaloes.

Keywords: *Buffalo, Feeding, rumination, resting, behaviour*

PP-5.15. Effect of FMD Vaccination on Milk Yield of Murrah Buffaloes

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Foot-and-Mouth Disease (FMD) vaccination is routinely practiced to prevent outbreaks and maintain the health and productivity of dairy herds. However, concerns are commonly raised by farmers regarding possible short-term reductions in milk production following vaccination. The present study was conducted to evaluate the effect of FMD vaccination on milk yield in lactating Murrah Buffaloes. Milk yield data were recorded for 10 days before and after vaccination, including both morning and evening milking sessions. A linear mixed model was applied to analyse repeated measurements, considering cow as a random effect and period (before vs after vaccination): day and milking time (AM/PM) as fixed factors. The estimated marginal means of milk yield before and after vaccination were 5.687±0.083 and 5.516±0.077, respectively. The comparison showed that the difference between periods was not statistically significant ($p= 0.268$). Likewise, AM-PM comparisons and daily post-vaccination trends did not demonstrate significant fluctuations in milk yield. These findings indicate that FMD vaccination does not adversely affect milk yield in dairy buffaloes. These results provide scientific evidence to support routine vaccination programs without concern for short-term milk yield losses.

Keywords: *Foot-and-Mouth Disease (FMD): Milk yield, Murrah buffalo, Vaccination*

PP-5.16. Effect of Varying Roughage Levels on Rumen Fermentation Parameters in Fistulated Buffaloes

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Roughage level in the diet of ruminants plays a crucial role in regulating rumen fermentation, nutrient utilization and overall metabolic function. Altering the roughage-to-concentrate ratio can markedly influence rumen environment, fermentation products and microbial activity. The present study evaluated the effects of two different dietary fibre levels on rumen fermentation parameters in fistulated buffaloes. Two treatment diets were formulated: T1 (60R:40C) and T2 (40R:60C). At the end of the feeding period, rumen fluid samples were collected and analysed for key fermentation parameters, including pH, ammonia-nitrogen (NH_3-N): lactic acid, total short-chain fatty acids (SCFA) and individual volatile fatty acids (acetate, propionate and butyrate). Results indicated that at the lower roughage, higher concentrate diet (T2) significantly reduced rumen pH ($P<0.05$) and increased lactic acid concentration ($P<0.05$): suggesting intensified fermentative activity. Ammonia-nitrogen levels were higher in T2, though not statistically significant ($P>0.05$). Total SCFA and acetate concentrations were significantly lower in T2 ($P<0.05$): while propionate concentration increased markedly ($P<0.05$): indicating a shift towards glucogenic fermentation. Butyrate concentrations remained unaffected by dietary treatments ($P>0.05$). Overall, variation in roughage level significantly altered several rumen fermentation indices, demonstrating that decreased roughage and increased concentrate content promote greater fermentative intensity and modify the acetate-propionate ratio in buffaloes. These findings highlight the importance of optimal roughage-concentrate balance for maintaining stable rumen function in buffaloes.

Keywords: *Roughage, fermentation, VFAs, rumen, buffaloes, concentrate*

PP-5.17. Comparative analysis on the reproductive performance of Murrah Buffaloes under three different rearing systems

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Reproductive performance in Murrah buffaloes is strongly modulated by nutrition, growth rate, management intensity and health inputs. This study compared key reproductive parameters among buffaloes reared under an institutional farm system (n=16): an organized commercial dairy farm at field conditions (n=24) and smallholder buffalo-production systems (n=24). Parameters evaluated include body weight and body condition score (BCS) at puberty, age at puberty, age at first estrus, age at first AI, number of AIs required for conception, age at successful conception and age at calving. Buffaloes reared in the institutional and organized farm systems showed no significant differences across all major reproductive indicators. Both groups exhibited comparable body weight at puberty (312.88±11.84 vs. 306.25±11.19 kg) and BCS at puberty (3.06±0.10 vs. 2.84±0.08): supporting similar ages at puberty (28.13±0.73 vs. 29.13±0.73 months) and first AI (29.25±0.74 vs. 30.13±0.73 months). Conception was achieved efficiently, requiring only 1.38±0.16 to 1.63±0.16 inseminations, resulting in early calving (43.98±0.69 and 44.96±0.69 months). In contrast, buffaloes raised in smallholder systems demonstrated significantly inferior reproductive performance. Lower body weight at puberty (271.88±4.16 kg) and BCS (2.46±0.06) were associated with markedly delayed puberty (36.50±0.76 months): later first AI (40.50±0.76 months): a greater number of inseminations for conception (2.63±0.16) and substantially delayed calving (56.50±0.76 months). Nutritional and health-management discrepancies strongly contributed to these differences. Institutional and organized farms provided 100% and 98% concentrate rations, periodic deworming and mineral-mixture supplementation, whereas 54-58% of smallholder animals were maintained primarily on household residues with no concentrate feeding or periodic deworming or mineral support, impairing growth and delaying reproductive maturation. These findings underscore the critical role of **nutrition, health care and structured management** in enhancing reproductive efficiency and highlight the urgent need to strengthen support programs for smallholder farmers.

Keywords: *Murrah buffalo, Puberty, Rearing systems, Sexual maturity, Reproductive performance, Body condition score.*

PP-5.18. Body Condition Score (BCS) and its Association with Milk Production Traits in Murrah Buffaloes

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Under tropical conditions body condition score (BCS) influences feed intake and metabolic status which can affect economically important production traits in dairy cattle and buffalo. This study examined the role of BCS as a regulator of milk yield and composition in dairy buffaloes. Data were collected from 51 lactating Murrah buffaloes maintained at the Livestock Production and Management Section of IVRI. Animals were scored for BCS on a 1 to 5 scale and were divided into two groups: Group I with BCS ≤ 3.5 (n=18) and Group II with BCS>3.5 (n=33). Milk production traits were obtained from farm records. Total daily milk yield, lactation milk yield, peak yield and days to reach peak did not differ significantly between the two groups. However, group II buffaloes with higher BCS had significantly higher fat percentage (P=0.014) along with higher solids not fat ($P<0.001$) and lactose ($P<0.001$). Correlation analysis indicated strong positive associations of BCS with fat (r=0.343): solids not fat (r=0.670) and lactose (r=0.886). BCS showed weak correlations with production traits while total lactation milk yield and 305-day milk yield were strongly correlated with each other. The findings indicate that although BCS does not markedly influence milk yield under tropical management, it is an important determinant of milk composition. Maintaining higher BCS can therefore contribute to improved milk quality and better economic returns in dairy buffalo enterprises.

Keywords: *dairy buffaloes, milk yield, milk composition, tropical dairy management, lactation performance*

PP-5.19. Reducing the Age at Puberty by Nutritional Interventions in Female Surti Buffalo Heifers

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The present work was conducted on female Surti buffalo calves (n=18) on attainment of six months age under three groups, Treatment-I (n=6) basal green and dry fodder with concentrate having 18% crude protein, Treatment-II (n=6) basal green and dry fodder with concentrate having 22% crude protein and chelated mineral mixture and Treatment-III (n=6) basal green and dry fodder with concentrate having 25% crude protein and chelated mineral mixture, were fed and managed individually from six months onward and concentrate feed was increased every month @ 1% of body weight. Animals were followed for body weight gain at monthly interval up to the age of puberty. All animals were also subjected for blood collection at monthly interval for estimation of the blood glucose by using Accu-Check Integra Kit and blood plasma was separated and stored at -20°C for leptin estimation using ELISA assay kits (KRISHGEN BioSystems, India). The result showed that, animals under treatment groups–II and III, attained more body weight at puberty (251.33±4.81 and 265.50±7.93 kg) and, attained early age at puberty (23.67±0.95 and 20.83±0.48 month): as compared to group–I (250.00±4.28 kg and 27.00±1.32 month): respectively. Treatment group–III significantly ($P<0.05$) advanced age at puberty compared to group–II. Both the treatment groups I and II showed significant ($P<0.05$) reduction in the age at puberty as compared to the group–I. At the age of puberty, the levels of blood glucose was 62.50±0.72, 62.50±0.92 and 63.50±1.12 mg/dl and levels of Leptin hormone was 2.18±0.91, 1.81±0.47 and 1.49±0.33 ng/dl, in treatment group I, II and III, respectively, having non-significant difference between treatment groups. Based on the results it was concluded that the feeding of female Surti buffalo calves with high protein concentrate diet having 25% crude protein and chelated mineral mixture along with basal green and dry fodder, starting from the age of six month onwards, reduces the age at puberty and sexual maturity.

Keywords: *Puberty, Heifers, Protein, Mineral mixture, Leptin*

PP-5.20. Estimation of bovine urine metabolites using paper strips to evaluate health parameters

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Urine testing plays a crucial role in healthcare, as it provides a fast and non-invasive method for detecting and monitoring various health conditions. It helps identify abnormalities, such as early indicators of kidney disorders, urinary tract infections, acid-base balance disturbances, hydration status issues and liver problems. A set of paper-based strips has been developed to enable non-invasive monitoring of animal physiology, offering a cost-effective, portable and user-friendly solution for real-time assessments. Using in-house protocols and specific chemical dye combinations, the nitrite, protein, pH, specific gravity and bilirubin strips were designed to detect a broad range of metabolite levels under laboratory conditions as well as in urine samples. This capability is particularly significant for on-site monitoring, as urine pH is a critical parameter in evaluating the physiological and metabolic health of animals. The strips provide immediate results, reducing the need for complex laboratory equipment and enabling timely interventions in animal care. These test strips work by dipping them into a fresh urine sample, allowing the chemical pads on the strip to react with specific components present in the urine. After a few seconds, each pad changes colour based on the concentration of the corresponding analyte. The colours are then matched with a standard reference chart provided on the strip container, enabling quick and easy interpretation of the results.

Keywords: *Metabolite, paper Strips, Nitrite, pH, Bilirubin, specific gravity, Protein*

PP-5.21. Salivary Metabolomic Profiling of Pregnant and Non-Pregnant Buffaloes using 1H-NMR Spectroscopy

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In livestock management, particularly for water buffaloes (*Bubalus bubalis*): early and accurate diagnosis of pregnancy is pivotal for maintaining reproductive efficiency and economic viability. Metabolomics offers a promising, non-invasive alternative by providing a comprehensive snapshot of the biochemical phenotype. This study aimed to characterize and compare the salivary metabolite profiles of pregnant and

non-pregnant buffaloes using Proton Nuclear Magnetic Resonance (1H-NMR) spectroscopy to identify potential biomarkers associated with gestation. The experiment was conducted on a total of 10 clinically healthy adult female buffaloes maintained under identical husbandry and dietary conditions to minimize environmental variations. The animals were divided into two distinct groups: a control group consisting of non-pregnant animals (n=5) and a test group of pregnant animals (n=5) confirmed via standard veterinary procedures. Saliva samples were collected from all subjects. The samples were processed and analysed using high-resolution 1H-NMR spectroscopy. The resulting chemometric analysis revealed a distinct variations in several key metabolite classes, indicating a significant shift in the salivary metabolome during pregnancy. The PLS-DA model demonstrated high predictive accuracy, suggesting robust metabolic differentiation. These differential metabolites hold promise as non-invasive candidate biomarkers which, upon validation in larger cohorts, could aid in the development of rapid, point-of-care diagnostic tools for pregnancy detection in the buffalo industry.

Keywords: *Bubalus bubalis, Metabolomics, 1H-NMR, Pregnancy diagnosis, Saliva, Biomarkers*

PP-5.22. Evaluation of Serum Interleukin-6 in Cattle Calves Fed Buffalo Colostrum

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Colostrum feeding immediately after birth is necessary for the optimal growth and disease prevention of calves. Buffaloes are significant contributors in dairy industry and many a times raising of cattle along with buffalo is seen. Twelve Vrindavani calves were fed dam's and Murrah buffalo colostrum for three days and cow milk for 60 days by following the standard feeding schedule practised at Cattle and Buffalo Farm, ICAR-IVRI. The IL-6 in serum was determined using Bovine IL-6 (Interleukin 6) ELISA Kit; Cat. No. EB0028; Finetest, Wuhan) and blood samples were collected on day 3 and day 7 for analysis. The serum cytokine concentrations including IL-6 are influenced by colostrum cytokines which help new-born calves adapt to the environment and the development of their immune system. It was evident that there was no difference in the concentration of IL-6 observed in the serum of calves in the control and treatment group respectively. Moreover, there was no difference in morbidity of calves of both groups, suggesting that buffalo colostrum possesses almost similar biological capability to maintain the health of cattle calves. Altogether, dam's colostrum is best for their calf but in case of unavailability or deficit of colostrum; buffalo colostrum may be a good alternative for the survival and sound health of cattle calf.

PP-5.23. Ultrasonographic Evaluation of Rib Eye Area and Back Fat Thickness in Murrah Buffalo Calves Supplemented with Palm Stearin

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Buffalo meat has gained increasing significance in India due to its export potential and cultural acceptance. However, the major challenge in buffalo development is the availability of feed and the lack of awareness among farmers about the benefits of palm oil by-products. Plant oils and oil seeds are the primary sources of supplemental fatty acids in ruminant diets and when processed at a controlled temperature (fractionated fat): they become a superior and more affordable fat source. Palm stearin (PS): the solid fraction of palm oil obtained by partial crystallization, is one such valuable by-product. A feeding trial was conducted to evaluate the influence of palm stearin supplementation rib eye area (REA) and back fat thickness (BFT) using ultrasonography in live animals. In this context, ultrasonography provides a rapid, non-invasive approach for assessing carcass traits in live animals, particularly REA and BFT, which indicate carcass yield and finish, respectively. Twelve Murrah buffalo calves between the age of 10-14 months were separated in two groups: a control group (basal diet) and a treatment group receiving a baseline diet with a 3% inclusion of palm stearin for 120 days. REA and BFT measured using ultrasonography were significantly higher ($P<0.05$) in palm stearin-supplemented calves, indicating improved muscle development and subcutaneous fat deposition. Overall, palm stearin supplementation enhanced economically important carcass attribute and ultrasound measurement of REA and BFT thickness can be utilised to estimate carcass.

PP-2.24 Assessment of Phyto-chemicals and Free Radical Scavenging Capacity of *T. cordifolia*, *W. somnifera* and *A. racemosus*

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The study was conducted to assess the total flavonoid, total phenolics and free radicle scavenging capacity herbal feed additives. Total phenolic content present in the herbs was estimated by spectrophotometry by using Folin-Ciocalteute technique. Total flavonoid content in the herbs was estimated by $AlCl_3$ method with slight modifications. The antioxidant capacity of *T. cordifolia*, *W. somnifera* and *A.racemosus* was assessed by DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radical scavenging assay. Total phenolic content of *T. cordifolia*, *W. somnifera* and *A. racemosus* were 7.3, 5.0 and 3.4 (mg of TAEg^{-1} of extract), respectively. The Total phenolic contents were significantly ($P<0.01$) higher in *T. cordifolia* followed by *W. somnifera* and *A. racemosus*, respectively. Total flavonoid content of *T. cordifolia*, *W. somnifera* and *A. racemosus* were 3.8, 2.2 and 1.4 (mg of QEg^{-1}extract), respectively. The total flavonoid values were significantly ($P<0.01$) higher in *T. cordifolia* followed by *W. somnifera* and *A. racemosus*, respectively. The DPPH inhibition percentage of different herbal feed additives were significantly ($P<0.001$) different at different concentrations. The IC$_{50}$ value of *T. cordifolia*, *W. somnifera*, *A. racemosus* and ascorbic acid (standard) were 47.3, 57.6, 61.2 and 16.1 (µgmL^{-1}), respectively. The IC$_{50}$ values of *T. cordifolia* was significantly ($P<0.001$) lower followed by *W. somnifera* than *A. racemosus*, respectively. The percentage inhibition of DPPH free radical activities at different concentrations of *T. cordifolia* was significantly ($P<0.001$) lower followed by *W. somnifera* and *A. racemosus*, respectively. It may be concluded that *T. cordifolia* exhibited higher flavonoid and phenolic contents as compared to *W. somnifera* and *A. racemosus*. Additionally, the percentage inhibition of DPPH free radical activity was lower in *T. cordifolia* followed by *W. somnifera* and *A. racemosus*.

Keywords: *A. racemosus, phytochemicals, W. somnifera, T. cordifolia*

Session-VI

Production Technology & Extension Practices

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LP.-6.1. Buffalo Meat Production in India: Opportunities and Challenges in a Changing Climate

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Introduction

India's livestock economy has experienced substantial transformation over the past decade. Between 2014-15 and 2023-24, the sector's gross value added (GVA) expanded by almost 195% and livestock now accounts for roughly 31% of the agricultural GVA and 5.5% of the national GVA (DAHD, 2025). In livestock sector, buffalo meat represents a particularly important component of the value chain, reflecting both its economic weight and its centrality to India's bovine meat sector. India has one of the world's largest buffalo populations (109.85 million head) as per the 20th Livestock Census 2019, up from roughly 43 million in 1951reflecting sustained long-term growth in the buffalo sector (DAHD, 2024).Buffalo accounts for around 18% of national meat output, compared with about 3-4% for cattle, with poultry contributing roughly half of total meat production. In export context, buffalo meat ("carabeef") has long been the backbone of India's animal-product exports: APEDA's Red Meat Manual reports that in 2018-19, India was the world's largest exporter of buffalo meat, shipping 1.24 million tonnes valued at US$3.61 billion (Bajaj & Ranjhan, 2020). India currently ranks around 10thamong global buffalo/beef exporters, yet buffalo meat still dominates its animal-product export basket and generated about US$3.74 billion in 2023-24, with major destinations in Southeast Asia, West Asia and North Africa (APEDA, 2024). As per recent report of DAHD (2024),buffalo meat contributed over 82% of India's total "animal products" export value in 2023-24, depicting its economic importance.

Beyond its role in export earnings, buffalo meat is closely linked to India's broader goals of *Viksit Bharat 2047*ensuring protein availability and sustaining rural livelihoods. Animal husbandry as a whole is a critical income source for small and marginal farmers; policy assessments suggest that around 70% of national livestock holdings are in the hands of smallholders and landless households, who rely on animals for regular cash flow, draught power and risk buffering (NAAS, 2016). Buffalo in particular are appraised for their multi-purpose role in milk, meat and work and have been described as "indispensable" to the Indian rural economy because they can thrive on crop residues and low-quality forages while producing high-value outputs (El Debaky *et al.*, 2019). From a macro perspective, the surge in bovine meat exports (dominated by buffalo) is one of the contributors to the livestock sector's growing share in agricultural GVA, thereby reinforcing feedbacks between export performance, on-farm investment and rural employment (DAHD, 2025).

However, these gains are emerging in an era where climate change is increasingly recognised as a risk multiplier for livestock systems. The IPCC Sixth Assessment Report projects a marked increase in the frequency and intensity of extreme heat events in South Asia, with large parts of the subcontinent expected to experience dangerous heat-stress conditions under mid- to high-emission pathways (Shaw *et al.*, 2025). Indian assessments echo these concerns: national reviews on climate-resilient livestock production classify India as one of the most climate-vulnerable regions, emphasising that smallholder-dominated livestock systems face heightened exposure to heat waves, erratic rainfall, water scarcity and emerging diseases (NAAS, 2016; Pankaj *et al.*, 2021). Empirical studies show that increases in the number of days with temperature-humidity index (THI) above critical thresholds are already depressing milk yields and reproductive performance in cattle and buffalo and are projected to cause additional losses in the coming decades (Kaur *et al.*, 2019; Manjunath *et al.*, 2024).

Household-level vulnerability analyses from coastal and semi-arid regions reveal that a substantial share of livestock rearers fall into medium or high climate-vulnerability categories, with clear implications for income stability and food security (Devi et al, 2021; Maiti *et al.*, 2014). In this context, buffalo offer an important case for understanding how large ruminants respond to a warming climate. They possess several adaptive features—such as the ability to make efficient use of fibrous, low-quality feeds and behavioural traits like wallowing—that help them cope with hot, humid environments and marginal lands (Chiariotti *et al.*, 2025; Yáñez-Pizaña *et al.*, 2020). At the same time, their dark skin, limited hair cover and relatively poor sweating capacity make them vulnerable to heat when shade or adequate water is unavailable. These physiological constraints make it essential to provide suitable microclimatic conditions and management support if buffalo productivity is to be maintained under increasing thermal stress (Ximenes *et al.*, 2024; Yadav *et al.*, 2016).

In India, buffalo becomes more important than cattle in view of legal and religious context. Anti-cattle-slaughter legislation is in force in most Indian states, often imposing complete bans on cow slaughter and stringent restrictions on bulls and bullocks, whereas buffalo slaughter is generally permitted under specified conditions. As a result, nearly all of India's formal bovine meat sector is based on buffalo and "beef" exports from India in international statistics are in practice exports of processed buffalo meat (APEDA 2024).This chapter therefore reviews the current production systems and the opportunities and challenges that buffalo meat faces in a changing climate, linking production trends with emerging climate risks to outline possible routes for resilient and inclusive growth in buffalo sector.

2. Buffalo Meat Sector in India: Current Status and Production Systems

2.1 *Buffalo Population Dynamics and Geographical Distribution:* USDA projections indicate that India's beef output—largely represented by carabeef—is expected to reach about 4.64 million metric tonnes (carcass weight equivalent) in 2025, a modest increase of around 2% over 2024 levels (Baloni& Agarwal, 2025). The pattern of buffalo meat production in India is closely linked to how buffalo populations are distributed across different agro-ecological zones. National census figures provide an overall picture, but district-level data from the Department of Animal Husbandry and Dairying (DAHD) show clear regional concentrations. Buffaloes are not spread uniformly throughout the country; rather, they tend to cluster in well-irrigated regions with better fodder availability, strong dairy activity and easier access to markets (DAHD, 2023). These regional trends are consistent with breed distribution maps published by ICAR-NBAGR (2025): which indicate that major riverine breeds such as Murrah, Mehsana, Jaffarabadi and Bhadawari are predominantly found in areas with established dairy systems and reliable feed resources. Pundir *et al.* (2022) further validate this spatial relationship by demonstrating that the principal breeding tracts of these buffalo breeds coincide with districts that supply a substantial proportion of animals entering regulated meat procurement chains. The co-location of improved breeds, irrigated crop systems and peri-urban markets thus provides the demographic foundation for modern buffalo meat production.

2.2 *Structure of Buffalo Meat Production:* Buffalo meat production in India operates predominantly within a mixed-purpose, smallholder-based system in which animals are reared primarily for milk, draught power and manure, with meat production functioning largely as a secondary or terminal-output component. India's buffalo meat production system remains deeply embedded within the dairy economy, where smallholder farmers raise animals primarily for milk and channel surplus male calves and culled females into slaughter markets. In general, surplus male calves and culled adult females constitute the major share of animals entering slaughter channels, reflecting the predominance of dairy-linked by-product system rather than specialised meat-herd development (Kumar and Singh, 2010; Jairath *et al.* 2025) noting that the flow of slaughter animals depends more on dairy herd dynamics than on specialised meat breeding. Further, carcass quality and dressing percentages vary substantially depending on whether animals originate from culling or are finished under controlled feeding (Muthukumar *et al.*, 2018). Meat-quality studies such as those by Naveena *et al.* (2014) confirm that carcass composition, muscle traits and dressing yields vary depending on age, physiological status and production background at the time of slaughter, with culled dairy females differing significantly from younger animals raised under more uniform management. Value-chain assessments, including Bardhan (2019): highlight that buffalo meat supply in India continues to be shaped by the informal nature of farm-level sales, limited organised finishing capacity and the predominance of smallholder households with restricted access to balanced feed resources.

2.3. *Slaughterhouse Infrastructure and Processing Capacity:* India's slaughterhouse infrastructure operates through a dual system: a highly regulated export segment and a large, uneven domestic network. According to the APEDA Integrated Abattoir-cum-Meat Processing Plant List (11 June 2024): the export sector consists of 72 approved integrated abattoir-cum-meat-processing plants, authorised to produce chilled and frozen boneless buffalo meat and buffalo offals (APEDA, 2024a). These plants, concentrated in Uttar Pradesh, Maharashtra, Telangana, Punjab and Haryana, function under strict requirements that include segregated slaughter lines, dedicated ante-mortem and post-mortem inspection areas, hygienic carcass-handling systems and temperature-controlled deboning rooms. Export-compliant operations also require potable water systems, stainless-steel working surfaces and a continuous cold chain, from slaughter to container loading The prescribed process flow begins with animal receiving and lairage, followed by washing, humane slaughter, bleeding, hide removal and evisceration, under veterinary supervision. Carcass washing, proper drainage, waste-segregation and sanitation infrastructure are mandatory to reduce contamination risks. Rapid carcass chilling is designated a Critical Control Point (CCP) because of its importance in controlling microbial proliferation and preserving meat quality (Bajaj &Ranjhan, 2020). As per recent publication of Kumar et al (2024): these export-oriented plants also operate cold storage or blast freezers, maintain in-house quality control laboratories and have designated areas for packaging and dispatch, all of which help maintain traceability and hygienic handling. In contrast, the domestic slaughter sector looks very different. India has roughly 8,000 registered slaughterhouses and more than 25,000 unregistered slaughter points serving local markets and many of these facilities lack even basic infrastructure such as proper flooring, ventilation, effluent treatment systems, overhead rails, or reliable electricity. In several areas, slaughter still takes place on open ground or on unlined floors, conditions that increase the likelihood of contamination from dung, soil and unhygienic handling. Inconsistent ante-mortem and post-mortem inspection practices further compromise meat safety (Kumar *et al.*, 2024).This contrast between modern export units and inadequately equipped domestic slaughterhouses emphasizes the need for systematic upgrading. Improvements in sanitation, cold-chain development, waste management and regulatory compliance remain central to strengthening India's overall meat safety and processing capacity.

3. Climate Change and Buffalo Production: Opportunities

3.1 ***Buffalo as a climate-resilient large ruminant resource:*** Although climate change is usually framed in terms of risks to livestock, it also creates a strategic opportunity to reposition buffalo as a key climate-resilient species in tropical and subtropical production systems. Compared with cattle, water buffaloes show a strong capacity to utilise fibrous forages and agricultural by-products, combined with longevity, rusticity and the ability to perform in both hot-humid and relatively cool environments (Chiariotti *et al.*, 2025). A synthesis of buffalo adaptability notes that they outperform cattle in converting low-nutrient roughages into milk and meat and can be maintained on crop residues and low-quality fodder with relatively modest external inputs, which is particularly attractive under conditions of feed scarcity and rising feed costs (Chiariotti *et al.*, 2025; **ICAR-CCARI, 2025).** In India, ICAR-CCARI emphasises that buffaloes are more efficient converters of low-quality feeds, thrive under harsh climatic conditions and already contribute the majority of national bovine milk, underlining their centrality to mixed crop-livestock systems in the Indo-Gangetic and arid-semi-arid regions (**ICAR-CCARI, 2025).** From an adaptation perspective, these traits offer policy space to gradually shift part of the demand for bovine meat and milk towards buffalo-based supply chains that are better suited to hot, humid and variable climates, especially in irrigated belts and canal command areas where water for drinking and wallowing can be integrated into farm design. Recent physiological work on global warming in buffalo farming describes morpho-anatomical features such as thick, highly vascularised skin and relatively large sweat glands that support sensible heat loss, while behavioural adaptations like wallowing and shade-seeking enable animals to dissipate heat loads when ambient temperatures approach or exceed 40-45 °C (Napolitano *et al.*, 2023; Garcia *et al.*, 2022). These studies suggest that, provided animals have access to water bodies, sprinklers or wallows, buffalo can maintain thermoregulation and productive performance under thermal environments that would severely constrain Bos taurus dairy and beef herds. At the same time, their ability to work in flooded rice fields and wetlands—where mechanisation is often difficult—creates an opportunity to integrate buffalo-based traction, manure recycling and meat/milk production into climate-smart landscape management in low-lying and flood-prone regions (Chiariotti *et al.*, 2025). For India, where climate-resilient livestock roadmaps already prioritise species and systems that can cope with heat stress, feed volatility and water constraints, buffalo production can thus be positioned as a core pillar of adaptation strategies that seek to stabilise protein supply and smallholder incomes under warming climates (Sahoo *et al.*, 2024).

3.2. ***Climate-smart feed resources, crop residues and circular bioeconomy:*** Climate change magnifies the pressure on India's already tight feed and fodder balance, but buffalo production offers an avenue to convert large volumes of crop residues and agro-industrial by-products into high-value animal protein, thereby linking adaptation with waste minimisation. National feed-fodder assessments project persistent deficits in both dry and green fodder and underline that crop residues such as cereal straws and stovers constitute a dominant share of ruminant diets in India (Patil *et al.*, 2021). Buffaloes are particularly suited to these conditions: they can efficiently utilise coarse crop residues and tolerate fluctuations in forage quality, allowing farmers to rely more heavily on residues from rice, wheat, sorghum and oilseed crops without excessive concentrate use (ICAR-CCARI, 2025).This biological advantage aligns closely with emerging climate-smart feeding strategies that seek to densify and balance residue-based rations. The global enteric methane emissions from ruminants and reported that buffaloes contribute about 10.5% of the total ruminant enteric CH_4 pool; yet, they emphasise that mitigation at the animal and herd level—through better feeding, health and herd structure—can substantially lower emissions intensity, especially in South Asian buffalo systems where productivity gaps remain large (Prathap *et al.*, 2021). Complementary interventions such as feed additives (e.g. monensin) have also been shown to decrease methane and nitrous oxide emissions from Murrah buffaloes under controlled conditions, although their broader on-farm applicability and regulatory acceptance require careful evaluation (Gupta *et al.*, 2018; monensin trial in Murrah buffaloes). Further, crop-residue-based complete feeds additionally point out that stabilised rumen fermentation and improved digestibility can lower methane yield per unit of digested organic matter, suggesting that feed-processing innovations can deliver both economic and climate benefits (Patil *et al.*, 2023). When combined with improvements in reproductive efficiency, disease control and reduction of unproductive animals in the herd, as advocated in climate-resilient livestock strategies in India, these measures create an opportunity to expand buffalo meat production while keeping the carbon footprint per kilogram of carcass within acceptable bounds and, in some systems, potentially lower than that of low-productivity cattle herds (Sahoo *et al.*, 2024).

3.5. Lower Emissions: Emerging evidence suggests that buffalo production systems may present certain environmental advantages that can be strategically leveraged to position buffalo meat as a climate-compatible protein source. Controlled comparative studies indicate that buffaloes produce **lower absolute enteric methane emissions per animal per day** than cattle under identical feeding conditions, approximately **93 g/day in buffaloes versus 141 g/day in cattle,** reflecting nearly a one-third reduction in per-head emissions (Malik *et al.*, 2021). This lower methane value is largely explained by differences in feed intake and body size between the two species rather than any inherent advantage in methane yield per unit of feed. Even so, the per-animal reduction can matter in regions where smallholder buffalo systems dominate. Farmers also note that buffaloes cope better with climatic stress, particularly in terms of heat tolerance, water-use patterns and their ability to adjust to shifts in forage quality during dry or erratic seasons (Escarcha *et al.*, 2018). These traits help maintain production under warming conditions.

3.6. Nutritive Value of Buffalo Meat: Several inherent qualities make buffalo meat an important prospect within India's red-meat sector. A major advantage is its acceptance in many international markets, where it does not face the religious limitations that often restrict the consumption of cattle beef. Research comparing carcass traits shows that buffaloes produce a higher proportion of edible muscle and relatively less bone and fat than cattle, resulting in a leaner product and better dressing percentages. From a nutritional standpoint, buffalo meat is low in fat and cholesterol and contains a high proportion of lean tissue and protein, features that contribute to its recognition as a healthier red-meat choice. Although similar to beef in flavour and texture, it usually contains fewer calories and offers a more favourable lipid profile (Jerome *et al.*, 2022). Meat from young buffaloes has also been reported to possess an advantageous (n-6):(n-3) fatty acid ratio of about 7.0, better than that of bovine calves and adult buffaloes (Dimov *et al.*, 2012). Its total lipid content is among the lowest recorded in red-meat species (around 1.37 g/100 g) and conjugated linoleic acid levels are higher than those found in zebu cattle (de Mendoza *et al.*, 2005). Buffalo meat also presents attractive visual characteristics; its colour is often more uniform and brighter than beef, influenced by myoglobin levels that vary with age and muscle location (Naveena and Kiran, 2014). The species' generally calm temperament further supports the production of tender meat under typical handling and slaughter conditions (Jerome *et al.*, 2022). Together, these nutritional, sensory and behavioural traits provide strong grounds for promoting buffalo meat as a premium, health-focused red-meat option in both domestic and export markets.

3.7. Export Performance and Global Demand for Indian Buffalo Meat: The continued strength of international markets for Indian buffalo meat has become an important source of stability for the livestock sector, especially as climate conditions grow more variable. Despite concerns about heat stress, water scarcity and feed fluctuations, the export segment has remained steady. Official data show that India exported about 1.295 million tonnes of buffalo meat in 2023-24, generating USD 3,740.53 million in foreign exchange earnings (APEDA, 2024). These volumes have been maintained by long-standing demand in countries such as Vietnam, Malaysia, Egypt, Iraq and the United Arab Emirates, which have continued to import Indian buffalo meat even during periods of global uncertainty (APEDA, 2024). Studies on trade competitiveness point out that India retains an advantage in world markets because of its relatively low production costs, dependable supply chains and the lean nutritional profile of the meat (APEDA, 2025; Kaur *et al.*, 2021). The significance of this trade is strengthened by India's large buffalo population and the species' ability to cope with hot, humid climates when adequate water is available. These characteristics help explain India's sustained position as a major supplier of buffalo meat, a trend reflected in comparative analyses of production and global trade flows (Di Stasio and Brugiapaglia, 2021; Kaur *et al.*, 2021). The export sector also benefits from an established APEDA-regulated processing network with hygienic slaughter facilities, temperature-controlled deboning units and reliable cold storage systems, which are essential for maintaining product quality in warmer conditions (APEDA, 2024; 2024a). As climatic pressures increase, there is growing interest in expanding value-added products, including chilled and frozen cuts, deboned portions, halal-certified preparations and ready-to-cook items. Together, these developments position buffalo meat as an important contributor to foreign exchange earnings and as a sector with strong potential for climate-resilient growth within the broader livestock economy.

3.8. Socio-Economic Resilience: Buffalo husbandry offers important socio-economic advantages in regions facing increasing climate variability, particularly within mixed crop-livestock systems where household incomes are becoming more unstable due to irregular rainfall, rising temperatures and repeated crop losses. Evidence from South and Southeast Asia indicates that buffaloes often maintain steadier milk yields under heat stress and fluctuating forage conditions, allowing families to rely on a more predictable income stream when crop revenues decline. Their feeding pattern, which depends mainly on crop residues, stovers and agro-industrial by-products rather than expensive compound feeds, also reduces the financial pressure on farmers during years of fodder scarcity or price spikes. In many rural settings, buffaloes serve as a form of household capital; the sale of animals during droughts or other climatic shocks helps families absorb economic stress without resorting to migration or selling essential assets. Women play a central role in buffalo-based dairying and small-scale meat enterprises and their daily involvement in feeding, milking and manure management provides access to small but steady earnings. Taken together, these characteristics make buffalo production an important source of livelihood resilience in a warming climate.

4. Challenges

Climate change presents a set of interconnected challenges for buffalo meat production that are widely recognised in recent research. Prolonged periods of high temperature place buffaloes under acute heat stress, which in turn lowers feed intake, slows weight gain and reduces carcass yield, effects that are particularly evident in males raised for meat (Garcia *et al.*, 2022; Napolitano *et al.*, 2023). These physiological pressures coincide with shifts in feed availability, as cereal residues and stovers—the main roughages used for buffalo fattening—tend to show higher fibre content and poorer digestibility under warmer and drier conditions, leading to less efficient feed conversion and lower finishing weights (Patil *et al.*, 2021; Sahoo *et al.*, 2024). Disease risks also increase with climatic fluctuations; higher humidity and rainfall variability have been linked to greater incidence of haemorrhagic septicaemia, lumpy skin disease and parasitic infections, all of which undermine growth and raise the likelihood of carcass rejection (Escarcha *et al.*, 2018). Elevated ambient temperatures further affect the processing environment by accelerating microbial spoilage and placing additional pressure on already stretched cold-chain systems, with implications for both meat safety and export standards (APEDA, 2024; Di Stasio & Brugiapaglia, 2021). As growth slows and animals require longer finishing periods under heat or feed stress, greenhouse-gas emissions per unit of meat produced rise, creating concerns about the future emissions intensity of buffalo meat systems under continuing climate change (Prathap *et al.*, 2021; Malik *et al.*, 2021).

5. Conclusion

Buffalo meat production plays a vital role in India's livestock economy, supporting agricultural output, export earnings and the livelihoods of millions of smallholders. Its strength lies in buffalo's population and its ability to utilise crop residues and other low-quality roughages. Further, the presence of a well-established export-oriented processing system makes it's a economic hub. In climate change scenario, buffaloes' capacity to maintain performance under hot and humid conditions when provided adequate water, their suitability for crop-by-product-based feeding systems and their lean meat profile collectively position them as a comparatively climate-resilient source of animal protein. At the same time, rising temperatures, declining feed and fodder availability and increasing pressure on slaughterhouse hygiene and cold-chain management present significant challenges. Strengthening feed resources, improving thermoregulation and water access, enhancing disease surveillance and upgrading domestic slaughter infrastructure will be the way-forward. With targeted interventions and climate-smart management, India can sustain and expand buffalo meat production while safeguarding livelihoods and maintaining export competitiveness.

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LP. 6.2. Revisiting Extension Approaches in the 21st Century

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ABSTRACT

Agricultural Extension and Advisory Services (AEAS) are undergoing a profound transformation as global food systems confront climate variability, digital disruption, market volatility and the rising demand for sustainable production. Traditional top-down, Technology of Transfer (ToT) models such as the Training and Visit (T&V) system proved effective for uniform Green Revolution technologies but failed to address the growing complexity, diversity and risk-prone environments of smallholder agriculture. This paper revisits extension approaches for the 21st century, highlighting the urgent need for a shift toward demand-driven, participatory, climate-responsive and digitally empowered systems. It critically examines climate-smart extension, ICT-enabled advisory services, community-based and pluralistic models and gender- and youth-responsive strategies. The paper underscores the emergence of digital tools, AI-powered platforms, value chain integration and public-private partnerships as key drivers of modern AEAS. Central to this transformation is the evolving role of the extension agent from a mere technology disseminator to a facilitator, innovation broker, network weaver and risk manager equipped with advanced technical, digital and entrepreneurial competencies. The article concludes that resilient, inclusive and future-ready extension systems depend on institutional pluralism, strengthened human capacity, continuous professional development and the integration of climate adaptation and digital innovation, positioning AEAS as a critical engine for sustainable and equitable agricultural growth.

Introduction

The global agricultural landscape is facing unprecedented complexity, marked by the concurrent pressures of climate change, rapid population growth, resource depletion and a transformative digital revolution (FAO, 2021). Feeding an estimated 10 billion people by 2050 while simultaneously safeguarding environmental sustainability requires a systemic overhaul of how knowledge, technology and innovation are managed and shared within food systems. Central to this challenge is the role of Agricultural Extension and Advisory Services (AEAS).

Traditionally conceived as the crucial link between agricultural research and farmers, AEAS has historically operated on a Technology of Transfer (ToT) model, exemplified by the centralized, top-down approaches of the mid-to-late 20th century (Rivera and Qamar, 2003). While models like Training and Visit (T&V) were instrumental in disseminating standardized Green Revolution technologies and boosting production, they proved structurally rigid and ill-equipped to handle site-specific, complex problems, ultimately leading to concerns over sustainability and high recurrent costs (Birner *et al.,* 2009).

The 21st century demands a radical paradigm shift. Farmers today require more than just technical input packages; they need access to timely market information, adaptive climate risk management strategies, financial services and complex knowledge for navigating diverse and fragmented value chains (World Bank, 2017). The focus must move away from simply diffusing a predetermined technology "supply" towards facilitating a continuous, demand-driven process of innovation, knowledge co-creation and systems learning (Garforth *et al.*, 2018).

This article revisits the foundational principles and operational mechanisms of extension, arguing that its relevance and effectiveness in the modern era hinge upon three critical transformations: digital enablement for scale and precision, robust integration with climate adaptation strategies and institutional pluralism for diversified service delivery. We will critically examine the shift from a conventional ToT model to one centered on Facilitation, Innovation Brokerage and Knowledge Management. By exploring these dynamics, this paper aims to define the contours of an effective, resilient and equitable extension system that can support sustainable global food security in an era of profound change.

The Traditional Extension Model: A Critical Review

The conventional approach to Agricultural Extension and Advisory Services (AEAS) is rooted in the Transfer of Technology (ToT) paradigm, which became dominant globally following the successes of the Green Revolution in the mid-20th century. This model views agricultural development as a linear process, flowing from research institutions to extension agents and finally to the farmer (Rivera and Qamar, 2003).

The Linear Model: Training and Visit (T&V)

The most prominent example of the ToT approach is the Training and Visit (T&V) system, widely promoted by the World Bank between the 1970s and 1990s(Tiwari and Singh, 2005). Its core features were designed to address perceived weaknesses in earlier, fragmented public extension services:

- **Top-Down Structure:** Technology and recommendations were centrally developed by research institutions and Subject Matter Specialists (SMSs).
- **Methodology:** The system relied on a strict schedule of regular training for field-level extension workers and frequent, systematic visits to a select group of "contact farmers" (Benor and Baxter, 1984).
- **Focus:** The primary goal was the rapid and uniform dissemination of standardized, divisible and high-yielding technologies, such as improved seeds, fertilizers and irrigation practices.

Strengths:

- **Effectiveness for Uniform Technologies:** T&V proved highly effective in boosting productivity where agro-ecological conditions were homogenous and technologies were well-suited (e.g., irrigated wheat and rice in parts of Asia).
- **Professionalism and Accountability:** It introduced a single line of command, continuous staff training and time-bound work plans, significantly improving the discipline and professionalism of public extension staff (Feder *et al.,* 2004).

Inherent Weaknesses and Failures of the ToT Model

Despite its initial successes, the structural rigidity and conceptual limitations of the ToT model led to its eventual decline in many regions. The model's reliance on standardized solutions proved profoundly inadequate for the complex and diverse reality of smallholder agriculture (Birner *et al.*, 2009).

1. Lack of Contextual Relevance and Adaptability

- **"One-Size-Fits-All":** The model assumed that technologies developed in research stations were universally applicable. This ignored local knowledge, varying soil types, diverse micro-climates and socio-economic constraints faced by farmers, particularly in rain-fed and marginal areas (Van den Ban, 1999).
- **Limited Feedback Loop:** The flow of information was overwhelmingly unidirectional (top-down). Mechanisms for transmitting farmer feedback, especially regarding technology constraints or adaptation needs, back to research were either weak or non-existent, resulting in a disconnect between supply-side research and farmer demand.

2. Fiscal Unsustainability and Equity Issues

- **High Recurrent Costs:** T&V was expensive to maintain due to its large, hierarchical staff structure and the need for frequent, subsidized transport for continuous training and visits. When donor funding (such as the World Bank's) ended, many national governments were unable to sustain the high recurrent costs, leading to collapse (Feder *et al.,* 2004).
- **The "Contact Farmer" Bias:** By focusing on a select group of "contact farmers" (often those who were wealthier, more educated, or better connected): the system inadvertently marginalized smallholders, women farmers and those in remote communities, exacerbating existing social and economic disparities (Birner *et al.,* 2009).

3. Failure to Address Complex Issues

The T-T model was production-focused and technology-driven. It was ill-suited to address new, complex and process-oriented challenges that require a systems approach:

- **Natural Resource Management (NRM):** Sustainable NRM, soil health and water conservation require long-term, adaptive learning, not simple technology packages.
- **Market Linkages:** The model offered minimal guidance on post-harvest handling, quality standards, or connecting farmers to value chains and markets, which became increasingly important (World Bank, 2017).

The Shift to Participatory Paradigms

By the late 20th century, the recognized shortcomings of the linear model spurred a corrective shift towards more process-oriented and farmer-centric approaches. This led to the emergence of new paradigms:

- **Farming Systems Research and Extension (FSR&E):** Advocated for starting research by understanding the whole farm-household system and identifying constraints in consultation with farmers, moving towards a more holistic view.
- **Participatory Approaches (e.g., Farmer Field Schools–FFS):** Methods like FFS emphasized experiential learning and farmer-led experimentation, moving the extension agent's role from a teacher to a facilitator of learning and innovation (Braun *et al.,* 2006).

While these participatory models provided a crucial conceptual and methodological counterpoint, they often struggled with scalability and fiscal sustainability within large public systems, leaving a persistent gap in the delivery of widespread, quality advisory services (Garforth *et al.,* 2018). It is this remaining gap, the need for scaled, context-specific and resilient services, that drives the contemporary need to revisit and fundamentally transform extension approaches in the 21st century.

Extension Approaches for the 21st Century

A systematic revisiting of approaches to meet **climate-resilient buffalo production** needs:

Climate-Smart Extension (CSE)

Climate-Smart Extension (CSE) is an advanced and contemporary extension approach that helps farmers and livestock owners, especially smallholders, adapt to climate change, reduce vulnerability and build resilience. These extension services are critical in providing climate education, capacity building, timely climate information and designing area-specific adaptation strategies for livestock farmers. The core objectives of Climate-Smart Extension (CSE) focus on empowering farmers to respond to the increasing challenges posed by climate change effectively. CSE aims to strengthen farmers' awareness about climate variability and its impacts on livestock production, health and farm resources. By promoting climate-smart livestock practices, such as improved housing, heat stress mitigation, efficient fodder and water management and disease-prevention measures, CSE helps farmers adopt technologies and behaviour that enhance resilience.

A key objective is to provide location-specific climate advisories, ensuring farmers receive timely and relevant weather, disease and management information tailored to their Agro-ecological conditions. CSE also focuses on supporting preparedness for climate risks and disasters by enabling farmers to anticipate extreme events, develop contingency plans and minimize losses. Importantly, it seeks to integrate scientific knowledge with indigenous practices and ICT-based innovations, ensuring that farmers receive holistic, practical and culturally appropriate solutions. Ultimately, CSE works to enhance farmers' adaptive capacity, reduce climate-induced production losses and build more resilient, sustainable livestock farming systems.

Climate-Smart Extension (CSE) relies heavily on a wide range of mass awareness and communication strategies to build climate literacy and promote adaptation among livestock farmers. Large-scale awareness campaigns, utilizing Kisan Melas, Gosthis, health camps, street plays, folk theatre, puppet shows, hoardings, posters and social media or electronic media platforms, help create substantial behavioral and attitudinal change by delivering coordinated climate-smart messages to diverse audiences. These are supported by mass meetings, exhibitions, road shows, climate fairs, wall posters, AV aids, short films and public displays at railway stations, malls and airports, ensuring widespread outreach across rural and semi-urban populations. CSE also emphasizes the integration of indigenous knowledge with scientific knowledge, enabling farmers to utilize traditional climate indicators and community wisdom in conjunction with scientific advisories for more accurate and culturally relevant adaptation decisions. Strengthening rural institutions and village knowledge centres such as SHGs, cooperatives, FPOs, ClimaAdapt centres and Rythu Bharosa Kendras (RBKs) provides farmers with trusted, localized and continuous access to climate-smart information and services. Practical learning is advanced through Climate-Smart Demonstration Units in KVKs, particularly under NICRA, where farmers observe proven technologies such as drought- or flood-resilient fodder production, improved shelters for heat stress, water harvesting structures and methane-reducing feed practices. Finally, visually engaging platforms such as road shows, climate exhibitions and mobile exhibition units, including buses and train-based mobile showcases, bring technologies directly to communities, creating curiosity, dialogue and a deeper understanding of climate-resilient livestock innovations across blocks and districts.

Initiatives Supporting Climate-Smart Extension

Several national initiatives support Climate-Smart Extension by strengthening farmers' resilience to climate risks. The National Innovations on Climate Resilient Agriculture (NICRA): launched in 2011 and implemented by the Indian Council of Agricultural Research (ICAR): focuses on climate-resilient demonstrations in vulnerable districts covering crops, livestock and natural resource management. The National Mission for Sustainable Agriculture (NMSA): initiated in 2014-15 under the Ministry of Agriculture and Farmers Welfare, promotes land and water resource conservation through components like Rainfed Area Development, Soil Health Management and on-farm water-use efficiency. The National Livestock Mission (NLM): restructured and relaunched in 2014 under the Department of Animal Husbandry and Dairying (DAHD): aims to enhance fodder production, improve feed and breed quality and strengthen livestock health and productivity with a focus on climate resilience. The National Animal Disease Control Programme (NADCP): launched in 2019 by DAHD, provides nationwide vaccination for FMD and Brucellosis to build climate-resilient animal health infrastructure and reduce climate-linked disease outbreaks. Complementing these, the India Meteorological Department's (IMD) Agromet Advisory Services (AAS): operational since 1976 and expanded under the Gramin Krishi Mausam Sewa, delivers location-specific weather forecasts, early warnings and management advisories to farmers through KVKs, DAMUs, mobile apps, SMS portals and digital platforms, enabling timely climate-smart decisions at the farm level (Tiwari *et al.,* 2005).

ICT-Enabled and Digital Extension

ICT-enabled and digital extension has become a transformative pillar of Climate-Smart Extension, offering rapid, personalized and scalable solutions for livestock farmers facing climate variability. A wide range of digital tools, including mobile applications for buffalo health management, ration balancing, heat-stress alerts and farm decision-making, enables farmers to receive real-time, need-based guidance directly on their smartphones. Tele-veterinary services and AI-driven diagnostic platforms further strengthen last-mile support by providing expert advice, remote disease assessment and round-the-clock assistance, reducing delays in treatment and preventing climate-aggravated livestock losses. Digital communication networks, especially WhatsApp and Telegram groups, have emerged as efficient channels for sharing localized weather alerts, disease warnings, feeding advisories and best practices among farmers, veterinarians and extension workers.

ICT-enabled and digital extension for farmers encompasses a wide range of digital information services, consulting platforms, service-delivery tools, e-governance systems and e-learning solutions that enhance access to knowledge, facilitate informed decision-making and promote market participation in Indian agriculture. Information services include web portals such as the Farmers' Portal, e-Choupal, Crop Insurance Portal, PGS-India, VISTAAR, e-Pashu Haat, AGMARKNET, IMD Portal and ICAR's Krishi-DSS, which provide real-time updates on markets, weather, schemes, certification, livestock and satellite-driven agricultural insights. Mobile applications, including e-Gopala, Pashu Poshan, KISAAN 2.0, BAIF Godhan Seva, Krish-e, Krishify and Kisan Suvidha, offer personalized advisories on livestock health, feeding, crop management, weather forecasts and access to expert services. Chatbots such as IVRI SHRIA, AgronomoBot and Milchbot provide 24/7 automated support using conversational AI. Social media platforms, such as YouTube, Facebook, Twitter, LinkedIn and Instagram, used by institutions like ICAR, MANAGE and IVRI, disseminate educational videos, advisories and success stories. Television and radio programs, including DD Kisan, Krishi Darshan, Green Radio and Kisan Vani, extend agricultural information to diverse audiences. Consultancy services such as Kisan Call Centers (KCC): mKisan and Kisan SARATHI deliver expert guidance through calls, SMS and digital platforms. Service-delivery tools like Kisan Rath, e-NAM and e-RaKAM support transportation, market access and transparent trading. Finally, e-learning platforms such as SWAYAM, KrishiKosh, Digital Green and MOOCs provide agricultural education and skill development through digital courses, repositories and community-based video learning. Together, these e-resources enhance efficiency, knowledge sharing, market integration and resilience among India's farming communities (Tiwari *et al.,* 2025).

Advanced technologies, such as drones, are increasingly being used for fodder mapping, monitoring pasture availability and inspecting livestock sheds for ventilation and heat stress risks. Similarly, decision-support tools (DSTs) help farmers assess climate risks, plan for fodder resources, manage water and make informed climate-smart choices. Digital dashboards used by cooperatives, FPOs and dairy organizations enable continuous monitoring of milk yield trends, disease occurrence, reproductive performance and farm-level vulnerabilities, supporting data-driven climate resilience.

Complementing these tools, national ICT initiatives, such as the Kisan Call Centers (KCC): offer expert teleconsultation in local languages. At the same time, the mKisan SMS advisory service ensures that every farmer receives timely, location-specific messages on climate and livestock management. Kisan Sarathi is a vital ICT-enabled digital extension platform developed by India's MeitY and ICAR, designed to deliver timely, personalized agricultural advisories directly to farmers across the country in their preferred regional language. It facilitates direct multimodal communication (text, voice, image, video) between farmers and a vast network of over 731 Krishi Vigyan Kendras and agricultural experts, moving beyond traditional extension models to offer location-specific, expert guidance via mobile app, web portal and call centers. The platform significantly enhances decision-making by integrating weather data, market information and a comprehensive knowledge base, effectively bridging the digital divide and empowering farmers to adopt more efficient and climate-resilient practices.

Modern AI-powered chatbots provide interactive advisory support to livestock farmers, responding instantly to queries on climate-smart practices, disease prevention, feeding and shelter management. Climate-sensitive diseases are addressed through forecasting apps, such as NIVEDI's LDF App, which provide early warnings and help farmers prepare for outbreaks two months in advance. Social media platforms, including YouTube, Facebook, Twitter and Instagram, further expand the reach of climate adaptation messages through videos, infographics and short educational content. Additionally, hybrid digital models integrating cloud-based advisory systems facilitate personalized recommendations by combining big data analytics, weather information, extension expertise and local context. Together, these ICT-based strategies significantly strengthen farmers' adaptive capacity, improve response time and enhance the overall climate resilience of buffalo and livestock production systems.

Community-Based Extension Models

Community-based extension models play a crucial role in advancing Climate-Smart Extension by grounding climate resilience efforts within local institutions, knowledge systems and farmer networks. A key component is the integration of indigenous knowledge with scientific information, enabling farmers to combine traditional climate forecasting, local indicators and pastoralist wisdom with modern climate advisories for more reliable and culturally relevant decision-making. Strengthening village-level institutions such as Village Knowledge Centres, ClimaAdapt centres, SHGs, FPOs, cooperatives and Rythu Bharosa Kendras (RBKs) ensures that farmers have continuous access to trusted, localized climate-smart information, inputs and services (Balasubramani and Dixit, 2016). Practical learning and technology adoption are further supported by climate-smart demonstration units in KVKs, where farmers can observe and learn about resilient fodder practices, heat-stress mitigation, improved shelters, water harvesting and feeding innovations under real farm conditions. Participatory approaches, such as Farmer Field Schools (FFS): encourage experiential learning, group analysis and collaborative problem-solving, enabling farmers to test and refine adaptation practices. Local leaders, including Gram Pradhans, teachers and progressive farmers, act as climate influencers, motivating communities to adopt new technologies and practices. The involvement of para-vets, Pashu Sakhis and local resource persons (LRPs) significantly enhances last-mile connectivity, ensuring timely disease management, climate advisories, emergency support and dissemination of climate-smart livestock practices at the doorstep of farmers. Community resilience is further reinforced through community disease surveillance systems, where farmers and local institutions jointly monitor climate-sensitive diseases, report outbreaks early and strengthen preparedness. Establishing community fodder banks provides a buffer during droughts, floods and heatwaves, ensuring fodder availability and reducing climate-induced feed scarcity. Farmer-to-farmer extension strengthens peer learning through field visits, group discussions and experience sharing, accelerating horizontal knowledge flow within villages. Events such as climate fairs at village, block and district levels provide platforms for farmers to interact with scientists, extension agencies and private stakeholders, promoting broader awareness.

A major recent initiative strengthening this participatory ecosystem is the **Viksit Krishi Sankalp Abhiyan**, which actively engaged communities by collecting farmer feedback, identifying local challenges and documenting grassroots innovations developed by farmers themselves. This campaign played a pivotal role in amplifying the voices of farmers, highlighting indigenous solutions and ensuring that extension interventions are more need-based and locally grounded. Finally, the establishment of **Climate-Smart Villages** (CSVs) integrates all these community-driven approaches by combining climate information services, local knowledge, institutional strengthening, village development planning and climate-resilient technologies into a holistic village-level model. Together, these community-based extension models create empowered, informed and climate-resilient farming communities.

Pluralistic Extension and Market Integration

Agricultural Extension and Advisory Services (AEAS) in the 21st century have evolved into a complex, "pluralistic" landscape, moving far beyond the traditional model of a single, government-run entity. This paradigm shift recognizes that the delivery of crucial knowledge and services is now a shared responsibility, involving diverse stakeholders such as the private sector, non-governmental organizations (NGOs): farmer-based organizations (FPOs) and media outlets. This multifaceted system leverages the unique strengths of each actor: the public sector typically focuses on providing essential public goods like foundational research, regulation, policy framework and reaching marginalized or remote communities; the private sector drives innovation and delivers input-specific, commercial services (e.g., specific seed or fertilizer advice); while NGOs and FPOs are instrumental in community mobilization, advocacy and specialized training tailored to local needs. Effective coordination among these diverse players is paramount to avoid duplication and ensure seamless service delivery (Singh *et al.,* 2004).

A key feature of this modern extension approach is its transition to a demand-driven model, which fundamentally shifts the power dynamics and positions the farmer at the center of the advisory system. Unlike the former supply-driven "transfer of technology" approach, which often pushed generalized information, contemporary extension must be highly responsive to specific farmer requirements and, critically, to market demands. This involves understanding what farmers want to know to improve their livelihoods and what the market requires in terms of quantity and quality. The focus is on empowering farmers to make informed decisions that align with economic opportunities, rather than merely maximizing physical yields without considering market viability.

Furthermore, pluralistic extension integrates "value chain extension," which expands the scope of advisory services beyond just production advice. It now encompasses the entire agricultural value chain, from pre-production planning to post-harvest handling, quality control, value addition and logistics. Extension agents help farmers meet stringent quality standards, acquire certifications and adopt improved processing techniques to access higher-value markets. This holistic approach is crucial for enhancing farmer incomes and resilience, ensuring that increased production translates into tangible economic benefits.

The practical implementation of this pluralistic system relies on robust collaboration among all stakeholders, including research institutions, extension services, farmers and the private sector. Public-Private Partnership (PPP) models have emerged as critical mechanisms, particularly in the dissemination of complex, climate-smart technologies that require significant investment and expertise. These partnerships can accelerate the uptake of innovations that build resilience to climate change. Dairy cooperatives and producer companies play a vital role in this ecosystem, serving as practical knowledge hubs that pool resources, facilitate collective learning and leverage economies of scale. These organizations act as intermediaries, bridging the gap between cutting-edge research and the everyday challenges faced by smallholder farmers, making knowledge dissemination more practical, efficient and impactful. This integrated, collaborative and market-oriented approach is essential for a resilient and profitable agricultural future. The BAIF model is a highly successful Public-Private Partnership (PPP) for livestock development in India, centered on providing high-quality Artificial Insemination (AI) services. It operates through Technical Hubs and a network of trained village-level entrepreneurs, known asGopals, who deliver doorstep services, ensuring last-mile connectivity for small farmers. The Public Sector (Government) provides initial funding, regulatory oversight and a social mandate. BAIF (the NGO) provides the technical expertise, efficiency and human capital management. The Farmers ensure sustainability by paying a nominal fee-for-service, making the model demand-driven and achieving the public goal of enhancing cattle genetics and milk productivity.

Gender-Sensitive and Youth-Oriented Extension

Women play a central role in India’s agri-food systems, making significant contributions across crop production, horticulture, livestock, fisheries and natural resource management. Despite comprising nearly half of the population and a significant share of the agricultural workforce, they continue to face systemic disadvantages in accessing land, inputs, credit, technologies and decision-making. National data reveal women’s extensive engagement: 76.9% of rural female workers are involved in agriculture; they constitute 31% of the crop production workforce; dominate horticultural tasks across many states; form about 70% of the dairy workforce; and play a significant role in post-harvest fisheries, where they account for up to 90% of processing workers. Their contributions are especially prominent in the Northeast and under subsistence systems, while states with mechanized, commercial agriculture show lower female participation.

Women’s work extends far beyond production. They shoulder a disproportionate burden of unpaid domestic and care responsibilities, resulting in longer total work hours and reduced opportunities for health, education and economic advancement. Gendered inequities contribute to India’s persistent malnutrition challenges, with high rates of anaemia among women and widespread undernutrition among children. Addressing these issues requires gender-responsive agricultural and nutrition strategies such as diversification, biofortified crops, homestead nutrition gardens and targeted health education.

Recognizing both the challenges and the transformative potential of women in agriculture, gender equality has been placed at the heart of global development priorities under SDG 5, which contributes directly to goals related to hunger, poverty, health and climate resilience. To accelerate progress, ICAR has introduced its first institution-wide Gender Strategy for the National Agricultural Research, Education and Extension System (NAREES). This unified framework aims to mainstream gender equity across ICAR institutes, CAUs and SAUs, guiding research, education and extension toward greater inclusivity and impact. It complements existing organizational strategies and emphasizes stakeholder engagement to empower women, referred to as Krishika and strengthen the resilience and sustainability of India’s agricultural and food systems. In parallel, the strategy incorporates gender-sensitive and youth-oriented extension approaches. These initiatives include developing women-focused training modules, enhancing access to climate-smart technologies and leveraging the roles of women and youth in dairy, livestock and horticulture sectors. It promotes youth engagement through agri-startups, digital tools and entrepreneurship, supported by incubation and innovation platforms. Skill development in climate-resilient practices, value addition and digital literacy is prioritized to prepare both women and youth for emerging opportunities. Strengthening women's and youth participation in FPOs, dairy cooperatives and producer companies, which are positioned as local knowledge hubs, ensures better access to markets, information and technology.

The Strategy for a Gender-Equitable Extension System aims to ensure that women farmers gain equal access to technologies, resources, opportunities and decision-making that enhance productivity and contribute to food and nutritional security. It promotes an inclusive agri-food system through gender-sensitive social and institutional environments. Key interventions include nationwide awareness initiatives, such as the Krishika Shakti campaign, gender awareness programs, recognition of women's contributions and the dissemination of success stories. The strategy emphasizes building women's capacities through tailored training, convenient learning formats and gender-sensitive curricula for extension workers. It also focuses on improving women's access to government schemes, strengthening their participation in extension processes and establishing institutional support systems, such as Krishika Cells, at the block level. Women's collectives, SHGs, W-FPOs and federationsare to be strengthened through training, collective action, reduced licensing fees and targeted entrepreneurship support. The strategy promotes women-friendly technologies, digital literacy and women-led machine banks to reduce drudgery and improve access to tools and information. It further calls for exceptional support to marginalized women, including landless, tribal and single women, to enhance their livelihood opportunities through homestead farming and value addition. Market access is to be expanded through literacy programmes, e-commerce linkages and dedicated women's marketplaces. Finally, robust monitoring and evaluation mechanisms are recommended to track participation, empowerment and economic gains, as well as to document successful models for wider adoption (ICAR, 2025).

The Future of the Extension Agent: Role, Capacity and Competencies

The effectiveness of modern extension systems depends less on the simple delivery of pre-packaged advice and more on the agent's ability to act as a catalyst within a complex Agricultural Innovation System (AIS). This requires a profound transformation of the agent's identity and skill set.

I. From "Teacher" to "Facilitator and Broker": The traditional extension agent was a teacher and technical expert, responsible for transferring scientifically proven knowledge to farmers (Van den Ban, 1999). The modern agent must embody a multi-faceted professional identity:

- **Facilitator of Learning:** The role shifts from instructing *what* to do to facilitating *how* to learn. This involves guiding farmers through experiential learning processes, such as Farmer Field Schools (FFS): to enable them to experiment, adapt and co-create knowledge specific to their local conditions (Braun *et al.,* 2006).
- **Innovation Broker/Network Weaver:** In a pluralistic extension landscape, the agent is a broker, connecting farmers and Farmer Producer Organizations (FPOs) to a diverse range of non-traditional actors, including researchers, input suppliers, financial institutions, buyers and digital service providers. This requires skills in networking, negotiation and alliance-building (Chowa*et al.,* 2013).
- **Institutional Entrepreneur:** The agent must identify and address systemic constraints (e.g., policy barriers, market failures). This involves leveraging resources to create or transform institutional arrangements, moving beyond individual farm-level advice to address systemic impact (Liao*et al.,* 2024).
- **Risk Manager:** Given the increasing volatility of climate and markets, the agent's advisory role must expand to include guidance on risk mitigation, such as crop insurance, early warning systems and diversification strategies (Priya*et al.,* 2025).

II. Required Competencies for the 21st-Century Extension Agent

To execute these new roles effectively, the modern extension professional requires a blend of technical expertise, process skills and business acumen.

A. Process and Facilitation Skills (Soft Skills):

These competencies are paramount for managing group dynamics and fostering local innovation:

- **Facilitation and Group Management:** The ability to organize and lead practical group discussions, manage conflicts, encourage collaboration and ensure the participation of diverse groups, including women and marginalized farmers (Priya *et al.,* 2025).

- **Communication:** Effective communication must be tailored to different formats (oral, written, digital) and different audiences (farmers, policymakers, private sector): emphasizing clarity, active listening and persuasive framing (Pathak *et al.,* 2023).
- **Systemic Thinking:** Understanding that agriculture is a complex system influenced by ecology, economics and policy and the ability to diagnose problems at multiple levels, not just the technical farm level.

B. Technical and Digital Competencies

While process skills are critical, a foundational technical base remains necessary, complemented by digital proficiency:

- **Integrated Technical Knowledge:** Deep understanding of complex, sustainable practices like Climate-Smart Agriculture (CSA): Integrated Pest Management (IPM) and Natural Resource Management (NRM): which requires systems-level understanding rather than single-technology focus (Davis *et al.,* 2021).
- **Digital Literacy and Data Interpretation:** Proficiency in using, promoting and training farmers on digital tools (mobile apps, weather alerts, digital marketplaces). This includes the ability to interpret data outputs from precision farming tools (such as GIS and sensors) into practical, local recommendations.

C. Business and Entrepreneurial Acumen

The shift toward market-led extension demands strong business skills:

- **Market and Value Chain Analysis:** The ability to analyze local, regional and global market trends, quality requirements and price information to guide farmers' production choices (Ferris*et al.,* 2014).
- **Entrepreneurship Mentoring:** Skills to help farmers develop farm business plans, access financing and credit and transition from subsistence farming to market-oriented agripreneurship.
- **Negotiation and Financial Literacy:** Assisting farmers in negotiating with buyers, understanding contract terms and managing farm finances.

III. Reforming Extension Education

To produce agents with these complex competencies, a substantial revitalization of extension education institutions is required.

- **Curriculum Overhaul:** Curricula must move beyond traditional agronomy, animal husbandry and social science to incorporate modules on digital technology, climate risk management, agribusiness, systems thinking and facilitation skills.
- **Pedagogical Shift:** Training must adopt a problem-based, experiential learning approach, mirroring the facilitative methods agents are expected to use in the field. This includes internships with diverse stakeholders (NGOs, private firms) to build brokerage skills.
- **Continuous Professional Development (CPD):** Given the rapid pace of technological and climate change, in-service training must be continuous and competency-based, moving beyond basic technical updates to cover entrepreneurial leadership, conflict management and digital security.

Conclusion

The journey of Agricultural Extension and Advisory Services (AEAS) in the 21st century is marked by a fundamental shift away from the rigid, top-down Technology of Transfer (ToT) models of the past. As demonstrated throughout this paper, the convergence of profound global challenges, the climate crisis, rapid digital transformation and complex market demandshas rendered traditional approaches obsolete and necessitated a comprehensive strategic pivot.

The revitalization of extension rests on three core pillars that collectively define the new paradigm: Digital Enablement: The integration of Digital AEAS (DAEAS): leveraging mobile technology, remote sensing and AI, offers the unprecedented potential for scale, speed and precision. However, this transformation is contingent on actively bridging the digital divide, ensuring equitable access, promoting digital literacy and establishing robust data governance to maintain farmer trust. Climate-Adaptive Focus: Extension must transition from being purely production-centric to knowledge-intensive and risk-centric. The promotion of Climate-Smart Agriculture (CSA) requires agents to become facilitators of co-learning and local adaptation, focusing on resource management, resilience-building and systemic risk mitigation, rather than relying on standardized technology packages. Institutional Pluralism and Innovation Brokerage: The public sector can no longer sustain the full burden of AEAS delivery. A viable system is pluralistic, with clear roles for private companies, NGOs and farmer organizations (FPOs). The frontline agent’s role has evolved into that of an innovation broker and network weaver, connecting farmers to diverse knowledge, market opportunities and financial services.

Ultimately, the effectiveness of the reformed extension system hinges upon strategic investment in human capital. The future extension agent must be a highly skilled professional with competencies spanning digital literacy, business acumen and sophisticated facilitation skills, demanding a complete overhaul of extension education curricula (Source 3.4).Achieving this systemic transformation requires sustained political will and investment in policy frameworks that foster collaboration, mobilize public-private partnerships (PPPs) and ensure that extension services remain demand-driven and accountable to the end-user- the farmer.

AEAS remains an indispensable engine for achieving the Sustainable Development Goals, particularly in areas such asglobal food security, poverty reduction, gender equality and environmental sustainability. By embracing pluralism, leveraging the digital revolution responsibly and prioritizing climate resilience, extension services can successfully navigate the complexities of the 21st century and fulfill their mandate as key drivers of agricultural innovation and change.

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LP 6.3. Proactive efforts for sustaining buffalo production and growth in buffalo meat exports

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ABSTRACT

Buffalo meat exports are important for sustaining buffalo production as value addition of culled buffaloes with modern meat export plants provides demand driven situation for increased earnings by the farmers. However, further increase in meat exports and thereby further increase in farmers income is possible only with salvaging of male buffalo calves which are neglected at present. There are an estimated 15-20 million male calves that need to be adequately utilized for contributing to buffalo productivity. Buffalo meat exports earnings could be increased from present 30000 crores to Rs 135000 crores after meeting domestic supply. Model projects with relevant schemes for popularization of male buffalo calf utility are essential. ICAR-Animal Science Institutes and Veterinary colleges should develop relevant economic practices for preventing early age mortality of male buffalo calves. Investments in buffalo meat production and export would be a win win situation for farmers, industry and Government. Hence deserves a relevant scheme with pragmatic policies and regulations.

Buffaloes are unique Livestock asset of India sustaining their growth in population since 1951 as a result of improved utility of the species to the farmers. Growth in buffalo meat exports since 1970 has accelerated buffalo utility as value added exports of the culled stock of the farmers. Demand driven production has sustained farmers interest in the species. Indian buffaloes are unique in the world with large variety of breeds and successful adaptation in all the agro-climatic zones of the country. Though there are a number of socio-economic and land utility constraints exerting pressure on livestock species prospects in particular loss of draught function of cattle, bans and restrictions on slaughter utility but in respect of buffaloes negative pressures are much limited and shown sustained prospects. Production and processing opportunities are enormous in buffalo meat sector with need for diversification of agriculture for better income and employment. Export opportunities have increased in the recent years with growth in demand for animal products in the developing countries.

Indian buffalo meat popularity among world countries:

Buffalo meat is popular among 70 countries of the World with exports starting from 2000 MT in the 1970s and reaching number one status in the export trade of bovine meat category (Indian bovine meat is only from buffaloes as beef from cattle is banned for export/import) with buffalo meat shipments at 1.476 million MT valued at $4.78 billion (2014-15). What are the reasons and basis for such popularity of buffalo meat among the consumers of the large number of the world countries. Some of the reasons include economical cost due to traditional natural rearing; desirable compositional characteristics (lean meat of higher quality protein, lower fat and cholesterol, desirable fatty acids, minerals and vitamins etc.); good health and nutrition contributory aspects; safe meat and desirable public health aspects; ethical and traditional considerations etc. Buffaloes produce tender meat by virtue of their docile habits, slow movement and energy saving specialities. A detailed basis of these unique aspects of buffalo meat in comparison to beef of world countries will go a long way in better understanding of Indian buffalo meat and mutually benefitting societies of buffalo meat producing and consuming countries.

Need for Improving Meat Productivity of Indian buffaloes:

Table 1: Buffalo meat production and productivity in the top 10 countries of the World

Countries	Buffalo popu- lation (Rank)	Meat prodn.(t) (Rank)	Meat Productivity* (RANK)
India	112049996 (1)	1854640$^{\#}$ (1)	**1.66 (8)**
Pakistan	44974000 (2)	1220000 (2)	**2.71 (3)**
China	31527876 (3)	753144 (3)	**2.39 (4)**
Nepal	3081062 (4)	116503 (5)	**3.78 (1)**
Philippines	2776115 ((5)	63251 (7)	**2.28 (5)**
Vietnam	2136009 (6)	67600 (6)	**3.16 (2)**
Myanmar	2075000 (7)	28618 (8)	**1.38 (10)**
Egypt	1445764 (8)	210859(4)	**1.46 (9)**
Indonesia	1016596 (9)	22110 (9)	**2.17 (6)**
Thailand	889775 (10)	19091 (10)	**2.16 (7)**

**Meat Productivity= meat production(t)/population multiplied by100*
Data source: FAO Stat 2023 # data as per BAHS-DAHD-GOI 2024

With male buffalo calf salvaging and rearing. India buffalo Meat productivity would substantially increase and the available potential is 128 percent increase even to reach upto Nepal level or 63 percent increase to reach the level of Pakistan which has Riverine buffaloes as of India. The present buffalo meat productivity is low because male buffalo calves are neglected resulting high early age mortality and not contributing to meat production. As a result the meat productivity of Indian buffalo population is lower in the rank at 8 among the top 10 countries of the World having buffalo populations. Hence all efforts are required to prevent early age mortality of buffalo calves and rear them to optimum slaughter weights of upto 350 kgs in 18-24 months period with balanced feeds at different stages.

The following aspects need consideration for enhancing production potential of buffaloes:

- Pragmatic slaughter policy and free movement of animals
- Salvaging of buffalo calves from early death
- Studies on buffalo colostrum and its quality improvement and appropriate feeding to calves
- Growing of excess calves to larger weight (200-300kg liveweight) for meat production
- Intensive and semi-intensive system of meat animal production
- Supply of critical feed inputs in the lean season for efficient animal production
- Popularizing silage production and feeding practices (urea, molasses, chaffing crop residues) for sustained feed supplies
- Crop residue based decentralized rural feed processing units for balanced feeds
- FMD-Disease Free Zones in buffalo populous states to promote meat and dairy exports
- Development of livestock markets and transport facilities
- Involvement of stake holders in proposing development programmes and policy proposals
- Development of extension literature on different aspects of buffalo productivity- in particular salvaging buffalo calves from early age mortality at low cost including soya milk replacement of whole milk.

Essentiality of buffalo Meat exports: Meat exports contributes for Direct and indirect benefits:

a. Forex earning to the extent of Rs 30,000 crores per annum and potential for Rs one lakh crore.
b. Beneficial and easy disposal of culled animals and better returns to producer
c. Promotes effective culling–reduces unproductive and stray animals and the associated social, environmental, economic and public health implications
d. Increases herd productivity and the associated gains
e. Contributes for better efficiency in meeting Food and nutrition security needs
f. Employment contributor
g. Promotes gain from Technologies and value addition from the modern meat export plants
h. Contributes good quality hides and skins to leather industry
i. Private investments promote innovative marketing and fetch better returns to farmers
j. Facilitates import of essential items

Issues adversely affecting supply chain of Buffalo meat export:

1. Regulations:

a. The Prevention of Cruelty to animals (slaughterhouse) Rules 2001- (PCA (slaughterhouse Rules 2001) framed under the Prevention of cruelty to animals act,1960 has no jurisdiction of Ante-mortem and post -mortem inspection for hygienic meat production with a large number of (a to y) points of post-mortem. Least importance is given to animal welfare aspects at slaughterhouse in the PCA (SH) Rules 2001 and need to be repealed. One of the provisions is limiting the capacity of the vet to examine only 12 animals in an hour or 96 animals in 8 hours a day which has adverse economic implications to meat sector. Other provisions are either against animal welfare or not related to prevention of cruelties to animals.

Adequate animal welfare measures are listed under FSSAI regulations (Food Safety and Standards (Licensing and Registration of Food Businesses) Regulations, 2011) and BIS standards (IS: IS14904:2007, Transport of Livestock-Code of Practice; and IS: 1982:2015 Indian Standard Ante-Mortem and Post-Mortem Inspection Of Meat Animals — Code Of Practice (Second Revision).

None of the international regulations related to Animal Welfare or Meat inspections subjects have prescribed the number of animals to be inspected by a Vet in one hour or in 8 hours a day. FSSAI regulation, 2011 has adequate provisions hence, withdrawal of the Notification would not compromise with any aspect of prevention of cruelty to animals. As per the data from a Report of Canada the number of animals (animal unit equivalent to a Cow) inspected per hour comes to 55 and for 8 hours a day 440 which is far higher than the 96 animals per 8 hours a day of the PCA (SH) Rules 2001.(Report of the

Meat Regulatory and Inspection Review by The Honourable Ronald J.Haines, Ontario, 2004. Chapter 6–Abattoirs). Recommendations of Codex, EU and OIE: None of the International Organizations have stipulated any particular number of animals to be inspected by a vet for Meat Inspection purpose or animal welfare purpose. FSSAI has not stipulated any fixed number of animals to be inspected by Veterinary Doctors for animal welfare, ante-mortem and PM inspection. Following are the other inappropriate, untenable, uneconomic, conflicting and anti-animal welfare provisions of the Prevention of cruelty to animals (slaughterhouse) rules 2001 which justifies total repeal of PCA (SH) Rules 2001:

(1) Meat Inspection- not covered under PCA Act 1960
(2) No mention of specific animal cruelties or welfare measures at slaughterhouses:
(3) Conflict on the recognized place of slaughter:
(4) Conflicting with State Animal Preservation act provisions on Vet Capacity
(5) Conflicting with FSSAI Regulations which mention animal welfare measures in detail
(6) Excess resting period in lairage- anti-animal welfare and against WOAH recommendation
(7) Undesirable excess lairage space not considering variation in size of animals
(8) Separate section for slaughter of individual Animal- an impractical clause
(9) More Emphasis On Post-Mortem and Least Importance to Animal Welfare
(10) Undesirable Interference of Awbi or tts Representative

A scientific view is necessary in order to have effective meat inspection for the consumer safety with the new approaches of HACCP and other quality standards being implemented in modern meat plants. There is need to repeal the untenable aspects and the need for withdrawal of the Notification in view of the present FSSAI regulations.

b. **‘Central Motor Vehicles (Eleventh Amendment) Rules, 2015’:** Road transport of animals is regulated as per the provisions of the Motor vehicles Act, 1988 and the Central motor vehicles rules 1989 rules made there under. An amendment has been made to the Rules namely ‘Central Motor Vehicles (Eleventh Amendment) Rules, 2015’ to be effective from Jan 2016. (http://morth.nic.in/showfile.asp?lid=1753). A critical examination of above amendment rules indicates following deficiencies/ difficulties and animal cruelties for reconsideration and repeal:

Due to large variability in types, breeds, sizes and condition of animals it is necessary to take utmost care in proposing any interventions in the livestock related activities based on a thorough analysis of implications and interventions.

- Provision of permanent partitions in the body of the vehicle for individual animal transport need to be deleted as it is against animal welfare. Partitions have to be as per IS14904: 2007.
- Space requirements which are same for small and big size animals of the species is against animal welfare and should be same as in IS 14904: 2007.
- Space requirements given in the Amendment Rules are in conflict with the space requirements mandated in Transport of Animals (Amendment) Rules,2009 made under Prevention of cruelty to animals Act 1960, which provides different space requirements for different size/weight animals.
- None of the international standards/ regulations/ guidelines- stipulated requirement of permanent partitions for carrying individual animals, nor such space requirements for different species without considering size/weight consideration;
- The provision of ‘livestock transport vehicles should not be used for carrying other goods’ when there are no animals in the vehicle is against the farmer interest. Livestock transport vehicles after proper cleaning could be used for transporting approved goods in return journey as the economics alone would ensure success of livestock transportation. *The above adverse aspects demonstrates beyond doubt the repeal of the Notification GSR 546ECentral Motor Vehicles (Eleventh Amendment) Rules, 2015.*

c. **Buffaloes and slaughter policy**: Buffalo slaughter policy primarily contributes for productivity improvement by way of effective culling for improving herd average. As culled animals bring adequate returns to farmers by way of slaughter disposal, farmers need to be facilitated for practicing effective culling of unproductive, poor and low producers. Also excess and defective animals that can not be retained in the herd also need to get culled. Such culling (removal) of stock is essential for increasing productivity potential. In the absence of effective slaughter policy and meat export for higher returns through economic demand driven growth in buffalo production and utilization, there would be increased number of aged and unproductive buffaloes which would affect sustained production but also results in breed deterioration and adverse effect on buffalo prospects. Salvaging buffalo calves through early weaning and rearing on weaning diets has been proposed for increasing buffalo production potential.

d. **Amending States' Animal Preservation Acts:** In respect of buffalo slaughter regulations states of UP, Rajasthan, Haryana, Punjab, Delhi, Tamil Nadu, Odisha etc. have no bans or restrictions. In these states despite no ban/restrictions buffalo prospects have substantially increased and playing an important role in society for last 5-6 decades. Hence, Altogether 'buffalo' reference could be deleted from the States Animal Preservation Acts, as that of U.P, Rajasthan, Punjab, Haryana, Orissa, T Netc..by all other states as well. In case of AP and Telangana there is ban on slaughter of male buffalo calf but age of calf has not been mentioned hence, age may be mentioned as 12 months so that it will be clarity to farmers for rearing MBCs for meat production.

2. **Improvements in Livestock markets services:**

Livestock markets do not have even basic facilities though the fees is collected. While agri-markets have been developed to a large extent none of the livestock markets have been improved. For implementing animal welfare measures as well as facilitating farmers and livestock traders with ease basic facilities need to be developed at least in major livestock markets. Important Requirements For Livestock Markets: Loading and Unloading facilities; Securely holding of animals; Protecting animals from extremes of weather with shade and shelter; Feeding and watering facilities; Persons for animal handling; Disease implications- Fit for slaughter certification; Isolation facilities for sick animals; Truck cleaning and washing; Attending to emergencies and treatment facilities; Animal welfare measures

3. **Augmenting buffalo supplies to promote buffalo meat exports:**

With the present buffalo population further increase in quantum export of buffalo meat is possible only with salvaging buffalo calves from early age death and rearing to larger weight. GOI-DAHD has to promote a modified scheme under National Livestock Mission to include farmers even having one to five male buffalo calves to participate and benefit from the scheme. There are about 15-20 million male calves that could be gainfully utilized for increasing quantum quality buffalo meat exports.

Table 2: Estimated availability of buffaloes and meat production potential in 2025

Category	**Est. Population in 2025as per 20th Census (m)**	**Est. buff-aloes for Meat (m)**	**Meat Production (lakh MT)**	**Value Realization**	
				$ (m)	Rs.(Crores)
MBC under 1 year	27.00	23.63	23.52	12936	107369
FBC under 1 year	27.00	5.00	5.00	2750	22825
Adult males- over 2 years	3.59	0.54	0.54	243	2017
Adult females-over 3years	56.00	12.32	12.73	5728	47547
Total	**113.59**	**41.49**	**41.79**	**21657**	**179753**

Assumptions and estimates:

Each 10 lakh MBCs reared for meat 1/3rd number each to 250 kg, 300 kg and 350kg live wt (boneless meat yield of 82.5, 100 and 116kg respectively) would produce about one lakh MT boneless meat.

Rates for veal taken as US$ 5500/MT and buffalo meat from adults at US$4500/ MT as boneless.

US$ conversion at Rs 83 per US$. With FMD Control and eradication programme becoming successful about 20 percent of quality tender meat cuts would fetch price of 6500 US$ per tonne. Hence, concurrent efforts are necessary to realize the full export potential of buffalo meat export by 2025-2030.

4. Why subsidy scheme for salvaging and rearing male buffalo calves (MBC):

The export of buffalo meat has reached from a mere 2000MT in 1969 to about 14 lakh MT in the recent years. Any further growth in buffalo meat quantum exports is possible only with the salvaging and rearing buffalo male calves to optimum weights. As an initial step rearing 10 lakh MBC with a subsidy of Rs 7000 per calf over a period of 5 years at an estimated cost of Rs 700 crores would result one lakh MT of boneless quality veal that would fetch about Rs 5376 crores (10 lakh MBC when reared one third each to 200, 250, 300 kg live weight would produce about one lakh MT boneless veal and edible offals; at an average rate of US$5500 per MT and Rs85 per US$ would result 4675 crores and from by products at 15% of export value Rs701 cr with a total Rs 5376 crores). The scheme to benefit individual farmers having 1-5 MBC need to be implemented along with restoring of the earlier entrepreneur scheme under NLM (National Livestock Mission) providing Rs 7000 per MBC salvaged and reared to optimum weight at 18-24 months and live weight of 200-350 kg. Export plants shall pay to farmer account (based on animal identification and Adhar linked bank account) price of MBC as per its live / carcass wt. Meat plants communicate details of calf identification, weight, farmer details to the DAH (State) for paying subsidy amount to farmer bank account.

The subsidy component shall be reimbursed to the State (DAH) through Apeda/ NABARD/ from the GOI-DAHD. This scheme would be a win win situation with establishing FMD- disease free zones and justifying investments.

5. **Establishment of model buffalo calf salvaging units in urban dairies:**

Elite female buffaloes yielding 15-20 litres of milk per day are brought to urban dairies from rural areas along with young calf or during late stage of pregnancy. Due to lack of facilities and neglect calf mortality is about 95-98% in urban dairy complexes. There is depletion of superior buffalo germplasm. Efforts to salvage calves from early death to sustain superior germ plasm need to be reared in rural areas. Model units for salvaging urban and periurban buffalo calves from early death with package of practices are not available. Extension programmes on these aspects are also necessary. Salvaging units should comprise:

- Calf collection and early rearing centres in urban areas and,
- Calf growing units in rural areas

Dairy cooperatives could establish more such units throughout the country once success of these units is established. Financial support to popularize the programs should be provided.

6. **ICAR Animal Science Institutes R and D programs for increasing farmers income:**

Following projects need to be considered by ICAR-Animal Science Institutes/ Vety. Univs in particular Central Institute for research on Buffaloes (ICAR_CIRB-Hissar): National Dairy Research Institute (ICAR-NDRI) Karnal; IVRI-Izatnagar; ICAR-DFMD; ICAR-NRCM. DAHD-GOI need to impose target oriented programs of strategic research to ICAR for promoting need based R and D activies from ICAR-Animal Science Division.

a) Nutritional interventions for low cost-efficient meat production from male buffalo calves
b) Studies on replacing milk with soya milk at optimum level during early age calf nutrition
c) Crop diversification for fodder production and value addition through buffalo production
d) Evaluating sweet potato crop for augmenting livestock feed resources-
e) Supporting extension programs and Model field demonstration of male buffalo calf rearing.
f) Studies on Pre-slaughter interventions for improved glycogen status.
g) Taking proactive role in establishment of FMD disease free zones in the prospective states
h) Demonstrating risk free status of RT-PCR positive frozen deboned buffalo meat samples.
i) Identifying prospective processed meat products for export.
j) Conducting National Residue Monitoring of Meat and Meat products
k) Program for developing auxiliary (supportive) staff to assist official vets-in meat inspection.

LP 6.4. Recent Advances in Buffalo Meat Processing in India and the Role of AI/ML in Enhancing Global Competitiveness

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ABSTRACT

India is the world's largest producer and exporter of buffalo meat and buffalo (carabeef) has become one of the country's most important livestock-based export commodities. Over the last two decades, the Indian buffalo meat sector has undergone a remarkable transformation—from largely unorganized slaughter practices to modern, export-oriented abattoir-cum-processing plants operating under HACCP, ISO 22000, FSSAI regulations and importing-country standards. Significant advances have occurred in hygienic slaughterhouse design, ante-mortem and post-mortem inspection, chilling and deboning technologies, value-added product development, advanced packaging, cold-chain logistics and by-product utilization.

At the same time, the global meat sector is transitioning into the era of "Meat Industry 4.0", where artificial intelligence (AI): machine learning (ML): computer vision, spectroscopy, robotics and Internet of Things (IoT) systems are being deployed to optimize productivity, standardize quality, enhance traceability and ensure compliance with increasingly stringent sanitary and phytosanitary (SPS) standards. For India's buffalo meat sector—traditionally competitive on price and volume—embracing these frontier technologies is essential to achieve quality differentiation and sustain global competitiveness.

This article reviews recent advances in buffalo meat processing in India across the value chain, critically analyses strengths, weaknesses, opportunities and challenges and discusses in depth how AI and ML can be integrated into farm management, slaughter and processing operations, quality and safety assurance, supply-chain management, export compliance and strategic market positioning. A practical roadmap is proposed for transforming the Indian buffalo meat sector from a cost-driven commodity exporter to a digitally enabled, quality-focused global leader in safe, sustainable, high-value buffalo meat products.

Keywords: *Buffalo meat, carabeef, India, meat processing, value addition, artificial intelligence, machine learning, computer vision, hyperspectral imaging, carcass grading, traceability, global trade.*

1. Introduction

Buffaloes (*Bubalus bubalis*) are central to India's livestock economy, contributing significantly to milk production, draught power and meat. While cows enjoy legal and cultural protection in many Indian states, buffaloes constitute the major source of bovine meat for both domestic use and export. Indian buffalo meat—often referred to internationally as carabeef—is valued for its relatively lean character, halal suitability and competitive pricing. In the early 1990s, buffalo slaughter and meat trade in India were dominated by small, unorganized slaughterhouses with limited hygienic control. Since then, driven by export opportunities, regulatory pressures and changing consumer expectations, the industry has progressively upgraded to modern abattoir-cum-processing plants equipped with mechanized slaughter lines, rapid chilling systems, hygienic deboning rooms, advanced packaging equipment and integrated cold-chain infrastructure. Parallel advances in meat science—such as understanding muscle biochemistry, rigor mortis, tenderization, oxidative stability and microbiological ecology—have been applied to optimize product quality and shelf life. However, the competitive landscape is changing. Importing countries are demanding higher levels of traceability, animal welfare, residue control and sustainability. Consumers increasingly expect transparent information, consistent quality and convenient, ready-to-eat meat products. Major meat-exporting nations are investing heavily in digitalization and AI-based solutions for carcass grading, quality prediction, robotics and logistics optimization. India's buffalo meat sector can no longer rely solely on low cost; it must also demonstrate technological sophistication and data-driven quality assurance.

The objective of this article is threefold:

1. To summarize recent advances in buffalo meat processing in India, from slaughter to export.
2. To examine the strengths, weaknesses, opportunities and challenges of the sector in the present global context.
3. To explain how artificial intelligence and machine learning can be used strategically to enhance quality, efficiency, traceability and global competitiveness.

2. Global and Indian Context of Buffalo Meat

2.1 Global Importance of Buffalo Meat

Globally, beef and buffalo meat together constitute a major proportion of red meat consumption. While beef dominates in many Western countries, buffalo meat has a strong presence in South and Southeast Asia, parts of the Middle East and North Africa. Buffalo meat is particularly favoured where:

- Halal requirements are mandatory.
- Lower-cost red meat is preferred by middle and lower-income consumers.
- Lean meat with relatively lower intramuscular fat is desired for health reasons.

As global incomes rise and urbanization increases, demand for animal protein—including red meat—continues to grow, especially in developing economies. However, this growth is accompanied by heightened concerns about food safety, zoonotic diseases, antimicrobial resistance and environmental sustainability. Exporting countries are therefore under pressure to demonstrate compliance with higher-level standards relating to animal health, slaughter hygiene, cold-chain integrity and traceability.

2.2 India's Role in the Global Buffalo Meat Trade

India has emerged as the largest producer and exporter of buffalo meat. The export-oriented buffalo meat industry developed rapidly from the late 1990s onwards, largely based in states such as Uttar Pradesh, Maharashtra, Andhra Pradesh, Telangana and Bihar. Export plants are registered with national agencies, certified for HACCP and ISO 22000 and approved by importing countries after rigorous inspection.

Key features of India's buffalo meat export profile include:

- High volumes of boneless, frozen buffalo meat (mostly primal and sub-primal cuts).
- Major export destinations in Southeast Asia, Middle East and Africa.
- Strong price competitiveness compared to beef from Brazil, Australia and the USA.
- Halal slaughter and certification as standard practice.

This strong baseline position provides India with an excellent platform to build a next-generation, high-value buffalo meat industry, provided that it addresses existing weaknesses and adopts cutting-edge technology.

3. Recent Advances in Buffalo Meat Processing in India

In recent years, significant progress has been made at almost every stage of the buffalo meat value chain. The following sections outline the technological and infrastructural advances that have improved safety, quality and marketability.

3.1 Modern Abattoir Design and Hygienic Slaughter

3.1.1 Shift from Traditional to Scientific Slaughter

Traditional municipal slaughterhouses were often characterized by:

- Poor lairage and animal handling facilities.
- Inadequate separation of clean and dirty operations.
- Manual hoisting and floor dressing of carcasses.
- Minimal record-keeping and quality monitoring.

In contrast, modern export abattoirs now incorporate:

- Scientific lairage design with non-slip flooring, adequate space, water availability and stress-minimizing handling.
- Mechanized slaughter lines where carcasses are suspended on overhead rails, minimizing floor contact and cross-contamination.
- Dedicated zones for skinning, evisceration, washing and chilling, with strict one-way flow of product and personnel.
- Comprehensive sanitation programs, including clean-in-place (CIP) systems, hot-water knife sterilizers and verified sanitation standard operating procedures (SSOPs).

These improvements have directly contributed to lowering microbial loads and enhancing the hygienic quality of buffalo carcasses.

3.1.2 Ante-Mortem and Post-Mortem Inspection

Veterinary inspection at the plant is now more systematic, covering:

- Ante-mortem inspection: assessment of animal behaviour, signs of disease, injuries and compliance with welfare considerations.
- Post-mortem inspection: visual, palpation and incision-based examination of carcasses and organs, with condemnation of diseased parts and carcasses as per regulations.

Increasingly, plants maintain digital records of inspection outcomes, which can later be integrated with AI-based traceability systems.

3.1.3 Environmental Management and By-Product Recovery

Modern abattoirs have moved towards:

- Effluent treatment plants (ETPs) to treat blood, fat and organic waste, ensuring compliance with discharge norms.

- By-product processing units to convert hides, bones, offal, blood and fat into value-added products (gelatin, bone meal, tallow, pet food ingredients, etc.).
- Controls on odour emissions, solid waste and noise pollution, improving community acceptance and environmental sustainability.

3.2 Advances in Chilling, Freezing, Deboning and Portioning

Temperature management from the moment of exsanguination is crucial for meat safety and quality.

3.2.1 Rapid Chilling and Control of Rigor Mortis

Indian plants now use:

- Pre-chillers and blast chillers to rapidly bring down carcass temperatures from ~37°C to below 7°C within specified time limits.
- Optimized chilling regimes to avoid cold shortening and maintain tenderness.

Some plants have experimented with electrical stimulation of buffalo carcasses to accelerate post-mortem glycolysis, improve sarcomere length and reduce variation in tenderness.

3.2.2 Deboning and Trimming Under Controlled Conditions

Deboning is typically done in specially designed rooms maintained at 10-12°C. Improvements include:

- Hygienic stainless-steel tables, conveyor belts and specialized knives.
- Standard cutting patterns (primal and sub-primal schemes) tailored to export markets.
- Ergonomic design to reduce worker fatigue and potential for contamination.
- Digital yield records to monitor and improve cutting efficiency.

Trimmings are segregated by fat content to ensure consistent raw material for minced meat and emulsion-type products.

3.2.3 Portion Control and Retail-Ready Cutting

For higher-value segments, plants increasingly produce:

- Steaks and portion-controlled cuts with defined thickness and weight.
- Ready-to-grill or ready-to-cook cuts marinated or spiced, targeting domestic supermarkets and export retail markets.

Computerized portioning systems and inline weighing scales assist in meeting tight specifications demanded by international buyers.

3.3 Development of Value-Added and Convenience Buffalo Meat Products

Historically, the bulk of export buffalo meat trade involved frozen boneless blocks. Recent years, however, have seen diversification into value-added products, both for domestic and export markets.

3.3.1 Emulsion-Type Products

Buffalo meat has been extensively used in:

- Frankfurters and other sausages.
- Nuggets, patties, meatballs and spreads.

Advances in formulation include:

- Use of various binders and extenders (soy proteins, starches, fibres) to improve texture and yield.
- Incorporation of functional ingredients (omega-3 oils, plant fibres, probiotics) to support health claims.
- Optimization of emulsification, chopping and cooking processes to enhance emulsion stability and sensory quality.

3.3.2 Restructured and Marinated Products

Buffalo meat, especially from older animals, can be tenderized and restructured to produce:

- Restructured steaks using binders (e.g., transglutaminase, alginate-calcium systems).
- Tumbled and injected products, where brines containing salt, phosphates, spices and functional ingredients are infused to improve juiciness and flavour.
- Vacuum-marinated buffalo meat cubes and strips for stir-fry and ethnic dishes.

3.3.3 Ready-to-Cook (RTC) and Ready-to-Eat (RTE) Products

A major trend is the development of:

- RTC products: marinated buffalo kebabs, burger patties, spiced mince, etc.
- RTE products: retort pouched curries, biryanis, stews and gravies with extended ambient shelf life.

Retort processing, sous-vide cooking and controlled thermal processes are used to achieve microbial safety, sensory stability and long shelf life.

3.4 Packaging Innovations for Buffalo Meat and Meat Products

Packaging is central to quality maintenance, especially for long-distance exports.

3.4.1 Vacuum Packaging (VP)

Vacuum packaging removes headspace air, thereby:

- Reducing oxidative rancidity and pigment oxidation.
- Extending refrigerated shelf life by inhibiting aerobic spoilage flora.
- Minimizing freezer burn during frozen storage.

High-barrier multilayer films with good sealing properties are standard in export plants.

3.4.2 Modified Atmosphere Packaging (MAP)

For chilled retail cuts and high-value products:

- High oxygen MAP (e.g., 70% O_2 / 30% CO_2) can maintain a bright red colour, though care is needed to control oxidation.
- Low oxygen MAP with CO_2 and N_2 is used for extended shelf life, especially for processed buffalo meat products.

MAP systems must be designed carefully considering buffalo meat's darker pigmentation and lipid composition.

3.4.3 Vacuum Skin Packaging (VSP)

VSP tightly molds a clear, high-barrier film to the surface of the product placed on a tray:

- Enhances visual appeal and premium perception.
- Reduces drip loss and purge accumulation.
- Improves shelf life and merchandising value.

It is particularly useful for branded buffalo steaks and portion-controlled cuts.

3.4.4 Retort Pouch Packaging

Retort pouches made of multilayer laminates (e.g., polyester/aluminium foil/cast polypropylene) allow:

- Thermal sterilization at 115-121°C to achieve commercial sterility.
- Ambient shelf life of 1-2 years for RTE buffalo meat curries and gravies.
- Lightweight, flexible and space-saving packaging ideal for exports, institutions and defence rations.

3.5 Strengthening of Cold Chain and Logistics

Buffalo meat exports depend critically on an unbroken cold chain from plant to destination.

- Refrigerated trucks are now widely used to transport carcasses/blocks from abattoirs to ports.
- Modern plants use loggers and data recorders to monitor product temperature continuously.
- Export consignments are shipped in reefer containers with automated temperature control, alarms and remote monitoring options.

The cold chain has improved significantly, but gaps still exist in domestic distribution and in linking small farmers and municipal slaughterhouses to modern processing units.

3.6 Quality Assurance, Certification and Regulatory Compliance

Modern buffalo meat plants operate under:

- Good Manufacturing Practices (GMP) and Sanitation Standard Operating Procedures (SSOPs).
- Hazard Analysis and Critical Control Point (HACCP) systems that identify and control biological, chemical and physical hazards.
- National regulations (e.g., FSSAI standards) and importing-country requirements (residue limits, microbiological criteria, etc.).

Certification against ISO 22000 / FSSC 22000, Halal standards and sometimes BRCGS or equivalent standards has become common, particularly for plants targeting high-end markets.

4. Sectoral Strengths, Weaknesses, Opportunities and Challenges

4.1 Strengths

- Large and growing buffalo population, providing a stable supply base.
- Competitive production and processing costs.
- Longstanding experience in exporting to multiple markets.
- Established Halal certification and practices.
- A core of modern, export-oriented abattoir-cum-processing plants.

4.2 Weaknesses

- Fragmented production: many smallholder farmers with limited integration into organized supply chains.
- Variable animal quality and age at slaughter, leading to inconsistent meat traits.
- Lack of a standardized, widely-accepted carcass grading system specifically for buffaloes.
- Limited digitalization and data capture at the farm and slaughter levels.
- Domestic slaughterhouses and retail channels still oftenlag behind in hygiene and infrastructure.

4.3 Opportunities

- Development of premium young buffalo meat segments with assured tenderness.
- Expansion of value-added and branded RTE/RTC buffalo meat products domestically and for export.
- Use of AI, ML, computer vision and spectroscopy to standardize quality and build trust in high-value markets.
- Positioning buffalo meat within sustainable livestock and circular economy narratives via by-product utilization and improved environmental performance.

4.4 Challenges

- Sanitary and phytosanitary (SPS) barriers, including concerns about FMD or other transboundary diseases.
- Increasing global competition from Brazil, Australia and other exporters.
- Heightened consumer scrutiny on animal welfare, antibiotics usage and environmental impact.
- Rapid technological advancements requiring capital investment, skilled manpower and institutional support.

5. Role of Artificial Intelligence and Machine Learning in Buffalo Meat Processing

AI and ML can be deployed throughout the buffalo meat value chain. Below, we discuss key domains where these technologies can make a transformative impact.

5.1 AI/ML Fundamentals in the Meat Context

AI refers broadly to computational systems that perform tasks typically requiring human intelligence, such as pattern recognition, decision-making and prediction. ML is a subset of AI where algorithms learn patterns from data and improve performance with experience. In meat processing, typical data inputs include:

- Images and videos (carcass surfaces, meat cuts).
- Spectral data (NIR, hyperspectral, fluorescence).
- Sensor data (temperature, humidity, vibration, pressure, energy usage).
- Traceability data (animal ID, weight, health records, transport history).
- Quality and safety measurements (pH, colour, microbial counts).

Common techniques include:

- Supervised learning (regression, classification) for quality prediction, grading and defect detection.
- Unsupervised learning (clustering, anomaly detection) for identifying unusual patterns, process drift, or emerging problems.
- Deep learning (especially convolutional neural networks, CNNs) for processing images and spectral data.
- Reinforcement learning for optimizing complex processes and control systems.

5.2 AI for Buffalo Production and Pre-Slaughter Management

Although this article focuses on processing, AI and ML can also support production and pre-slaughter management:

- Predictive models for growth, body condition and carcass yield based on breed, diet and management.
- AI-enabled health monitoring, including behaviour analysis, early disease detection and reproductive management using sensors and video analytics.
- Decision-support systems for selecting animals for slaughter at optimal age and body condition, improving consistency of meat quality.

Well-managed, AI-assisted pre-slaughter systems create a more uniform raw material base for the processing sector.

5.3 AI-Enabled Carcass Grading and Classification

One of the most promising applications is computer vision-based carcass grading.

5.3.1 2D and 3D Imaging

High-speed cameras placed along the slaughter line capture images of carcasses from multiple angles. These images are processed using ML models to extract features such as:

- Carcass length, width and surface area.
- Fat cover, lean-fat distribution and external defects.
- Bruises, lesions and contamination spots.

3D imaging systems can reconstruct carcass geometry, providing more precise measures of volume and shape.

5.3.2 Prediction of Yield and Quality

AI models can be trained to predict:

- Primal and sub-primal yields from each carcass.
- Meat-to-bone ratio, trimming losses and boneless yield.
- Potential tenderness based on conformation, fat score and other features.

Objective, automated grading enables:

- Transparent payment systems (e.g., paying suppliers based on quality grades rather than just weight).
- Consistent allocation of carcasses to different product lines (premium steaks vs. processing meat).
- Building of buffalo carcass grading standards comparable to beef grading systems in other countries.

5.4 Non-Destructive Quality Assessment Using AI

Conventional meat quality assessment relies on destructive sampling and laboratory analysis, which are slow and expensive. AI combined with non-destructive sensing technologies offers real-time alternatives.

5.4.1 Hyperspectral and Multispectral Imaging

Hyperspectral imaging captures spectral information at hundreds of narrow wavelengths for each pixel in an image. ML algorithms can link spectral signatures to:

- pH, colour and water-holding capacity.
- Fat content and oxidized pigments.
- Surface contamination or incipient spoilage.

In buffalo meat, hyperspectral systems can be used to:

- Detect DFD-like (dark, firm, dry) or PSE-like conditions.
- Monitor colour changes during storage.
- Sort cuts based on predicted quality traits.

5.4.2 Near-Infrared (NIR) Spectroscopy

Portable NIR devices can be used inline or at-line to predict:

- Moisture, protein, fat and collagen content.
- Adulteration of buffalo meat with other species.
- Degree of cooking and water activity in processed products.

AI models trained on calibration datasets can instantly infer these properties, aiding process control and product standardization.

5.4.3 Electronic Nose and Tongue

Electronic noses and tongues use arrays of chemical sensors and pattern-recognition algorithms to mimic human smell and taste. They can:

- Detect volatile compounds associated with spoilage.
- Distinguish between fresh, chilled and marginally spoiled buffalo meat.
- Evaluate flavour profiles and batch-to-batch consistency in processed products.

5.5 Robotics and Automation in Buffalo Meat Processing

Robotics combined with AI can address labour, safety and yield challenges.

5.5.1 Vision-Guided Cutting and Deboning

Robotic systems equipped with cameras and depth sensors can:

- Identify anatomical landmarks on buffalo carcasses and cuts.
- Execute precise cutting paths using ML-trained models.
- Adjust in real time to variations in carcass size and shape.

Such systems can increase yield, reduce repetitive strain injuries for workers and maintain high-speed processing.

5.5.2 Robotic Handling and Packaging

Robots can be used to:

- Load/unload trays, cartons and pallets.
- Place products accurately into thermoformed packs or trays.
- Seal and label packages with minimal human contact.

This improves hygiene, reduces labour dependency and enhances consistency.

5.5.3 Predictive Maintenance

Sensors attached to motors, compressors, conveyors and refrigeration units capture vibration, temperature and other performance indicators. ML algorithms:

- Identify patterns preceding equipment failure.

- Trigger maintenance alerts before breakdown occurs.
- Optimize maintenance schedules and spare-part inventory.

In buffalo meat plants, predictive maintenance reduces unscheduled downtime, protects product from temperature abuse and saves energy.

5.6 AI for Process Optimization and Energy Management

AI models can analyse multivariate plant data to:

- Optimize chiller temperatures, air flow and loading patterns.
- Adjust cooking, smoking, or retort schedules for consistent product outcomes.
- Minimize energy consumption while maintaining product safety and quality.

For example, reinforcement learning algorithms can gradually fine-tune chiller set points to balance microbial safety, weight loss and energy costs.

5.7 AI in Supply-Chain Management, Traceability and Export Strategy

5.7.1 IoT-Enabled Cold-Chain Monitoring

Sensors in refrigerated trucks and containers transmit:

- Real-time temperature and humidity data.
- Door-open events and location updates.

AI algorithms can:

- Detect anomalous patterns (e.g., intermittent warming events).
- Estimate cumulative thermal abuse and its likely impact on shelf life.
- Recommend corrective actions or re-routing if problems arise.

5.7.2 Blockchain and Digital Traceability

Blockchain-based systems, when combined with AI:

- Provide tamper-resistant records of animal origin, slaughter date, processing steps and logistic history.
- Allow buyers to verify compliance with halal standards, animal welfare and residue limits.
- Support recall management by rapidly identifying affected batches.

AI can analyze traceability data to identify patterns of quality problems, high-risk suppliers, or routes with frequent cold-chain breaches.

5.7.3 Demand Forecasting and Pricing

Using historical trade data, currency movements, seasonal consumption patterns and competitor activity, AI models can:

- Forecast demand for buffalo meat in different countries.
- Suggest optimal product mix and shipping schedules.
- Enable dynamic pricing strategies to maximize margins while maintaining competitiveness.

Such tools help exporters move from reactive to proactive market management.

5.8 AI for Regulatory Compliance and SPS Risk Management

AI can assist in:

- Early detection of disease outbreaks by analysing livestock health data, surveillance reports and environmental factors.
- Predicting risk of border rejections based on microbiological testing trends, residue reports, or non-compliance history.
- Automating documentation and validation for export certification, reducing administrative workload and errors.

In the long run, regulators may also use AI to monitor compliance, making it imperative for industry to adopt compatible digital systems.

6. Strategic Roadmap for Integrating AI/ML into India's Buffalo Meat Sector

To move from isolated pilot projects to large-scale transformation, a structured roadmap is needed.

6.1 Building the Digital Foundation

- Standardized data collection: just as HACCP requires documented procedures, AI requires well-structured, quality-checked data.
- Electronic animal identification and farm-to-plant data transfer systems.
- Implementation of Manufacturing Execution Systems (MES) that integrate production, quality and maintenance data.
- Cloud-based platforms where data from multiple plants can be aggregated (with appropriate privacy safeguards) for model training.

6.2 Collaborative R&D and Pilot Projects

- Joint projects between ICAR institutes, veterinary universities, engineering colleges and industry to develop buffalo-specific AI models.
- Pilot deployment of carcass imaging systems, NIR/HSI sensors and predictive maintenance tools in selected export plants.
- Validation of AI predictions against conventional laboratory and sensory data to build confidence.

6.3 Capacity Building and Human Resource Development

- Inclusion of AI/ML, data analytics and digital quality systems in meat science curricula at undergraduate and postgraduate levels.
- Short courses and certification programs for plant managers, quality assurance personnel and veterinarians.
- Encouraging a new profile of "meat data scientist" capable of bridging animal science and data science.

6.4 Policy and Incentive Mechanisms

- Government schemes that support investments in sensors, imaging equipment and digital infrastructure.
- Recognition of AI-based quality assurance and traceability systems in export promotion schemes.
- Development of buffalo-specific carcass grading standards and regulatory frameworks that leverage AI for implementation.

6.5 Branding and Communication

- Positioning Indian buffalo meat as "digitally assured", emphasizing traceability, consistency and safety.
- Use of QR codes and digital labels that allow importers and consumers to view product journey, quality metrics and certifications.
- Participation in international food expos with live demonstrations of AI-enabled grading and traceability to signal technological leadership.

7. Conclusion

India's buffalo meat sector has travelled a long path—from unorganized, small-scale slaughter to modern export-oriented processing under stringent hygiene and safety standards. Advances in abattoir design, chilling and processing technologies, packaging, value addition and cold-chain management have enabled India to become a major global supplier of buffalo meat. However, the next phase of global competition will be shaped not just by cost and volume but by data, digital assurance and technological sophistication. AI and ML provide a powerful set of tools to:

- Objectively grade carcasses and standardize quality.
- Predict and control meat quality and shelf life using non-destructive sensing.
- Automate cutting, deboning and packaging, improving yield and worker safety.
- Optimize processes and energy usage.
- Enhance cold-chain monitoring, traceability and regulatory compliance.
- Support intelligent market forecasting and strategic export management.

By systematically building digital infrastructure, investing in collaborative research, training human resources and aligning policies and branding with this technological trajectory, India can transform its buffalo meat industry from a cost-driven commodity exporter into a globally respected, quality- and technology-led powerhouse. This transformation is fully aligned with the broader vision of Viksit Bharat, where advanced technologies are harnessed to ensure food security, economic growth and international leadership in the protein economy.

LP 6.5. Ethno-Veterinary practices for sustainable buffalo development in India

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Introduction

Buffalo (*Bubalus bubalis*) keeping contributes substantially to household food security, employment generation and poverty reduction. It has the ability to convert the locally available biomass not suitable for human consumption (grass, leaves, twigs, agricultural crop residues) into useful products such as milk, yogurt, cheese, paneer, lean meat etc. required for better human health. Besides, buffaloes provide nutrient rich manure for growing crops and provide draught power as an important source of energy. Buffalo milk, richer in fat and protein, supports dairy-based economies, while their resilience to poor-quality feed and resistance to diseases enhances their suitability for smallholder farming systems. Furthermore, buffalo meat is leaner and more nutritious compared to beef. (Singh, I., Kaur, S. (2025); https://doi.org/10.1007/978-981-96-8552-3_1. Because of its extremely nutritive milk, leaner meat and top draught power for wet conditions, buffalo proposes extreme ability for the enhancement of living standard of mankind Buffaloes are well known for their diet versatility, remarkable resistance to diseases and flexibility to a wide range of dwelling, feeding and managemental circumstances (Surendra Ku etal-2021 J.of Animal Production). Male buffaloes are strong and persistent draft animals, particularly useful for pulling heavy loads in muddy, swampy or deep soil conditions. A key advantage is their stamina, which can allow them to work longer and more consistently than bullocks in specific environments. With 109.85 million heads, India ranks first in world buffalo population. Buffaloes serve as security for difficult times, like a saving account, to be used in times of crisis.

Background

In last two decades, the use of modern technologies have resulted in increased milk production and contributed significantly to the national economy. But the research has developed technologies for intensive and industrialized dairy product system in urban and semi-urban area benefiting rich livestock farmers. The generated technologies have either not reached the deep rural poor farmers or the farmers have not adopted them due to high cost involved in formal health system, lack of text materials in local language, inability of the trained modern animal health practitioners to reach the needy animal keepers at the time of emergency or non-availability of the medicines and veterinary aids in proper time, cost and place.

In the other hand, over hundreds of years, indigenous animal healing systems have been evolved as simple solutions to produce livestock in a sustainable manner. These practices relating to animal healing and many other allied activities are diffused to people over the years and the system became more refined and time tested in the hands of millions (Anil Gupta- Honey Bee,Vol-4). Repeated and successful uses of such indigenous knowledge system such as ethno veterinary practices have been popular and beneficial. People get benefited by these readymade simple solutions. This indigenous knowledge system contributes immensely to the rural economy as it involves very low and affordable cost to the poor farmers by use of local herbs and other natural resources.

Hence, in last decade, efforts at different Governmental and non Governmental level have been made to diffuse the traditional animal healing system/ ethno veterinary medicines of India (Honey Bee, Anil Gupta etal 1998; Nature Heals, Jayaveer Anjaria et al 2000, Indigenous Veterinary Medicine, Vivekananda 2005) for sustainable livestock development.

a. Why Herbal healings for livestock?

Herbal/traditional remedies are practiced since time immemorial. A number of such herbs and plants are available, but lack of documentation of successful remedies and awareness is causing lack of interest by the successive generation. Most of the animals are herbivorous, easily accept herbs and are self-healers.

Herbs are part of our environment and indigenous herbal formulations form the bedrock of our medicine system, which is rooted back to thousands of years. Herbal medicines are tested over hundreds of years in the hands of millions of people. Herbal remedies are low cost, have no side effects, address AMR issues and needs simple preparation skills. Uneducated animal keepers of rural areas, especially women, can prepare herbal formulations for their sick animals easily with little of training as they are grown up with these herbs and spices since birth.

Religious attachment of rural mass of our society with the plants increases faith on herbal formulations. Use of herbal formulations by people increases biodiversity products use, which ultimately leads to better natural resource management by people. Most importantly, antibiotic resistance issues will be not there in this ethno-vet practice adoption.

b. **Herbs Are Neutraceuticals**

- Animals easily accept herbs
- Herbs are natural
- Easy available
- Many domestic animals including buffaloes are self-healers
- Herbs are part of our environment. Indigenous herbal formulations form the bedrock of our medicine system, which is rooted back to thousands of years.
- Herbal medicines are tested over hundreds of years in the hands of millions of people.
- Herbal remedies are low cost.
- These have no side effects.
- Needs simple preparation skills.
- Uneducated buffalo keepers of rural areas can prepare herbal formulations for their sick animals easily with little of training as they are grown up with these herbs since birth.
- Religious attachment of rural mass of our society with the plants increases faith on herbal formulations.
- As it is low input based technology, it will have sustainable effect on livestock development.
- It discourages subsidy dependent and project dependent livestock development
- In remote villages, Tribal area Where formal education is yet to reach, the lower strata of our society, gets benefitted
- Indigenous herbal technology helps organic production of animal food and fibers like meat, milk, eggs, wools skin etc.
- There is increase in worldwide demand for consumption of organic milk, meat, eggs and other animal products.
- This has increased the avenues for export of organic animal based food and fiber to other countries and earn foreign currencies.

c. Antimicrobial resistance (AMR) and Herbal Medicines: In view of increase in per capita consumption of milk and milk products animal food industry has been rapidly influenced by changes in livestock management, including a rapid transition towards concentrated animal-feeding operations (CAFOs): antibiotics, growth stimulating factors and other intensive forms of livestock agriculture, This leads to an important phenomenon of Antimicrobial resistance (AMR) in Humans and invading our soil, air and water system. This needs to be addressed by use of alternate medicines like Herbal and Ethno veterinary medicines. These alternate medicines are not only naturally available but also are user friendly and often low cost. This system of alternate medicine will not only reciprocate the human and animal welfare but also lead to biodiversity and plant health conservation

Issues Addressed

A. Buffalo Bio Diversity- The Indian states come under the tropical geo -climatic condition and are famous for its indigenous cattle resources in one hand and its abject poverty in other. About 27-30% of rural households keep buffaloes as an alternate source of income generation and are like “Bank Accounts” for these local poor. Buffalo keeping has been a strong weapon in poverty reduction, labour migration as well as a source of food security. India homes 22 numbers of defined buffaloes (Native breeds by NBAGR). The most buffalo habitating Indian states are Uttar Pradesh, Rajasthan, Gujarat, Haryana, Punjab and some NE states. Due to the tropical climatic condition and lack of suitable and timely animal health services to rural poor at low cost, the productive capacity of livestock decreases causing huge economic loss.

B. Sustainable Animal Health Services- At present, the animal health system is controlled by the state Animal Husbandry Dept. who provides animal health services through veterinarians, formally trained Para Veterinary workers who also perform multifarious activities and are not available at time of urgency

C. Food Security-It has been observed that buffalo keeping in small holding is the key answer to poverty reduction in rural areas. Small is beautiful. These small milch animal units should be self-sustainable and low cost based. Low external input based animal husbandry is the best practice of sustainable buffalo development. Animal rearing based and relying on price consuming health care system will be contrary to the sustainability.

D. Climate Change-Climate change has badly affected agriculture including dairy where weather has been unpredictable. It has also affected buffalo keeping, causing occurrences of more seasonal pests and diseases, disappearance of swampy, fallow lands, less availability of food grains, more demand on low cost food materials, nutriceuticals etc. There is need to reach people with simple solutions like low cost based animal herbal healing and natural food formulations for animals.

E. **Resilience among men and women in villages-**In villages, small livestock keepers, pastoralists, school drop outs and women keep buffaloes for earning livelihood. But they are not aware of many diseases, scientific feed management and rational approach in many climate change related health problems like worm infestations, bloat, diarrhea, mastitis etc for which they lose livestock. This herbal system helps them.

F. **Women and Buffalo Keeping-** Role of women in cow keeping is very important. Usually women folk rear buffaloes, feed them and look to their well being. To empower the rural women by adding to their income, attention should be given to build up their capacity. Generally women folk are shy in approaching formal animal health providers, in fear of high cost for treatment of their animals. They always prefer low cost animal treatment based on herbal and indigenous knowledge. Due to their strong belief and easy access to this system, it is required to have an in-depth study.

Some outstanding ethno-veterinary practices used in Buffaloes

1. Diarrhoea

Unhygienic food and water causes diarrhea. Organisms, such as bacteria, enter the body, replicate in intestine and cause diarrhoea. It is associated with foul smell and shooting loose motion. Diarrhoea causes loss of water and salts from the body of the animals which make them dull, inactive and lying low. Diarrhoea can be treated with an easy yet effective practice. For this Harida/Chebulic myroban (*Terminalia Chebula*)is used along with yogurt or curd from cow/Buffalo milk or goat milk. *Terminalia chebula* contains chebulin, chebulic acid and yogurt contains RNAse enzyme. Both elements help stop the activities of the pathogenic organism and stop diarrhoea. (Pathe Pathshala-FAO- TECA

Path: https://teca.apps.fao.org/teca/en/technologies/10055)

2. Anoestrus

A heifer or an adult buffalo, comes to heat or oestrus for breeding. If a heifer, buffalo does not come to heat, then there is a problem due to lack of hormones and as a consequence they cannot become mothers. This condition of not coming to heat iscalled as anoestrus.

Herbally the cases of anoestrus in animals is treated with seeds of nutmeg. Nutmeg (*Myristica fragrans*) seeds are also used as spices forfood.

Scientifically nut meg contains myristic acid, elemicin, safrole, trimyristin, macelignan and other phytohormones like molecules which help inbringing heifers and cows to heat.

One nutmeg seed (*Myristica fragrans*) locally called Jaiphal is grounded to powder and mixed with 20 to 25 g of jaggery. A bolus from themixture is prepared and offered to the cow. After a month (21 to30 days): the animal comes to heat. If not, the process can be repeated.

Path: https://teca.apps.fao.org/teca/en/technologies/10057

3. Mastitis

Milk is secreted from the udder of the mother cow. Due to infections, the udder becomes swollen leading to blood coming out with the milk.

Mastitis can be treated effectively using betel leaves (*Piper betel*) and castor oil prepared from castor seed (*Ricinus communis*). The two components are mixed and offered to treat mastitis.

Scientifically, castor oil contains ricinoleic acid, methyl ricinoleate, ricinolein. And betel leaves contain piperine and piperidene. These bimolecular prevent and controls infection and mastitis is controlled.

Path: https://teca.apps.fao.org/teca/en/technologies/10058

4. Repeat breeding and early abortion

Repeat breeding and early abortion cases in Buffaloes are due to fewer hormones and/or due to infection. These two cases are treated by *Aloe vera* juice. *Aloe vera* juice-50 grams mixed with 50gms of jiggery and 50 gms of wheat flour and a bolus is prepared. This bolus is offered orally once a day for continuous 3-5 days

5. Bloat/ Tympani

Bloat/Tympani condition in consequence to over feeding, faulty feeding is treated by Betel leaves and Ginger. Betel leaves 5 numbers and Ginger of 20 gms are made to paste separately and mixed together. This is offered once and repeated after 2 hours. Piperene, Piperidine in betel leaves and Gingine, Gingiverine in ginger helps reducing gasses (Anjaria etal -2000 Nature Heals). Including above practices there hundreds of ethno veterinary practices, documented and practiced across India.

Conclusion:

All dairy animals including buffaloes, cows, camel and sheep goats are herbivorous and adapt well to regenerative medicines like ethno veterinary medicines. These medicines are mostly herbal regimens and are easily available and at low cost. Goal of sustainable buffalo development can be reached with ethno veterinary medicines.

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(About the author- Dr Balaram Sahu is a veterinarian with expertise on Veterinary Virology and ethno veterinary medicine practices. He has been bestowed with a National Award instituted by DST, GOI for his outstanding contribution in the field of Communicating Science and Technology in 2011-2012. He has authored 14 books out of which a book called " Agar Aaspas Pashu Doctor Na Ho" in Hindi on Ethno-Veterinary Practices is very popular in India. He conducts road side classes, Pathe Pathshala- A school on move for communicating simple and effective Herbal Healing Technologies to livestock keepers in different parts of India).

Oral Presentation

OP-6.1. State-wise Assessment of Age at First Calving in Murrah Buffaloes Under Field Conditions in India

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Age at First Calving (AFC) is a key fertility trait influencing lifetime productivity, generation interval and economic returns in dairy buffalo systems. This study evaluated the variation in AFC among Murrah buffaloes reared under smallholder conditions across ten major buffalo-rearing states in India. A dataset of 527 Murrah heifers with complete AFC records was categorized into two biological classes: AFC between 2-3 years (ideal to acceptable range) and AFC >3 years (delayed calving). Descriptive and state-wise distribution analyses were performed to quantify the extent of delay as well as identify regional patterns in management-related fertility constraints. Results revealed that only 12.3% (n=65) of Murrah buffaloes achieved AFC within the biologically desirable range of 2-3 years, while a large proportion (87.7%, n=462) calved for the first time beyond 3 years, indicating a serious delay in sexual maturity and successful conception across states. Uttar Pradesh (n=265) and Maharashtra (n=51) contributed the highest number of delayed AFC cases due to their large sample representation. Mean AFC estimates showed considerable regional variability, ranging from 1493 days in Rajasthan to 2799 days in Karnataka. Across all states, the overall mean AFC was 1599 days (≈4.38 years): significantly higher than the recommended benchmark of 36-40 months for Murrah buffaloes. The findings highlight substantial management-driven delays in achieving optimal AFC, likely associated with nutritional deficiencies during early growth, inadequate heat detection, poor estrus expression in buffaloes, delayed breeding decisions and field-level reproductive inefficiencies. States with higher mean AFC also showed smaller proportions of animals calving within the ideal biological window. This study underscores the urgent need for targeted interventions such as improved heifer nutrition, systematic reproductive monitoring, early-age estrus detection training for farmers and structured breeding advisory services. Establishing region-specific heifer development programs and strengthening AI delivery systems will be essential to reduce AFC and enhance lifetime productivity of Murrah buffaloes in smallholder dairy systems across India.

OP-6.2. Effect of *Moringa oleifera* leaf meal supplementation on the performance and economical returns of Murrah buffaloes under loose housing system.

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The present investigation has been undertaken to study the effect of *Moringa oleifera* leaf meal supplementation on performance of lactating Murrah buffalo under loose housing system. Eighteen lactating Murrah buffaloes were assigned to three dietary treatment groups (T_1, T_2 and T_3) resulting in six lactating buffaloes per treatment based on parity. The experiment was conducted for a period from 21 days prepartum to 5 months of lactation to find the effect of supplementation of MOLM in following treatments, namely, T_1(control)- Feeding as per ICAR standards (2013): T_2- Feeding as per ICAR standards (2013) + 75 g MOLM/animal/day, T_3- Feeding as per ICAR standards (2013) + 150 g MOLM/animal/day. Significantly ($P<0.05$) higher milk yield (kg) and 6% FCM (kg) were reported in T_3 treatment group as compared to T_1. The statistical analysis of the data revealed that daily dry matter intake was significantly ($P<0.05$) higher at 4th, 5th and 6th months in experimental buffaloes of treatment groups than the control group. The perusal data on economics demonstrated that in terms of feed cost/kg milk yield, there was a net profit of Rs. 0.30 in T_2 treatment and Rs. 1.94 in T_3 treatment as compared to T_1 treatment and in terms of feed cost/kg 6% FCM, there was a net profit of Rs 1.39 in T_2 treatment and Rs. 3.03 in T_3 as compared to T_1 treatment. The findings of the present study inferred that MOLM supplementation increases production performance and is economic in lactating Murrah buffaloes. By supplementation of MOLM in ration it is possible to increase the income of dairy farmers owing to more milk production and 6% FCM content.

Keywords: Moringa, leaf meal, milk yield, feed cost and economics

OP-6.3. Male Buffalo Farming: A Low-Input Livestock Enterprise for Marginal and Resource Poor Farmers

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Male buffalo farming has emerged as a resilient and economically viable enterprise for marginal and resource-poor households across semi-urban and rural regions of India. Despite minimal investment in concentrate feeding, healthcare inputs, or structured management, this system continues to generate stable income due to strong market demand for male buffalo meat. The present analysis compared production and economic indicators from three farmer categories male buffalo rearers, rural conventional farmers and peri-urban smallholders to assess the viability and efficiency of low-input male buffalo farming systems. Male buffalo farmers exhibited substantially higher performance across key parameters, with mean sale weight of 269±5.38 kg, selling price of ₹33,625±671.98 and net income of ₹170,475±8,127.15, despite modest input expenditure (₹14,400±364.35) and zero concentrate feeding in most units. Their cost-benefit ratio (2.35±0.08) was markedly superior to rural conventional (1.30±0.07) and peri-urban farmers (1.73±0.17). Lower input dependence, longer rearing duration and access to low-cost crop residues contributed to higher profitability. In contrast, rural and peri-urban farmers faced higher proportional input costs, smaller herd sizes and shorter rearing periods, resulting in reduced income. The findings reinforce that male buffalo farming represents a practical, low-risk and scalable livelihood strategy for economically weaker households. Strengthening this sector requires targeted extension interventions focused on improved health care (deworming, vaccination): optimized housing and better market linkages to enhance profitability while retaining its low-input character.

Keywords: *Male buffalo farming, Livestock systems, marginal farmers, Extension strategies, Resource-poor households*

OP-6.4. Influence of Age on Physico-chemical Attributes of Buffalo Meat

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Meat physico-chemical characteristics—pH, WHC, cooking loss, tenderness and collagen—are key determinants of technological quality and consumer acceptance. Age-related changes in muscle physiology necessitate scientific evaluation. Longissimus dorsi and Biceps femoris muscles from buffaloes of different age groups were analyzed for pH, water-holding capacity (WHC): cooking loss, Warner-Bratzler shear force (WBSF): myofibrillar fragmentation index (MFI): muscle fibre diameter, fascicle dimensions and collagen content. Ultimate pH values showed no significant variation across age groups. WHC decreased with increasing age, while cooking loss exhibited minor fluctuations. WBSF increased significantly in older buffaloes, indicating reduced tenderness. Muscle fibre diameter, fascicle perimeter and collagen content increased progressively with age ($p<0.05$): contributing to tougher meat texture. Buffalo age significantly impacts key physico-chemical parameters, especially tenderness-related indices. Meat from younger buffaloes exhibits superior WHC, lower shear force and better tenderness, making them more suitable for high-value meat products and export markets.

OP-6.5 Identification of Upper Critical THI in *Murrah* Buffalo Calves

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The present study was conducted to determine the Upper Critical Temperature-Humidity Index (THI) in *Murrah* buffalo calves using an integrated approach comprising physiological, biochemical, infrared thermography, immune and heat-shock markers. Six healthy *Murrah* buffalo calves (8-12 months; 100-150

Kg) were subjected to controlled cyclic heat-stress (HS) conditions in a psychrometric chamber. Animals were exposed to six progressively increasing temperatures—25, 28, 31, 34, 37 and 40 °C—corresponding to THI values ranging from 73 to 90. Each temperature was maintained for 12 hours daily (0600-1800 h): while the remaining time animals were kept at 25 °C. Calves were maintained at each temperature for 10 consecutive days. Physiological variables including pulse rate (PR): respiration rate (RR): rectal temperature (RT) and body surface temperature (BST) were recorded at 1500 h on the 10th day, coinciding with blood sampling. Blood was collected for hematological profiling, plasma biochemical analysis and peripheral blood mononuclear cell (PBMC) isolation. PBMCs were used for RNA extraction, followed by qPCR using SYBR Green chemistry to quantify gene expression of Toll-like receptors (TLRs) and heat-shock proteins (HSP70, HSP90). UCT values were estimated using segmented regression analysis (SegReg Software). A clear heat-stress response was observed, with significant alterations ($p<0.05$) across multiple physiological and molecular parameters. Upper critical THI values for physiological traits were identified at 78.37: PR; 78.03: PR; 78.71: RT; and 84.93: feed intake**,** while no UCT was detected for water intake. Hematological indices exhibited UCTs at 77.36 for lymphocytes and WBC **and** 82.41 for granulocytes**.** Biochemical parameters showed UCTs for total protein and globulin at 77.36, albumin at 81.74, ALKP at 80.73, LDH at 77.87, glucose at 82.07, creatinine at 80.73 and ALT at 86.95. Redox biomarkers indicated UCTs at 78.03 (ROS) and 83.76 (SOD): while cortisol exhibited a UCT of 77.36**.** Heat-shock proteins displayed high UCTs at 85.44 (HSP70) and 84.43 (HSP90). Overall, the study demonstrates that *Murrah* buffalo calves begin to exhibit pronounced physiological, biochemical and molecular stress responses once the THI exceeds approximately 78. These findings provide strong evidence for defining a species-specific Upper Critical THI for *Murrah* buffalo calves, which can be used to implement timely heat-stress mitigation strategies and improve their welfare during summer conditions.

OP-6.6 Successful management of Hydroallantois in Buffalo: Case Report

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Developmental abnormalities have been reported in domestic animals from time to time. Fetal anomalies and monstrosities of various type have been reported in cow. Hydroallantois is a gestational disorder in which sudden increase in allantoic fluid due to foetal membrane pathology leads to bilateral enlargement of abdomen and more common in third trimester of gestation and subsequently converted into dystocia. A case of pluriperous upgraded buffalo of second parity with nine and half months of gestation. On visual examination enlargement of both side abdomen was observed. After the per-rectal examination there was balloon like distended and tense abdomen was found without the palpation of fetus. On per-vaginal examination tense balloon like protruding structure was palpated covering the almost whole vaginal space. On the basis of breeding history and clinical examination case was tentatively diagnosed as hydrollantois. Induction was done for the parturition by giving the Valthamate bromide, Dexamethsasone, Cloprostenol sodium and Estradiol benzoate as per standard dose. Fluid therapy was also started to cope up with expected sudden removal of large amount of water as generally occurred in this type of cases. After 18 hrs of induction therapy a gentle massaging of cervical os was done and that leads to opening of birth canal within two hrs. A dead male fetus was extracted out with manual interventions. A large amount (approx. 70-80 lit) of fluid was also drained out. Examination of placenta reviled the degenerated and liquefied cotyledons. Buffalo was uneventfully recovered with panty of fluid therapy along with other supportive medicines.

Key word: Hydroallantois. Buffalo, Cotyledones, Dystocia

Poster Presentation

PP-6.1. SWOT-Based Assessment of Farmers' Perceptions on Service Delivery by Animal Husbandry Assistants in Rythu Bharosa Kendras

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Rythu Bharosa Kendras (Farmers assurance centres) became a one stop solution for livestock farmers in Andhra Pradesh state. Through these centres, Animal Husbandry Assistants (AHAs) provide essential livestock and dairy-related services directly at the farmers' doorstep, enhancing accessibility and support. The present study is conducted to identify the strengths, weaknesses, opportunities and threats faced by AHAs as perceived by farmers during the service delivery. The data was collected from a total of 90 farmers through simple random sampling from nine districts of Andhra Pradesh and responses were recorded through direct interview. *Ex-post facto* research design is used for the study. The swot analysis revealed that major strength of AHAs was their greater acceptance with the local community and promptness in responding. The key weaknesses identified were the inability of AHAs to meet farmers expectations due to insufficient technical knowledge, inadequate training. Among opportunities, farmers perceived that effective use of mass and social media for information dissemination as the most promising, followed by enhanced social participation of farmers in departmental programmes. With respect to challenges, farmers ranked their preference for veterinarians over AHAs and inadequacy in the skill sets of AHAs. Overall, the findings highlight both the potential and limitations of the service delivery system and stress the need for continuous capacity building, adequate input supply and structured training programmes to strengthen the effectiveness of AHAs operating through Rythu Bharosa Kendras.

Keywords: *Animal Husbandry Assistants, dairy animals, farmers, SWOT analysis*

PP-6.2. Determinants, Constraints and Conservation Imperatives of Swamp Buffalo Rearing in North-Eastern India

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According to the 20th Livestock Census (2019): India recorded 109.85 million buffaloes achieving the largest buffalo population globally with an increase of 1.1% compared to the 2012 census. In contrast, several North-eastern states show a declining trend. In Manipur, the buffalo population dropped to 0.036 million, reflecting a sharp decline of 45.41% from 2012. Assam also recorded a reduction, with its buffalo population decreasing to 4.22 million, a decline of 3.11%. Swamp buffaloes are recognized as a distinct germplasm in the Northeastern Region; however, their numbers remain low and insufficiently documented, making them a priority for conservation interventions. The present study aims to investigate the livelihood significance, management practices and key constraints associated with swamp buffalo rearing. It also examines farmers' perceptions related to conservation perspectives. The study was conducted in Manipur and Assam, where a sample of forty farmers was selected using a combination of purposive and random sampling techniques. Data were collected through a semi-structured interview schedule and the responses were systematically scored and analyzed using appropriate statistical methods. The findings indicated that almost all swamp buffaloes are reared traditionally under zero-input management conditions. Adult males and females are commonly used as single animals for ploughing paddy fields with locally designed wooden ploughs. Inadequate veterinary manpower and limited farmer awareness, economic low returns, lack of government incentive, insufficient boundary fencing leads to loss of animals through straying, low productivity are among the main challenges identified. Given the severe decline in the swamp buffalo population, immediate intervention through targeted breeding and conservation programs is essential. Only a limited number of swamp buffaloes remain in the country, making the preservation of their distinct germplasm an urgent priority.

Keywords*: Disease resistance, draught, government incentives, grazing lands, wetlands*

PP-6.3. Ultrasonography Led Field Extension Interventions for Strengthening Farmer's Income by Enhancing Reproductive Efficiency

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Reproductive disorders remain a persistent constraint for smallholder buffalo herds in the sub-Himalayan Tarai region of Uttar Pradesh, where field-level incidence typically includes anoestrus (10-30%): repeat breeding (8-16%): uterine infections (6-15%): ovarian cysts (4-10%): hydrometra (1-4%) and early embryonic death (4-16%). Routine per-rectal (PR) examination, though widely practiced, frequently misses early pregnancies, underestimates ovarian and uterine pathology and contributes to repeated inseminations, delayed treatment and preventable economic losses. Portable ultrasonography (USG): with its real-time visualisation and superior tissue differentiation, offers both diagnostic precision and strong extension value by enhancing farmer understanding and confidence.

To evaluate the effectiveness of portable ultrasonography, lead reproductive health camps as extension services across multi-district rural dairy systems, with specific aims to:

1. Compare the diagnostic performance of USG versus per-rectal examination for common reproductive disorders in buffaloes.
2. Assess farmer's perception towards ultrasonography-based reproductive examination.

Over a three-year period, reproductive camps were conducted across Bareilly and adjoining districts, examining *n=368 buffaloes* through paired evaluation initial PR examination followed by portable USG using established reproductive criteria. Farmer perception was assessed through a structured Likert-scale questionnaire administered before and after USG demonstration. Diagnostic agreement and improvement were analysed using McNemar's test, whereas perception change was evaluated using paired t-tests with descriptive statistics. Participation was voluntary and anonymised.

Ultrasonography significantly outperformed PR across virtually all reproductive conditions. Pregnancy detection improved from 258/368 (70.1%) by PR to 345/368 (93.8%) by USG ($\chi^2=62.1$; p<0.0001). Similar gains were observed for anoestrus (14.1% vs 22.8%; p<0.0001): repeat breeding (11.1% vs 14.7%; p=0.0062): uterine infections (4.9% vs 11.1%; p<0.0001): ovarian cysts (3.8% vs 8.7%; p=0.0003): hydrometra (1.1% vs 4.1%; p=0.0086) and early embryonic death (1.9% vs 5.7%; p=0.0031). USG provided definitive differentiation between early pregnancy, hydrometra, pyometra, mummification, adhesions and cystic ovarian disorders conditions that frequently overlap on PR examination. Field impact included early pregnancy diagnosis in repeat breeders, avoidance of culling or sale of pregnant animals and detection of contextual contributors such as high RBS in sugarcane-fed herds. Farmer feedback showed substantial improvement in trust, clarity of decision-making and compliance with AI and treatment schedules following visual USG explanations.

Portable ultrasonography markedly enhanced the accuracy of reproductive diagnosis and reduced misclassification in smallholder buffalo herds of western Uttar Pradesh. Coupling PR with USG forms a low-cost, high-impact, scalable strategy that strengthens reproductive efficiency, minimises economic losses and elevates the effectiveness of buffalo-focused extension services.

Keywords: *Buffalo; Diagnostic accuracy; Field extension; Pregnancy diagnosis; Ultrasonography.*

PP-6.4. Influence of microclimatic modifications on THI and physiological parameters in loose housed lactating buffaloes

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Heat stress adversely affects the physiological mechanisms governing thermal regulation and maintenance energy. To mitigate these effects, this study was designed to evaluate the impact of microclimatic modifications on the physiological parameters of Murrah buffaloes. Eighteen lactating Murrah buffaloes were divided into three treatment groups (n=6) in a loose housing system: (T_1) Concrete flooring with corrugated asbestos roofing (control); (T_2) Glass wool (50 mm) on the false ceiling and white paint on the upper side of the roof; (T_3) Expanded polyethylene sheet (70 mm) on the ceiling and white paint on the upper side of the roof. The study was aimed to assess the effect of microclimatic modifications on the THI and physiological parameters of Murrah buffaloes. THI were significantly ($P<0.05$) lower in both T_2 and T_3 treatments as compared to control group. Physiological parameters including respiration rate, pulse rate and panting score, were significantly lower ($P<0.05$) in microclimatic modified sheds as compared to Control. The elevated THI results in increased physiological strain, leading to heightened respiratory and circulatory activity to dissipate heat. In conclusion, microclimatic modifications, significantly reduced heat stress, improved thermal comfort and enhanced the overall performance of lactating Murrah buffaloes.

Keywords*: Murrah buffaloes, microclimatic modifications, asbestos, glasswool, physiological parameters, panting score.*

PP-6.5. Ecological and Economic Gradients in Buffalo Rearing: A Comparative Sustainability Assessment of Peri-Urban, Urban and Rural Systems in the Tarai Belt of Uttar Pradesh

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Buffalo rearing in the Tarai region operates under contrasting ecological and market pressures. Peri-urban herds benefit from proximity to natural water bodies and access to both green fodder and urban markets, urban systems are restricted by space and environmental stress, while rural herds remain limited by poor market linkages despite good fodder availability. These systemic differences influence sustainability, economic returns and long-term germplasm quality.

To characterise and compare rearing patterns, resource utilisation, cost-benefit ratio, sustainability and system fragility across three buffalo-rearing strata peri-urban, urban and rural and to identify critical policy gaps through SWOT analysis.

A cross-sectional survey was conducted in the Tarai belt region of Uttar Pradesh. Three subgroups were selected: peri-urban (n=25): urban (n=25) and rural (n=25) buffalo farmers. Data were collected using structured questionnaires, on-farm observations and SWOT evaluation. Quantitative parameters included herd size, annual milk yield, cost-benefit ratio, calf retention rate, green-fodder availability, access to natural water resources and market distance.

Peri-urban farmers showed the strongest performance across all measurable indicators. Their mean cost-benefit ratio (1:1.72) exceeded that of urban (1:1.39) and rural (1:1.18) systems, supported by superior access to riverbank resources (84%) and year-round green fodder (76%). Urban farmers remained heavily constrained, with only 8% having access to natural water bodies and just 14% reporting reliable green-fodder availability. Calf retention patterns highlighted severe germplasm loss in urban dairy units (22%) compared with peri-urban (54%) and rural (61%). Milk price realisation was highest in urban areas (56.8±4.5 INR/L) but was offset by high production costs, frequent drainage blockage (72%) and dependence on purchased feed (91%). Rural systems, though ecologically favourable, received the lowest milk prices (38.7±2.6 INR/L) due to poor market connectivity. Overall system fragility was greatest in urban dairies (stress index

0.78): moderate in rural (0.54) and lowest in peri-urban units (0.32): confirming a clear gradient of sustainability: peri-urban>rural>urban.

Peri-urban buffalo rearing emerged as the most sustainable and economically efficient system, benefiting from both natural resource availability and proximity to high-value markets. Urban dairies were the most fragile, with environmental stress, germplasm wastage and poor sustainability indicators. Rural systems remained under-utilised due to weak market access despite favourable ecological conditions. Policy interventions including germplasm protection norms, urban waste-management strategies, rural milk-collection infrastructure and strengthening peri-urban green fodder buffers could substantially enhance the overall dairy ecosystem in the Tarai belt.

Keywords: *Buffalo production systems, Tarai region, peri-urban dairy sustainability, germplasm conservation, cost-benefit dynamics.*

PP-6.6. Advancing Buffalo Productivity Through Nanotechnology

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Nanotechnology provides innovative approaches to enhance ruminant nutrition by improving feed efficiency, nutrient utilization, animal health and overall sustainability. This review highlights the role of nano-minerals, nano-encapsulated bioactives, nano-enzymes and nano-sensors in optimizing rumen function, digestion and immune response.

Nano-minerals exhibit greater bioavailability at lower doses and may help reduce antibiotic dependence. Nano-encapsulation of essential oils, probiotics and vitamins enhances rumen fermentation, nutrient absorption and product quality. Additionally, nanotechnology enables precise nutrient delivery through encapsulation, chelation and nano-packaging without affecting the sensory properties of feed.
Nanoparticles can be inorganic, organic, or complex in nature and are synthesized using physical, chemical, or biological methods. Buffaloes play a crucial role in global food systems by converting low-quality fibrous feeds into nutrient-rich animal products. However, challenges such as rising feed costs, low nutrient efficiency, greenhouse gas emissions and increasing demand for sustainable animal-source foods necessitate the adoption of innovative, science-based solutions.

Nanotechnology—working with materials sized between 1-100 nanometers—offers promising structural and functional advantages in animal agriculture. Nevertheless, its application in buffalo nutrition requires careful evaluation of safety, environmental impact and economic feasibility. Comprehensive risk assessments and well-defined regulatory frameworks are essential for its responsible implementation.

Keywords: buffalo nutrition, nano-minerals, nano-encapsulation, feed efficiency, methane mitigation, livestock sustainability

PP-6.7. High Definition (WiFi)-Assisted (AI): A Smart Teaching and Reproductive Diagnosis Vaginal Scope for Buffalo

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The High Definition (WiFi) -Assisted Artificial Insemination device is a cutting-edge smart vaginal scope that transforms buffalo reproductive management by providing unparalleled clarity, real-time learning support and highly accurate diagnostic capabilities. This innovative AI Scope is equipped with a WiFi-enabled high-definition camera that provides real-time visualization of the vaginal and cervical structures on Android smartphones, tablets, laptops and computers without requiring an internet connection. This internal view enables accurate cervical location and precise semen deposition, ultimately improving AI efficiency and conception rates. The integrated high-intensity LED illumination and fully rechargeable design allow clear visualization of internal reproductive tissues, even in low-light conditions. Its diagnostic capability makes the device valuable for identifying reproductive abnormalities and diseases such as lesions and pus due to bacterial or viral infections, vaginal and cervical inflammation, duplex cervix and cervical stenosis (closed cervix). With a WiFi transmission range of 10-20 meters and camera resolutions of 640 × 480 and 1280 × 720 pixels, the device provides high-quality imaging in a waterproof environment, ensuring performance even in the presence of mucus or fluid. As a teaching tool, the vaginal scope supports hands-on learning by allowing students, AI workers and veterinarians to observe the insemination process and cervical morphology in real time. The device operates through the freely available "Inskam" application, enabling photo and video recording directly into the device gallery without internet access, making it ideal for demonstrations and training sessions. Its portability, ease of handling and cost-effectiveness make this technology suitable for field use, particularly in rural and tribal areas with limited connectivity. Affordable and requiring no specialized training, the HD WiFi-assisted AI vaginal scope offers a practical advancement in teaching, artificial insemination and reproductive diagnosis in buffalo.

Keywords: *HD WiFi, Vaginal scope, Reproductive diagnosis, Teaching tool*

PP-6.8. Digital Engagement in Indian Dairy Farming: ICT Accessibility, Usage Patterns and Implications for Sustainable Livestock Management

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The dairy sector in India plays a crucial role in rural livelihoods, directly supporting over 80 million farmers and contributing nearly 5% to the national GDP. As the largest employer in agriculture, dairy farming has become an essential secondary income source, especially empowering women, marginal farmers and landless laborers. Importantly, about 73% of India's milk production comes from small and marginal farmers, with output growth mainly driven by increasing herd size rather than productivity improvements. However, the sector faces ongoing challenges, including insufficient feed and fodder resources, rising input costs, low genetic potential of animals, poor breeding practices, limited extension services, delayed veterinary care and variable milk prices. The study explores the adoption and accessibility of Information and Communication Technologies (ICTs) in dairy farming, showing YouTube as the most popular platform for dairy-related information, followed by television, social media, mobile apps, websites, radio and the Kisan Call Centre (KCC). While mobile applications and YouTube were consistently accessible, platforms such as radio, TV, websites, KCC and social media were accessed only intermittently, with 28% of respondents finding KCC completely inaccessible. YouTube was the most frequently used tool daily, while television, radio, websites, social media and mobile apps were mostly used weekly. The findings highlight the transformative potential of digital tools in sharing knowledge, expanding market access and improving veterinary services. The study recommends targeted policy actions, enhancement of digital infrastructure and AI-enabled advisory systems to maximize the use of ICT and support sustainable and inclusive dairy farming in India.

Keywords: *Small ruminants, ICT adoption, digital tools, ICT Utilization pattern*

PP-6.9. Urinary Retention in Buffalo Calves Under Changing Climatic Conditions: Risk Factors, Health Impacts and Surgical Interventions

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Retention of urine in neonatal and young buffalo calves is an emerging clinical concern, with multifactorial roots including early-age castration, hormonal disruption, nutritional imbalance and climate-driven stressors. This study- synthesizes observed patterns, production and health impacts, common complications and practical interventions to build resilience in buffalo production under changing climatic conditions. Clinical caseloads from field clinics and polyclinic IVRI indicate an increased frequency of urinary retention episodes following pre-pubertal castration (in goat) and in buffalo calves exposed to heat stress and suboptimal diets. Early castration may alter androgenic support for lower urinary tract development; concurrent protein-energy deficits and mineral imbalances (notably calcium and chloride) compound neuromuscular dysfunction. High ambient temperatures and water scarcity are hallmarks of climate variability exacerbate dehydration, concentrate urine and predispose calves to urolithiasis and obstructive retention. Affected calves show reduced growth rates, poor feed conversion, decreased future fertility potential and elevated mortality risk where complications occur. Socioeconomic consequences for smallholders include treatment costs, lost rearing investment and reduced draft/milk prospects. Prompt

decompression via tube cystostomy or catheterization is effective for acute retention; tube cystostomy is recommended when urethral passage is impossible or when long-term drainage is required. Adjunctive medical therapy includes urinary acidifiers and correction of electrolyte/mineral deficits to dissolve calculi and restore urine flow. Nutritional rehabilitation, balanced energy, adequate protein and appropriate mineral supplementation prevents recurrence. Farmer awareness campaigns about timing of castration, hydration strategies during heat, balanced feeding and early veterinary referral are critical. Complications such as cystorrhexis, uroperitoneum and septic peritonitis demand urgent surgical intervention and carry guarded prognosis. Integrating surgical readiness (tubecystostomy capability): targeted nutritional programs, climate-adaptive husbandry and owner education offers a pragmatic pathway to reduce urinary retention incidence and improve buffalo calf resilience.

Keywords: *Acidifier, Retention of urine, Early castration, Climate change, Tube cystotomy*

PP-6.10. Integrating Artificial Intelligence and Climate-Smart Extension Approaches for Sustainable Buffalo Production: A Comprehensive Review

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The water buffalo (Bubalus bubalis) is vital for the global protein supply, but its production faces increasing threats from climate change, mainly through heat stress, feed shortages and disease risks. Conventional extension methods struggle to deliver the timely, precise and context-specific management strategies required to build resilience against these challenges. This paper offers a comprehensive review of how integrating Artificial Intelligence (AI) and Climate-Smart Extension (CSE) can enhance the sustainability and resilience of buffalo production systems. The review carefully examines how AI technologies, such as machine learning (ML): computer vision and IoT-enabled precision livestock farming (PLF): can transform buffalo management. Specific focuses include developing early-warning systems for heat stress using behavioral and physiological data, predictive analytics to improve feed efficiency and reproductive health and strategies for reducing greenhouse gases. Importantly, the review explains how CSE serves as a vital link, leveraging AI-based, data-informed insights to provide tailored, real-time advisory services to smallholder farmers via mobile and digital platforms. The combination of AI's prediction capabilities and CSE's localized knowledge sharing creates a promising pathway toward climate-resilient buffalo farming. Although challenges like data infrastructure, technical skills and digital literacy are acknowledged, this review emphasizes the need for targeted policy support and capacity-building efforts to ensure fair access and adoption of these integrated solutions among vulnerable farming communities.

Keywords: Artificial Intelligence, Climate-Smart Extension, Sustainable Buffalo Production

PP-6.11. Role of Male Buffalo Calves in Strengthening the Indian Meat Sector

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Buffaloes are highly adaptable, disease-resistant and can thrive on diverse diets and management systems, making them well-suited for meat production. India, the world's largest buffalo meat exporter, home to 54.6 % of the world's buffalo population, produces over 1.6 million tonnes of buffalo meat annually, which accounts for roughly 43% of global production. Although buffalo meat production and exports have increased, the industry primarily relies on spent and unproductive animals and per-capita consumption is only 2 kg per year. Male buffalo calves, about 10 million each year, are largely culled because they do not contribute to milk production, causing a huge economic loss. Male buffalo calves, in particular, are often undervalued and have traditionally received little attention from dairy farmers. These calves exhibit a high growth rate of 478 g per day and an efficient feed conversion ratio of 6.0-6.5, achieving a body weight of 350 kg within 16-18 months under intensive feeding conditions. Meat from these calves provides a dressing percentage of 50-55 %, with low fat (~4 %): high protein content, desirable texture, making it suitable for premium export cuts. A calf purchased for Rs 3,000-4,000 can be fattened to 350 kg at a cost of Rs 9,000-10,500, yielding a net profit of Rs 7,000-10,000, recoverable over 2-3 production cycles. Government initiatives, including the SMBC scheme under the National Livestock Mission and NABARD subsidies, aim to encourage buffalo rearing and strengthen associated infrastructure. However, major constraints such as insufficient cold-chain infrastructure, limited slaughterhouse capacity and low farmer awareness keep the national slaughter rate at only 10 percent. There is an urgent need to implement a pragmatic policy for the slaughter and utilization of male buffalo calves for meat, without imposing restrictions on their disposal or use. With proper management and feeding practices, slaughter rates of up to 20 percent can be maintained without negatively impacting population sustainability.

PP-6.12. AI and sensor-based approaches for enhancing and optimizing buffalo production

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Artificial Intelligence (AI) and sensor technologies are rapidly gaining attention and are now widely used in the livestock sector. Their application in buffalo farming has led to significant improvements in reproduction, productivity and animal welfare, while also addressing critical issues such as heat stress. Wearable sensors, thermal cameras and real-time physiological monitors allow continuous tracking of temperature, rumination, locomotion and heart rate, enabling faster interventions when animals are stressed, ill, or in estrus. AI-guided estrus detection systems have increased insemination accuracy and improved conception rates by up to 25%, while fixed-time artificial insemination protocols have boosted reproductive scheduling efficiency by 30-40%. Smart cooling systems using infrared thermography and automated ventilation have reduced heat-stress-related productivity losses by 35-40% and mortality rates during peak summer have declined by up to 20% on farms using such technologies. Milk yields have increased by 15-30%, with sensor-based monitoring enabling early disease detection, reducing treatment costs by 12-20% and minimizing milk withdrawal periods. Precision feeding systems powered by AI have improved feed conversion efficiency by 10-18%, reducing feed wastage by nearly 25%. These tools also contribute to environmental sustainability by lowering methane emissions per liter of milk by 8-15%. While barriers such as initial investment costs, digital literacy and infrastructure gaps remain, the growing availability of user-friendly, cost-effective AI platforms is closing the adoption gap. With the right support, AI and sensor integration holds the potential to modernize buffalo production into a smarter, more profitable and climate-resilient system.

Keywords: Precision Livestock Farming, wearable sensors, thermal camera, milk production, estrus detection

PP-6.13. Effect of Age on Carcass Characteristics of Female Buffaloes

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[1A]Presenter

Buffalo meat constitutes a major share of India's red-meat export sector. However, carcasses derived predominantly from older, spent animals often exhibit poor quality attributes. Understanding the influence of slaughter age on carcass traits is essential for improving yield and market value. Female buffaloes of four age groups were evaluated for live weight, carcass weight, dressing percentage, carcass length, backfat thickness and rib-eye area. Standard carcass measurement protocols were followed in a commercial abattoir. Advancing age significantly increased live weight, carcass weight, carcass length and rib-eye area ($p<0.05$). Backfat thickness showed a progressive rise with age, indicating greater subcutaneous fat deposition in older buffaloes. Dressing percentage demonstrated minor fluctuations across age groups but did not show statistically significant variation. Age markedly influences carcass development in female buffaloes. Younger buffaloes yield smaller but more uniform carcasses, while older animals produce heavier carcasses with larger rib-eye areas and thicker fat cover. These findings highlight the need for age-based segregation at slaughter for optimizing carcass value in the buffalo meat industry.

PP-6.14. Trend Analysis of Artificial Insemination, Calving Performance, and Female Calf Births in Buffaloes under the BAIF in Uttar Pradesh

Vinod Potdar*, Amit Kundalkar, Shivaji Sontakke, Vikas Tiwari, Akshay Bind, Vinod Shende, Prabhat Singh, Santosh Bansal, Abhishek Pande, V.B. Dyasa, Pratik Kudale, Sachin Joshi and Jayant Khadse
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The present study aimed to analyse long-term trends (2015–2025) in artificial insemination (AI), calving performance (CP), and female calf birth ratios in buffaloes under the different BAIF Project in Uttar Pradesh. Data compiled from district and zonal reports were used to evaluate the year-wise and zone-wise efficiency of reproductive interventions. The analysis revealed a substantial rise in AI and CP activities, with improved female calf ratios during recent years, indicating enhanced genetic and managerial efficiency. The AI-to-calving performance ratio, used as an efficiency indicator, improved by nearly 15 % over the decade, suggesting better conception and calving outcomes per insemination. Growth rate analysis showed positive trends in all parameters, with the eastern and central zones showing the highest improvements. These findings highlight the effectiveness of sustained AI programs and field management under BAIF's integrated breeding interventions.

Keywords: *Buffalo, Artificial Insemination, Calving Performance, Female Calf Ratio, Trend Analysis, Uttar Pradesh.*

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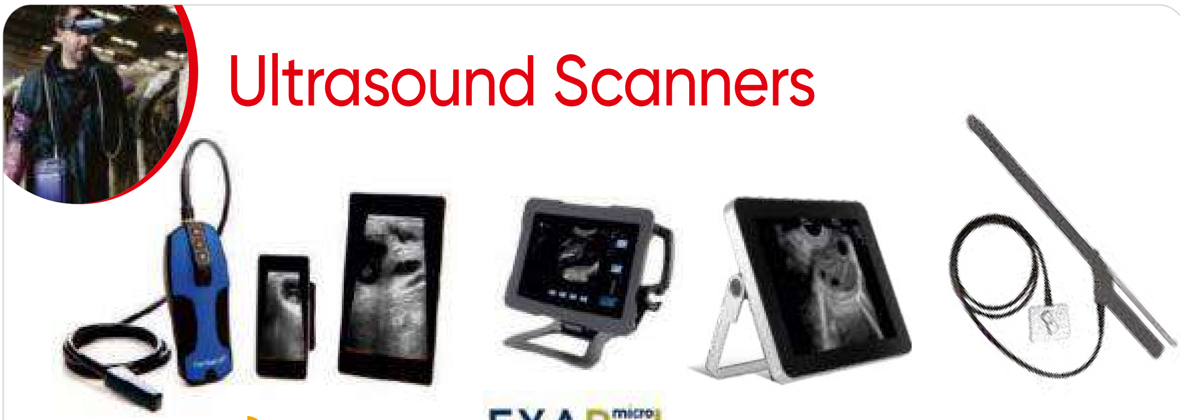

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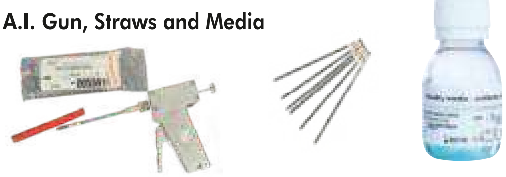

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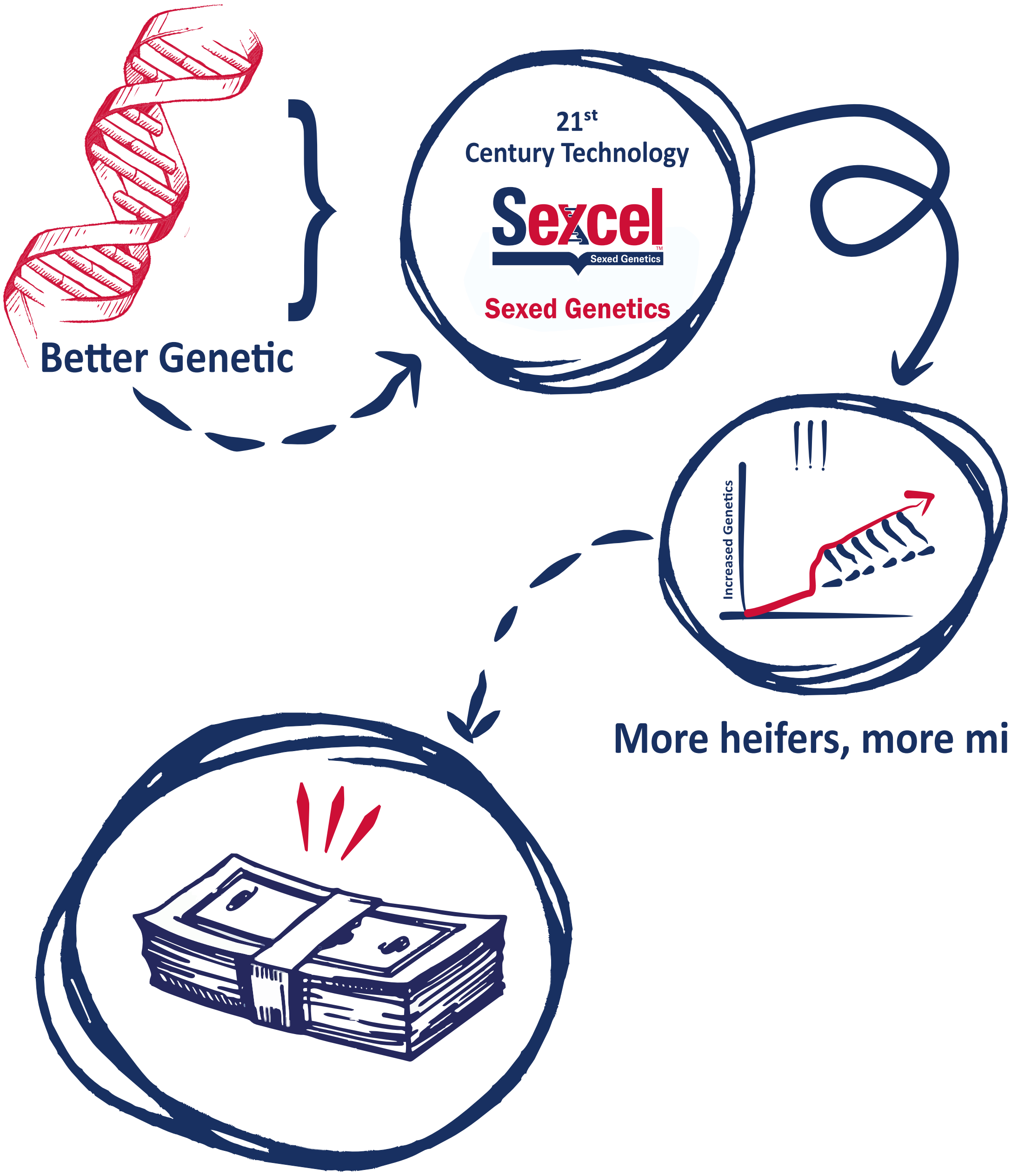
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