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ABSTRACT E-BOOK

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Buffalo Milk as a Functional Shield for Metabolic Health and Defense Against Micro- and Nanoplastics

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The global rise in metabolic disorders, including insulin resistance, type 2 diabetes mellitus (T2DM), and colorectal cancer (CRC), represents a major public health challenge with profound clinical, social, and economic implications. Recent evidence suggests that the burden of these conditions can be further exacerbated by exposure to micro- and nanoplastics (MNPs), pervasive environmental pollutants recognized as severe a threat to human health. MNPs exposure through food, water, air, and consumer products is a potent driver of oxidative stress, inflammation, endocrine disruption, metabolic dysregulation, and carcinogenic processes. These converging
challenges underscore the urgent need for innovative nutritional interventions capable of preserving metabolic homeostasis while mitigating the adverse health effects associated with environmental exposure to MNPs.
The latest advances in the use of buffalo milk as a functional food to preserve metabolic health and counteract the adverse effects of MNPs will be discussed. Emphasis will be placed on the molecular and cellular mechanisms through which buffalo milk and dairy products exert beneficial effects in the prevention of metabolic diseases. Owing to its distinctive composition, including betaines, carnitines, bioactive peptides, essential fatty acids, antioxidants, and microRNAs, buffalo milk has the potential to support metabolic homeostasis and attenuate MNPs-induced toxicity by modulating epigenetic and redox-sensitive pathways. Harnessing this potential requires multidisciplinary and translational research to validate the efficacy of buffalo milk-derived bioactive compounds as evidence-based nutritional strategies for disease prevention and health promotion. In this context, farming practices, animal nutrition, and environmental conditions play a pivotal role in shaping the bioactive composition of buffalo milk, reinforcing a holistic farm-to-health perspective aligned with the One Health approach.

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Strategies to enhance the functional profile of buffalo milk through innovative management and Precision Livestock Farming techniques

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The buffalo plays a pivotal socio-economic role in both developed and developing countries, particularly due to its remarkable adaptability to harsh environmental conditions and climate change. It is a triple-purpose animal that supplies milk, meat and draught power, supporting all-round agricultural development. Furthermore, they are highly efficient at utilizing low-quality roughage, are highly resistant to disease, and produce milk with a higher nutritional value than cattle. In this context, buffalo milk is of great importance in tropical agricultural systems, supporting the livelihoods of small-scale farmers, while representing a premium raw material for high-value dairy products such as Mozzarella di Bufala Campana PDO. In Italy, however, buffalo farming has reached a high level of innovation compared to dairy cattle farming: for this reason several challenges persist, including issues related to production efficiency, reproductive performance, and animal welfare. Consequently, extensive research has been carried out to face with these problems and enhance the functional profile of buffalo milk through technological advancements. Functional foods are defined as foods that offer specific health benefits beyond mere nutrition, and have received significant attention from consumers. Recent research has highlighted the presence of bioactive compounds in buffalo milk that are associated with functional food properties. Among these, betaines and carnitines (such as *γ-butyrobetaine*, *acetylcarnitine*, *propionylcarnitine, glycine betaine* and *δ-valerobetaine*), which are produced during anaerobic fermentation in the rumen have attracted increasing attention. δ-Valerobetaine (N,N,N-trimethyl-5-aminovaleric acid) is synthesised by rumen microbiota during anaerobic oxidative deamination of Nε-trimethyllysine and glycine. It is found in aqueous extracts of buffalo milk and has antioxidant and anti-inflammatory properties. Experimental evidence indicates that it can reduce the inflammatory damage caused by high glucose concentrations in endothelial cells through the modulation of the SIRT1/SIRT6-NFκB metabolic pathway. In addition, emerging studies suggest that betaines may exert cytotoxic activity against several cancer cell lines, including breast, tongue, larynx and colon cancers. The presence of δ-valerobetaine and γ-butyrobetaine (the immediate biosynthetic precursor of L-carnitine) in ruminant milk suggests that buffalo milk may have broader health-promoting properties. An interesting aspect is that the concentration of these functional biomolecules can be modulated by applying innovative management techniques that improve animal welfare. For example, providing 10–15 m² of space per head does not affect milk yield, but increases the concentration of these molecules in milk. Furthermore, several nutritional strategies, such as the administration of green forage, have been shown to improve the functional properties of buffalo milk. The inclusion of fresh green forage has been associated with improvements in the functional profile of milk, likely due to its influence on rumen microbiota composition and activity. In particular, shifts in microbial populations capable of producing carbohydrate-active enzymes (CAZymes) may promote the synthesis of bioactive metabolites. Similarly, the use of feedstuffs rich in simple sugars, which partially replace dietary starch in the diet, has been reported to positively influence the metabolic pathways involved in the production of these compounds, thereby improving the nutritional and functional characteristics of milk. In this scenario, Precision Livestock Farming (PLF) technologies represent a promising frontier for optimizing buffalo production systems. PLF can be defined as the continuous, automated, real-time monitoring of the physiological, behavioural and productive parameters of livestock using advanced sensor technologies and information systems. By systematically collecting and integrating high-resolution data at herd and individual levels, PLF enables data-driven decision-making, which may improve production efficiency, animal welfare and environmental sustainability. The application of PLF tools may therefore facilitate the implementation of targeted nutritional and management strategies that enhance the functional quality of buffalo milk in a sustainable manner. Overall, improving the health-promoting properties of buffalo milk through integrated approaches that combine animal nutrition, welfare, and technological innovation represents a key strategy to meet the growing consumer demand for natural and sustainable dairy products. Enhancing milk quality directly at the farm level offers a valuable alternative to post-processing interventions, contributing to the development of clean-label dairy products with increased functional value.

Keywords: Water Buffalo Milk, Functional Foods, Precision Livestock Farming (PLF), Animal Welfare.

Buffalo Dairy Products as Functional Foods: From Nutritional Value to Human Health

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Buffalo milk and its derivatives, including mozzarella, yogurt, fermented products, and whey fractions, represent nutrient-dense foods with potential benefits for human health. Compared with bovine milk, buffalo milk generally contains higher concentrations of proteins, lipids, calcium, phosphorus, and fat-soluble vitamins. Its proteins may also release bioactive peptides during digestion or fermentation, with preliminary evidence suggesting antioxidant, antimicrobial, immunomodulatory, and blood-pressure-regulating activities. Fermentation can further enhance functionality by generating bioactive metabolites, improving digestibility, and delivering beneficial microorganisms that may support intestinal barrier function and microbiota homeostasis. These effects may promote gastrointestinal well-being by supporting a balanced gut microbiota and preserving intestinal barrier integrity. However, the higher energy and saturated-fat content of some buffalo dairy products require appropriate portioning, particularly in individuals at cardiometabolic risk. Although experimental findings are promising, robust human intervention studies remain limited. Future research should therefore define clinically relevant outcomes, identify the most active components, and evaluate how processing, fermentation, and dietary context influence their effects. Buffalo-milk derivatives should be regarded not only as traditional foods of high nutritional value but also as promising candidates for the development of evidence-based functional foods.

Introducing Genomics in Buffalo Breeding: From Enabling Technologies to Global Opportunities

Stefano Biffani

The introduction of genomics in buffalo breeding represents one of the most relevant methodological transitions for the genetic improvement of this species. For many years, buffalo selection relied almost exclusively on pedigree, phenotypic recording, and conventional genetic evaluations, while genomic tools remained largely underdeveloped compared with dairy cattle. This gap was particularly limiting for a species of major economic, cultural, and social importance, ranging from highly specialized dairy systems to smallholder and multipurpose production systems across different continents.

A first decisive step was the development and validation of buffalo-specific SNP genotyping platforms. The design of the Axiom Buffalo Genotyping Array 90K provided, for the first time, a medium-density SNP tool suitable for genomic analyses in river buffalo populations. This resource enabled studies on population structure, genomic relationships, genetic diversity, and genome-wide associations for production traits, while also providing preliminary information on its possible use in swamp buffalo. Although only a subset of markers was polymorphic in swamp populations, this work demonstrated that genomic information could become a practical tool not only for selection, but also for understanding and managing buffalo biodiversity.

In the Italian Mediterranean Buffalo, genomics has now moved from possibility to implementation. A major contribution to this transition was provided by the national BIG project, funded by European resources within the framework of the Italian National Rural Development Programme for Biodiversity, Sub-measure 10.2, supporting the conservation, sustainable use and development of genetic resources in agriculture. The project enabled the systematic collection of genotypes across the Italian buffalo population and the recording of innovative phenotypes, creating a unique platform for the implementation of genomic selection. The availability of these new genomic and phenotypic resources, together with large-scale pedigree and routine performance recording, has allowed the transition from traditional BLUP evaluations to single-step genomic BLUP. The first genomic evaluation for production and type traits showed that ssGBLUP can be successfully integrated into the existing breeding program, increasing the accuracy of breeding value prediction while maintaining continuity with the previous genetic evaluation system. This is particularly important in buffalo, where the number of genotyped animals is still limited compared with cattle, but where single-step methods allow all available information, genomic, pedigree, and phenotypic, to be combined in a unified framework.

The impact of genomics is not limited to milk yield or cheese-related traits. Recent developments in the Italian Mediterranean Buffalo breeding program are expanding the selection objective toward fertility, functionality, welfare, sustainability, and resilience. Genetic studies on reproductive traits have shown that fertility is complex, lowly heritable, and unfavorably correlated with milk production, highlighting the need for balanced selection strategies. Genomic tools can help address this challenge by improving prediction accuracy, enabling earlier selection, and supporting the construction of more comprehensive selection indices.

The next frontier is to move beyond genomic breeding values and exploit SNP effect estimates derived from ssGBLUP evaluations. These estimates may provide biological and practical information that can be useful beyond the reference population in which they were obtained. In this presentation, I will also discuss how SNP effects estimated in the Italian Mediterranean Buffalo could be evaluated through simulation as a potential source of information for other river buffalo populations and, more cautiously, for swamp buffalo. Such an approach may help assess the degree to which genomic signals are transferable across populations with different genetic backgrounds, demographic histories, and production systems.

This perspective is particularly relevant for countries where structured breeding programs, pedigree recording, or large reference populations are still unavailable. If properly validated, genomic information generated in well-recorded populations could contribute to accelerating the implementation of genomic tools elsewhere, supporting both genetic improvement and conservation. The future of buffalo genomics will therefore depend not only on larger reference populations and more dedicated SNP resources, but also on international collaboration, data sharing, and methods able to connect local breeding goals with global genomic knowledge.

1000 Buffalo Genomes Project Phase 2

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The water buffalo underpins the livelihoods of more people than any other domesticated species, yet it remain poorly characterised genetically compared with other major livestock species. To bridge this critical gap, the 1000 Buffalo Genomes Project (1000BGP) was established to provide comprehensive genomic resources for both river and swamp buffaloes, delivering key tools to breed more sustainable and productive livestock. The 1000 Buffalo Genome Project (1000BGP) is an international consortium formed in 2022, comprising 46 researchers who work on water buffalo from 16 countries. Phase 1 of the 1000BGP project released a new swamp buffalo genome and discovered ~41 million single nucleotide polymorphisms (SNPs) from whole genome sequencing (WGS) data of 140 buffalo. Comparison between river- and swamp-type buffalo revealed 1.5% genetic differentiation with an estimated divergence 3.1 million years ago (95% CI, 2.6–4.9).

In phase 2 of 1000BGP, we aim to analyse global genetic diversity of buffalo. We extended the number of buffalo samples by gathering 1190 publicly available whole genome sequences and 121 newly generated WGS data including Australian and Philippines buffalo. So far, 398 of these WGS data have been processed for SNP calling against the river buffalo genome assembly, UOA_WB_1. The current samples are spread across 18 countries and represent 81 breeds/populations. From this dataset, we discovered ~49 millions SNPs and ~7 millions indels. The transition to transversion ratio is 2.05. Principal component analysis (PCA) using the SNPs revealed distinct clusters separating the river-type from the swamp-type buffalo as well as clusters for a few breeds/populations e.g. Mediterranean, Indonesia, Nepal, Australia. The Australian swamp buffalo are feral animals which clustered closest with Indonesian swamp buffalo. This is consistent with the history of the introduction of Indonesian buffalo to Australia. We will use this dataset to investigate potential signals of adaptation to a feral existence in the Australian buffalo genome.

One of the goals of 1000BGP is to create a cost effective and flexible genotyping tool that is suitable for genotyping diverse buffalo breeds. Using one of the 1000BGP runs that has 225 samples (107 swamp, 118 river), we extracted biallelic autosomal and X chromosome SNPs as input dataset for creating a new buffalo ~50K SNP genotyping panel. The allele frequencies for river- and swamp-type buffalo samples were calculated separately with plink2.0.0 and were filtered for minor allele frequency of 1%, sample completeness 90% and SNP completeness 90%. The SNPs were further filtered for their surrounding bases GC content to be between 40% to 60%. We retained 16,630 highly polymorphic and overlapping SNPs with Axiom 90K SNP array to ensure compatibility of our new panel with existing genotypes on earlier array. To enable sex prediction, six Y chromosome markers that are compatible with array were included in our genotyping panel. To create a ~50K panel, SNPs from our WGS were used to fill in missing SNPs and selection was done by optimising inter-SNP distance and allele frequency of both river- and swamp-type buffalo. This new target capture based genotyping panel achieved average call rates of 99.2% across 95 test samples representing both river- and swamp-type buffalo. We are in the process of testing the genotype concordance of river- and swamp-type buffalo using this new genotyping panel.

Reproductive Biotechnologies in Water Buffaloes under Tropical Conditions in Venezuela: Current Advances and Biological and Technical Challenges

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Reproductive biotechnologies have played a crucial role in accelerating genetic gain and improving productive efficiency in the livestock industry. In cattle, the combined use of artificial insemination (AI), embryo transfer (ET), and *in vitro* embryo production (IVEP) has achieved outstanding figures in developed economies, where IVEP embryos exceeded two million in 2024, with North America clearly leading global production and Europe showing sustained growth (Viana, IETS, 2024). In that context, AI has also become highly consolidated, particularly in the U.S. dairy sector, where sexed semen is widely used. However, in buffaloes under tropical conditions, and especially in Venezuela, the application of these technologies requires, first, substantial adaptation to the species' biology and, second, to the technological characteristics and capabilities of the local production systems.

In Venezuela, a leading country in buffalo production in the Americas with over 3,000,000 heads, farms' buffalo herd sizes are often about 500 animals, and farm sizes usually exceed 150 ha (Source: CRIABÚFALOS). These systems are largely managed under semi-intensive or extensive grazing over large areas, where estrus detection becomes a major limiting factor for conventional AI application, severely restricting its use as a mass genetic improvement tool. This limitation is compounded by buffalo reproductive physiology, characterized by seasonality, low expression of estrous signs, nocturnal estrus behavior, infrequent homosexual behavior, variable heat duration, and unpredictable ovulation timing, as well as logistical difficulties in estrus detection in grazing herds.

In this scenario, timed artificial insemination (TAI) has become the most practical and scalable alternative for introducing superior genetic materials without relying on estrus detection. TAI protocols allow efficient control of follicular dynamics, synchronization of ovulation, and insemination at a predetermined time for a large number of animals, thereby facilitating the use of genetically proven sires and improving reproductive coverage in extensive and semi-intensive herds. In water buffaloes, TAI regimes combining progesterone, GnRH, and PGF2α-based protocols have produced satisfactory pregnancy rates when adapted to the species' reproductive physiology during the favorable and non-favorable breeding season (Gutiérrez-Añez et al., 2022: https://doi.org/10.1007/978-981-16-7531-7_15). Under this program, TAI has become an essential tool for genetic improvement in Buffalo systems. Conception rates are approximately 50% during the non-breeding season and around 60% during the breeding season. Additionally, pregnancy rates exceed 75% after two TAI procedures are performed (Gutiérrez-Añez, data not published). By overcoming the limitations of estrus detection observed in this species and allowing the systematic use of genetically proven sires, fixed-TAI has been shown to increase the proportion of pregnancies sired by elite bulls, accelerate genetic gain, and improve reproductive efficiency (Gutiérrez-Añez, 2018). In semi-intensive Venezuelan herds, this technology has proven particularly valuable for reproductive management enhancement and genetic progress, thereby transforming breeding programs into more predictable and productive farming systems. By enabling the use of genetically proven sires at scale, TAI has not only improved reproductive efficiency but also translated into measurable gains in milk production. Venezuelan water buffalo herds under TAI showed a clear shift away from natural mating and toward AI-sired offspring (from 5% to 75%), alongside progressive increases in total milk yield across generations, with reported milk yield gains of about 22.6%, 24.4%, and 52.2% in the first, second, and third AI generations compared with the natural mating baseline (Gutiérrez-Añez, data not published).

On the other hand, IVP faces greater challenges. Water buffalo have a particular ovarian physiology, with a smaller follicular reserve than that observed in cattle, estimated at approximately 10,000 to 14,000 primordial follicles, together with greater negative sensitivity to seasonality, heat stress, and nutritional variation. These characteristics reduce the availability of competent oocytes and limit the efficiency of IVP programs, both at the level of oocyte maturation and during fertilization and early embryonic development. Current figures for IVP embryos are still suboptimal, with 6- 8 suitable cumulus-oocyte complexes per donor recovered and a blastocyst rate of around 20%. Ultimately, the figures show fewer than one embryo per OPU per donor IVP session, with a pregnancy rate of around 40% with fresh IVP embryos. Thus, although IVEP is a strategic tool for multiplying high-value genetics, its use in buffaloes under tropical conditions, particularly in Venezuela, still faces important biological and technical barriers.

Experience in Venezuela indicates that TAI can generate real genetic progress in buffalo herds when applied on a large scale and under strictly organized management. In systems with adequate body condition, proper and robust health management, and appropriate hormonal synchronization protocols, TAI allows reductions in days open and increases in productivity, providing a concrete path to improve the profitability of buffalo production systems. By contrast, IVEP requires not only specialized infrastructure and highly trained personnel, but also a more favorable biological baseline and overcoming the technological limitations that have not yet been fully achieved in this species worldwide.

In summary, under Venezuelan conditions, conventional AI has limited practical application, whereas TAI is recognized as the popular technology with the greatest immediate impact for genetic improvement and productivity. On the other hand, though IVEP is promising and in demand, it remains a biotechnology with limited significant efficiency and transferability challenges still to overcome. The future of buffalo reproduction in tropical regions will therefore depend on integrating robust TAI protocols, species-adapted reproductive management, and research aimed at overcoming the physiological constraints that still limit the application of more advanced biotechnologies.

Keywords: Water Buffaloes, Reproductive Biotechnologies, AI, TAI, IVEP

Actual Technological Status and Future Reproductive Challenges in Egyptian Buffalo

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Egyptian buffalo (*Bubalus bubalis)* is the cornerstone of the livestock sector, with significant contribution to the national milk and meat production and food security especially in the rural livelihoods. Despite its remarkable adaptability and high-quality dairy and meat products, reproductive inefficiency remains a major problem limiting productivity and genetic progress. The major factors for reproductive frailer in buffaloes are delayed puberty, prolonged postpartum anestrus, silent heat, reproductive diseases, and heat stress.

Over the past two decades, Egypt has made significant progress in application of reproductive biotechnologies to improve buffalo fertility and to increase profit of buffalo farming. These technologies include reproductive ultrasonography, artificial insemination, fixed timed insemination programs, estrous synchronization protocols, semen evaluation and preservation, embryo transfer, *in vitro* embryo production, molecular diagnosis and control of reproductive diseases.

The national strategy of buffalo breeding focuses on strengthening genetic improvement programs, expanding the use of advanced reproductive technologies, establishing integrated recording systems, enhancing farmer extension services, and integration of international collaboration. The successful implementation of these strategies improve reproductive efficiency, accelerate genetic gain, increase productivity, and enhance the sustainability and resilience of Egyptian buffalo production systems under changing environmental conditions.

Antimicrobial Resistance in Buffalo Mastitis Pathogens in Pakistan: Current Status and Drug Repurposing Strategies

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Antimicrobial resistance (AMR) poses a serious global threat of growing concern to human, animal, and environmental health due to persistent failure to develop new antibiotics and imprudent use of antibiotics. This current study was designed to investigate the molecular prevalence of *Staphylococcus aureus, Streptococcus spps, E. coli, and Klebsiella spps,* antibiotic resistance profiling, and drug repurposing strategies from mastitis in buffaloes.

A total of 384 individual buffalo milk samples were collected from five dairy farms including Premium dairy farm, Mian dairy farm, Sab dairy farm, Aulakh buffaloes, Anwar sons dairy farm and these milk samples were screened for subclinical mastitis (SCM) by the California Mastitis Test (CMT). The SCM samples were further streaked on mannitol salt agar (MSA) for isolation of S. aureus, blood agar for Streptococcus spp., and MacConkey agar for E. coli, and Klebsiella spp. After this, genotypic confirmation was done by targeting the respective genes S. aureus (nuc), Streptococcus spp. E. coli, (16sRNA), and Klebsiella spp (gyrA) by using PCR. Furthermore, by using the Kirby-Bauer disc diffusion method, the sensitivity and resistance of antibiotics against each pathogen (n=10) were determined, and resistant antibiotics were further modulated by using non-antibiotics by the well diffusion method. Moreover, the prevalence of MDR isolates was also investigated.

The results revealed that 54.43% (209/384) of the samples were positive for subclinical mastitis. Among 209 SCM positive samples, the prevalence of *S. aureus, Streptococcus spps, E. coli, and Klebsiella spp.* was noted as 57.90% (121/209), 47.37% (99/209), 19.62% (41/209), 13.88% (29/209), respectively. The antibiogram of each pathogen showed a similar resistance pattern, with higher resistance to oxytetracycline, followed by gentamicin and vancomycin, while moxifloxacin and linezolid were among the susceptible antibiotics. A total of 14.35% local isolates showed resistance to various antibiotics and were classified as multidrug-resistant (MDR).

The findings of the well diffusion assay demonstrated synergistic interactions between penicillin and ketoprofen, gentamicin and flunixin meglumine, and oxytetracycline and *N*-acetylcysteine. The study underscores the increasing occurrence and dissemination of antimicrobial resistance with subclinical mastitis in buffaloes and highlights the potential of drug repurposing strategies to mitigate multidrug resistance.

Keywords: Buffalo, AMR, Subclinical mastitis, MDR, Synergy

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The current study was conducted in the Medicine Research Laboratory, Department of Veterinary Medicine, UVAS, Lahore, Pakistan.

Efficacy of calfhood RB51 vaccination against Brucella abortus challenge in pregnant buffaloes years later

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A challenge trial was carried out to assess the long-term efficacy of the RB51 vaccine in adult pregnant water buffaloes (*Bubalus bubalis*). According to the Italian authorized protocol (triple bovine dose and booster), ten animals were vaccinated at 6–9 months of age, while six remained unvaccinated controls. At challenge, performed in two experimental phases, all sixteen buffaloes were 3–5 years old and in mid-gestation (second trimester). In the first phase, conducted 4 years post-vaccination (ypv), 6 vaccinated and 2 control animals were transferred to a high-containment facility and challenged conjunctivally with 10^7 CFU of *B. abortus* strain 2308, following an acclimation period. The second phase, 5 ypv, included 4 vaccinated and 4 control buffaloes, managed under identical housing and challenge conditions. Clinical monitoring, serology (RBT, CFT, RB51-CFT, i-ELISA, c-ELISA) and IFN-gamma release assay were applied to evaluate health status and humoral and cell-mediated responses. Bacteriological investigations focused on late gestation, parturition and early lactation, as critical windows for Brucella excretion. All buffaloes seroconverted in RBT, CFT, i-ELISA and c-ELISA, with absent or low RB51-CFT reactivity. Among vaccinated animals, 8 delivered viable calves and 2 experienced premature parturition with calves not able to survive; placental expulsion was normal in all vaccinated buffaloes and *Brucella* was not isolated at parturition nor from calves that died postnatally. All calves acquired maternal anti-*Brucella* antibodies *via* colostrum and milk. Shedding was minimal, with low levels of *B. abortus* 2308 detected in milk from one buffalo 4 ypv, and positive vaginal swabs from one buffalo 5 ypv between days 2 and 17 post-partum. In contrast, among controls, 4/6 aborted around 2 months post-infection and 2 delivered normally. *B. abortus* 2308 was consistently isolated from placenta, foetus and milk of aborting controls, but not at parturition from the two full-term controls. Overall, the RB51 regimen currently adopted in Italy for young water buffaloes confers long-term protection against abortion, although infection may still occur, and with decreased effectiveness observed in some animals 5 ypv. Vaccination substantially reduces Brucella shedding, lowers reservoir potential, and decreases horizontal transmission from vaccinated animals, supporting the use of RB51 to accelerate brucellosis eradication in buffalo herds.

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Antimicrobial stewardship and integrated approch in livestock: the Italian strategy reducing antimicrobial use

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Antimicrobial resistance (AMR) is a public health priority within the One Health framework, and livestock production contributes to selective pressure. In Italy, ClassyFarm, developed by IZSLER for the Ministry of Health and integrated into the National Veterinary Information System, enables risk-based categorisation of farms. It combines data on antimicrobial use, biosecurity, animal welfare, and health and production parameters. Animal welfare and biosecurity are collected by national check list. Health and diagnostic data specific for each farm are included using national network of IIZZSS. Antimicrobial use is derived from the Electronic Veterinary Prescription (REV), mandatory since 2019, and expressed as DDDAit (Defined Daily Dose Animal for Italy) normalised by biomass at risk. This allows standardised comparison across farms, species and geographical areas. Benchmarking against national reference values and stratification by AMEG category guide targeted interventions, with particular attention to critically important antimicrobials (CIAs) such as fluoroquinolones, 3rd–4th generation cephalosporins and polymyxins. The system gives farm veterinarians, farmers and competent authorities performance indicators for designing reduction plans. These plans build on improved animal welfare, biosecurity, vaccination and laboratory diagnostics to support therapeutic choices. Improving the living conditions of animals is an essential prerequisite for achieving the social, environmental and economic sustainability of livestock farms. Improved animal welfare and reduced antimicrobial use are associated with a lower environmental impact, assessed through the carbon footprint (kg CO_2eq/kg FPCM), and with improved economic profitability, expressed as margin per unit of product (€/L of milk). Classyfarm is also used by MASAF (Italian Ministry of Agriculture, Food Sovereignty and Forestry) for the allocation of payments linked to the Common Agricultural Policy (CAP), and by certification bodies for the SQNBA voluntary labelling scheme (National Quality System for Animal Welfare), which covers the sustainability attributes of animal production.

Harmonizing Passive Surveillance and Early Detection Across the Italian IIZZSS Network: A Diagnostic Appropriateness Model for principal Water Buffalo Syndromes

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In veterinary medicine, diagnostic workflows are increasingly complex and costly due to evolving farming systems and opportunistic pathogens. Aligning with the European Animal Health Law (Reg. UE 2016/429), animal health must shift from a reactive to a proactive prevention approach based on risk analysis. In this context, "diagnostic appropriateness", rooted in Evidence-Based Medicine (EBM), is crucial to optimize resources, ensure clinical efficacy, and guarantee economic sustainability through an integrated investigation framework.
This strategic national project, funded by the Italian Ministry of Health, aims to achieve five core objectives: 1) define Appropriate Diagnostic Pathways (PDAs) for specific symptomatic or syndromic forms in major livestock species, focusing on listed infectious diseases; 2) manage the systematization of PDAs across the IIZZSS multi-site network; 3) guarantee uniformity of passive monitoring data at regional and national levels, leveraging territorial diagnostics as a privileged epidemiological observatory for early detection; 4) harmonize and visualize data through ClassyFarm by developing a shared LIMS thesaurus to translate diagnostic reports into uniform nationwide information; 5) fulfill official disease surveillance obligations mandated for the competent authority.
The methodology involves technical groups and National Reference Centers. Crucially, the layout of each PDA is structured based on three technical pillars: 1) defining the specific interest level (Reg. UE 2018/1882 categorization, zoonotic risk, or zootechnical interest); 2) establishing an execution hierarchy (mandatory, optional, or conditioned by territorial exposure scenarios); 3) identifying exact execution criteria, such as animal age, specific lesions, mortality levels, and restriction or surveillance zones.
The project delivers validated PDAs and data-processing systems with Business Intelligence dashboards. Notably, for the water buffalo species, PDAs have already been successfully developed for enteric, respiratory, and abortive syndromes. This eliminates regional assessment biases and maximizes resource efficiency. Implementing PDAs provides a homogeneous diagnostic model that bridges private farm veterinarians and official laboratories, optimizing early detection of infectious diseases in full alignment with the One Health paradigm.

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Buffalo Milk: From Gourmet to Functional Food

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Historically, the perceived value of buffalo dairy products in Western markets has been linked to the success of the Mozzarella di Bufala Campana PDO, which reached 57,584 tons in 2025, consolidating its position as the third best-selling geographical indication cheese worldwide. This excessive dependence on a single product category has created structural vulnerabilities, particularly in light of margin compression imposed by retail distribution networks. A strategic reassessment of the value proposition of buffalo dairy milk is therefore required.

The global health landscape is dominated by the rising prevalence of non-communicable diseases (NCDs), responsible for approximately 74–75% of global mortality (about 43 million deaths per year). Cardiovascular diseases, cancer, chronic respiratory diseases, and diabetes share common pathophysiological mechanisms — oxidative stress, low-grade chronic inflammation, dyslipidemia, and gut dysbiosis — which can be modulated by targeted dietary interventions. This understanding has established the rationale for functional foods as essential tools in preventive health promotion.

Buffalo milk presents a significantly higher nutritional density than conventional bovine milk, with higher concentrations of protein (4.0–4.5 g/100g), calcium, magnesium, and total solids. Its A2A2 genotype eliminates the production of the inflammatory peptide BCM-7 associated with A1 beta-casein, which is linked to gastrointestinal discomfort and systemic inflammation. Furthermore, buffalo milk is a rich source of bioactive compounds, notably δ-valerobetaine, which has demonstrated potent antioxidant and anti-inflammatory activity in in vitro studie. The digestion and fermentation of buffalo milk release specific bioactive peptides, such as VLPVPQK and NAVPITPTL, with ACE-inhibitory, antioxidant, and osteogenic activities. The lipid fraction offers a superior profile of conjugated linoleic acid (CLA) and polar lipids from the milk fat globule membrane (MFGM), with prebiotic effects and promotion of intestinal barrier integrity.

Consumer behavior in the mid-2020s reflects a prioritization of health and sustainability. Surveys across 18 European countries indicate that health is the primary factor influencing food choices, with sustainability ranking second. The global cheese market reached approximately US$ 173.8 billion in 2025, with a modest annual growth rate of 3%. In contrast, the functional food market, valued between US$ 373 billion and US$ 423 billion, is growing at rates of 6.6% to 8.8% per year. The dietary supplement market, valued at US$ 203.4 billion, is growing at 7.8% per year. These data indicate that the greatest growth potential for buffalo breeding lies at the intersection between dairy and functional products.

The transition to a functional platform opens strategic opportunities: formulation of specialized products for the elderly (sarcopenia and osteopenia); A2 whey supplements for sports nutrition; clinical nutrition products for cancer patients or interventions for childhood malnutrition. Fermentation and aging of ripened cheeses lead to the progressive release of ACE-inhibitory peptides, suggesting that aged buffalo cheeses could be marketed not only for their sensory profile but also for their cardiovascular benefits. Buffalo whey, rich in β-lactoglobulin and α-lactalbumin, can be concentrated into functional pastes or powders for the clinical and sports nutrition markets.

To capture these opportunities, investment in rigorous scientific validation is necessary. Much of the available research remains mechanistic, in vitro, or based on animal models — randomized clinical trials are essential to substantiate functional claims. The formation of consortia and public-private partnerships can make the costs of regulatory research viable for small producers.

Buffalo breeding offers sustainability advantages: buffaloes thrive on low-quality forages and are resilient to adverse environmental conditions, aligning with clean label and naturalness trends in the functional food sector. Ethical considerations, particularly regarding animal welfare and the management of male calves, are critical to the sector's long-term success.

The evolution of buffalo milk from a gourmet niche to a broad-spectrum functional platform represents a strategic opportunity for the production chain. This transition does not imply abandoning the gourmet market, but rather expanding its reach through product diversification and clinical validation. By combining traditional artisanal excellence with cutting-edge nutritional science, the sector can ensure its long-term profitability and sustainability, offering products that promote healthy aging while preserving the cultural and biological heritage of the water buffalo.

Buffalo Meat Through an Integrated Species Perspective: Functional Molecules, Biochemical Transformations and Nutraceutical Potential

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Buffalo meat has traditionally been considered a leaner alternative to beef due to its lower intramuscular fat content, reduced cholesterol concentration and high protein profile. However, recent advances in animal physiology and food biochemistry suggest that buffalo meat should not simply be regarded as a conventional red meat with improved compositional parameters, but rather as a biologically distinct food matrix characterized by species-specific molecular traits potentially associated with relevant nutraceutical properties. The organoleptic characteristics of buffalo meat also reflect the uniqueness of this product, including its dark color, compact muscle structure, characteristic flavor and peculiar biochemical behavior during post-mortem transformation, storage, cooking and digestion. Nevertheless, the biological significance of buffalo meat extends far beyond conventional compositional and sensory parameters. Increasing evidence suggests that buffalo-derived products may provide a wide range of functional biomolecules related to lipids and fatty acids, proteins and functional peptides, glycoproteins, glycolipids and specific enzymes involved in metabolic regulation, oxidative balance and biochemical processes. Among the most interesting compounds are carnitine and its related metabolites, particularly δ-valerobetaine. Carnitine plays a central role in mitochondrial fatty acid transport and cellular energy metabolism, whereas δ-valerobetaine has recently attracted growing scientific interest because of its potential involvement in metabolic regulation, mitochondrial function and redox balance. Although trimethylamine-related compounds are often discussed in relation to cardiovascular risk, their physiological significance appears considerably more complex than previously believed. Buffalo-derived products, especially buffalo milk, have shown higher concentrations of carnitine-related metabolites than bovine products, suggesting the existence of species-specific metabolic pathways that may also characterize buffalo muscle tissue.

Another promising field of research concerns the generation of bioactive peptides from buffalo proteins during ageing and food processing. These peptides may exhibit antioxidant, antihypertensive, antimicrobial, anti-inflammatory and immunomodulatory activities. Of particular interest are peptides with angiotensin-converting enzyme (ACE)-inhibitory properties, which may contribute to blood pressure regulation and cardiovascular protection. Although studies specifically focused on buffalo meat peptides are still limited, the high biological value of buffalo proteins and the peculiar muscular organization of this species support the hypothesis that buffalo meat may represent an important reservoir of functional peptides.

A particularly innovative area concerns glycobiology and the biological role of glycoproteins and sialylated compounds. In recent years, increasing attention has been devoted to the physiological significance of glycoconjugates in food systems and to their interactions with immune pathways, gut microbiota and inflammatory regulation. While scientific discussions regarding red meat and sialic acids have often focused on the possible implications of Neu5Gc, a broader interpretation also highlights the beneficial physiological functions of sialylated glycoconjugates, particularly Neu5Ac, of which buffalo meat appears to be rich.

Recent advances in transcriptomics and epigenetic regulation further support the concept that buffalo muscle is biologically distinct from bovine muscle at the molecular level. In addition, non-coding RNAs appear to play important regulatory roles in buffalo muscle physiology. These molecular mechanisms reinforce the hypothesis that buffalo meat should be evaluated through a systems biology perspective rather than through conventional compositional analysis alone.

Particular attention should also be devoted to iron metabolism and oxidative balance. Buffalo meat is generally characterized by high myoglobin levels and elevated iron bioavailability, which contribute to its darker color and nutritional value. At the same time, species-specific regulation of ferritin, iron-binding proteins and endogenous antioxidant systems may influence oxidative stability and redox balance within the meat matrix. Although information regarding specific oxidation-derived metabolites is still limited, preliminary evidence suggests that the interaction among phospholipid composition, hemoproteins, endogenous antioxidants and oxidative metabolism may differ substantially from that observed in beef.

Collectively, these observations support the emerging concept that buffalo meat represents a biologically specialized food matrix shaped by species-specific molecular and metabolic adaptations extending far beyond traditional nutritional descriptors. The interplay among oxidative metabolism, iron homeostasis, non-coding RNA regulation, proteolytic dynamics and glycoconjugate biology may contribute to the formation of a unique biochemical ecosystem capable of modulating not only nutritional and potential health-related functionalities, but also the technological and biochemical behavior of buffalo meat. Within this broader perspective, buffalo meat should be interpreted as a dynamic biological system in which post-mortem transformations, oxidative pathways and molecular interactions continuously evolve throughout the different stages of the food chain, potentially generating distinctive functional and nutraceutical traits. A deeper understanding of these interconnected mechanisms may therefore provide new insights not only into meat quality and oxidative stability, but also into the development of innovative health-oriented foods and species-specific strategies in the context of modern precision nutrition and functional food science.

Nutrition and metabolic condition during critical windows for reproductive success

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Nutrition and metabolic condition have a central role in reproductive success in buffalo. The fundamental role of nutrition is to achieve the required isocaloric (energy) and isonitrogenous (protein) balance, together with minerals and vitamins, for optimal reproductive function. This can be achieved with improved pastures in extensive systems, with total mixed rations in intensive systems, and a mixture of pasture and supplementation. The most intensively studied role of nutrition in reproduction is during the pre-, peri- and post-partum periods. It is now well established that nutritional management for body condition and metabolic health before calving has a major impact on the re-sumption of cyclic ovarian activity and re-conception after calving. Less well studied, but arguably just as important, is the role of nutrition during other windows for reproductive function in females. This includes the foetal period when the primordial follicle pool is established, the period from birth to six months, and from six months to puberty. Liveweight gain in the pre-pubertal period is particularly important for both age at puberty and lifetime fertility in females. Another under-researched area is the role of early life nutrition on age-at-puberty in male buffalo. The manipulation of crude protein and net energy early in life has the potential to enhance testicular growth and advance the onset of spermatogenesis in bulls. This is important for reducing generation interval and accelerating genetic gain using bulls, and complements oocyte recovery and *in vitro* embryo production using pre-pubertal heifers. The study of nutrition has moved into the realm of metabolomics. This field has the potential to identify certain metabolites that have key signalling roles that link nutrition with specific reproductive processes. Included in this area is the role of nutrition in altering the ruminal microbiome. This determines the contribution of the ruminal metabolome to the whole-body metabolome, and the impact this has on reproductive processes in males and females. Buffalo are a globally important livestock for milk and meat across diverse environments with differing economies and production systems. There are different buffalo types and feeding systems rely largely on local resources which can be limiting. Irrespective of the production circum-stances, it is important to manage nutrition according to reproductive stage in both males and females. In all situations, nutritional manage-ment for reproduction ensures optimal benefit is derived from the production system platform created by genetic selection and breeding.

The 'One Nutrition' approach for buffalo feeding

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The One Nutrition approach requires a holistic consideration of human, animal and plant nutrition; the increased demand for products of animal origin needs for a sustainable system to ensure productive supply chains with minimal environmental impact. Even if improvements in production efficiency reduce environmental impact, increased productivity still heightens demand for feed, energy and water. In addition, intensive genetic selection has reduced animals' longevity, resulting in fewer lactations and increase of culling rate, contributing to greenhouse gas emissions and manure production. Moreover, the high proportion of concentrates in the diet results in the emergence of several technopathies involving rumen fermentation, whit detrimental effects on the nutritional quality of milk. Therefore, efficient management animal nutrition can significantly influence animal welfare, its productivity and their environmental impact, contributing to the achievement of the 17 Sustainable Development Goals of the ONU 2030 Agenda. In this context, several opportunities as modification of diet forage:concentrate (F:C) ratio, use of alternative feeds or phytogenic-based additive have been investigated.

In Italy, corn silage is the main forage in the diets of dairy buffaloes, even if the high cost of irrigation and the contamination risk by mycotoxins. Thus, the growing interest in other forage, such as sorghum which tolerate drought better than corn thanks to its root system, able to penetrate very deep into the soil providing water and nutrients. According to buffalo cows fed sorghum silage showed an average milk yield higher than group fed corn silage (kg/d 10.120 vs. 9.270; $P< 0.05$), due to the lower lignin content (31.0 g/kg DM vs 47.0 g/kg DM) thus to the higher digestibility of sorghum diet. The omega 6/omega 3 ratio was significantly lower in milk from buffalo cows fed sorghum than those fed corn silage (7.8 vs. 12.9; $P< 0.01$) while serum biochemistry was unaffected by the treatment. Partial replacement of corn silage (C) with green sorghum (S) did not affect milk yield (C: 10.4 kg/head/d vs. S: 10.4 kg/head/d) or milk composition, except for fat content, significantly higher in the S group (71.2 g/kg vs. 85.0 g/kg; $P < 0.01$). This increase was associated with improved digestibility of NDF and ADF ($P < 0.01$). The economic assessment revealed that the use of green sorghum should reduce feeding costs by 0.27 EUR/head/day, making this strategy economically advantageous. Finally, substituting maize silage for sorghum silage determined a significant 39.6% reduction in CH4 yield (103.00 kg vs 63.48 kg CH4/head/year) was observed with no effect on weight gain or microbial population.

A higher F:C has been shown to enhance fibre intake, which in turn has been demonstrated to promote improved rumen function and milk quality. Comparing diets differing for F:C ratio (H, 58:42 vs. L, 45:55), buffalo cows of group H showed significantly higher milk fat content and increase of oleic acid, CLAs, and omega-3, all these fatty acids linked to health benefits in humans, including anti-inflammatory, cardioprotective, and anticarcinogenic effects. While decreasing the F:C can shift VFAs production towards propionate and reduce CH4 yield, this strategy must be applied cautiously in buffaloes. Their physiological adaptation to high-fiber diets and specific rumen environment may render them more susceptible to ruminal acidosis compromising animal health and productivity.

Plant-derived secondary metabolites, specifically essential oils (EOs), have emerged as promising candidates to manipulate the rumen environment. The inclusion of a blend of EOs in the diet of dairy buffaloes resulted in an increase in milk yield with no effect on milk composition. In contrast, milk urea was consistently lower in the treated group throughout the trial while higher were the levels of trans vaccenic acid and eicosapentaenoic acid in the treated animals. In accordance, the administration of a phytogenic-based feed additive has been in vivo evaluated in lactating buffaloes, achieving a significant reduction in CH4 concentration in exhaled air due to their activity against methanogenic Archaea.

Buffalo in the world: sustainability, management, products, economic trends

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The domestic buffalo (*Bubalus bubalis*) plays a strategic role in the world both for the economy and society. This species is represented by 204 mil heads on the planet and produces more than 144 mil T of milk and 7 mil T of meat. Buffalo products are important for the human requirements of proteins of high nutritional value, requirements that are going to increase rapidly in the future with the growth of the human population. Therefore, the buffalo has and will have more a strategic role in Governments' economic programs in the planet. The buffalo is a long-living ruminant, with a high capacity to adapt in difficult areas such as marshlands or in hot and humid climates as humid savannah, where other ruminants cannot survive. Buffalo is a social and family animal in small villages in Southeast Asia, where it is used mainly in the rice fields as a draught animal producing milk, important for the children's nutrition, meat, leather, manure, horns, and bones. Thanks to its great adaptability, buffalo can be bred both in intensive systems as well as in extensive systems, as in South American countries where there is a high availability of natural pastures.

The present abstract aims to provide an updated and comprehensive overview of local breeds and cosmopolitan dairy breeds to sustain protein production, maintain the sustainability and the biodiversity of the land, and to sustain the livelihood of populations, are reported according to the different farming systems in all the countries of Europe, Asia, Africa and America.

Selecting for meat production in Italian Mediterranean Buffalo (IMB)

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Selecting for meat production in Italian Mediterranean Buffalo (IMB) is increasingly important because buffalo meat has competitive potential based on nutritional value, territorial identity, and versatility. It is generally perceived as leaner than beef, with lower fat infiltration and high protein content, and it is suitable for fresh consumption and processed products. However, premium market development is still limited by variability in carcass and meat quality. Therefore, a buffalo meat production requires measurable and traceable quality traits, objective selection criteria, and economic valorization linked to carcass and meat performance. In the IMB, females are destined for milk production, making males the main candidates for meat-oriented pathways. In this context, the aim of the present study was to estimate genetic parameters and breeding values for muscling in IMB. Muscling is a morphological trait defined as the conformation of the thigh and rump according to the development, convexity, length, and downward extension of the muscle masses. The trait is recorded on a linear scale with values ranging from 1 to 50. The model included contemporary group (herd, year, classifier), parity-age, days in milk class, and milking number as fixed effects. In order to increase the accuracy of genetic parameter estimation by exploiting the genetic and residual correlations among traits, a multi-trait animal model was applied including locomotion, body condition score, and muscling (31.831 records), with a pedigree of 151.223 animals. Results showed a mean muscling score of 24.48 (SD =4.76), indicating moderate phenotypic variability across the population. Heritability was low ($h^2 = 0.09$), suggesting that muscling in IMB is influenced by environmental factors, although the additive genetic variance (1.64) indicates that genetic improvement remains feasible through selection. Estimated breeding values for muscling ranged from 83 to 131, where higher values indicate greater genetic merit. The genetic trend showed an increasing pattern up to 2010, followed by a decline in subsequent years, suggesting that selection pressure for muscling has not been consistently maintained over time. These results indicate exploitable genetic variability for muscling in IMB, supporting its inclusion in breeding programs aimed at improving meat aptitude and establishing a consistent, traceable, and value-oriented buffalo meat chain.

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Only You-psilon: an alternative strategy to sequence the Y chromosome alone

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The Y chromosome is a key determinant of male fertility, sex determination, and spermatogenesis, making its characterization biologically interesting. In Mediterranean river buffalo (*Bubalus bubalis,* 2n=50), a species of major economic importance in Italy due to its role in the production of *Mozzarella PDO*, breeding programs have mainly focused on milk traits, while the Y chromosome remain understudied. This is partly due to its intrinsic complexity, including high repeat content, extensive heterochromatin, and complex palindromic structures. Although a chromosome-level Y sequence has been reported for swamp buffalo, no equivalent reference is currently available for the river type. To overcome this gap, peripheral blood from 10 buffalo bulls was cultured, and Y chromosomes were physically isolated by laser microdissection, followed by whole-chromosome amplification. Fluorescence *in situ* hybridization (FISH) was performed to assess the specificity of produced Y probes. Subsequently, the same Y-whole chromosome probes were sequenced by Illumina NovaSeq 6000, assembled into contigs and analysed through read processing, *de novo* assembly, repeat identification, sequence alignment and variant detection. Comparative bioinformatics analyses involved aligning contigs to the swamp buffalo Y chromosome, annotating Y-linked genes through BLAST searches against the *Bos taurus* reference genome and identifying gene-associated contigs and CNVs.
FISH analysis confirmed the specificity of the isolated material, showing strong signals on the Y and the X chromosome corresponding to the PAR and heterochromatic region. Subsequent sequencing generated over 240 million paired-end reads, and *de novo* assembly yielded 566,815 contigs. Repeat analysis indicated that repetitive elements accounted for 3.91%, mainly SINEs, while variant calling identified over 23,000 variants. Comparative analyses further revealed multiple contigs mapping to the swamp buffalo Y chromosome and to Y-linked genes. Annotation against the *Bos taurus* genome identified 26 unique genes, some showing homology to both X and Y chromosomes and gene copy number analysis revealed duplications in the MSY region, including 10 copies of *TSPY* and 3 of *HSFY*. These findings show that laser microdissection with NGS enables effective access to the buffalo Y chromosome, representing a milestone in the characterization of the river type genome, and providing a basis for studies on buffalo male fertility and breeding programs.
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A Cross-Validated framework for STR imputation from SNPs and its impact on Parentage Assignment in Italian Mediterranean Buffalo

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In recent years, genomic technologies in livestock have progressively evolved, with microsatellite markers (STR) gradually being replaced by high-density SNP arrays. Although STRs have long been the reference standard for parentage testing, allele scoring remains a largely manual and error-prone process, whereas SNP platforms offer broader genomic coverage, improved inter-laboratory standardization, and lower genotyping costs. In Italian Mediterranean Buffalo (IMB), STRs are still used for parentage verification and pedigree management, making the integration of both platforms strategically important to preserve genealogical continuity during this technological transition. Therefore, the aim of this study was to develop and validate a machine learning framework to impute STR genotypes from SNP data, evaluating its impact on parentage assignment. A total of 64,028 animals with STR profiles (15 loci) and 6,780 animals with SNP genotypes (72,434 markers) were available. Genomic coordinates from ENSEMBL and FAO sources were used to define informative SNP windows surrounding each STR locus. An XGBoost gradient boosting model was trained to impute STR alleles from SNPs. Complete trios with full SNP and STR genotypes were identified. The analysis was structured in 10 independent bootstrap replicates, each based on a different random selection of 100 complete trios, with all trio members strictly excluded from the training set to ensure fully independent validation and prevent family leakage. Results showed high genotypic concordance between imputed and observed STR (≈98–99% for most loci). Paternity analysis using Mendelian compatibility confirmed that imputed STR genotypes reliably support parentage verification, particularly after excluding two poorly imputed loci. Removal of these markers substantially increased the proportion of correctly assigned trios, especially under the most stringent threshold (THR0), with values increasing from 42% to 79%. These findings demonstrate that XGBoost-based STR imputation from SNP data is accurate, robust, and applicable at population scale. This approach provides a methodological framework for integrating historical STR records with modern genomic data in routine parentage certification for Italian Mediterranean Buffalo.

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Genomic Selection of buffaloes in small holder conditions

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Buffaloes are unique animals that convert coarse roughages in to highly nutritious milk. Dairy farming in India is much dependent on buffaloes. India hosts 110 million buffaloes with huge genetic diversity of 22 registered distinct breeds. Of the total milk produced in India buffalo milk is around 45%. Genetic Improvement of buffaloes is done through Artificial Insemination in India.

Artificial Insemination services are well developed in India. Of the 135.7 Million Frozen semen doses produced and used for Artificial Insemination in India, 53.2 million (39%) doses are from 2136 buffalo bulls under collection, of this major is from Murrah (46.8mill, 88%) and Mehsana (3.4mill, 6%) breeds. Owing to small holder conditions, traditionally record keeping for breeding and production data by farmers was practically non-existent. Genetic evaluation and selection of bulls for frozen semen production in such conditions was quite challenging.

After successful initial pilot in some pockets, with the help of funding from Government of India, National Diary Development Board of India is implementing a nationwide genomic selection program for buffaloes. The program covers activities like creating national database for bovines, implementing Progeny Testing Projects for two major buffalo breeds, performance recording of sizeable number of buffaloes for various production and reproduction traits, development of Genome Assembly and Genotyping Microarrays suitable for Indian buffalo population, collection of biological samples and genotyping from recorded animals and bulls. This enabled to create a reference population for Genomic Selection. It was found that average Milk Yield (305 D ME) is 2744 Kg in Murrah breed and 2508 Kg in Mehsana breed, from the 19495 and 3206 recorded animals respectively in the year 2025.

With establishment of sizeable refernce population, and adjusting models to account for typical production system of small holder dairying, it was feasible to estimate Genomic Breeding Values (GBV). Upon validation, it was found that the GBVs was 2.5-3 times more accurate than traditional selection based on Dam's own performance for young bull selection. The system for Genomic Selection for young bulls is fully operational in India with GBVs for milk yield, Fat and Protein yields, Age at First Calving and Days Open. All the young bulls are now selected based on GBVs expecting faster genetic progress.

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Genome-Wide Association Study and Genomic Selection for Important Economic Traits in Buffalo

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Low milk yield remains a major constraint on buffalo industry development. Understanding the molecular mechanisms underlying important economic traits and developing genomic selection (GS) technologies are essential for accelerating genetic improvement and breeding high-yielding buffalo. Using BaseVar-STITCH and low-coverage resequencing, this study constructed a buffalo genetic variation reference panel, performed genome-wide association studies (GWAS) on 12 traits using phenotypic data, and developed GS models. The main findings are as follows:

Low-coverage resequencing of 1,743 buffalo generated 9.66 Tb of clean data and identified 11,773,341 SNPs. Genotyping accuracy reached 98.31% compared with high-coverage resequencing. Population genetic structure analysis revealed that samples from Pakistan exhibited a homogeneous genetic background, corresponding to Nili-Ravi buffalo, while domestic buffalo populations in China showed complex genetic backgrounds, including Nili-Ravi, Murrah, Mediterranean breeds and hybrids. GWAS and selective sweep analyses identified 241 SNPs for breed-specific genetic identification of Nili-Ravi buffalo.

The 12 traits in Nili-Ravi buffalo included 280-day milk yield (MY), body height, body length, chest circumference, rump height, median suspensory ligament (MSL), fore udder attachment angle (FUAA), fore teat spacing (FTS), fore teat length (FTL), rear udder attachment height (RUAH), rear udder attachment width (RUAW) and rear teat spacing (RTS). ESRRB was associated with MY, MSL, FTS, FTL and RTS; CD55, GPATCH2L and RBM46 with MY, FTS, FTL and RTS; and PCDH17 with MY, MSL, FTS and RTS. Coding-sequence SNPs in COL12A1 were associated with FTS and RTS, while those in ABCC6 were associated with MY and FTS. EGFR and ADAM12 were significantly associated with MY. These genes are important candidate targets for molecular breeding.

Genomic best linear unbiased prediction (GBLUP) models for seven traits (MY, body length, FUAA, FTS, FTL, RUAH and RUAW) achieved prediction accuracies of 0.045–0.689. SNP filtering using linkage disequilibrium (LD) thresholds from <0.1 to <0.9 yielded moderate accuracy for most traits at thresholds of <0.2–<0.9. Compared with single-trait models, dual-trait GBLUP reduced MY prediction accuracy to 0.253–0.267 but improved accuracy for the other five traits, excluding RUAH.

In summary, this study established a large-scale buffalo genetic variation reference panel using low-coverage whole-genome resequencing, identified genetic associations with important economic traits and preliminarily developed GS models. These findings provide a foundation for accelerating genetic improvement and developing new buffalo strains through genome-guided precision breeding in the era of artificial intelligence (AI) and big data.

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Genomic landscape of selection signatures in three Eastern European buffalo breeds

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Genetic variation in buffalo resulting from selection leads to distinctive fingerprints within the genome. Nonetheless, a comprehensive understanding of the selection responses for economic production traits in some Eastern European buffalo breeds is still unclear. This work aimed to detect selection fingerprints using runs of homozygosity (ROH), integrated haplotype score (iHS), standardized log-ratio of the integrated site-specific extended haplotype homozygosity (EHH) between pairs of populations test (Rsb), and cross-population EHH methods (XP-EHH), intending to identify candidate genomic regions and genes that are subject to positive selection associated with economic production traits in Bulgarian (BUL), Hungarian (HUN), and Romanian (ROM) Buffalo breeds. A total of 160 water buffalo genotypes from three Eastern European countries (Bulgaria, Hungary, and Romania) were used: 58 samples from two farms for the BUL Buffalo breed, 55 samples from three farms for the HUN Buffalo breed, and 47 samples from one farm for the ROM Buffalo breed, according to the Dryad information repository (https://doi.org/10.5061/dryad.9cnp5hqgc). The quality control and genotyping using the Axiom® Buffalo Genotyping Array 90K from Affymetrix1 were performed by ATLAS Biolabs GmbH (Berlin, Germany). Applying the four selection signatures (ROH, iHS, Rsb, and XP-EHH), we identified 22, 5, 15, and 15 genomic regions, containing 286, 35, 80, and 49 candidate genes, respectively, in the three Eastern European buffalo breeds. The annotation of these genomic regions revealed several genes associated with milk yield and composition (*ATE1, ADAM12, CHST15, UGT8, CAMK2D,* and *PRLR*), fertility and reproductive (*CSMD1, SPATA18, FANK1, SPAG17, PTGER3, PDE4D,* and *SAXO1*), growth and muscle development traits (*MYOM2, GNPDA2, WDR3, GHR, RPS6, RRAGA,* and *DENND4C*). This study reveals a comprehensive profile of the selection fingerprint in the BUL, HUN, and ROM buffalo breeds by combining the ROH, iHS, Rsb, and XP-EHH approaches. The results contribute significantly to improving economic characteristics in buffalo breeds worldwide, thus promoting sustainable livestock systems. The detected candidate genes highlight the response of selection to milk production, reproductive, and growth traits, providing crucial insights for improving BUL, HUN, and ROM buffalo breeding programs.

Keywords: Buffalo, SNP array, selection signatures, genomic regions

Multi-omics insights into heat stress responses in the Mediterranean Buffalo

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Heat stress represents a major threat for livestock production negatively impacting on animals' health, productivity, and physiology. Investigating the molecular bases of adaptation of Mediterranean buffalo (*Bubalus bubalis*) to high temperature is critical to optimize their resistance against changing climate conditions. The study analyzes seasonal responses to heat stress adopting an integrative approach by combining multiple omics technologies.

Eight buffaloes were sampled at three time points: T0 (July), T1 (September) and T2 (January). T0 and T1 represent the warm season (>30°C), while T2 represents a reference low temperature (3°C) season. Omics data relative to epigenome, transcriptome (RNA-seq), proteome, metabolome and lipids were collected. The epigenomic data collection involved Reduced Representation Bisulfite Sequencing (RRBS). Both proteomic and metabolomic analyses were carried out adopting Liquid Chromatography–tandem Mass Spectrometry (LC–MS/MS). The RRBS data were mapped and analyzed using Bismark and methylKit, while the RNA-Seq data were processed with edgeR for Differential Expression Analysis (DEA).

Epigenomic analysis showed slight but significant DNA methylation changes according to the seasons. Indeed, 8 differentially methylated CpG sites (DMCs) distinguish between T0 and T1; 17 DMCs differentiate T1 and T2, and finally 5 DMCs separate T0 and T2.

Greater differences among seasons, especially between warm periods (T0 and T1) and the reference condition (T2), were observed by the DEA. Indeed, when comparing T2 with both warm periods we found a total of 8,576 Differentially Expressed Genes (DEGs) in T2 vs T0 and 7,835 DEGs in T2 vs T1, while only 595 DEGs were found when comparing the two warm periods (T1 vs T0).

To gain a systems-level understanding of the heat stress response, the datasets were integrated using the DIABLO method. The multi-omics analysis identified highly correlated variables ($r > 0.93$), comprising markers involved in stress response and metabolism, and metabolites linked to energetic homeostasis and inflammation.

Converging immune related signals were identified across multi-omics datasets, and further corroborated by proteomic Gene Ontology analysis, which identified shared pathways linked to immune function and stress response, thereby strengthening integrative coherence.

Overall, these results provide insights into the molecular mechanisms underlying seasonal adaptation in Mediterranean buffalo.

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Performance of the Intradermal Test and Molecular Characterization of *Mycobacterium bovis* in Water Buffaloes (*Bubalus bubalis*) from Corrientes, Argentina

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Bovine tuberculosis (bTB), primarily caused by *Mycobacterium bovis*, affects cattle, buffaloes, and humans. In Argentina, control strategies are based on the intradermal skin test (IDR) and the culling of positive animals (SENASA 128/12), with inclusion of buffaloes since 2015 (SENASA 38/15). However, in *Bubalus bubalis*, there are ongoing controversies regarding the diagnostic performance of the IDR, with variable results compared to those observed in cattle. This study aimed to evaluate the diagnostic performance of the intradermal test (IDR) in water buffaloes and to characterize molecularly the Mycobacterium bovis isolates obtained from a herd in Corrientes Province, Argentina, in order to contribute valuable information for bovine tuberculosis surveillance and control in this species. A total of 422 buffaloes were evaluated, of which 5.21% (n = 22) tested positive to the IDR. Positive animals were slaughtered and subjected to post-mortem examination for tuberculosis-like lesions. Samples from lymph nodes and organs were collected for bacteriological culture and molecular analysis. Isolates were confirmed through amplification of IS6110 and *hsp65*. Spoligotyping identified three profiles: SB0140 (commonly reported in Argentina), SB0120 (low frequency and not previously reported in buffaloes in the country), and SB1055, previously described in cattle from central Argentina and now detected in the NEA region. The sensitivity, specificity, positive predictive value, and negative predictive value of the IDR were 0.89, 0.67, 0.80, and 0.80, respectively. The IDR demonstrated high sensitivity, supporting its use as a screening tool for bTB control in buffaloes. The detection of multiple spoligotypes within the same herd suggests both the introduction of infected animals and ongoing intra-herd transmission, underscoring the importance of implementing strict sanitary measures. Furthermore, the identification of new spoligotype profiles in buffaloes provides valuable insights into the regional epidemiology of *M. bovis*. These findings reinforce the need to develop species-specific strategies for buffaloes within national control and eradication programs, particularly considering their potential role in the persistence of bTB in mixed production systems.

Water Buffalo blood biobank as a supporting tool for the control of brucellosis

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The management of biobanks can represent a useful tool for the control of infectious diseases for livestock species. Water buffalo farming is an important livestock sector in many countries, especially in southern Italy, where milk from these animals is used to produce the worldwide famous mozzarella di bufala cheese. However, infectious diseases represent one of the main threats to health and productivity of water buffaloes. The management of a dedicated biobank can help prevent and control infectious diseases, by correctly identifying animals and related samples. In 2019 a water buffalo blood biobank has been established at the Istituto Zooprofilattico Sperimentale del Mezzogiorno. This biobank possesses a collection of blood samples available for diagnostic, research, and epidemiological surveillance purposes. The aim of this work is to describe the usefulness of this biobank as a supporting tool for the control of brucellosis. At present the biobank collects over 400,000 water buffalo blood samples from over 1100 farms, stored on vet-cards and suitable for analysis when required. Blood samples are genotyped by microsatellite (STR) analysis, with a validated procedure. Genetic profiles are used for identification purposes and parental diagnosis. All the genetic profiles are stored in a dedicated database. STR analyses are carried out to clarify demographic inconsistencies found in the farm or to certify the genealogy of calves. During the years 2023-2025 we performed 730 analyses to solve demographic purposes (match between blood from ambiguously or not identified animals and corresponding blood stored on vet-card), and 1407 parental diagnosis to certify the motherhood of calves, in agreement with the Health Department of the Campania region, which corresponds reimbursements to farmers slaughtering claves born from mothers tested positive to brucellosis. These measures allowed the correct identification of animals of dubious origin and the slaughtering of the correct calves to prevent the spreading of brucellosis within and across farms.
In a global strategy for the eradication of brucellosis in water buffalo, including numerous activities, mostly based on European and National Regulations, the management of a biobank proved to be an effective tool contributing to the control of this disease. Moreover, this biobank provides valuable samples useful for any future study based on water buffalo genetic characterization.

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Extracellular vesicle miRNA changes during experimental infection with Bubaline alphaherpesvirus 1 (BuAHV-1) in water buffalo

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Bovine alphaherpesvirus 1 (BoAHV-1) and bubaline alphaherpesvirus 1 (BuAHV-1) are major pathogens associated with respiratory, reproductive, and neurological diseases, with more severe clinical outcomes reported in water buffaloes. Extracellular vesicles (EVs) are lipid bilayer-enclosed nanostructures (30–150 nm) released by most cell types, playing key roles in intercellular communication through the transfer of bioactive cargo such as proteins, metabolites, and microRNAs (miRNAs). Increasing evidence suggests that EV-associated miRNAs (EV-miRNAs) can modulate host–virus interactions and play a role in disease development.

This pilot study aimed to evaluate whether experimental BuAHV-1 infection alters EV-miRNA cargo in vaccinated and non-vaccinated water buffaloes, and to explore the potential presence of viral miRNAs within EVs, as described for other herpesviruses. Thirteen buffaloes seronegative for BoAHV-1 and BuAHV-1 were allocated into three groups: animals vaccinated with a live IBR marker vaccine (gE-/tk-) (VAX-1), animals immunized with an inactivated IBR marker vaccine (gE-) (VAX-2), and non-vaccinated controls (CNT). At 55 days post-vaccination, all animals were experimentally infected with a wild-type BuAHV-1 field strain. Plasma samples were collected at the time of infection (T0) and 7 days post-infection (T7).

EVs were isolated from plasma using size-exclusion chromatography and characterized by nanoparticle tracking analysis and Western blotting. EV-miRNAs were extracted and profiled using next-generation sequencing. In the CNT group, BuAHV-1 infection led to the significant downregulation of 15 EV-miRNAs. Functional enrichment analysis revealed that these miRNAs are involved in angiogenesis and in the polarization of monocytes/macrophages, suggesting a potential role in modulating immune responses during infection. No statistically significant differences in EV-miRNA profiles were observed between vaccinated and control groups, nor between the two vaccination strategies. No viral miRNAs were found in purified EV.

Overall, these findings demonstrate that BuAHV-1 infection changes EV-miRNA expression profiles in water buffaloes, supporting their involvement in the epigenetic regulation of viral pathogenesis. Further studies are needed to validate these results and to investigate the functional roles of miRNA in the infected animals' EV cargo.

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Dynamics of total serum immunoglobulin E (IgE) in buffalo herds *(Bubalus bubalis)* adapted to the American tropic, and its role as a biomarker of physiological stress during weaning

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Over the past decade, animal welfare experts have debated how routine husbandry practices can negatively impact the physiological processes regulating immune response in large ruminants; even so, although the buffalo *(Bubalus bubalis)* has expanded considerably throughout Latin America, the scientific approach to understanding the complex metabolic pathways associated with immunological function remains very limited. In this regard, monitoring immunoglobulins (Ig), glycoproteins produced by plasma cells as part of the adaptive humoral immune mechanism against any immunogen, may prove a preventive diagnostic tool to consolidate the global "One Health" approach at the farm level, since the main immunoglobulin classes (IgG, IgM, and IgA) increase during the course of inflammatory pathologies; however, although IgE, the antibody with the lowest plasma concentration, acts as a biochemical signal transducer linked to allergic disorders and parasitic conditions, this Ig is not regularly referenced in the specialized literature. Therefore, given the deficit of studies on IgE in *B. bubalis*, 144 clinically healthy animals with excellent body condition were randomly selected, stratified by age, weight, and productive status, defining 12 age groups with 12 individuals per stratum in equal sex proportions, to quantify total serum IgE. For this purpose, 6 mL of blood was collected from each animal by venipuncture of the jugular vein using vacutainer needles and tubes without anticoagulant; samples were then centrifuged at 3600 rpm for 20 min and analyzed by enzyme-linked immunosorbent assay (ELISA). Results revealed 0.01 IU/mL of IgE across all evaluated animals, except buffalo at weaning age of 270 days; in this group, one female and two males reported levels of 3.54 IU/mL and 3.67 ± 0.30 IU/mL respectively, showing a significant difference ($p<0.05$) relative to the other age groups, which may indicate an alteration in immune response. For this reason, serum cortisol levels were measured using ELISA in these three individuals, yielding a mean of 37.25 ± 4.18 ng/ml. This exploratory finding, limited by the small affected sample size, suggests that chronic stress resulting from weaning could alter the humoral immune response. Consequently, further field studies with larger samples are needed to confirm the IgE-cortisol-weaning association and thus characterize the biochemical reactions involved.

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Development of an *in vitro* repellent bioassay to evaluate the efficacy of wood vinegar against the sucking lice *Haematopinus tuberculatus*

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Intensive water buffalo farming systems favor the proliferation of lice, particularly *Haematopinus tuberculatus,* causing direct damage characterized by clinical signs such as skin irritation, anemia, anorexia, restlessness, and loss of body condition (Shakya et al. Tropical Animal Health and Production 54, 66, 2022). In addition, lice infestations (pediculosis) are associated with reduced productivity in buffalo herds, which represent an important livestock resource in several countries (Bosco et al. Large Animal Review 24 (2), 73–79, 2018; Morgoglione et al. Frontiers in Veterinary Science 7, 410, 2020). The *in vitro* bioassay was performed in glass Petri dishes (ø 19 cm) divided into 3 separate sections, each covered with a layer of bibulous paper soaked with 3 mL of different solutions. The first (starting) and the third (finishing) area layers were soaked with distilled water. The layer of finishing area was previously vigor- ously scrubbed on the host skin and then covered with buffalo fur after soaking, to increase the attraction to the lice. The central (obstacle) area was impregnated with different concentra- tions of the WV to be tested (100%, 75%, 50% and 25%). In the positive and negative controls, the central layer was soaked with deltamethrin solution (BUTOX® 7,5 Pour-On, MSD Animal Health Srl) and distilled water, respectively. Each assay included three replicates, with 10 adult lice per replicate, maintained at 27 °C and 75% relative humidity. Observations were conducted at 5, 15, 30, 60, 90, 120, and 180 minutes post-application to evaluate lice movement, survival, and their ability to cross the treated area, expressed as avoidance percentage. In parallel, *in vitro* cytotoxicity tests were also performed. The results demonstrated a strong repellent effect, with 100% WV achieving 77% avoidance after 30 minutes of exposure, a value comparable ($P < 0.05$) to that observed with deltamethrin (77%). The bioassay developed in this study proved to be a robust and reproducible method for screening repellent compounds against lice under controlled conditions. Overall, wood vinegar showed promising potential as an alternative tool for the control of buffalo pediculosis. However, further *in vivo* investigations are required to confirm its efficacy and safety under field conditions.

Achieving helminth-free status in water buffalo farms through long-term surveillance

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Helminth infections are widely distributed across livestock farms worldwide, significantly impacting animal welfare and productivity, with subsequent economic losses for farmers. Routine farm practice often involves the use of anthelmintic drugs, frequently administered without prior diagnostic confirmation. This approach poses substantial risks, including drug residues in milk and meat, as well as environmental contamination due to the unjustified dispersion of drugs and the progressive emergence of anthelmintic resistance (AR) against different drugs.

In the Campania region, southern Italy, the Center for Monitoring of Parasites in livestock (CREMOPAR) has developed and implemented sensitive, specific, accurate and precise quali-quantitative copromicroscopic diagnostic tools (i.e. FLOTAC, Mini-FLOTAC and Fill-FLOTAC). These approaches, combined with effective evidence-based antiparasitic treatments, have significantly improved the health status of livestock. Trough continuous technical support to farmers, including regular parasitological monitoring and rational use of anthelmintics, it has been possible to substantially reduce and, in many cases, eliminate the helminth infections in buffalo farms. Data from our diagnostic activity over the last ten years show that, out of a total of 5,811 buffalo farms analysed, only 22 (0.4%; 95%CI= 0.2 - 0.6%) were positive at least to one helminth genus. Notably, in recent years the number of positive farms declined, reaching two (0.03%; 95%CI= 0.01 – 0.1%) in 2022 and 2023, one (0.01%; 0 – 0.1%) in 2024 and zero positive farms in 2025.

These findings demonstrate that the implementation of integrated, sustainable parasite control strategies can lead to the effective elimination of helminths in buffalo farms. Furthermore, they highlight the importance of combining advanced diagnostics, targeted treatments, and farmer engagement to ensure long-term sustainability. Such an approach provides a strong basis for the development of standardized protocols schemes for helminth-free farms, with added value in terms of product quality, food safety and environmental protection. In addition, the adoption of these practices may contribute to reducing drug dependence, limiting the spread of resistance and promoting more responsible and sustainable livestock production systems at both regional and global levels.

Some authors contributed to the development of the FLOTAC, Mini-FLOTAC and Fill-FLOTAC techniques used in this study. The authors declare that this involvement had no influence on the analysis or interpretation of the data presented.

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Predictive Andrology in River Buffaloes: Retrospective Heat Stress Analysis to Forecast Semen Cryotolerance

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While commercial cryopreservation of river buffalo (*Bubalus bubalis*) semen is a consolidated global reality, optimizing post-thaw viability remains a critical industry priority due to the species' inherent physiological challenges with thermoregulation and susceptibility to heat stress. Standard pre-freezing evaluations heavily rely on immediate kinetic parameters, inherently overlooking latent, sub-lethal cellular damage inflicted by climate variations weeks prior to collection. This study aimed to quantify the physiological cost of retrospective climate variations on buffalo sperm cryotolerance using a predictive modeling approach. Fresh and thawed river buffalo ejaculates (n=111) were analyzed via Computer-Assisted Sperm Analysis (evaluating up to 30 kinetic and morphological variables) and cross-referenced with localized meteorological records (maximum temperature and relative humidity) spanning 15, 30, and 60 days pre-collection. Utilizing the ReproSense platform and DeepRepro analytics, powered by Random Forest ensemble algorithms, non-linear relationships between climatic history and post-thaw survival were mapped. The physiological analysis revealed a distinct thermal vulnerability window. By evaluating climatic variables independently, the algorithm demonstrated that latent heat stress driven primarily by maximum temperatures during epididymal transit (15 days pre-collection) and the onset of spermatogenesis (60 days pre-collection) emerged as the predominant limiting factor for post-thaw viability. This delayed impact likely compromises critical lipid membrane remodeling during epididymal maturation. Surprisingly, historical maximum temperatures alone outperformed real-time linear velocities (VCL, VSL) in defining freezing success, with the ensemble framework forecasting batch approval with a predictive accuracy of 96% (AUC-ROC = 0.81). Furthermore, among immediate kinetic markers, Beat Cross Frequency (BCF) and Straightness (STR) were identified as the most reliable biophysical indicators of cellular membrane resilience against thermal shock. In conclusion, buffalo sperm cryotolerance is fundamentally dictated by prior thermal exposure rather than exclusively by pre-freezing motility. Implementing Predictive Andrology to monitor these environmental and biophysical dynamics allows for the early identification of compromised ejaculates, mitigating economic losses and elevating quality control standards in buffalo reproduction programs.

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Ovarian Response and Embryo Quality in Riverine Buffaloes Treated with a Wagyu Cattle -Based Superovulation Protocol

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Multiple ovulation and embryo transfer (MOET) is a promising technology for accelerating genetic improvement and is commercially applied in Wagyu cattle. This study evaluated the ovarian response and embryo developmental stages in riverine buffaloes treated with a Wagyu-based superovulation protocol. Fourteen buffaloes (4–8 years; BCS 3.0–3.5) from two farms received a progesterone implant (Ovapron®, Kyoritsu Seiyaku, Japan) on Day 1, decreasing doses of FSH (Antrin R10, Kyoritsu Seiyaku) from Days 7–9, prostaglandin (Dalmacin, Kyoritsu Seiyaku) injection and implant removal on Day 9, artificial insemination on Days 11 and 14, and GnRH (Espornen, Kyoritsu Seiyaku). Ovarian structures were examined by ultrasonography on Day 1 and Days 16–18. Baseline evaluation showed 5.6±2.1 small follicles and 0.2±0.4 CL. After superovulation, buffaloes developed 3.6±1.7 CL and 2.1±1.5 unovulated follicles, corresponding to an ovarian response of 108.8±47.9% and an ovulation rate of 78.0±48.7%. Statistical comparison showed significant variation between farms: Farm A (n=9) produced 2.9±1.4 CL (ovarian response: 95.1±48.7%; ovulation rate: 55.8±36.0%), while Farm B (n=5) produced 5.0±1.2 CL (ovarian response: 133.3±39.1%; ovulation rate: 118.0±44.8%). Age also influenced outcomes ($p<0.05$): buffaloes 4–<6 years (n=8) generated 2.9±1.5 CL (ovarian response: 86.2±43.5%; ovulation rate: 50.3±34.2%), whereas those 6–8 years (n=6) produced 4.7±1.4 CL (ovarian response: 138.9±37.5%; ovulation rate: 115.0±40.8%). Timing of ovarian assessment showed increasing response with later examination: Day 16 animals (n=4) showed 98.6±53.4% ovarian response and 61.1±44.9% ovulation rate; Day 17 (n=5) showed 92.4±50.9% and 51.5±32.2%; Day 18 (n=5) showed 133.3±39.1% and 118.0±44.8%. Embryo recovery occurred only on Day 16, yielding transferable embryos exclusively at the morula and mid-blastocyst stages. Overall, the Wagyu-based protocol stimulated follicular recruitment and ovulation in riverine buffaloes, with farm, age, and timing significantly affecting response. The recovery of morula and mid-blastocyst embryos supports the potential application of Wagyu-derived MOET strategies in buffalo genetic improvement programs.

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Association of the uterine microbiome with fertility in buffalo

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This work aimed to assess if the microbiome of reproductive organs is associated with the outcome of artificial insemination in Italian Mediterranean buffalo. For this purpose, 40 pluriparous Italian Mediterranean Buffaloes, homogenous (mean ± SD) per age (3.9 ± 1.4 years) and days in milk (58 ± 16 days) were enrolled. All the animals underwent clinical examination, and only healthy ones were included in the trial. Moreover, animals that received antibiotic treatment within the previous 12 months were excluded. The animals were synchronized using Ovsynch protocol, and artificial insemination (AI) was performed. Before AI, samples were collected for bacterial DNA isolation as follows: the external surface of the vulva was gently cleaned with 2% Chlorhexidine solution, and a double-guarded cytobrush (Minitube, German) was gently inserted into the uterine body. This was followed by a vaginal swab performed in a similar manner. Upon withdrawal, an operator wearing sterile gloves placed the uterine/vaginal samples into sterile tubes containing 2 ml of PBS. Tubes were kept on ice until transported to the laboratory. Pregnancy status was assessed at 30 and 45 days after AI by ultrasonography. The animals were retrospectively divided into pregnant (P) and non-pregnant (NP) groups, with 10 samples per group + 2 blank control samples randomly selected for further analysis. DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific) according to the manufacturer's instructions. Amplification and library preparation of the 16S rRNA V3-V4 gene region were performed using a two-step PCR approach as described in Illumina's application note. Sequencing was performed on a MiSeq (Illumina). Beta diversity was computed by the Bray-Curtis method, and differences between groups were evaluated by PERMANOVA and Pairwise tests. Significance was set at $p < 0.05$. Beta diversity analysis revealed differences between P and NP in the uterine microbial composition (PERMANOVA: $p = 0.035$ Pairwise: $p = 0.028$) but not in the vaginal one.
The findings highlight the presence of uterine-specific microbiota associated with early pregnancy in buffalo. Further studies are required to identify specific bacterial taxa involved and elucidate their role in modulating the uterine microenvironment. This knowledge could lay the ground for novel diagnostic tools or therapeutic strategies to enhance the AI success rates in this species.

Uptake of nanoparticles by buffalo spermatozoa: potential implications for male fertility

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Nanoplastics (NP) are emerging as a potential key driver of male infertility. However, there is still a lack of *in vitro* studies on the effects of NP on sperm. This study aimed to assess the internalization of NP and their consequences on buffalo frozen-thawed semen after 1 hour of exposure.

To evaluate NP internalization, five ejaculates were incubated at 38°C for 1 h either without (control; CTR) or with different concentrations: 10, 50, 100 and 200 µg/mL of 30 nm fluorescent yellow-green carboxylate-modified polystyrene beads (NP10, NP50, NP100 and NP200, respectively). Samples were then analyzed by flow cytometry (CytoFLEX® S) to assess fluorescence intensity (Arbitrary Fluorescence Units; AFU) within the live sperm population of each group. To analyze the effects of NP on semen, 14 samples (2 ejaculate/bull) were incubated for 1 h with identical concentrations of non-fluorescent 30 nm polystyrene NP. Then, sperm viability (DRAQ7), mitochondrial activity (JC-1), intracellular ROS (reactive oxygen species; CellROX green) and GSH (glutathione; Monochlorobimane) levels were assessed by flow cytometry. Groups differences were evaluated using one-way ANOVA with Tukey's post-hoc test or Kruskal-Wallis with Dunn's post-hoc, based on data normality. Results are given as mean ± SEM.

A dose-dependent uptake of NP was observed, with NP100 and NP200 groups showing higher green fluorescence than CTR and NP10 groups ($P<0.01$). Notably, NP200 demonstrated the highest internalization, significantly exceeding all other conditions ($P<0.0001$). Sperm viability and mitochondrial activity remained comparable across groups. Nevertheless, intracellular ROS and GSH levels showed a significant dose-dependent decline in live sperm, with a marked reduction in NP100 (ROS: 89.5 ± 4.4 AFU; GSH: 100.2 ± 2.2 AFU) and NP200 (ROS: 64.9 ± 5.9 AFU; GSH: 69.4 ± 6.6 AFU) groups compared to CTR (ROS: 132.7 ± 6.2 AFU; GSH: 117.6 ± 3.6 AFU) ($P<0.05$). Additionally, the NP200 concentration caused the most significant depletion of intracellular ROS and GSH levels relative to NP10 (ROS: 110.8 ± 6.1 AFU; GSH: 108.4 ± 3.3 AFU) and NP50 (ROS: 102.1 ± 3.3 AFU; GSH: 104.4 ± 1.9 AFU) ($P<0.05$).

This study demonstrated for the first time the successful internalization of NP in buffalo spermatozoa, revealing a clear dose-dependent uptake. Moreover, the depletion of intracellular ROS and GSH levels following even short-term exposure suggests an early interference with the sperm redox homeostasis.

Precision de-seasonalization in buffalo herds: a farm-specific production strategy to enhance farm competitiveness

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In Italy, buffalo milk is primarily processed into Mozzarella di Bufala Campana, a PDO cheese characterized by marked seasonal fluctuations in demand. Although the Mediterranean buffalo is a rustic and resilient species, its reproductive seasonality represents a major challenge for dairy farming. Reduced daylight duration during autumn and winter promotes reproductive activity, concentrating calvings in these months, when both consumer and dairy processor demand for milk decline substantially; moreover, the contractual milk price is also lower.

Our field experience, involving more than 70 buffalo farms and over 25,000 animals, shows how farm-specific de-seasonalization and planned calving distribution could improve milk production predictability and better align supply with market requirements. The most reliable strategy for obtaining pregnancies within predefined time windows is timed artificial insemination following synchronized ovulation, combined with early pregnancy diagnosis and prompt re-synchronization of non-pregnant animals. Two synchronization protocols are applied according to the season, maintaining similar pregnancy rates throughout the year in both buffaloes and heifers.

The number of animals enrolled in each synchronization and insemination group should be determined by herd size, production targets, farm-specific fertility rate, which averaged 65% across the farms involved, and marketing strategy. The program is therefore tailored to each farm, whether milk is processed on-farm or delivered to an external dairy processor.

With appropriate planning, more than 70% of annual calvings and the resulting milk production can be concentrated in the first six months of the year, shifting production towards spring and summer and improving alignment with market demand. Precision de-seasonalization should therefore be considered not simply as a reproductive management technique, but as a farm-specific production planning strategy.

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A Novel Weighted Cryopreservation Resistance Index (CRI) for Assessing Post-Thaw Semen Viability in Water Buffaloes

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Cryopreservation induces severe thermal and osmotic stress on buffalo spermatozoa, leading to significant declines in kinetic viability and substantial economic losses due to commercial batch rejections. Traditional pre-freezing evaluations relying on isolated kinetic parameters often fail to accurately predict post-thaw recovery and overall cryotolerance. This proof-of-concept study aimed to develop and calibrate a species-specific, weighted Cryopreservation Resistance Index (CRI) for water buffaloes by integrating multi-dimensional Computer-Assisted Sperm Analysis (CASA) data to proactively assess freezeability and stratify sires based on cellular resilience. A retrospective, paired analysis of 122 semen batches (fresh vs. thawed) from 9 buffalo sires was compiled via the ReproSense platform. The computational model evaluated relative percentage losses across seven key kinetic parameters, assigning specific physiological weights: total motility (20%), progressive motility (20%), beat cross frequency (BCF; 20%), straightness (STR; 15%), and velocities (VSL 15%, VCL 5%, VAP 5%). The inclusion of BCF and STR was based on prior machine learning validations highlighting their critical role in cellular resilience. To address predictive validity, a Leave-One-Sire-Out Cross-Validation (LOGOCV) using Random Forest algorithms was retrospectively applied to the fresh semen data. The model achieved an 81.6% global predictive accuracy for post-thaw approval, and its autonomous feature importance ranking closely corroborated the rationally assigned CRI weights. Notably, current batch rejections were mathematically isolated into the Low resistance category (approaching 30% loss). Furthermore, statistical comparisons confirmed that post-thaw rejection was dictated by individual biological resilience rather than dose hyper-concentration ($p > 0.05$). While the limited number of sires ($n=9$) and the natural dataset imbalance currently restrict broad generalization, this proof-of-concept establishes a robust and validated framework. As the data lake expands to include a wider genetic pool, the CRI thresholds will be dynamically refined and subjected to further external validation to maximize predictive accuracy.

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Influence of forage management on milk and buffalo mozzarella quality

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A research trial was carried out to evaluate the effect of different forage species and preservation methods on the yield and quality of PDO buffalo mozzarella cheese. The study was conducted on a dairy buffalo farm producing PDO mozzarella using only onfarm milk. In September 2023, two fields were sown with ryegrass (*Lolium multiflorum* L.) and a legumerich polyphytic mixture (grass/legume ratio 45/55). In March, two feeding trials were undertaken. Trial 1 evaluated fresh ryegrass versus ryegrass hay, while Trial 2 compared fresh ryegrass with fresh polyphytic forage. Two groups of fourteen lactating buffaloes, homogeneous for age (5.9 ± 1.8 years), days in milk (119 ± 29), and milk yield (10.4 ± 2.1 kg/d), were used. In Trial 1, one group received a basal diet with 3.5 kg ryegrass hay (HRy), partially replaced by fresh ryegrass (FRy; 12 kg fresh forage replacing 2 kg hay on an asfed basis) in the FRy group. In Trial 2, the hay in the HRy diet was substituted with 12 kg fresh polyphytic forage (FPo). Each trial included 10 days of adaptation followed by 2 weeks of milk recording. Milk samples were collected twice weekly and analysed for major components. At the end of each period, milk from each group was collected separately for three consecutive days and used to produce mozzarella, which was analysed for composition, fatty acids, sensory traits and consumer liking. Fresh forage species had no significant effect on milk yield or mozzarella quality. Likewise, no significant differences in milk composition were detected between FRy and HRy, although a tendency ($0.05 < p < 0.1$) toward higher yield was found in FRy (9.7 vs 10.5 kg/d, SEM = 0.21). Milk from FRy had higher ($p < 0.05$) αlinolenic acid and c9,t11 CLA than HRy. Mozzarella from FRy showed lower ($p < 0.05$) cohesiveness, graininess and moisture, with no differences in consumer liking. Overall, freshforage species did not influence productivity or cheese quality. Replacing ryegrass hay with fresh ryegrass improved the nutritional profile of mozzarella by increasing beneficial fatty acids while maintaining, or possibly enhancing, milk production.

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Mammary lipid metabolism in buffalo compared with other ruminants: a multivariate factor analysis of milk fatty acid profiles

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Milk fatty acid (FA) composition reflects the complex interplay between rumen microbial metabolism, mammary gland activity, and diet. While multivariate factor analysis (MFA) has previously been applied within single ruminant species, interspecific comparisons remain limited. This study aimed to identify common latent metabolic factors underlying milk FA composition across ruminants, with specific emphasis on buffalo milk.

A total of 1,960 mid-lactation animals reared in Italian commercial farms were included: cows (n=770), sheep (n=751), goats (n=209), and Italian Mediterranean buffalo (n=230). Milk FA profiles (49 FA) were determined by gas chromatography. MFA was applied to the correlation matrix to extract latent factors (eigenvalue >1). Factor scores were used to evaluate species effects.

Eleven latent factors explaining 72% of total variance were identified, representing distinct metabolic pathways, including ruminal microbial activity (OBCFA, BCFA, OCFA), mammary de novo synthesis (Mammary activity, SCFA), biohydrogenation pathways (BH_1, BH_2), β-oxidation, n-6 metabolism, milk fluidity, and FA elongation.

Buffalo milk showed a distinctive metabolic profile. It exhibited intermediate mammary activity (0.51), high short-chain FA synthesis (SCFA factor: 0.41), and markedly elevated β-oxidation scores (4.56), suggesting enhanced mitochondrial lipid metabolism compared with other species. Conversely, buffalo displayed the lowest values for the alternative biohydrogenation pathway (BH_1: −0.72) and negative scores for n-6 metabolism (−0.35), indicating reduced elongation of linoleic acid derivatives. Buffalo milk also showed moderate ruminal microbial signatures (OBCFA: −0.20; BCFA: 0.01; OCFA: −0.34), differentiating it from both cattle and small ruminants.

Compared with cows, buffalo demonstrated lower biohydrogenation variability but higher short-chain FA contribution, consistent with its higher milk fat content and energy density.

Multispecies MFA revealed shared metabolic structures underlying milk lipid synthesis while highlighting species-specific adaptations. Buffalo milk is characterized by a unique balance between mammary synthesis, β-oxidation, and short-chain FA production, supporting its distinctive nutritional and technological properties. This integrative approach provides a unifying framework for interpreting ruminant mammary metabolism and offers new perspectives for the valorization of buffalo milk within dairy production systems.

Serum biochemical parameters, antioxidant capacity markers, and lysosomal enzyme activities in peri-weaned buffalo calves supplemented with Tulsi (holy basil) extract

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Research has shown that Tulsi (*Ocimum tenuiflorum L.*) is effective in the prophylaxis of physiological and nutritional transitions associated with stress. Most crucial for farm animals is the adaptation to weaning – an energy shift from fats and carbohydrates to volatile fatty acids, acetate and ketone bodies.

The present aim was to analyse the effects of Tulsi liquid extract on serum biochemical parameters, enzymes and antioxidants in buffalo calves during the peri-weaning period, in association with health and adaptation.

Bulgarian Murrah calves from the farm of Agricultural Institute – Shumen were stratified into control (Ctr, n= 6) and experimental (Exp, n= 6) group 13 days before weaning. To the milk replacer of each animal in Exp was added 20 ml of Tulsi from Day 1 to Day 13. Individual serum samples were taken from both groups on Day 0, Day 13 (last suckling day) and Day 14 (first day after weaning).

Total polyphenols show significant increase by 27.0% ($P \leq 0.001$) in Exp in the 13-day period, in view of the slight decrease in Ctr, the significantly big difference in favour of Ctr on Day 0 (21.0%, $P \leq 0.001$) becoming a smaller one ($P > 0.05$) on Day 13 in favour of Exp. Significant increase in DPPH is observed from Day 0 to Day 14 – by 9.8% ($P \leq 0.001$) in Exp, while that in Ctr is smaller. A substantial and significant increase by 65.0% ($P \leq 0.05$) in Vitamin C is found in Exp compared to the little change in Ctr. On Day 13, in Exp HDL is by 22.5% significantly higher than Ctr ($P \leq 0.05$), compared with the small difference (10.7%) on Day 0. Triglycerides in Ctr is reduced by 44.0% ($P \leq 0.05$), while in Exp the drop is by 21.4% ($P > 0.05$) and by another 32.7% on Day 14.

In conclusion, Tulsi did not affect some biochemical hepatic parameters, like AST, ALT and ALP, but it had positive effect on Acid phosphatase, Cholesterol and Triglycerides. The 13-day supplementation showed a tendency to increase the activity of several serum glycosidases (β-glucuronidase, β-galactosidase and β-glucosidase) and lipase, suggesting a modulation of carbohydrate-related metabolic processes, but the effect ceases after weaning when Tulsi is removed. The effect of the basil extract is best expressed on total polyphenols, DPPH scavenging, and especially Vitamin C.

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Harnessing native ruminal bacteria to enhance fiber digestion, modulate volatile fatty acids, and reduce methane in tropical water buffalo

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Water buffalo production in tropical regions is constrained by low-quality forages, limiting nutrient utilization and increasing enteric methane emissions. This study evaluated the effects of native ruminal probiotic consortia on fiber degradation, fermentation dynamics, and methane production. Ruminal fluid samples were collected from sixteen buffalo calves (2–8 months old) and subjected to selective anaerobic culturing for microbial isolation. A total of 47 isolates were obtained, including 30 lactic acid bacteria (Lactobacillus spp.-like) and 16 yeast strains (Saccharomyces-like). From the microbial isolation, four bacteria were selected (*Lacticaseibacillus rhamnosus* (a), *Lactiplantibacillus plantarum* (b), *Lacticaseibacillus paracasei* (c), and *Pediococcus acidilactici* (d)) and assembled into 15 treatments (combinations), including single strains and multispecies consortia. *In vitro* fermentation assays were conducted using ruminal fluid and *Brachiaria humidicola* (CP: 5.8%; NDF: 58.4%). Multispecies consortia significantly improved degradability ($p<0.01$). The combination a+c+d showed the highest values (DM: 61.4%, NDF: 59.0%, ADF: 55.8%) compared to the control (58.0%, 55.4%, 51.6%). Methane production was modulated ($p<0.001$ at 24 h), with b+c showing the lowest emissions (~25.2% vs. 30.1% in control). Fermentation profiles revealed shifts in volatile fatty acids without affecting total gas production, indicating maintained microbial activity. Multifactorial analysis identified significant microbial interactions, particularly involving *L. rhamnosus* and *L. plantarum*, confirming that probiotic effects are driven by synergistic rather than additive mechanisms. A composite index integrating degradability, methane, and fermentation parameters ranked a+c+d as the best-performing consortium. These results demonstrate that native ruminal microbial consortia enhance fiber utilization, modulate fermentation, and reduce methane emissions. This approach supports precision microbiome strategies to improve productivity and sustainability in tropical buffalo systems.

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Probiotic microorganisms and ruminal microbiota populations in Italian buffalo

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The ruminal microbial population in Italian buffaloes is a diverse ecosystem essential for digesting fibrous feedstuffs. The primary functional populations include specific phyla of bacteria, archaea, fungi, and protozoa, which are crucial for nutrient breakdown and energy production. However, some microbial feed additives, such as Bacteria (*Lactobacillus plantarum* and *Lactobacillus fermentum, Limosilactobacillus reuteri*, *Ligilactobacillus salivarius*, *Bifidobacterium lactis, Streptococcus thermophilus, Enterococcus faecium* and *Enterococcus faecalis, and Enterococcus faecium*); Fungi (*Saccharomyces cerevisiae* and *Aspergillus oryzae*, are commonly used in Italian buffalo feeding. Probiotic microorganisms beneficially modulate the ruminal microbiota populations in Italian buffaloes by selectively promoting the growth of beneficial bacteria, enhancing fiber-degrading communities, and suppressing harmful pathogens. This modulation leads to improved nutrient absorption and overall animal health. *Saccharomyces cerevisiae* yeast and certain *Bacillus* strains (e.g., *Bacillus paralicheniformis* SN-6 isolated from buffalo rumen), stimulate the activity and population of these cellulolytic microbes (e.g., *Fibrobacter* succinogenes and *Ruminococcus flavefaciens*), making more energy available to the animal. Studies involving buffalo-derived probiotics, such as *Bacillus paralicheniformis* SN-6, have shown significant increases in the abundance of beneficial bacteria families and genera, such as *Clostridiaceae* and *Lachnospiraceae* (utilizing dietary polysaccharides and producing beneficial metabolites like short-chain fatty acids, which nourish the intestinal lining and support overall health*), Bifidobacterium* (play a critical role in maintaining intestinal health, supporting the immune system, and preventing disease*), Cellulolyticum* (specific cellulolytic genus also increases with probiotic supplementation*)*. The addition of probiotics in dietary buffalo has many nutritional benefits: enhanced digestion- improved fiber breakdown and nutrient utilization; improved performance- increased milk yield and body weight gain; reduced methane- modulation of rumen microbes to decrease methanogen populations; and as an Antibiotic alternative: Replaces banned antibiotic growth promoters.

Advancing the Characterization of Carcass and Meat Quality Traits of Water Buffalo (*Bubalus bubalis*) Raised under Philippine Conditions

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Water buffalo (*Bubalus bubalis*) remains an important livestock species in most East and Southeast Asian countries. It is primarily used for draft purposes and milk production but increasingly recognized for its potential as a meat source. This study advances the characterization of carcass and meat quality traits of fifty-one (51) male water buffaloes (Bubalus bubalis), with an age range of one to three years old, raised under Philippine conditions by evaluating its carcass yields, physico-chemical properties, and nutritional composition. Mean liveweight was 403.10±54.67kg, while hot and chilled carcass weights were 203.66±34.94kg and 194.75±33.60kg, respectively. Mean dressing percentage was 49.72±2.77% for hot carcass and 47.54±3.32% for chilled carcass, with a significant decrease from hot to chilled carcass weight ($p<0.001$). Postmortem pH showed a significant decline from 6.16±0.22 at slaughter to 5.65±0.28 at 24h ($p<0.001$), followed by stabilization at 48h with 5.66±0.19, indicating normal rigor mortis progression. Drip loss is at 1.35±0.62%, while tenderness averaged at 7.23±1.26kg shear force. Buffalo meat was characterized by 73.11±3.18% moisture, 19.02±2.49% protein, 4.14±3.13% fat, and 46.42±25.50% cholesterol. Iron content averaged 2.60±1.32%, supporting its value as a nutrient-dense red meat. The fatty acid profile was dominated by palmitic, stearic, and oleic acids, consistent with typical ruminant lipid profiles. Overall, the results demonstrate that that water buffalo under Philippine conduction is a lean, protein rich red meat with acceptable carcass yield. These findings provide baseline reference data and a scientific basis for future research on feeding systems and other production strategies aimed at improving buffalo meat quality.

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Antibiotic Resistance Profiling of Lactobacillus Strains Isolated from Buffalo Milk

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Species within the genus *Lactobacillus* are generally recognized as safe (QPS status) and widely utilized in the food and probiotic industries. However, recent large-scale assessments indicate that antibiotic resistance (AR) levels in these microorganisms often exceed recommended thresholds, requiring a reassessment of their safety profiles. The aim of this study was to characterize the AR profiles of five strains isolated from thermized buffalo milk, identified via MALDI-TOF/MS as *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum*, and *Levilactobacillus brevis*.

Phenotypic screening using the Kirby-Bauer disk diffusion method against 14 antimicrobial agents revealed a complex multi-drug resistance (MDR) pattern. All isolates (5/5) exhibited resistance to ampicillin, cefoxitin, ciprofloxacin, gentamicin, vancomycin, and trimethoprim-sulfamethoxazole. Of particular concern is the observed resistance to meropenem and imipenem; as carbapenems represent last-resort antibiotics in human medicine, their phenotypic failure in foodborne isolates poses a significant public health threat. Calculated per the Multiple Antibiotic Resistance (MAR) index of 0.60 and Antibiotic Resistance Pattern (ARPA) value of 8.4 significantly exceeded the critical environmental risk threshold of 0.2 reported in literature, suggesting either high selective pressure or notable genomic plasticity.

While certain resistances (e.g., vancomycin and aminoglycosides) may be attributed to intrinsic mechanisms, the high average resistance count per isolate (nR > 8) highlights the potential role of these strains as reservoirs for AR genes (ARGs). Consequently, the integration of Whole-Genome Sequencing (WGS) into the experimental workflow is established as the necessary diagnostic shift to characterize the *resistome*. By moving from phenotype-based to genotype-based assays, WGS allows for the discrimination between physiological traits and acquired determinants located on mobile genetic elements (MGEs), such as plasmids and transposons. This genomic surveillance is crucial for assessing the risk of horizontal gene transfer (HGT) within the gastrointestinal tract. In conclusion, the high phenotypic resistance observed underscores the need for rigorous molecular validation to ensure the safety of *Lactobacillus* strains along the food chain and to prevent the dissemination of mobile ARGs into the human gut microbiota.

Variation in composition of bulk tank milk in Italian buffalo herds

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Buffalo farming in Italy is mainly focused on the production of Mozzarella cheese. The quality of bulk tank milk is therefore of critical importance to monitor the efficiency of nutrient utilisation. Apart from the basic composition of milk (fat, protein, casein, lactose, urea), several other parameters can be used to assess more thoroughly the effect of nutrition, such as the fatty acid profile (included classes and some individual fatty acids). As traditional chemistry can be expensive and time-consuming, fast analysis, such as Mid-InfraRed (MIR) spectroscopy, can be used when properly calibrated. The objective of this work was to describe the variation of milk components in bulk tank milk from Italian herds measured with MIR technology.

Samples of bulk tank milk (n=117) from 73 buffalo herds located in different regions of Italy and collected between March 2024 and March 2026 were analysed in Nutristar's laboratory for milk composition and fatty acid content using a Lactoscope FT-A (Perkin Elmer) calibrated *ad hoc* for buffalo milk with a proprietary curve. Results were recorded in an Excel datasheet and a descriptive statistic of the results has been carried out to evaluate mean and variability (range and coefficient of variation, CV).

Fat, protein, casein and lactose showed a low variability (CV≤10%) among herds (CV 9.35%, 3.87%, 5.68% and 3.14% respectively). Urea, on the other hand, was very variable (CV 40.8%, range 6.2-83.8 mg/100ml). As regards fatty acids, a high variation was found for oleic acid (CV 26.8%, range 7.7-31.3, % total fatty acids). The other parameters showed CVs ranging from low to moderate (between 10 and 20%).

The difference in variability for the parameters we measured likely indicates that some components are more stable than others, regardless of the variability of herd characteristics (such as diet): others, however, can be more influenced by the characteristics of the herd, which will require further investigation to identify what herd-related factor has the highest impact. This will hopefully lead to a deeper understanding of the nutritional physiology of the lactating buffalo and to a more robust approach when formulating specific diets.

From Feeding Systems to Food Quality: Assessment of Buffalo Cheeses (Mozzarella, Scamorza, and Caciocavallo)

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Diet is a key extrinsic factor influencing several quali-quantitative parameters of buffalo milk and its derived products (Salzano et al., 2021). The aim of this study was to assess the physicochemical, rheological, and nutritional properties of buffalo cheeses produced from animals fed a novel functionalized diet. In this regard from September 2025 to February 2026, buffaloes were alternately subjected, on a monthly basis to a control diet and an experimental diet. Physicochemical, nutritional and rheological analyses, including pH, water activity (aw), fatty acid profile (FAME), chemical composition, texture profile analysis (TPA) and color, were performed on cheeses produced from functionalized (TRT) and non-functionalized (CTR) buffalo milk, namely mozzarella, scamorza, and caciocavallo. For each product type, raw milk, curd, and final products were analyzed. The obtained results highlight the beneficial impact of the novel functionalized feed, with TRT products exhibiting more favourable characteristics than CTR. Specifically, TRT cheeses showed an improved nutritional profile, characterized by a higher proportion of monounsaturated fatty acids (MUFA), mainly driven by an increase in C18:1 cis (oleic acid), which represents the major contributor to the enhancement of lipid quality. Conversely, a lower proportion of saturated fatty acids (SFA) was observed, primarily due to the reduction of short- and medium-chain fatty acids (SCFA and MCFA), as well as specific saturated fatty acids considered less favourable from a nutritional standpoint, such as C12:0 (lauric), C14:0 (myristic), and C16:0 (palmitic acids), known for their higher hypercholesterolemic potential. Accordingly, lower atherogenic (AI) and thrombogenic (TI) indices, together with higher HH and HPI ratios, indicated a more favourable cardiovascular profile. These nutritional improvements associated with their compositional characteristics, namely higher moisture content and lower fat percentage compared to CTR, also influenced rheological characteristics, with TRT cheeses exhibited lower hardness, adhesiveness, and gumminess and lower yellowness (b*) values. Overall, these findings indicate that the dietary strategy adopted effectively improved the quality of functionalized buffalo cheeses, supporting the use of such feeding approaches to obtain products with enhanced health-related properties and desirable quality traits that satisfy both industry requirements and consumer expectations.

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Functional evaluation of the corpus luteum by Doppler ultrasonography in buffaloes treated with $PGF_2\alpha$ at the time of artificial insemination

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The corpus luteum plays a fundamental role in the establishment and maintenance of pregnancy, as its functionality is closely associated with vascularization; increased blood perfusion reflects greater steroidogenic capacity and viability. This study evaluated the effects of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) administration at the time of fixed-time artificial insemination (FTAI) on luteal vascularization and its association with conception rates in buffaloes. Forty-five Murrah females were randomly allocated into a control group (T0; n=22), which did not receive treatment, and a treated group (T1; n=23), which received 0.5 mg of sodium cloprostenol on the day of FTAI (D11). All animals underwent a standard synchronization protocol using intravaginal progesterone device + GnRH on Day 0, followed by progesterone device removal + $PGF_2\alpha$ + eCG on Day 8, and GnRH injection on Day 10. The FTAI was performed on Day 11. Frozen-thawed sexed semen from one bull was used for all FTAI, which were all performed by the same technician. Ultrasound examinations (B-mode and color Doppler) were performed on days 15, 22 and 30 post-TAI. Ultrasonographic images were analyzed using ImageJ software under standardized conditions to quantify total luteal area, vascularization and perfusion percentage through pixel calibration. On day 30 post-TAI pregnancy rate was detected (P/AI 30d). Ultrasonographic evaluations revealed that, although the T1 group showed a numerical increase of 6.5% in pregnancy rate (56.52% vs. 50.0% in T0) and a higher mean luteal blood perfusion (23.51% vs. 18.28%), these differences were not statistically significant ($p=0.777$ and $p=0.058$, respectively). However, corpus luteum perfusion proved to be an extremely strong predictor of pregnancy, with each 1% increase in vascularization raising the chance of conception by 14% (OR=1.14; 95% CI: 1.07–1.22; $p<0.001$), and a highly significant difference ($p<0.001$) was observed in perfusion between pregnant females (26.06%) and non-pregnant females (14.86%). Additionally, a moderate and significant positive correlation ($r=0.428$; $p<0.001$) was identified between follicular diameter and corpus luteum diameter. In conclusion, the results suggest that PGF2α primarily acts indirectly by enhancing luteal vascularization.

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Enhancing reproductive efficiency: A comparative evaluation of Ovsynch and Double-Ovsynch protocols on estrus synchronization and conception rates in Nili-Ravi Buffalo

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Ovsynch and CIDR-based protocols, especially with pre-synchronization strategy improve buffalo fertility by optimizing ovarian response, elevated progesterone level resulted in precise ovulatory follicle size, and improved synchronization efficiency. Postpartum primiparous buffaloes were randomly selected at Buffalo Research Institute (3110N, 73510E), Pattoki, Kasur, Punjab, Pakistan, with DIM (104 ± 0.1) and BCS of 3.5 ± 0.5, split into treatment (n = 60, DO; G1) and control (n = 54, Ovsynch; G2) groups. G1 received Double-Ovsynch (DO) [GnRH–7d-$PGF_{2\alpha}$–3 d-GnRH, followed 7d later by GnRH–7d-$PGF_{2\alpha}$–56h-GnRH] while G2 received Ovsynch (GnRH–7d-$PGF_{2\alpha}$–56h-GnRH). In both groups, timed artificial insemination was performed 16 hours after the last GnRH administration. Follicular dynamics, ovulatory events, and luteal development were studied using ultrasonography. Blood samples were collected for ovarian steroids analysis. Estrus detection was carried out twice daily. Buffaloes were scanned for pregnancy within days 30 to 40 of artificial insemination. A randomized complete block design was used to compare different response variables between the groups. In this study, G1 markedly outperformed G2: estrus response (88.3%; 53/60 vs 61%; 33/54; $P < 0.05$) and estrus intensity score (3.8 ± 0.2 vs 3 ± 0.2; $P < 0.02$) were greater in G1 compared to G2. Ultrasonography revealed larger pre-ovulatory follicle in G1 (13.9 ± 0.3 vs 12.2 ± 0.3; mm; $P < 0.05$) and larger corpus luteum (15.8 ± 0.4 vs 14.1 ± 0.3; mm; $P < 0.05$) post AI at day 7. Circulating estradiol at estrus was higher (8.1 ± 0.2 vs 5.8 ± 0.2; pg/ml; $P < 0.05$) and progesterone at AI lower (0.4 ± 0.1 vs 0.9 ± 0.1; ng/ml; $P < 0.05$) with G1, indicating complete luteolysis and follicular maturation. Correspondingly, ovulation (88%; 53/60 vs 66%; 36/54; $P < 0.01$), synchronization (91%; 55/60 vs 74%; 40/54; $P < 0.01$) and conception rates (53%; 32/60 vs 35%; 19/54; $P < 0.05$) were significantly higher in group G1. In conclusion, the Double-Ovsynch protocol clearly outperformed the Ovsynch strategy by enhancing preovulatory follicle size, larger corpus luteum diameter, stronger estrus expression, and significantly boosting synchronization efficiency and conception rates in Nili-Ravi buffalo.

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Use of two different sources of chorionic gonadotropin in fixed-time artificial insemination protocols in water buffalo (*Bubalus bubalis*)

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Artificial insemination allows to obtain a rapid and efficient genetic and productive improvement; in buffaloes due to management characteristics and the problems to detect oestrus fixed-timed artificial insemination (FTAI) is an alternative using hormones such us equine chorionic gonadotropin (eCG), to improve ovulation rates. To evaluate the effect over the pregnancy rates of the use of recombinant or natural eCG in a FTAI program in buffaloes. FTAI was performed in an Argentinian Farm during the breeding season of 2024. on 151 buffalo heifers, with an age between 2-3 years. Ultrasound was performed to determine genital reproductive development grade (RDG) classified as 3 (cycling, with a corpus luteum and good uterine tone) or 2 (superficial anestrus, follicles ≥8 mm in diameter, and moderate uterine tone). The protocol used was a modification of Ovsynch protocol which consisted on day 7: TRT1, 400 IU of recombinant eCG (Folirec ZooVet®); TRT2, 400 IU of serum eCG (Sincrogest Von Franken®); and TRT3 no medication. FTAI was performed 16 hours after last hormone administration using refrigerated semen from proven bulls. Twenty-six days after FTAI, pregnancy was diagnosed by ultrasonography. Groups comparisons were performed using ANOVA. No statistical differences were found in the age of the buffaloes by treatment and RDG, 2.27 ± 0.05, 2.40 ± 0.04, and 2.50 ± 0.12 years (p= 0.11) and 2.45 ± 0.05; 2.40 ± 0.07; and 2.31 ± 0.12 (p = 0.55) for TRT1, TRT2, and TRT3, respectively. The overall pregnancy rate was 54.3% (82/151). In TRT1, the pregnancy rate was 55.7% (49/88); in TRT2, the pregnancy rate was 53.2% (25/47); and in the control group (TRT3), the pregnancy rate was 50% (8/18); these differences were not statistically significant (p=0.9). It has been demonstrated that the use of eCG in the modification of ovsynch protocol in FTAI programs in buffaloes but not show an improvement in pregnancy rates. This report contributes to obtain more information on the use of recombinant gonadotropin for ovulation synchronization in buffaloes. No differences in pregnancy rates were found among buffaloes inseminated at a fixed time when recombinant chorionic gonadotropin, equine chorionic gonadotropin, or the control group (without gonadotropin administration) were used; therefore, the use of both formulations is feasible, more data is needed of the use of recombinant eCG to confirm the results.

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Effect of injectable progesterone, GnRH, or flunixin meglumine on maternal pregnancy recognition in buffalo recipients of *in vitro* embryos in Colombia

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The efficiency of embryo transfer (ET) programs in buffaloes remains low, particularly when *in vitro*-produced (IVP) embryos are used, as this exhibit reduced embryonic competence and weaker signaling during maternal recognition of pregnancy. Although the physiological mechanisms associated with luteal function and maternal recognition of pregnancy have been extensively described in cattle, species-specific evidence in buffaloes remains limited.

The aim of the present study was to evaluate the effect of progesterone (P4), GnRH, or flunixin meglumine (FM) administration on pregnancy rate (PR) and pregnancy loss (PL) in buffalo recipients of IVP embryos.

A total of 800 Murrah buffaloes were synchronized, of which 534 were considered suitable for ET (response rate: 66.75%). Animals that responded to the synchronization protocol were randomly assigned to four experimental groups: control, P4 (150 mg), GnRH (0.01 mg buserelin), and FM (880 mg). Response to the synchronization protocol was assessed using color Doppler ultrasonography (Mindray Z60). PR was evaluated at 45 days post-transfer, and PL was assessed at 90 days of gestation using B-mode ultrasonography.

PR was 27.61%, 35.82%, 27.06%, and 22.55% for the control, P4, GnRH, and FM groups, respectively. PL at 90 days was 10.81%, 6.25%, 13.33%, and 8.11%, respectively. Supplementation with P4 increased PR and reduced PL, consistent with its role in regulating the uterine environment and providing luteal support, widely documented in cattle. In contrast, GnRH administration did not improve reproductive outcomes, which may be associated with variability in the formation of an accessory corpus luteum. Likewise, FM did not show positive effects on either PR or PL, suggesting that luteolysis may not be the primary limiting factor under the evaluated conditions.

In conclusion, P4 supplementation represents an effective strategy to improve reproductive efficiency in buffalo recipients of IVP embryos, whereas GnRH and FM did not demonstrate benefits. These findings provide relevant experimental evidence in a species where physiological and applied knowledge remains limited, contributing to the understanding of mechanisms associated with maternal recognition of pregnancy in buffaloes.

Endometrium/Myometrium ratio as an early non-pregnancy diagnosis in dairy buffaloes

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The morphophysiological dynamics of uterine layers are essential for understanding reproductive efficiency in mammals. Since hormonal fluctuations during the estrous cycle induce predictable structural changes in the endometrium, it could be an interesting diagnostic tool in early pregnancy stages to shortening re-insemination periods. This study evaluated the efficiency of the endometrium/myometrium ratio (E:M), measured via B-mode ultrasonography, as a non-pregnancy diagnostic tool in dairy buffaloes. For this purpose, 57 Murrah females (16 heifers and 41 multiparous) were subjected to an 11-day Fixed-Time Artificial Insemination (FTAI) protocol. All buffaloes were inseminated using frozen-thawed semen from fertility-proven sires.

Uterine evaluations were performed 24 days post-AI (D24) using a Mindray ultrasound with a 7.5 MHz linear transducer. Buffaloes were scanned in B-mode at the uterine body; endometrial and myometrial thicknesses were measured using ImageJ software to calculate the E:M ratio. Pregnancy status was confirmed via ultrasound on D32 post-AI (Gold Standard). Data were analyzed using Student's t-test for mean comparisons, while Receiver Operating Characteristic (ROC) curves determined the optimal cut-off point, sensitivity, and specificity for non-pregnancy diagnosis.

Significant differences ($P < 0.001$) were observed based on pregnancy status; pregnant buffaloes exhibited a lower endometrial thickness (0.55 ± 0.09 cm) and E:M ratio (1.56 ± 0.22) compared to non-pregnant females (0.69 ± 0.17 cm and 2.25 ± 0.64, respectively). When categorized by parity, non-pregnant multiparous females presented the highest endometrial thickness (0.71 ± 0.17 cm) and E:M ratio (2.20 ± 0.68), significantly differing from pregnant multiparous (0.34 ± 0.07 cm and 1.70 ± 0.53) and pregnant heifers (0.53 ± 0.08 cm and 1.57 ± 0.23). No significant variations were found in myometrial thickness across groups ($P > 0.05$). The E:M ratio demonstrated high diagnostic accuracy (AUC = 0.85; $P < 0.001$); using an optimal cut-off of 1.84, the ratio identified non-pregnant buffaloes with 94.4% specificity, 69.2% sensitivity, and 84.0% NPV (Overall Accuracy: 78.4%). In conclusion, the E:M ratio is an effective ultrasonographic marker for early non-pregnancy diagnosis in dairy buffaloes, allowing for high-certainty identification of empty females at day 24 post-AI.

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Title: AgriGenomics innovations for a large-scale adoption of genomics in Buffalo

Xavier David is Associate Director in Product Management – Global Collaborations, at Illumina, with extensive experience in applied genomics and biotechnology. His work focuses on the development and implementation of genomics solutions for large scale research initiatives and applied genomics projects in both human and Agri genomics

This presentation explores how genomic innovations are transforming smart breeding by enabling faster, more accurate, and sustainable genetic improvement in agriculture. It highlights the maturation and scalability of genomic technologies, particularly SNP genotyping arrays, which have become cost-effective tools for genomic selection across livestock and crop species. The presentation emphasizes the importance of high-quality phenotypes, well-designed reference populations, and continuous data updates to achieve accurate genomic predictions, especially for complex fitness and disease-resistance traits. Using examples from cattle breeding, including genomic selection for production performance, product quality , health, it demonstrates the practical impact that genomics can have on Buffalo herds worldwide on their performance and resilience. But the genomic revolution extends now to multiomics approaches, integrating genomic, epigenomic, transcriptomic, and proteomic data to unlock deeper biological insights and accelerate breeding innovation. Together, these advances position genomics and multi-omics as key enablers of smart agriculture, supporting food quality and security, improved animal health, and more sustainable production systems.

Modern Strategies for Buffalo Farming: From Smart Monitoring to Smart Feeding

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Feeding behavior in Mediterranean Italian Buffalo represents a sensitive proxy for physiological status and management performance, particularly during the dry period, early lactation and heat-stress conditions. Recent advances in precision livestock farming enable continuous, high-resolution monitoring at both individual and herd levels, supporting early detection of deviations associated with nutritional changes or operational inefficiencies.

This study evaluates data from advanced behavioral monitoring systems to determine their ability to detect anomalies related to total mixed ration preparation and delivery, as well as group-management practices, in both lactating and non-productive animals. The overarching aim is to identify a set of keys, practical, and easily deployable behavioral indicators specifically suited to Mediterranean buffalo farming. Such indicators have the potential to enhance nutritional consistency, promote animal welfare, and improve overall production efficiency in modern buffalo enterprises.

Bimodal milk flow patterns and their effects on milk emission, milking duration, and milk quality in Italian Mediterranean buffaloes

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Although milking dynamics have been widely investigated in dairy species due to their effects on milking efficiency, udder health, and milk quality, they remain poorly characterized in dairy buffaloes, particularly regarding bimodal milk flow patterns. This study evaluated the effect of bimodality on individual milk emission, milking duration, and milk quality traits in Italian Mediterranean buffaloes. Data were collected from 786 lactating animals reared in 4 commercial farms in Campania region (March-September 2024). A total of 2,536 individual milking events were monitored using *Lactocorder*® to record milk flow and milking-time traits. Milk flow variables included milk yield per session and day, and milk yield in the first 1-3 minutes (1MG, 2MG, 3MG). Milking-time traits comprised total milking time (tMGG), time to milk peak flow (tMHG), and the duration of ascending (tAN), plateau (tPL), and descending (tAB) phases, as well as overmilking duration. Test-day records provided milk quality traits (fat, protein, lactose, urea, SCS and milk electrical conductivity). Bimodality was defined as a Lactocorder®-detected (official software) two-peaked milk flow curve with a >0.3 kg/min drop between peaks, visually confirmed by a trained technician. The effect of bimodality was analysed using a linear mixed model including month, parity, DIM, production class, milking session (morning/afternoon), bimodality, and their first order interactions, with animal and herd test-day included as random intercepts. On average the 25% of milking events were classified as bimodal. Bimodality significantly reduced early milk emission (1MG: 0.79 *vs.* 1.12 kg/min; 2MG: 2.11 *vs.* 2.33 kg/min; $P<0.01$). However, milk yield per session was slightly higher under bimodality (4.88 *vs.* 4.78 kg), with no effect on daily milk yield ($P>0.05$), suggesting a compensatory milk removal despite delayed milk ejection. Milking duration was also influenced by bimodality, greater tMHG, tAN were observed in bimodal curves ($P<0.05$). Moreover, tPL was greater in normal milking curves (97 *vs.* 68 s, respectively), indicating a shorter plateau phase under bimodal conditions. Bimodality was also associated with higher SCS (2.96 *vs.* 2.87) and average milk electrical conductivity (5.80 *vs.* 5.65 mS). Overall, bimodal patterns were associated with less efficient milk removal and impaired udder health, highlighting the importance of adequate pre-milking stimulation and optimized milking settings in dairy buffaloes.

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A bio-based approach to udder health: Antimicrobial peptides as next-generation sanitizers in buffalo milking systems

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Subclinical mastitis remains a persistent threat to dairy farm profitability, causing significant economic losses and public health concerns due to biofilm-forming and antibiotic-resistant bacteria. To address the failure of traditional sanitizers against biofilms, the aim of this study was to develop an eco-friendly disinfectant based on the *in silico*-designed peptide BC_1, which demonstrated robust, broad-spectrum efficacy against both planktonic and sessile foodborne pathogens.

Laboratory-scale analyses were performed to evaluate its antimicrobial efficacy on contaminated surfaces. Petri dishes (64 cm²) were intentionally contaminated with a bacterial polyculture of *Staphylococcus hyicus*, *S. chromogenes*, and *Streptococcus parauberis* (5×10^4 CFU/cm²). Two concentrations of BC_1 (25 and 50 µM) were tested across three contact times (10'', 1', 10'). Subsequently, field trials were conducted at buffalo farm, where an aqueous BC_1 solution (50 µM) was aerosolized (~700 µL) onto milking liners post-milking without prior cleaning. The solution was allowed to act for 30''. Efficacy was compared against standard daily alkaline detergent washes. Surface sampling was conducted in accordance with ISO 18593:2018, while microbial enumeration (Total Viable Count) was performed following ISO 4833-1:2022; 320 isolates were identified via MALDI-TOF mass spectrometry.

Laboratory assays demonstrated dose- and time-dependent activity. The 50 µM concentration showed superior efficacy, achieving a 98.81% reduction after only 10 seconds and reaching 99.84% at 10 minutes. In field trials, liners showed an initial mean contamination of 4.12±0.55 Log CFU/cm². While routine alkaline washing produced no significant reduction, BC_1 treatment resulted in a statistically significant ($p<0.05$) reduction of approximately 1 Log, effectively decreasing the microbial load tenfold. The mean bactericidal efficacy in the field was 88% (from 50 to 99%). Identification confirmed a relative prevalence of *S. chromogenes* (~50%).

The BC_1 peptide proved to be a highly effective sanitizing agent, drastically reducing bacterial loads on contaminated surfaces. Notably, the formulation maintained high activity even on uncleaned surfaces in the presence of organic residues, where traditional detergents failed. The rapid onset of action and successful field validation suggest that BC_1 is a promising candidate for integration into or replacement of standard sanitation protocols in buffalo and dairy farming.

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Influence of reproduction traits on lactation curve shape in Mediterranean buffalo

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In the present study, records from 16,003 heifers across 135 Italian Mediterranean Buffalo herds were used to investigate the association between age at first calving (AFC), season at first calving, and their interaction on Lactation Curve (LC) shape. The animals were classified into eight AFC classes according to age at first calving (1= ≤30; 2 =30–32; 3 = 32–34; 4 = 34–36; 5 = 36–38; 6 = 38–40; 7 = 40–42; 8 = >42 months). Test-day (TD) milk yield records were used to characterize LCs using the Wood equation. Only animals with at least five TD records, distributed throughout lactation at 70-day intervals, were included to ensure a reliable representation of the LC and an accurate estimation of the *a*, *b*, and *c* parameters. Subsequently, linear mixed-effects models were applied to evaluate the effects of AFC class, season at first calving and their interaction on the lactation curve parameters considering the random effect of the herd. In the Wood equation, *a* reflects production scale, *b* the pre-peak increase rate, and *c* the post-peak decline (persistency). Of 16,003 heifers, 17% calved at ≤30 months (Group 1) and 10% at > 42 months (Group 8), while more than 70% calved between 30 and 42 months of age (Groups 2-7). The results indicated that the LC in Mediterranean buffalo heifers was differentially shaped by AFC and season at first calving, through distinct effects on Wood's coefficients. Indeed, the *a* parameter was significantly higher in Group 8 (6.63 ± 0.16) compared to Group 2 (5.97 ± 0.14) and 3 (6.09 ± 0.14) ($p < 0.05$), probably due to their greater physiological maturity and consequently higher milk production. Parameter *b* registered the lowest value (0.26 ± 0.01) in spring compared with winter and summer (0.29 ± 0.01) and autumn (0.30 ± 0.01) ($p < 0.05$), suggesting potential issues in transition management. Finally, regarding the calving season, parameter *c* was significantly higher in winter (0.0054 ± 0.0001) compared with spring and summer (0.0051 ± 0.0001 and 0.0050 ± 0.0001, respectively) ($p < 0.05$). This pattern was also evident at a fixed AFC level (Group 8), where *c* values were significantly greater in winter (0.0058 ± 0.0002) compared with summer (0.0044 ± 0.0002) ($p < 0.05$). This result can suggest that for persistency (*c*), cold stress may accelerate post-peak decline by inhibiting prolactin production.

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An ICT-Enabled School-on-the-Air Program for Dairy Buffalo Production: Effects on Farmer Knowledge, Attitudes, and Practices in the Philippines

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Dissemination of dairy buffalo production technologies in smallholder farming systems is often constrained by limited extension reach and resources, hindering the adoption of recommended practices and innovations. This study aimed to evaluate the effectiveness of the School-on-the-Air on Dairy Buffalo Production (SOA-DBP), an ICT-enabled distance education and extension program, and its effects on farmers' knowledge, attitudes, and practices (KAP) in the CALABARZON region of the Philippines. Specifically, it sought to determine differences in KAP between program participants and non-participants and to identify demographic and farming-related factors associated with these outcomes. A pre- and post-intervention evaluation design was employed involving 111 SOA-DBP participants and 42 non-participating farmers as a comparison group. The program consisted of sixteen instructional episodes delivered through radio broadcasting, simultaneously streamed on the Facebook page of the Agricultural Training Institute (ATI)-CALABARZON and its *AgriStudio Live* YouTube channel, and complemented by interactive text-based question-and-answer sessions that enabled real-time clarification and guidance. Data were collected using structured questionnaires covering KAP and technology perception dimensions and analyzed using independent sample t-tests, chi-square tests, and Mann-Whitney U-tests. Results showed that participants scored significantly higher than non-participants in knowledge, attitude–persuasion, attitude–decision, practice–implementation, and practice–confirmation dimensions ($p < 0.01$). Educational attainment and geographic location were significantly associated with KAP outcomes, while years of farming experience influenced persuasion and confirmation stages of technology adoption. No significant differences were observed in perceived technology attributes or technology perception behaviors. These findings indicate that SOA-DBP primarily enhanced farmers' capacity to implement and sustain recommended dairy buffalo production practices rather than substantially altering pre-existing attitudes toward technology adoption. As an ICT-enabled extension approach integrating radio, online streaming, and SMS-based interaction, SOA-DBP offers a scalable and inclusive mechanism to improve technology adoption in settings with limited access to face-to-face extension services.

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The Economic Viability of Dairy Buffalo Farming Businesses: An Ex-Ante Analysis

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The Philippine dairy buffalo industry remains constrained by low private-sector investment. A key limitation is the lack of empirically grounded investment analyses to guide investors, policymakers, and development institutions. Bridging this gap, this study analyzed the economic viability of dairy buffalo farming businesses in the Philippines using an Ex-ante Cost Benefit Analysis following the circular economy framework. Specifically, it aimed to: (1) document the recommended production, technical, market, and economic assumptions for the enterprises; (2) analyze and compare the selected enterprises' economic viability using standard investment indicators; and (3) provide policy recommendations for industry stakeholders and program implementers.

The study examined eight enterprise modules i.e. 5, 10, 15, 20, 25, and 50-cow start-up dairy buffalo farms, silage production, and vermicast production. Primary data were generated through a two-day focus group discussion involving 13 industry experts and two model dairy buffalo farmers, while secondary data were obtained from the Philippine Carabao Center's (PCC) Dairy Buffalo Production Handbook and farm-level production and financial records from PCC's client database. Annual financial projections were done in accordance with the Philippine Accounting Standards and stretched to 10 years. Investment evaluation considering time value of money was employed with Net Present Value (NPV), Internal Rate of Return (IRR), Payback Period (PP), and Profitability Index (PI) as indicators, using 14% as hurdle rate.

Results showed that all enterprise modules were economically viable. The 50-cow module generated the highest NPV (PHP 8.24 million), while vermicast production achieved the highest IRR (40.04%) and shortest PP (3.81 years). Overall, the 15-cow module emerged as the most economically viable, recording the highest PI (2.85) and the best balance among profitability, investment recovery, and risk. Integrating dairy farming with silage and vermicast production in a circular economy effectively maximizes resource-use and diversifies income. Furthermore, the choice of enterprise scale and target efficiency significantly influences projected investment performance. The investor's technical and risk-bearing capacity are also important considerations aside from economic viability. It is also recommended that the enterprise assumptions and investment models are updated regularly by PCC to reflect current market and production conditions. Favorable improvements include ex-post analysis, inclusion of additional financial and risk indicators, and pursuing economic viability of other buffalo-based enterprise opportunities to enrich the portfolio.

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Upgrading Thai Swamp Buffalo: A Premium Branding Strategy and Global Market Positioning

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Thai swamp buffalo represent a unique indigenous livestock resource with significant cultural heritage, genetic value, and potential for transformation into premium products within global agri-food markets. However, these products have traditionally remained within low-value commodity chains due to limited product differentiation, branding strategies, and international market positioning. This study aims to develop an evidence-based premium branding framework and identify strategic market opportunities for Thai swamp buffalo products.

A mixed-methods research design was employed, integrating qualitative interviews with 35 key stakeholders across the buffalo value chain and a quantitative survey of 420 premium food consumers from Thailand, Japan, Singapore, and selected European markets, which were selected due to their high purchasing power, growing demand for premium and culturally distinctive food products, and strong potential for international market expansion. The qualitative findings identified five critical dimensions for premium brand development: cultural heritage, authentic product quality, sustainability, traceability, and strategic distribution channels, including premium retailers, gourmet restaurants, and international specialty food platforms.

Quantitative results revealed that consumers highly valued cultural heritage (Mean = 4.41), product authenticity (Mean = 4.36), and perceived quality (Mean = 4.32). Structural Equation Modeling demonstrated that cultural heritage value ($\beta = 0.43$, $p < 0.001$), traceability and certification ($\beta = 0.35$, $p < 0.001$), and product authenticity ($\beta = 0.29$, $p < 0.001$) significantly enhanced consumer trust, while consumer trust strongly influenced purchase intention ($\beta = 0.47$, $p < 0.001$).

The findings suggest that successful premium positioning requires the integration of heritage storytelling, quality assurance, sustainable production, and transparent supply-chain systems. The proposed Thai Swamp Buffalo Premium Branding Model provides practical guidance for farmers, entrepreneurs, and policymakers to increase global competitiveness, strengthen rural economies, and conserve indigenous livestock resources through sustainable value-chain upgrading.

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The Impact of Social Entrepreneurship on Inclusive Growth in the Carabao Sector in the Philippines

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This study examines social entrepreneurship as a strategic mechanism for inclusive growth in the Philippine carabao sector. It evaluates how the Philippine Carabao Center's (PCC) integrative interventions—including enterprise clustering, genetics upgrading, and market linkages—improve smallholder productivity and income.

Using a mixed-method approach, findings reveal that social enterprise models increased average milk yield from 4.96 liters/day (2023) to 5.17 liters/day (2025). Assisted cooperatives recorded a 20–35% increase in annual household income, with dairy-derived earnings rising from Php 45,000–Php 60,000 to Php 65,000–Php 90,000 per lactating animal annually.

These results align with broader national trends; domestic milk production rose from 29.10 million liters (2023) to 43.30 million liters (2025), with carabao milk production alone expanding by 24%. Beyond economics, these initiatives strengthened cooperative governance and increased the participation of women and youth, who now comprise 30–40% of membership in assisted sites.

The study concludes that reinvesting revenues into animal health and community development creates a self-reinforcing cycle of productivity and social equity. Social entrepreneurship is affirmed as a transformative framework for livestock-based rural development, provided it is supported by science-based technology and strong institutional partnerships.

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Effect of the Implementation of 170-2013: Ordinance Regulating the Slaughter of Female Carabaos in the Province of Pangasinan, Philippines

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This study evaluated Pangasinan Ordinance No. 170-2013, which regulates the slaughter of breedable female carabaos and establishes a buy-back program to support herd conservation, rural livelihoods, and dairy development. Using mixed methods, researchers analyzed slaughter numbers, farmgate prices, inventory trends, reproductive and production records from 2013 to 2023. Eighty-five recipients of distributed female carabaos in various Pangasinan municipalities were included, supplemented by interviews with farmers, owners, technicians, and program implementers. Quantitative data were analyzed using descriptive statistics and trend analysis, while qualitative responses were analyzed using thematic analysis to capture stakeholder perspectives.

Results showed that, despite the ordinance's conservation aims, carabao population growth was not achieved. Few entrusted females produced calves, and most farmers reported negligible milk production—some beneficiaries averaged only 2–3 liters daily, or less than 480–720 liters annually, far below dairy-type expectations. Poor reproductive outcomes limited milk supply and hampered dairy enterprise development. Farmgate meat prices rose from ₱88.83/kg (2013) to ₱164.39/kg (2023), with a 43% spike between 2021 and 2022, strongly incentivizing animal sale or slaughter.

Inventory declined and extraction rates rose, meaning more animals were disposed of than replaced. Farmers blamed low productivity, lack of dairy expertise, and reduced carabao use due to mechanization, decreasing the value of maintaining females for milk. Rising meat prices further encouraged extraction over retention. Interviews revealed challenges: inconsistent enforcement, weak monitoring, inadequate support, limited veterinary services, and insufficient production assistance, all limiting program impact.

The study concludes that slaughter regulation and buy-back schemes alone are insufficient for herd growth or dairy development. Investments in reproductive improvement, technical services, training, market links, and incentives to make milk production more attractive than disposal are needed. A data-driven, market-oriented policy would better align objectives with farmer behavior and sustain Pangasinan's carabao industry.

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Buffalo Sector In Kayseri Region Of Türkiye: Challenges And Strategic Solutions*

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Turkey ranks 19th worldwide in terms of water buffalo population, with approximately 162,000 head. Kayseri Province ranks 4th nationally, with approximately 8,278 buffaloes. This study aimed to assess the current status of the buffalo sector in Kayseri, identify its major constraints, and propose strategic solutions to support its sustainable development. The study was based on data of Kayseri Buffalo Workshop on 14 July 2025, post-workshop field visits, training programs.

The main challenges affecting the buffalo sector in Kayseri were identified as limited participation of young people and women in buffalo farming; insufficient technical knowledge on herd management, nutrition, and reproductive practices; environmental and legal constraints of buffalo breeding areas; high feed costs, low milk yield of native Anatolian buffaloes; limited use of estrus synchronization and artificial insemination, and legal restrictions on the importation of buffalo and sperma; deficiencies of milk transport and storage and processing; inefficient conventional animal identification systems; lower value of male buffalo carcasses compared with cattle; the absence of buffalo-specific standards in the Turkish Food Codex; shortage of veterinarians and technicians specialized in buffalo production.

Enhancing the competitiveness and long-term sustainability of the buffalo sector in Kayseri requires a comprehensive and coordinated strategy focusing on the following priorities. Expand crossbreeding programs using Italian buffalo genetics, increase the adoption of estrus synchronization and artificial insemination, and establish disease-free breeding centers; improve milk collection and processing infrastructure; uses of smart based identification systems like boluses or subcutaneous microchips; revisions of Turkish Food Codex to include buffalo product specific standards; strengthen collaboration among relevant institutions to solve the problems; promote the transition from traditional family-based farming to modern commercial enterprises, encourage branded and value-added buffalo products, ensure year-round market availability; integrate buffalo products with regional gastronomy and rural tourism.

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Beyond production: actor articulation in a tropical livestock system

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Tropical livestock systems face the challenge of maintaining productivity in contexts of high pressure on ecosystems, particularly in regions where agricultural expansion has contributed to biodiversity loss and the transformation of natural landscapes. In this context, sustainability does not depend solely on the implementation of production practices, but also on the articulation among actors and their ability to shape the dynamics of the system. This study aimed to analyze the relational structure of actors and their patterns of articulation in a tropical livestock system in Colombia. The study was conducted at Hacienda Cuba (Córdoba, Colombia), a cattle and buffalo production system under agroforestry conditions. A total of 61 actors were identified and classified into 11 functional categories according to their role within the system. The articulation capacity of actors was analyzed from a structural perspective, understood as their position within the network of alliances reported among categories. To do so, social network analysis was applied using density, centrality, and betweenness metrics to identify the actors with the greatest articulation capacity within the system. The results showed a highly connected network, with a structural core composed of governmental, academic, and research actors that showed higher levels of centrality and betweenness, suggesting a greater capacity for articulation and coordination within the system. Likewise, the functional block analysis showed that approximately 71% of the relationships occur between different blocks, evidencing a predominantly intersectoral level of articulation. However, a weak connection was identified between the productive block and social and community actors, representing a gap in the relational structure of the system that may limit the implementation of sustainable strategies and territorial governance. We concluded that the articulation capacity of actors in tropical livestock systems is determined by their position within the network of relationships. Furthermore, structural analysis allows the identification of strategic nodes and articulation gaps, providing key elements to strengthen governance and advance towards more sustainable production systems.

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Diagnostic Potential of Milk-Derived Exosomal miRNAs for Early Detection of Subclinical Mastitis to Enhance Production and Buffalo Herd Health

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Milk-derived exosomal microRNAs (miRNAs) are stable bioactive molecules involved in post-transcriptional regulation and intercellular communication. This study evaluated the expression of exosomal miR-148a, miR-186, miR-223, and miR-21 in normal milk, sub-clinical mastitis milk, and commercially pasteurized milk to assess their potential as biomarkers for early detection of sub-clinical mastitis in buffaloes. Additionally, the stability of these miRNAs under extreme boiling conditions was investigated.

A total of 30 milk samples were collected from the Mumbai region, including 20 buffalo milk samples categorized as normal (n=10) and sub-clinical mastitis (n=10), along with 10 commercial pasteurized milk samples, of which half were subjected to boiling. Exosome size, morphology, and integrity were characterized using transmission electron microscopy (TEM). Somatic cell count (SCC) varied significantly among groups prior to boiling ($p<0.001$).

Expression analysis revealed that miR-148a, miR-186, and miR-223 were upregulated in sub-clinical mastitis and pasteurized milk compared to normal milk, both before and after boiling. Significant differences ($p<0.001$) were observed for miR-148a and miR-186 prior to boiling, while post-boiling analysis showed miR-186 remained significantly different ($p<0.001$) across all groups. No significant changes in miRNA expression were detected before and after boiling, indicating high thermal stability. TEM imaging further confirmed the structural integrity of exosomes in both boiled and pasteurized milk. In contrast, miR-21 showed no significant variation among groups.

A strong positive correlation between SCC and the expression levels of miR-148a, miR-186, and miR-223 supports their potential as reliable biomarkers for early detection of sub-clinical mastitis. The persistence of miR-148a and miR-186 following pasteurization and boiling also raises important considerations regarding milk safety and cross-species biological effects. These findings emphasize the need for improved milk hygiene practices and further investigation into the biological implications of dietary miRNA transfer.

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Innovative Intramammary Oxygen-Ozone Therapy for Clinical Mastitis in Mediterranean Buffalo

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This study aims to describe the effects of two oxygen-ozone (O_2O_3) based therapeutic protocols, used either as integrative therapy in association with antibiotics (Ab) or as monotherapy, on udder health in dairy cows affected by clinical mastitis.
A prospective, randomized, and comparative clinical trial was conducted on 45 mammary quarters with clinical mastitis (grades M1–M2) in multiparous buffaloes (70–120 days in lactation), raised in a dairy farm. The quarters were randomly assigned to three groups (n=15/group): 1) Ab-conventional antibiotic therapy (ampicillin + dicloxacillin, intramammary, 2 administrations); 2) Ab+O_2O_3-the same antibiotic regimen followed by 4 intramammary infusions of O_2O_3; 3) O_2O_3 – monotherapy with 4 intramammary infusions of O_2O_3. The O_2O_3 mixture was produced using a certified medical ozone generator, immediately prior to administration. For the insufflation procedure per quarter, a total volume of 150 mL was administered at a concentration of 35 µg/NmL, with an infusion time of 100 seconds.
Diagnosis was carried out through clinical examination, somatic cell count (SCC) and bacteriological culture (BC). Evaluations were performed at T0, T1 (7 d), and T2 (14 d). The considered outcomes included: etiological cure (negative BC), clinical cure (absence of clinical signs), variation of SCC and milk yield.
A total of 135 clinical evaluations, 135 BC and 270 SCC were collected. At T2, etiological cure rates were: Ab = 43.8%; Ab+O_2O_3 = 73.3%; O_2O_3 = 60.0% (all groups vs. T0, $p<0.01$). Clinical cure rates at T2 were: 75.0% for Ab ($p < 0.001$) and 100.0% for both Ab+O_2O_3 and O_2O_3 groups ($p < 0.00001$ for both). SCC showed a significant reduction compared to T0, with decreases observed in the Ab ($p < 0.05$), O_2O_3 ($p < 0.001$) and Ab+O_2O_3 ($p < 0.01$) groups.
Intramammary ozone therapy, either in combination with antibiotics or as monotherapy, demonstrated clinical and etiological efficacy comparable or superior to conventional therapy, with positive effects also on milk yield. Considering the absence of withdrawal times, the O_2O_3 approach may represent a promising and sustainable therapeutic option for the management of buffalo clinical mastitis, applicable in both conventional and organic dairy farms, where antimicrobial use is strictly regulated and reduction of antibiotic treatments is a primary objective. However, these findings should be interpreted with caution and require further confirmation through larger-scale randomized studies.

Mastitis Control Strategy in Dairy Buffaloes in Guangxi, China's "Hometown of Buffaloes": From Pathogen Tracing and Virulence Dissection to Natural Product-Based Intervention

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Guangxi is renowned as China's "Hometown of Water Buffaloes," and buffalo milk is hailed as the "king of milk." However, mastitis has become an industrial bottleneck severely constraining its yield and milk quality. This study conducted an epidemiological investigation of 1,971 lactating dairy buffaloes across 33 farms in Guangxi, revealing that the prevalence of clinical mastitis (CM) and subclinical mastitis (SCM) was 3.4% and 19.79%, respectively. From SCM milk samples, 460 bacterial strains were isolated, among which Escherichia coli (E. coli) was the predominant pathogen (isolation rate 15.22%), followed by Staphylococcus chromogenes (12.61%), Staphylococcus spp. (6.74%), Acinetobacter towneri (5.87%), Klebsiella pneumoniae (5.00%), Bacillus subtilis (5.00%), Streptococcus uberis (4.78%), and Enterococcus faecalis (4.78%). For the predominant pathogen E. coli, further analysis of 70 clinical isolates revealed high genetic homology with the zoonotic O26 serotype and notable phenotypic traits: 98.57% exhibited strong motility, 87.14% were capable of biofilm formation, and 85.72% were multidrug-resistant (MDR). Genotypic analysis showed that 100% of the strains carried multiple virulence genes (bor, papA, ompA), and 98.57% carried MDR-associated genes (gyrA, parC, marA); in the Galleria mellonella larval model, over 80% of the strains were classified as hypervirulent.

To combat mastitis caused by multidrug-resistant pathogens, whole-genome sequencing was performed on the most virulent and resistant E. coli representative strain GXU-23-25-310. Integrated analyses of GO, COG, and KEGG functional annotations and virulence factors revealed that fimbriae and flagella are key virulence factors mediating biofilm formation and pathogenicity. Targeting this virulence determinant, we conducted high-throughput screening of components from Guangxi's indigenous medicinal herbs (including but not limited to plant polysaccharides, aldehydes, phenolic acids, flavonoids, and saponins). Considering Guangxi's regional characteristics and resource advantages, Illicium verum polysaccharide (IVP) was ultimately selected as the optimal candidate, possessing both antibacterial activity (MIC 12.5 mg/mL) and biofilm inhibition and eradication capacity. Following extraction optimization and purification, a homogeneous polysaccharide fraction, IVP-3, was obtained as an acidic pectic polysaccharide (Mw 35.566 kDa). Its monosaccharides were mainly GalA, Ara, and Xyl (accounting for 81.51% of both total mass and total molar ratio), containing free and methyl-esterified GalA. The backbone consisted of galacturonic acid/galactose residues, with arabinan side chains linked to the O-3 position of the backbone. Mechanistic investigations revealed that IVP-3 directly disrupts fimbrial and flagellar structures, inhibits bacterial motility, prevents early biofilm adhesion, and eradicates preformed mature biofilms. Meanwhile, IVP-3 damages cell membrane integrity, dissipates the proton motive force, induces metabolic dysregulation and oxidative stress, and impairs efflux pump function, ultimately leading to bacterial lysis through multi-target synergistic actions. In summary, IVP-3 holds promise as a novel active candidate for the prevention and treatment of mastitis in dairy buffaloes.

This study establishes a complete translational chain from pathogen tracing and pathogenic mechanism dissection to natural product development and validation, providing a scalable scientific framework for green control of mastitis in dairy buffaloes.

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Characterization of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* from Feces of Italian Mediterranean Buffalo

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Water buffaloes (*Bubalus bubalis*) are a key livestock species in Italy, particularly in the dairy sector, where they play a central role in producing Mozzarella di Bufala DOP. Despite increasing concern about antimicrobial resistance (AMR) in food-producing animals, data on resistant bacteria in buffalo farming systems remain limited compared with those available for cattle. This study investigated the occurrence and molecular characteristics of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* on Italian buffalo farms.
A total of 130 fecal samples were collected from buffaloes housed on 13 farms and subjected to microbiological analyses to isolate ESBL-producing *E. coli*. Isolates were characterized by antimicrobial susceptibility testing (AST) and molecular detection of β-lactam resistance genes. Selected positive isolates were further analyzed by whole-genome sequencing (WGS) to investigate resistance determinants and genomic background.
ESBL-producing *E. coli* were detected in 12 of 130 fecal samples (9.23%), with positive animals identified across multiple farms. Phenotypic AST confirmed ESBL production, and all isolates were fully susceptible to carbapenems. Molecular analyses revealed that all isolates carried *bla*CTX-M genes, while seven also harbored *bla*TEM. No isolates tested positive for *bla*SHV or *bla*CMY genes. Preliminary WGS analyses confirmed the presence of ESBL-associated resistance determinants. Although the prevalence of ESBL-producing *E. coli* appeared lower than that commonly reported in cattle, these findings indicate that buffaloes may represent a source of clinically relevant AMR bacteria. The results highlight the importance of including buffalo production systems in national and international AMR surveillance programs and support the development of targeted strategies to monitor and mitigate AMR dissemination within the buffalo dairy chain.

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Seasonal Dynamics of Bacterial Contamination and Antimicrobial Resistance in Buffalo Milking Parlors of the Campania Region, Italy: A One-Year Surveillance Study

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Maintaining adequate hygiene in milking parlors is fundamental to ensuring raw milk quality and safeguarding animal welfare. This study investigated the seasonal dynamics of bacterial contamination in buffalo milking parlors across the Campania Region (Southern Italy) over a full year, combining adenosine triphosphate (ATP) bioluminescence assay, bacteriological analysis (MALDI-TOF MS identification), and antimicrobial resistance (AMR) profiling of Gram-negative isolates.

Ten Mediterranean buffalo farms were selected through the Italian ClassyFarm system. Farm owners completed a questionnaire on milking practices and hygiene; comparable cleaning procedures were reported, yet a lack of uniform protocols was identified. Sampling covered all four seasons and included teat cups, teat skin swabs, bulk tank milk, and operator nasal swabs (360 samples total).

ATP bioluminescence revealed peak contamination in the internal milking area during summer. Bacteriological analysis showed marked seasonal fluctuations: Bacillaceae predominated in autumn (64%), while summer showed higher Enterobacteriaceae (38%). Gram-negative prevalence was 41.5% in summer and 45.1% in autumn, with greater species diversity in the cooler season. In summer, *Escherichia coli* predominated in teat cups, alongside *Citrobacter freundii*, *Serratia nematodiphila*, *Pseudomonas aeruginosa*, and *Aeromonas veronii*. In autumn, diversity increased markedly, with *Acinetobacter* spp., *Klebsiella oxytoca*, *Kluyvera ascorbata*, and other Enterobactera. Operator nasal swabs yielded *Citrobacter koseri* in summer and *Klebsiella aerogenes* in autumn.

Preliminary AMR testing revealed variable resistance profiles, indicating circulation of resistant Gram-negative bacteria across seasons and sample types, including operator nasal swabs.

In conclusion, integrating ATP luminometry with microbiological and AMR analyses provides a powerful approach for buffalo milking parlor surveillance, supporting the implementation of standardized, season-adapted hygiene protocols.

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Are improved biosecurity and welfare levels sufficient drivers to reduce antimicrobial use in ruminants? the Case study of Italian buffalo farms

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Biosecurity in ruminant's farming is often an overlooked area, but it is a key aspect in a One Health approach. The scenario models aimed at reducing multidrug-resistant bacteria pose a challenge for Antimicrobial Use (AMU). Animal welfare (AW) and biosecurity are also essential tools to achieve this goal. ClassyFarm (CF) is an integrated system for categorizing farms according to the risk assessment. CF collects and processes data related to AMU, AW and biosecurity. In CF, AMU is estimated using the Defined Daily Dose (DDDAit). The AW assessment for water buffalo includes 62 non-animal-based measures (N-ABMs) and 17 animal-based measures (ABMs). The N-ABMs were divided into 'management' (32 items) and 'housing' (30 items). Biosecurity is assessed using 15 items. AW and biosecurity scores are expressed as percentages. The aim is to describe the correlation between biosecurity, AW and AMU in buffalo farming. DDDAit, AW and biosecurity were assessed on 382 buffalo farms with an average size of 374 heads. The overall mean DDDAit was 0.40±0.04, with 43% of the farms scoring DDDAit = 0, while the mean of the only farms with DDDAit ≠ 0 was 0.7±0.07. The AW score was 76.9±9.8% and the biosecurity score was 68.9±15.3%. Statistical analysis was performed using Spearman's rank correlation in GraphPad Prism 8.0.1. Biosecurity and AW were positively correlated (ρ=0.42; $p<0.001$). A positive correlation was also observed between biosecurity and both management (ρ=0.61; $p<0.001$) and housing scores (ρ=0.36; $p<0.001$). No significant correlation was found between AMU, biosecurity and AW, probably due to the low levels of AMU found. Contrary to the linear narrative often suggested between AMU, biosecurity and AW, the dynamic system we described is far more complex and articulated. Knowing the level of biosecurity and AW, their relationship and critical points on livestock farms, are tools for planning strategic priorities in human resources and investments. It also helps to improve farmers awareness and animal health. However, while these tools are necessary, they are not sufficient to significantly reduce AMU; other socio-economic factors must be considered to achieve this goal. Achieving high biosecurity and AW levels reduces exposure to pathogens and improves the host immunity. We believe it is essential to integrate mitigation measures into decision-making processes to implement an appropriate diagnostic and treatment protocols involving breeders, vets and farm workers.

Analysis of flavor and metabolites changes during production of Ginger milk curd and Double-Layer Steamed Milk Custard from buffalo milk

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In southern China, buffalo farming has a long history and offers significant economic benefits through the production of milk, meat, leather, and draft power. Buffalo milk has a higher fat content, making it particularly suitable for producing high-fat dairy products. Ginger Milk Curd (GMC) and Double-Layer Steamed Milk Custard (DLSMC) are traditional Guangdong dairy products made from buffalo milk, prepared using different processes. Samples were collected at four key processing stages: raw buffalo milk, boiled buffalo milk, sweetened buffalo milk after sugar addition, and the final ginger milk curd for GMC; and raw buffalo milk, thermal processing, first-layer and second-layer milk skin formation for DLSMC.

The results in GMC revealed significant alterations in volatile organic compound (VOCs), resulting in flavor transitioning from the original flavor to fruity, sweet, green, and citrus flavor. The E-Nose and E-Tongue effectively differentiate the aroma and flavor characteristics of samples at four key processing stages. GMC contains higher levels of alcohols, aldehydes, ketones, and sulfides compared to samples from other stages, and it shows significant differences in umami, saltiness, and astringency taste characteristics. 13 key VOCs were identified via GC-IMS, including 1-octen-3-one, 1,8-cineole, dimethylsulfide, and methyl 2-methylbutanoate, contributing to the flavor variations in GMC. Meanwhile, protein degradation, lipid oxidation, and the Maillard reaction were identified as key factors significantly impacting the levels of metabolites and VOCs. The enhancement of flavor characteristics in GMC was attributed to the rise in taste-active peptides, aromatic amino acids, and free fatty acids during processing.

DLSMC demonstrated increased sweetness, saltiness, and umami compared to buffalo milk, along with heightened levels of nitrogen oxides, methane, alcohols, aldehydes, ketones, and sulfur-containing compounds. The thermo-processing and second-layer milk skin formation were pinpointed as critical for flavor alterations. GC-IMS detected a total of 46 VOCs, with 8 compounds identified as key flavor components. Untargeted metabolomics revealed 98 differential metabolites, including amino acids, lipids, and nucleotides, that were significantly linked to changes in key flavor compounds. The fluctuations in the levels of amino acids, lipids, and nucleotides play a crucial role in influencing flavor changes during the production of DLSMC made from buffalo milk.

This study provides new insights into the formation of flavor and nutritional properties during GMC and DLSMC processing, enhances understanding of buffalo milk dairy flavor formation, and has the potential to improve buffalo milk processing and product development on a broader scale.

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Formulation and Evaluation of Homogenized Buffalo Ricotta with Native Probiotic Protective Cultures

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The application of protective cultures (PC) in food has expanded substantially, providing a natural substitute for chemical preservatives. Ricotta is a suitable matrix for transporting lactic acid bacteria and other protective cultures. The aim of this study was to evaluate the shelf-life of homogenized buffalo ricotta cheeses produced with three PCs isolated from milk (*Lactiplantibacillus plantarum*, LC44 and LC11) and from buffalo whey (*Leuconostoc mesenteroides* ssp. *mesenteroides*, SS14). The characterization of the isolated strains was carried out through *in vitro* studies of probiotic, antimicrobial and antioxidant properties. A total of four experimental groups of ricotta were prepared: a control without PCs (CTR) and three with LC44 (BRM44), LC11 (BRM11) and SS14 (BRW14). The production technology included a modification of the standard processing flowchart at the cooling stage (35±2°C) for PC inoculation. The ricotta samples were analyzed for nutritional, physicochemical, sensory and microbiological characteristics during storage at 4±1°C. Analyses were performed at T0 (1 day), T1 (8 days), T2 (15 days), T3 (18 days), T4 (22 days) and T5 (25 days). PCs isolated showed greater survival (> 50 %) in simulated gastric and intestinal juice with bile salts. The supernatants of PCs exhibited antimicrobial activity against all indicator microorganisms, with *Listeria monocytogenes* being the most sensitive species. All strains demonstrated a survival rate > 45% when exposed to 1.0 mM hydrogen peroxide showing a high antioxidant activity. The application of PCs in ricotta confirmed greater ($p < 0.01$) resistance and adaptability of milk-isolated strains compared to whey-derived ones. Samples with PCs showed lower ($p < 0.01$) pH values than CTR, contributing to the inhibition of microbial population growth. The addition of PCs did not affect the nutritional composition or the oxidative status of the ricotta. The supplemented ricotta showed a more intense dairy aroma ($p < 0.01$) and a more yellow color ($p < 0.05$), as also confirmed by colorimetric analyses. From T2 onward, the emergence of excessive acidity and bitterness compromised product acceptability. The study showed that protective cultures can enhance buffalo ricotta with functional, antimicrobial, and antioxidant qualities. Future work should establish specific technological protocols and robust shelf-life evaluations for probiotic dairy products.

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Buffalo milk yogurt produced in the province of Salerno: evaluation of chemical-physical, nutritional and organoleptic characteristics

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Buffalo yogurt, a traditional product included among the PATs (Traditional Agri-food Products) of the Campania Region, is emerging as a promising functional dairy food due to its rich nutritional profile and probiotic potential. However, systematic characterization of its compositional and microbiological variability remains limited.

This study provides an integrated assessment of physicochemical, microbiological, and sensory properties of buffalo yogurt produced in the province of Salerno. Ten plain yogurt samples, collected on the same production day from different dairies, were analyzed. Composition (fat, protein, total solids, sugars, lactic acid, and fatty acid profile) was determined by FT-IR (Milkoscan FT3), pH measurement by Mettler Toledo pHmeter, while lactic acid bacteria (LAB) were enumerated (ISO 9232:2003) and identified using MALDI Biotyper,). Sensory attributes were also assessed through a structured panel test.

Despite heterogeneous production practices, samples exhibited a consistent compositional profile (average fat 5,53%, protein 4,62%, sugar 5,76 % with lactose as the predominant sugar. Fatty acid composition aligned with established buffalo milk profiles, confirming expected biological variability linked to production conditions. All samples showed LAB counts > 6 log CFU/g, indicating robust microbiological quality. Microbial profiling revealed dominance of *Streptococcus thermophilus* (50%) and *Lactobacillus delbrueckii* (30%), alongside the detection of *Clavispora lusitaniae*(20%), highlighting a potentially broader functional microbiota than traditionally assumed. The average lactic acid content (0,71 %) and pH (4,88) were consistent with optimal fermentation dynamics. Buffalo yogurt samples were assessed by colour, odour, syneresis and rope before the tasting, and after the tasting for thickness, creaminess, lumpiness, melting, sweetness, acidity and overall acceptability. Sensory evaluation indicated overall good acceptability of the product.

Collectively, these findings position buffalo yogurt as a microbiologically stable and nutritionally dense product with emerging functional attributes. The detection of non-conventional microbial species, combined with compositional variability, underscores the need for targeted standardization and deeper investigation of microbiota–matrix interactions. This could support the development of buffalo yogurt as a next-generation functional dairy product with enhanced health value.

Modelling the Risk of listeriosis associated with Water Buffalo Mozzarella consumption: A Quantitative Approach

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Buffalo mozzarella is a fresh pasta filata cheese of global relevance, with Italy as its main producer. Thermal steps such as pasteurization and curd stretching markedly reduce microbial loads and are generally effective against *Listeria monocytogenes* (LM), the agent of severe foodborne listeriosis affecting vulnerable populations. However, mozzarella can undergo post-processing contamination through contact with surfaces, water, or the brine and preserving liquid. A recent meta-analysis reported an expected LM prevalence of 1.2% in ready-to-eat mozzarella (Condoleo et al., 2025). Although initial contamination levels are typically low, the product's characteristics support the growth of LM, allowing it to reach concerning concentrations before the end of its shelf life. Our study aimed to assess the risk of invasive listeriosis associated with the consumption of packaged buffalo mozzarella in Italy, evaluating the risk per serving across different consumer subpopulations.

We simulated the final steps of the supply chain, from retail storage to domestic consumption, through a stochastic model (R software) using distributions for time–temperature conditions derived from recent Italian data (Iulietto et al., 2024). An "ad hoc" predictive growth model was applied to estimate LM dynamics until consumption. Additional simulations assessed the effect of short-term temperature abuse occurring within shelf life.

We estimated that the median concentration of LM in contaminated portions of mozzarella was 0.08 Log CFU/g (95% CI: -1.05 to 1.20 Log CFU/g). The predicted risk of listeriosis per random serving ranged from 2.21×10^{-15} in the adult population to 8.07×10-13 in vulnerable populations, such as immunocompromised individuals. Alternative scenarios involving temperature abuse yielded slightly higher, yet still very low, risk estimates, ranging from 1.78×10-12 (short abuse) to 3.42×10-12 (extended abuse) in high-risk individuals.

Overall, the risk of listeriosis associated with buffalo mozzarella consumption appears to be negligible to very low, as the simulated values indicate an extremely small number of expected cases even under high-consumption conditions. This is primarily attributable to the product's acidic pH of buffalo mozzarella, which greatly limits the ability of LM to replicate. Therefore, it is crucial to ensure adherence to proper manufacturing practices and maintain the typical pH levels of buffalo mozzarella.

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Brucella ovis as a model to study the survival of *Brucella abortus/melitensis* during the manufacture of buffalo mozzarella cheese

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Brucellosis is a zoonotic disease, and consuming unpasteurized milk and dairy products can lead to human infection. This study aims to assess the survival rate of Brucella ovis during mozzarella cheese production, using it as a model for Brucella abortus/melitensis. This was done by inoculating fresh unpasteurized milk collected from brucellosis-free farms in Salerno, one of the provinces in the Campania region known for producing Buffalo mozzarella cheese. The milk was divided into two aliquots of 5 L and inoculated with B. ovis at approximately 10 x 103 and 10 x 10 5 colony-forming units per millilitre, respectively. The mozzarella cheese was produced in full compliance with traditional production specifications, as commonly practised in cheese factories. The experiment was performed once, and the detection of B. ovis was analysed in the milk after contamination, in the whey after curd maturation, and in the finished product. Before contamination with B. ovis, the milk was analysed for physicochemical characteristics to determine quality parameters and total bacterial load. On the finished product, the total mesophilic count was analysed. Samples were tested for B. ovis using bacterial isolation and polymerase chain reaction, and pH, acidity (SH), and temperature were concurrently measured at different stages of the process. Our results showed that B. ovis can survive throughout the cheese-making process until the stretching phase, in which the curd is stretched in water at 100 °C, reaching a core temperature of about 62.5 °C. After this phase, no viable cells were detected, and only biomolecular analyses identified B. ovis DNA in the finished product. Our findings indicate that temperature and possibly also pH levels can influence the survival of Brucella. In conclusion, our experiment demonstrated that Brucella spp. cannot survive in the final product due to the characteristic cheese-making process, indicating that mozzarella cheese, an Italian gastronomic excellence, does not pose a tangible danger to consumers. The experiment also highlights the importance of closely monitoring critical parameters during the process, such as temperature and pH. Nonetheless, infected milk must always be avoided due to the potential risk to food-processing operators and the possibility of recontamination of the finished product.

Characterization of detailed protein composition in buffalo milk

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Proteins are among the most valuable constituents of buffalo milk, due to their central role in the nutritional and technological properties of dairy products, particularly Mozzarella di Bufala Campana PDO. Despite its importance, studies investigating the detailed protein profile of buffalo milk at the individual animal level remain limited. This research aimed to characterize individual milk protein fractions in Italian Mediterranean buffalo and to assess the effects of days in milk, parity, and season of sampling on protein composition. Individual milk samples were collected from 415 lactating buffaloes (one sample per animal) reared in 18 commercial farms within the Mozzarella di Bufala Campana PDO area. Milkoscan FT6000 (Foss Electric A/S, Hillerød, Denmark) was used to measure milk chemical composition and to collect spectral data. Detailed milk protein profile, including casein (α-, β-, and κ-casein) and whey (α-lactalbumin and β-lactoglobulin) fractions, were determined by reversed-phase HPLC. Sources of variation were investigated using a mixed linear model including fixed effects of lactation stage, parity order, and season of sampling, with herd-test day as random effect. Casein fractions averaged 25.30, 12.69, and 5.98 mg/mL for α-, β-, and κ-casein, respectively. On the other hand, whey fractions averaged 2.25 and 6.04 mg/mL for α-lactalbumin and β-lactoglobulin, respectively. Protein fractions measured using HPLC method were positively correlated with protein content measured using infrared spectroscopy analysis and negatively correlated with milk yield. Days in milk significantly affected the concentration (mg/mL) of all measured protein groups, in a way that both casein and whey protein fractions significantly increased along lactation. In terms of protein composition (%), whey proteins showed a slight decreasing trend which was opposite to the increasing trend observed for κ-casein. Parity order and season of sampling had minor effects on the variation of detailed milk protein profile. Future research should aim to assess how and to what extent the detailed milk protein profile is influenced by farm management practices and animal health, as well as to elucidate the contribution of individual proteins to milk technological properties and cheese yield.

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Smart monitoring of Italian Mediterranean dairy buffaloes through reticulo-rumen bolus technology

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Precision livestock farming enables continuous monitoring of physiological and behavioural parameters in farm animals. These technologies are limited in dairy buffaloes. This study aimed at evaluating the applicability of intraruminal sensor boluses in dairy buffaloes under commercial farming conditions. The study was conducted on a dairy buffalo farm in central Italy (Lazio) housing 140 lactating Italian Mediterranean buffaloes. A total of 70 animals were equipped with smaXtec® intraruminal boluses (smaXtec Animal Care GmbH, Austria) in February 2025, and a further 40 animals in December 2025. Data were continuously collected from March 2025 to March 2026. The boluses recorded rumen temperature (RT), rumination time (RuT), reticulorumen motility (RM), and activity at 10-minute intervals, water intake (WI) was estimated daily. Environmental temperature, humidity, and temperature-humidity index (THI) were recorded by a meteorological station positioned within the barn. The system generated automated alerts for estrus and calving, which were validated through veterinary inspections. Out of 154 estrus alerts, 62 animals were examined and 56 were confirmed in estrus, resulting in a detection precision of 90.3%. The remaining 92 alerts were not clinically verified and therefore could not be classified as true or false positives. Calving alerts correctly predicted 68% of parturition events. Physiological and behavioural responses were analysed across three THI classes (<60, 60-74, >74) using mixed models including THI class, days in milk (DIM), and parity as fixed effects, with animal as a random effect. Only lactating animals (n=68) were considered. Mean DIM was 149±78 days and mean parity was 3.9±1.9. Average RT was 39.35±0.21°C, RuT 432±81 min/day, RM 1.09±0.09 cycles/min, and WI 77.8±16.0 L/day. Compared with buffaloes exposed to THI ≤ 74, water intake decreased by 5.1%, RT increased (0.33%; $p<0.01$), RM decreased (-2.7%; $p<0.01$), while activity increased by 8.0% ($p<0.01$), whereas RuT showed a slight reduction under THI > 74 condition. After species-specific calibration, including reduction of the estrus detection threshold (from 50 to 30), and extension of the calving window to 30 days, intraruminal bolus sensors originally developed for cattle proved reliable for monitoring dairy buffaloes under field conditions. These systems may enable early detection of elevated THI conditions, support timely management interventions and improve animal welfare.

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Rumination time in Mediterranean Buffalos: Using Cowtech® sensors to monitor rumination

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Efficient heat detection is a critical challenge in Italian Mediterranean buffalo farming, as this species exhibits pronounced reproductive seasonality and a weak, often scarcely observable, behavioural expression of oestrus. In this context, rumination, being closely linked to metabolic, physiological and reproductive status, may serve as an objective and continuous proxy for identifying behavioural changes potentially associated with heat. This study aimed to evaluate the use of Cowtech® ear-tag accelerometers for automated rumination monitoring in Italian Mediterranean buffaloes and to develop a species-specific predictive model as an initial step towards sensor-based support for reproductive management, in a field where dedicated buffalo-oriented tools remain limited.
The trial was carried out on six lactating buffaloes reared on two farms in the province of Salerno, Italy, from March to September 2025. In total, 161 observation days and approximately 2,730 hours of video recordings were collected and behaviourally annotated as reference data. Real-time accelerometric signals were processed to train a Convolutional Neural Network for binary classification of rumination versus non-rumination. In total, 39,672 behavioural segments were analysed, including 20,117 rumination events and 19,555 non-rumination events.
The model achieved an overall accuracy of 87%, with balanced class performance (precision 0.89, recall 0.86, and F1-score 0.87 for rumination; precision 0.86, recall 0.89, and F1-score 0.87 for non-rumination). High discriminative power was confirmed by an ROC-AUC of 0.923 and a Precision-Recall AUC of 0.903. The bimodal distribution of prediction probabilities indicated limited model uncertainty across behavioural classes, supporting the robustness of the classification process. These findings demonstrate that accelerometer-based systems can reliably detect rumination in Italian Mediterranean buffalo and provide a robust foundation for future integration of reproductive, health, and welfare indicators. With further validation on larger populations and across different physiological stages, such tools may support non-invasive heat detection, reduce dependence on hormonal synchronisation protocols, and strengthen the implementation of Precision Livestock Farming in buffalo dairy systems.

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Pilot study on growing and morphological buffalo calves assessment by image analysis

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Image analysis in livestock farming can increase efficiency and welfare. A laser telemeter (Zoometer®) was used to indirectly determine Body Condition Score (BCS) and Body Weight (BW) in Mediterranean buffalo calves. Eighteen four months male were weaned and allocated into two pens (9 each). Groups were balanced for age and initial BW for a performance trial (growing and fattening), focusing on meat production. Trial started after two month adaptation and groups received the same diet, balanced for nutritional requirements. Animals were weighed at the beginning and every two weeks thereafter; BCS and BW were evaluated by both a trained operator (using a scale for BW and a 1–9 scoring system for BCS) and the Zoometer (via top-down image analysis). Once a month, official linear morphometric measurements was individually taken; the same measurements were then calculated by the Zoometer using a top-down imagery. Our results showed high consistency between measured and predicted data. Specifically, for 91 observations, the upper surface area of animals was highly correlated with BW ($r = 0.961$). The angle between the highest point of the rump and the head of the femurs, used to calculate BCS, showed a correlation > 0.96 with the operator's score. The regression models for BW ($y = 6.7844x + 1192.4$) and BCS ($y = 0.1872x - 3.6544$) provided high R^2 values, exceeding 0.92. We also found close correlations between the Zoometer response and operator's measurements (height at withers 0.982, height at rump 0.986, rump length 0.986, rump width 0.989, chest length 0.998) and a difference between the two measurements, always less than 3%. The average daily gain (Δ weight/Δ t) was 1.055 ± 0.321, with wide individual variation. This preliminary study, although limited by a small sample, provides interesting insight into automating the growth performance assessment of buffalo calves. Nevertheless, as the trial is still ongoing, the number of subjects will be progressively increased and a rigorous assessment of (co)variance between and within experimental subjects included, to make the models robust and applicable across different conditions.

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Fractal Dimension Analysis of Salivary Crystallization Patterns for Estrus Detection in Estrus-Synchronized Swamp Buffaloes (*Bubalus bubalis*)

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The inconsistent expression of estrous signs in swamp buffaloes necessitates simple, non-invasive detection methods. This study evaluated salivary crystallization as an objective estrus detection tool in ten female swamp buffaloes (*B. bubalis*), aged at least 2 years with a history of regular estrous cycles, synchronized using the standard Ovsynch protocol. On days 7, 8, 9, and 10 of the synchronization program, estrus behavior was observed, uterine horn tonicity was assessed, and follicular size was measured using ultrasonography. Saliva samples were microscopically examined to analyze fractal dimensions (FD) and morphometric parameters, including crystallization area, junction density, and endpoint frequency. Results revealed that structural complexity peaked during pro-estrus (Day 9). However, during estrus (Day 10), complexity dropped significantly ($p < 0.05$) with a reduced FD (1.73 ± 0.12), which corresponded to the formation of symmetrical typical fern patterns. Simultaneously, the crystallization area increased significantly to over 30% ($p < 0.05$), correlating strongly with the presence of large dominant follicles ($r = 0.431$, $p < 0.05$) identified via ultrasonography. In conclusion, integrating fractal dimension and structural morphometry provides a precise method for detecting estrus, particularly in buffaloes exhibiting silent heat. Future research, developing an application utilizing automated image analysis and machine learning to evaluate salivary crystallization patterns is highly recommended to facilitate real-time, on-farm estrus detection.

Keywords: Salivary ferning, Swamp buffalo, Fractal dimension, Morphometric parameters, Estrus detection

Methane Footprint and Productive Performance of Italian Mediterranean Buffalo

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Methane (CH_4) emissions are increasingly considered a relevant trait in ruminant production systems, with growing interest in their inclusion in genetic selection programs. On-farm technologies, such as methane sniffers integrated into automatic milking systems (AMS), enable the collection of individual CH_4 emission data under commercial conditions. However, information on methane emissions in buffaloes is still limited. The aim was to evaluate individual CH_4 emissions in lactating Italian Mediterranean buffaloes and their association with production and milking behaviour traits. The study was conducted on a commercial farm in Central Italy with 200 lactating buffaloes. Methane emissions were monitored over 4 months using a sniffer system installed on four AMS. The herd was divided according to lactation stage and assigned to specific robots; the sniffer was rotated monthly across robots. Emission data were recorded during milking and integrated with AMS performance data. Milk and TMR samples were collected monthly. Methane emissions were analysed using a linear model in R, including milk yield, lactation stage (Early vs Late), and their interaction as fixed effects. Animals were retrospectively classified into Early (22.9 ± 21.1 days) and Late (237.3 ± 48.0 days) lactation groups and further subdivided within each stage into High and Low CH_4 emitters using a quartile-based approach. Differences between High and Low CH_4 emitters were assessed by pairwise comparisons ($P < 0.05$). Mean CH_4 emissions tended to be higher in the Late group (819 ± 360 ppm) compared to the Early group (624 ± 285 ppm), although not statistically significant ($p = 0.08$). In early lactation, significant differences between emission groups were observed for milk composition traits, with lower protein and higher lactose content in High emitters compared to Low emitters ($p = 0.02$ and $p = 0.03$, respectively). The relationship between CH_4 emissions and milk yield was significantly influenced by lactation stage. A positive association was observed in the Late group, but not in the Early group. The interaction between milk yield and lactation stage was significant ($p < 0.001$), and the model explained 41% of the variability in CH_4 emissions ($R^2 = 0.41$; $p < 0.001$). These findings suggest a stage-dependent relationship between methane emissions and milk yield, potentially reflecting differences in feed intake and rumination patterns across lactation and warranting further investigation.

Successful Treatment of Snakebite Envenomation in a Buffalo Bull

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Snake envenomation in large ruminants is an acute, life-threatening emergency that is rarely documented in clinical veterinary literature during the monsoon season in Punjab. This case details the successful clinical management of an indigenous adult buffalo bull presented with sudden-onset hindlimb paresis, profound lethargy, and progressive neuro-paralytic signs. Upon physical evaluation, clinical manifestations included tachycardia and congested mucous membranes. Treatment prioritized venom neutralization via the slow intravenous infusion of polyvalent snake venom antiserum (ASV) along with fluid therapy, antibiotics, nerve tonics, and antihistamines. The bull demonstrated almost full recovery after extensive treatment and good management.

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Genomic identification of key genes underlying milk production and morphology in water buffalo

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Although water buffaloes are highly important livestock for milk and meat production in many tropical and subtropical regions worldwide, the genomic basis of most economic and morphological traits in the buffalo industry remains poorly understood yet. By integrating comparative population genomics, transcriptomics, and functional studies, we identified key genes underlying morphology and dairy performance in water buffalo. We pinpointed major genes and causal mutations for diverse coat-color phenotypes. In white swamp buffalo, a 2,809-bp LINE-1 transposon insertion upstream of ASIP creates an alternative promoter, markedly increases ASIP expression, and disrupts melanocyte maturation, causing the presence of white coat color. On the contrary, white spotting in Indonesian swamp buffalo is primarily due to loss-of-function variants in MITF and KIT, which impair melanocyte development and together explain ~95% of the spotted phenotype. In river buffalo breeds such as Murrah, Nili-Ravi, Mediterranean, solid black pigmentation maps to MC1R, with the missense variant (p.Met128Ile) showing the strongest association and functional evidence of increased receptor activity and eumelanin production. Considering milk production traits, dairy buffalo breeds exhibited strong selection sweeps on the casein gene cluster, particularly CSN2, identifying this gene as an important determinant of milk production. Single-cell transcriptomics reveals that CSN2 and related casein genes are highly expressed in mammary luminal cells, highlighting these cells as the key cellular basis of enhanced milk secretion. Together, these findings demonstrate that buffalo genomics can resolve genes underlying both visible morphology and dairy production, providing a solid foundation for the accurate application of marker-assisted selection to speed up the rate of genetic improvement in water buffalo populations.

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Pedigree and genomic inbreeding and their relationship with production traits in Italian Mediterranean Buffalo

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Increase in parentage relationships and inbreeding is a common consequence of animal breeding programs. In dairy species, production traits have been improved through the intensive use of sires with high genetic merit, resulting in an increase of the frequency of matings among related individuals. High levels of inbreeding can lead to the inbreeding depression, associated with increased morbidity and mortality, reduced production. Traditionally, inbreeding has been estimated using pedigree information (FPED), which provides expected relationships. However, it is limited by pedigree completeness and reliability. More recently, genomic data have been used to estimate inbreeding coefficients, either from the genomic relationship matrix (FGRM) or from runs of homozygosity (FROH). Genomic approaches do not require pedigree information and can therefore be applied to all genotyped individuals. The aim of this study was to compute different inbreeding coefficients in Italian Mediterranean Buffalo (IMB) and investigate their association with estimated breeding values (EBVs) for production traits, including milk yield (MY), fat yield (FY), protein yield (PY), and fat (FP) and protein (PP) percentages. A total of 7,069 animals were genotyped for 52,917 SNPs. FROH was calculated using different minimum ROH lengths: >2 Mb (FROH2), >4 Mb (FROH4), >8 Mb (FROH8), and >16 Mb (FROH16). Longer ROH are indicative of more recent inbreeding. EBVs were regressed on the different inbreeding coefficients using their reliability as weights. In addition, a bootstrap regression with 1,000 replicates was performed to obtain the 95% confidence intervals of the estimates.
As expected, the different FROH measures were highly correlated with each other ($R > 0.89$) and moderately to high with FGRM (>0.63). FPED showed moderate correlations with FROH (>0.42) and low with FGRM (0.15). The EBVs for the three yield traits were positively associated with FPED and negatively with FGRM. Opposite trends were observed for FP and PP, as expected. A negative effect of older inbreeding (i.e., FROH2 and FROH4) was observed for FP. When interpreting these results, it should be noted that FPED values equal to zero included in the analysis often correspond to animals with unknown parent(s), thus for which the inbreeding coefficient cannot be computed. Overall, this preliminary study suggests the presence of a possible genomic inbreeding depression affecting milk yield traits in IMB.

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Genetic evaluation of weaning weight in buffalo under conditions of limited information: a comparison of linear and mixed models in Colombia

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Weaning weight (WW) is a key indicator of productive performance in buffalo systems, as it integrates both genetic and environmental effects. In Colombia, the limited availability of records and low genetic connectivity due to small herd size, restrict the application of conventional statistical models for genetic evaluation. This study aims to compare the performance of linear and mixed models under different data structures and to propose a methodological approach applicable to systems with limited information. Data from of two comercial herds were analyzed: Middle Magdalena (2024: n=153; 2025: n=37) and Cordoba (2025, n=196). In all cases, linear and mixed models were fitted. In 2024, the models had low explanatory power (adjusted $R^2 < 0.18$), although the sire effect was identified as the main source of structured variation. In 2025, the dataset consisted exclusively of males, which reduced variability; the linear model identified a significant effect of season ($p<0.01$), while the mixed model detected no genetic variance ($\sigma^2 \approx 0$), highlighting the limited capacity for genetic inference under conditions of small sample size and lower data diversity. In Córdoba, both approaches identified relevant sources of variation; the mixed model revealed genetic variability among sires ($\sigma^2=96.52$), while the linear model showed environmental effects associated with lactation, where milk yields exceeding 1,700 L significantly increased the PD ($p<0.05$). Additionally, the estimation of genetic values (BLUPs) allowed for the classification of breeding stock and highlighted differences among sires. The results show that the models' ability to detect genetic variation depends critically on sample size, data structure, and data variability. Under limited conditions, the environmental effect predominates; however, as data quality improves, mixed models allow for a more robust genetic evaluation. It is concluded that it is possible to implement progressive genetic selection strategies in small herds through the combined use of linear and mixed models, adapting their application to data availability and improving their accuracy as records increase.

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A new SNP array to improve buffalo knowledge and performance

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The Italian Mediterranean buffalo (IMB) plays a key role in the European dairy sector, particularly in the production of high-value products with Protected Designation of Origin (PDO) status, driving the European market through the export of renowned products such as "Mozzarella di Bufala Campana" and "Ricotta di Bufala Campana". In recent years, the development of genomic selection, aimed at identifying single nucleotide polymorphisms (SNPs) associated with fertility, resistance traits and quanti-qualitative improvement of milk production, has enabled substantial acceleration of genetic gains. In this context, SNP array technology has become a cornerstone of genetic research, even if accurate and species-specific genomic tools remain lacking for IMB. To address this gap, we designed and evaluated the performance of a newly developed SNP array comprising 122,017 markers, including Y chromosome and functionally relevant variants, specifically tailored for the IMB. A total of 741 IMB samples (~38% male) were genotyped. After excluding low-confidence variants flagged by Affymetrix, over 87.7% of SNPs showed call rate (CR) >95% and minor allele frequency (MAF) >0.05, yielding a final dataset of 726 individuals and 107,040 SNPs. Compared to the previous array version (90k SNPs), the new tool provides an increased number of markers, with an improvement over 65% of high-quality controlled markers (MAF >0.01), and more evenly distributed markers throughout the genome. The new array was compared with its predecessor for genomic evaluation of milk and morpho-functional traits in the IMB, using the same animal set. Results in terms of accuracy and rank were evaluated against the official values from the IMB breeder association. Genomic relationships evaluation was also be checked. Finally, to assess biodiversity, 113 other river-type breeds (e.g. Nili-Ravi, Kundi, Azakheli, Murrah and Jafarabadi breeds), and 10 Swamp buffalo were also genotyped. Following quality control, 109,156 SNPs (89.5%) and 145 individuals were retained. Notably, the new array captured approximately 60,000 SNPs (MAF >0.01) in Swamp buffalo, compared to 23,938 SNPs in the previous array version, representing almost 61% improvement. These findings confirm the robustness and effectiveness of the new array as a valuable tool for genomic selection, biodiversity analyses, and the sustainable genetic improvement of IBM and other buffalo breeds.

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A novel probe-based genotyping method for thermotolerant gene- HSP70 in water buffaloes in the Philippines and its effect on milk production

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Identifying animals carrying thermotolerant traits is our foremost effort to develop climate-resilient animal production in the country. HSP70 is a biomarker for heat stress and has been identified as a tool for controlling heat tolerance in livestock species. This paper aims to develop an up-to-date, rapid, cost-effective genotyping protocol for thermotolerance in water buffaloes through probe-based assay. Specifically, to determine the population's carrier frequency of the HSP70 gene. A real-time PCR-based assay using TaqMan-MGB probes was designed to discriminate thermotolerant and non-thermotolerant alleles. SNP was located at the 55th base position of the HSP70 gene (55C>T), leading to a change of threonine (A) to methionine (B) in the amino acid sequence. Allele A was reported to be non-thermotolerant, while allele B is thermotolerant. Only one-step RT-PCR was required after the DNA extraction from the sample, and the time consumption was less than four hours. There are 157 water buffaloes from different breeds assayed. Genotypic frequencies are 1.77% AA, 56.7% AB, and 41.5% BB. The allelic frequencies are 22.5% for allele A and 77.5% for allele B. Analysis revealed that the HSP70 genetic frequencies do not fall to the Hardy-Weinberg Equilibrium, which can be due to natural selection or a product of the selective breeding program conducted for water buffaloes in the Philippines. The native breed, PC, exhibited the highest percentage of the thermotolerant genotype (BB), while only the Bulgarian Murrah breed was found to have the non-thermotolerant genotype (AA). Animals carrying the B allele presented an increase in milk production for both dry and wet seasons, overall implying the thermotolerant trait to be helpful in stabilizing milk yield even under heat stress. In conclusion, we have developed an effective method to identify SNPs through RT-PCR probe-based genotyping in the HSP70 gene for thermotolerance identification. To our knowledge, this is the first probe-based genotyping via RT-PCR and of thermotolerant traits in livestock species in the Philippines. This can pave the way for easily genotyping other economically important traits and the detection of genetic diseases.

As the author of the abstract and on behalf of all authors, I therefore grant the non-exclusive rights of use of our abstract, including tables and illustrations, for the purpose of publication, also in extract form, in printed and electronic media.

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Screening of Key Candidate Molecules for Buffalo Lactation Traits and Regulatory Mechanism of ESR1 Enhancer E3 Binding HMGA1 in Mammary Epithelial Cells

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Insufficient research on genes and regulatory elements related to buffalo lactation traits has restricted lactation breeding, resulting in low milk yield and short lactation period, which is a bottleneck for buffalo breeding and dairy industry. This study used RNA-seq, ChIP-seq and ATAC-seq to analyze mammary gland tissues of Murrah buffalo at dry and mid-lactation stages, and screened key candidate genes and regulatory elements for lactation traits.

A total of 4,900 differentially expressed genes (DEGs) were identified. Up-regulated DEGs including ESR1 were enriched in mammary epithelium development, prolactin, oxytocin and estrogen signaling pathways. Down-regulated DEGs were enriched in oxidative phosphorylation, protein transmembrane transport and ATP metabolism. ATAC-seq identified 37,296 and 42,386 typical enhancers at lactation and dry period, respectively. Combined analysis revealed 5,875 candidate active promoters and 4,439 candidate active enhancers. Candidate active regulatory elements marked by open chromatin and H3K27ac/H3K4me3 were detected near ESR1, INSR, GHR and PPARG.

Seven candidate enhancers upstream of ESR1 were verified; E3, E4, E5 and E7 showed enhancer activity, with E3 being the strongest. DNA-Pull-Down confirmed high-affinity binding between transcription factor HMGA1 and E3. CUT&RUN showed significantly reduced HMGA1 enrichment after E3 knockout ($P < 0.05$). Site-directed mutagenesis of the TTTTAA binding site markedly decreased dual-luciferase activity ($P < 0.01$), verifying that HMGA1 activates transcription via this site. 3C-qPCR confirmed strong spatial interaction between E3 and ESR1 promoter. CRISPRi silencing showed E3 knockout reduced ESR1 expression, inhibited BMEC proliferation, promoted apoptosis, and down-regulated milk fat genes (PPARG, VLDLR, PLIN2, CD36, FASN, FABP3) and milk protein genes (CSN2, RPS6, EIF4E) ($P < 0.05$).

In conclusion, HMGA1 binds the TTTTAA site on E3, mediates spatial interaction with ESR1 promoter, activates ESR1 transcription, and promotes lactation and cell proliferation via lipid metabolism genes and mTOR pathway. This study clarifies the molecular mechanism of buffalo lactation and provides a theoretical basis for genomic annotation and molecular breeding to improve milk yield.

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Comparison of ruminal wall transcriptomic profiles between dairy cattles and buffaloes

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Buffalo and cattle exhibit notable differences in feed utilization efficiency, fermentation patterns and resilience to low-quality diets, suggesting species-specific adaptations of the rumen ecosystem. To date, research has largely focused on microbial communities and metatranscriptomic activity, whereas the contribution of the host rumen transcriptome remains largely unexplored, especially in a comparative framework. To fill this gap, we performed an inter-specific comparative transcriptomic analysis in rumen of buffalo and cattle. After mRNA-sequencing, reads were aligned to the respective reference genomes and the differential expression analysis was restricted to genes exhibiting a one-to-one orthologous relationship between the two species. We found 7429 genes differentially expressed, of which 3687 genes were up-regulated and 3742 genes were down-regulated in buffalo vs cattle.
Gene Ontology Enrichment Analysis (GOEA) of up-regulated genes revealed enrichment of GO terms related to i) biological processes as fatty acid beta-oxidation, xenobiotic transmembrane transport, suppression of viral release by host, immune and inflammatory response, autophagy, response to toxic substance; ii) cellular components as apical junction complex, cis and trans-Golgi networks, endosomes; iii) molecular functions as fatty-acyl-CoA binding and phosphatase and kinase activities. GOEA of down-regulated genes highlighted enrichment of GO categories associated with i) biological processes as apoptosis, epithelial cell differentiation, isoprenoid biosynthesis, mitotic spindle organization, and response to oxidative stress; ii) cellular components as extracellular matrix and lysosomes; iii) molecular functions as extracellular matrix constituent, fibronectin, and collagen. KEGG pathway analysis of up-regulated genes showed enrichment of pathways related to aminoacid metabolism, CoA biosynthesis, fatty acid metabolism, immune system, and autophagy, while the analysis of down-regulated gene indicated enrichment of pathways linked to terpenoid biosynthesis, glutathione metabolism, and extracellular matrix. In conclusion, these findings suggest a more efficient and adaptive metabolic activity in rumen of buffalo vs cattle, especially in pathways related to nutrient metabolism, detoxification, and immune response. Although further functional studies are needed, our results are consistent with the previous observations of a higher digestive efficiency in buffalo.

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From sires to Genetic Diversity: how bull exchange networks influence inbreeding patterns in Italian Mediterranean Buffalo

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Effective genetic management of livestock breeds requires understanding both the current state of genetic diversity and the reproductive mechanisms driving it. In Italian Mediterranean Buffalo (IMB), sire exchange represents the primary vehicle of gene flow across geographically structured breeding groups; however, its influence on inbreeding dynamics remains poorly characterized. Therefore, the aim of this study was to integrate high-density genomic data with sire network modelling to simultaneously characterize population genetic structure and the reproductive processes shaping it. A total of 4,505 animals genotyped with the Axiom™ Buffalo Genotyping Array 90K (52,917 SNPs) were used and grouped into 10 geographic clusters based on geographical proximity and regional breeding structure. Sire-based gene flow networks were reconstructed from national Herd Book pedigree records using directed weighted graphs. To characterize reproductive structure, the following indices were estimated for each group: effective number of sires, Gini coefficient of sire usage, self-breeding rate, import rate, and source diversity. Exploratory linear models were used to assess associations between sire usage patterns and group-level FIS. The results showed that mean observed and expected heterozygosity were 0.384 and 0.380, respectively, with positive FIS in six areas, mainly in central and northern Italy. Three core groups, Caserta, Salerno, and Latina, were identified as dominant sire exporters and network hubs, concentrating most reproductive output, while peripheral groups depended on few or single sire sources. Higher within-group mating rates and reproductive skew were positively associated with FIS. Increased paternal diversity reduced inbreeding, but not substantially; therefore, adding more breeding males originating from the core groups provided limited additional benefit. Source diversity alone did not guarantee effective genetic renewal, as importing genetically similar sires contributes little to allelic diversification. These findings highlight the importance of targeted sire selection based on genetic complementarity, controlled sire rotation in core areas, and urgent intervention in isolated peripheral groups to limit inbreeding accumulation and ensure the long-term genetic sustainability of the breed.

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Water Buffalo As A Non-Lethal Defense Strategy For Jaguar Conservation In Productive Ranching Landscapes

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Throughout Latin America, livestock predation by jaguars (Panthera onca) often leads to retaliatory killing, threatening jaguar conservation. This presentation describes a field-tested, non-lethal alternative based on integrating Asiatic water buffalo (Bubalus bubalis) into livestock herds as a defensive species.

At San Miguelito Jaguar Conservation Ranch in eastern Bolivia, in a landscape with high jaguar density, Murrah-based dairy buffalo have been incorporated into mixed herds with cattle and horses. During an observation period exceeding three consecutive years, no jaguar attacks were recorded on domestic animals within the managed herds.

Water buffalo exhibit strong maternal defense, group cohesion, and active protective behavior toward calves and other herd members. These traits can deter jaguar predation, positioning buffalo as biological bodyguards within livestock systems and reducing the conflict associated with vulnerable calves. The experience is observational rather than a controlled evaluation of effectiveness.

The system combines habitat conservation, small-scale artisan buffalo dairy production, and low-impact wildlife tourism. Buffalo are adapted to wetlands, floodplains, and heterogeneous landscapes, offering production opportunities compatible with retaining native forest and lowland habitats. Morning milking and on-site mozzarella and burrata production provide educational experiences that connect sustainable production with jaguar conservation.

This ranch experience suggests that water buffalo can improve livestock security, support dairy production, and reduce incentives for retaliatory jaguar killing. Integrating buffalo husbandry, habitat protection, and tourism offers a conservation-based economy that supports long-term ranch viability.

The Future of Buffalo Breeding in Brazil and Latin America: Prospects, Challenges and Commitments

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The future of buffalo breeding in Brazil and Latin America is shaped by the global expansion and increasing socio-economic significance of water buffalo (Bubalus bubalis) at the regional level. This species has shown remarkable adaptability and contributes substantially to rural economies through the production of milk, meat, draught power, hides, and manure. In recent years, buffalo milk has gained recognition due to its higher concentration of total solids, fat, protein, lactose, and ash compared to cow's milk. It has also been acknowledged as a superior nutritional component for humans, largely owing to the presence of A2A2 proteins in buffalo milk, which contrast with those found in cow's milk. Since the 1970s, it has seen significant research efforts focused on buffalo management, nutrition, infectious diseases, reproduction and Assisted Reproductive Technologies. These initiatives have led to progress, such as deep-freezing semen, artificial insemination, and in vitro embryo production, all intended to improve the genetic quality of local herds. These technological advancements have positioned Brazil as a leading nation, possessing the largest buffalo herd in the Western world and employing advanced breeding technologies. Buffalo genetic progress has accelerated markedly, due to large decreases in generation interval and improvements in accuracy through the "**Estimated Breeding Values" and "Predicted Transmitting Ability**". Nevertheless, the major challenge currently facing the industry is reconciling the increased genetic knowledge of buffalo populations, especially given the presence of two distinct groups of water buffalo: river type (2n=50) primarily found in India, and swamp type (2n=48) originating from East Asia and introduced at the end of the 18th century. In the last decades many reports have been done on the presence of genetic hereditary diseases in buffalo local herds in through the identification of polymorphism in the osteopontin gene and their associations with certain semen production traits of buffaloes in the Brazilian Amazon, however recent studies on genome-wide association studies, gene expression profiling, next-generation sequencing (RNA and DNA) through whole-genome ChIP-seq scanning have been used to detect genetic variants and study gene regulation, gene function, and single nucleotide polymorphism sorting capabilities, aiming at whole-genome sequencing of the domestic buffalo have been performed.

Balancing Compliance and Sustainability in Livestock Systems: A Biogas-Based Solution for Soil and Water Governance

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This study explores the interplay between regulatory compliance, technological innovation, and farmer decision-making in manure management within the Campania buffalo milk production area. It introduces a simulation-based framework that integrates the compliance-enhancing potential of nitrate-stripping technologies in biogas plants with farmer behavior under risk aversion. The model highlights the moral hazard challenges faced by farmers managing manure, wherein compliance costs—driven by land rental, transport, and nitrogen-reduction technologies—often incentivize non-compliance.

By applying the Expected Utility Theory (EUT), farmer decision-making is modeled under uncertainty, accounting for the costs and risks associated with compliance and potential sanctions. Risk aversion parameters, calibrated using empirical data from Italian and European studies, underscore the sensitivity of farmer decisions to compliance costs and enforcement probabilities. The integration of a biogas plant with nitrate-stripping technology introduces a novel compliance mechanism, reducing the nitrogen content in digestate and, consequently, the land requirements for manure disposal.

Simulations conducted with data from the Food Accountancy Data Network (FADN) reveal that while baseline compliance is economically unfavorable for farmers, the inclusion of biogas technologies significantly reduces the profitability of non-compliance, thus narrowing the gap between compliance and non-compliance utilities. Under deterministic parameter settings, the model demonstrates that although full compliance remains economically challenging, the presence of a biogas plant decreases the economic advantage of non-compliance by 55%.

These findings emphasize the role of collective action and technological solutions in addressing environmental challenges in agriculture. By facilitating compliance with nitrate directives, biogas plants not only promote sustainable manure management but also enhance social welfare. This research provides actionable insights for policymakers, highlighting the importance of incentivizing collaborative and technological approaches to environmental compliance.

This framework advances our understanding of farmer decision-making in socio-ecological systems, offering a robust tool for assessing policy interventions and their implications for environmental sustainability in agricultural landscapes.

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Grazing dynamics in floodplains and their impact on the productive health of the water buffalo (Bubalus bubalis)

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Buffalo farming in the Venezuelan tropics faces significant challenges due to inefficient grazing management. This research aimed to evaluate the relationship between wet pasture management and overgrazing at the Santa Juana Ranch, Barinas State. A Murrah buffalo herd was analyzed on 675 hectares of sandy loam and clay soils under a stocking rate of 1 AU/ha. Probabilistic cluster sampling was used, selecting 40 paddocks (58.8% of the total population), using ordinal scales (0-3) to measure moisture and overgrazing. The procedure was validated using Z-scores at a 95% confidence level. The results were analyzed using Spearman's rank correlation coefficient ($r = -0.4226$). ($p < 0.05$) showed a moderate negative correlation, demonstrating that the underutilization of humid areas with Tanner grass (Brachiaria arrecta) leads to critical overgrazing of dry areas with Star grass (Cynodon nlemfuensis). This dynamic negatively affects the herd's body condition, the production target of 6 L/day, and the calving interval of 14 months due to negative energy balance and heat stress. It is concluded that the problem lies not in carrying capacity, but in the spatial distribution of grazing. The correct implementation of Voisin Rational Grazing is recommended to optimize forage harvest in humid areas and mitigate the degradation of upland soils.

Keywords: Grazing management, Overgrazing, Soil moisture, Livestock agroecosystems

Challenges on Sustainability of Swamp Type Buffaloes

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Majority of 40-45 million Swamp type buffaloes in the world are found in South East Asia and East Asia, and are mainly associated with the smallholders, primarily used for draught, and secondarily for meat. In most cases, swamp buffalo is one of the inputs in the crop-dominant, mixed farming system, and is seldom raised in a specialized production system.

There are concerns about the sustainability of these animals in the light of the observed general decline in population in most of the South East Asian Countries, measured to be on the average of -24.62 %, range of - 7.1 % to -84.7 %, 1970 vs 2024. Only Laos and Myanmar , whose labor force as of 2025 are in agriculture at the level of 68.9% and 48.0%, respectively, registered positive population growth of swamp buffaloes.

Against the background of its utility for draft, the aggressive farm mechanization program of concerned governments reaching > 90% in Thailand and Vietnam in 2025, is the primary reason for the large scale substitution of farm tractors in place of swamp buffaloes for draft. Measures in the early years to prevent the population decline include the imposition of slaughterer ban in some countries, which proved not successful at all.

The rapid industrialization and urbanization in mid 20th century have also massive effect on the decline of swamp buffalo population, such as in the case in Taiwan and Malaysia, with population decline of -98.3 % and -77.7 %, respectively, 1970 vs 2024. Such developments resulted in greater percentage of the work force to leave agriculture for higher income job in the industries. This is particularly true for the younger generation who prefer employment in manufacturing and in services, leaving the aging farmers in the farming communities, resulting in serious generational discontinuity in agriculture.

The increasing demand for food in view of the increases in human population, have posed another challenge, producing more food in less land and feed resources. This trend has led to the aggressive growth in monogastric animals, poultry and swine, as these animals a more efficient in feed nutrient utilization than ruminants. This then directed animal-derived food supply towards poultry and pork, leaving buffalo and bovine behind. And while the swamp buffalo registered negative population growth, chicken registered growth of 495.5% , and swine with growth of 161.0 %, 1970 vs 2025.

The logical future direction for the use of swamp buffalo to derive better benefits by farmers is crossbreeding with the riverine type buffaloes in order to develop milk-meat type crossbreds and backcrosses. Such approach has been widely adopted as early as 1970s in China with the infusion of Murrah breed, Nili Ravi, and Mediterranean Italian breed. The same is being done in the Philippines that started in 1996 with infusion of selected Bulgarian Murrah, Brazilian Murrah, and Italian Mediterranean buffaloes, coupled with massive artificial insemination and dairy herd improvement programs.

There are efforts for conservation and genetic improvement of pure swamp buffaloes being undertaken in Thailand through the creation of Thai Buffalo Conservation Village and the Surin Buffalo Center. In the Philippines, conservation of swamp buffalo genetics are in two forms, first is *in-vivo* genetic conservation where selected swamp buffalo are reared and utilized in designated genetic conservation island, and secondly through *in-vitro*, cryo-banking semen and embryos of selected swamp buffalo genetics. Indigenous swamp buffaloes in Indonesia has significant cultural values in some specific areas, and is seen to remain for generations to come. On the other hand, the swamp buffaloes in Australia that turned as feral buffaloes , with current population of about 300,000 are being depopulated due animal health issues, while the remaining clean stocks are facing domestic and export markets.

The water buffalo in the regeneration of tropical soils in Mexico

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In the humid tropics of Mexico, the main agricultural activity is livestock farming, traditionally practiced through extensive grazing. This leads to deforestation, loss of biodiversity, and soil degradation, causing pastures to become unproductive within a few years. Trampling by livestock and overgrazing cause soil compaction, reduce infiltration, deplete soil microorganisms, and contribute to erosion. In contrast, the water buffalo (Bubalus bubalis) is emerging as a grazing alternative with less environmental impact and greater ecosystem regeneration capacity. The objective of this study is to document and analyze the changes observed in the physical and biological properties of the soil after years of rotational grazing with water buffalo at Hacienda del Ranchero, Tacotalpa, Tabasco, Mexico. The system was established on 35 hectares of native and introduced pastures that had been grazed by cattle for more than three generations. A precision grazing model based on Voisin's four laws was implemented. Indicators such as vegetation cover, A horizon depth, water infiltration, presence of soil macrofauna, and organic matter content were monitored. Management practices include the use of low-lying areas prone to flooding. Results observed after grazing cycles include: (1) reduction of soil compaction, due to the buffalo hoof's unique anatomy, which distributes weight more effectively and massages the soil rather than cutting it; (2) enhanced organic matter levels via excreta deposition and trampling-induced plant matter integration; (3) regrowth of native species.
Grazing with water buffalo under precision grazing management serves as a regeneration tool for degraded tropical soils. Their ethological behavior, adaptation to wetlands, and gait pattern allow them to break up compacted layers, activate soil biology, and increase vegetation cover, closing the cycle between meat production and sustainability. Experience suggests that the water buffalo is not only a suitable species for aquatic environments but also a key ally for regenerative livestock farming in the Humid Tropics.

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System Dynamics modelling of sustainability in a tropical buffalo dairy system in Colombia

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Tropical buffalo dairy systems face mounting sustainability challenges driven by the tight coupling between climate variability, forage dynamics, animal productivity and socioeconomic constraints. Static indicator frameworks (SAFA, TAPE), while widely adopted, fail to capture the systemic picture of feedback loops, delays and nonlinear dynamics that govern long-term system viability. This study develops, calibrates and validates a System Dynamics (SD) model for a 4,440-ha tropical buffalo dairy operation (Hacienda Cuba, Montelibano, Cordoba, Colombia) integrating 10 heterogeneous empirical datasets spanning 50 years of climate records, 13 years of weekly production data (n = 660), complete nutritional balances, financial statements and biodiversity inventories. The model comprises seven state variables organised in four coupled subsystems (Climate–Hydrology, Forage–Soil, Animal–Production, Economy–Social Capital) and generates a composite Synthetic Sustainability Index (SSI) integrating five weighted dimensions. Calibration (2020–2022) and independent validation (2023–2025) yielded MAPE = 8.3% and weekly RMSE = 11.4%. ANOVA revealed highly significant differences among management phases ($F = 67.19$; $p < 0.001$; $\eta^2 = 0.235$), and cross-correlation analysis identified a 4-month lagged production response to rainfall ($r = 0.503$). Five dominant feedback loops were identified (Precipitation → Soil; BO → Stocking Rate; ST → Soil Fertility; Market Price (PL) → Revenue; Supplementation → Costs) the compound overgrazing–fixed-cost trap (B1–B4) constitutes the primary structural risk. A structural paradox was uncovered: record financial performance (33.2% operating margin in 2025) coexists with declining biophysical sustainability, invisible to static audits. Sensitivity analysis demonstrated that forage parameters (NAR_max elasticity = 0.41; consumable fraction = 0.37) exert approximately twice the leverage of market price (0.19) on the SSI. Biodiversity functions as active systemic capital, preventing the SSI from falling below the collapse threshold. Scenario analysis (2025–2045) showed that only the sustainable management pathway (pasture rests ≥45 days with soil reinvestment) projects SSI > 0.50, while business-as-usual leads to system collapse by 2040. The SD approach provides an actionable, feedback-aware methodology for sustainability assessment in tropical buffalo dairy systems.

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Keywords: system dynamics; sustainability assessment; buffalo dairy; Bubalus bubalis; Vensim PLE; feedback loops; tropical livestock; Colombia

Comparative evaluation of *in vitro* methane production in buffaloes and beef cattle with tropical pastures

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Livestock farming is a cornerstone of Costa Rica's socioeconomic landscape, with cattle accounting for over 90% of the national herd. While the buffalo population has expanded exponentially in recent years, research regarding its role as a source of enteric methane remains limited, particularly when compared to the more extensive data available for cattle. To address this gap and the lack of comparative data between these species, this study examined the effect of including a legume (Cratylia argentea at 60 days of regrowth) on in vitro methane production of two tropical grasses: Urochloa decumbens (D, 60 days of regrowth) and a Urochloa hybrid known as Cayman (C, 30 days of regrowth). Inocula were sourced from three Brahman steers (IV) and two buffalo (IB). Treatments involved incubating each grass, both pure (D, C) and in a 20% legume mixture (D/C, C/C), with each inoculum type. All treatments were performed in triplicate using a factorial design, with methane production measured after 48 hours of incubation using Ankom RF equipment. (Currently, bromatological and secondary compound of the forages used are still under analyses.) Results indicated significant differences ($P<0.05$) in methane production between forage types, but no between animal species. When using IV, more methane was produced ($P<0.05$) with D (27.2±1.0 mg g^{-1} DM) than with C (18.7±0.5 mg g^{-1} DM). With D/C, the methane produced was lower ($P<0.05$) (27.1±1.0 mg g^{-1} DM) than with C/C (18.6±0.5 mg g^{-1} DM). With IB, D produced more methane ($P<0.05$) (26.1±1.0 mg g-1 DM) than with C (17.8±0.5 mg g-1 DM); D/C produced a greater amount ($P<0.05$) 27.7±1.0 mg g-1 DM than with C/C (19.9±0.5 mg g-1 DM). The study concludes that while methane production differs significantly among forage species, there is no significant difference between the animal species evaluated. Further research is recommended, incorporating a broader range of grasses and leguminous or non-leguminous forages currently used in Costa Rican bovine production systems.

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SWATH-MS-based proteome profiling of buffalo milk under heat stress

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Heat stress is a critical factor affecting the nutritional value of buffalo milk, particularly by modulating the protein production and profile. Advanced proteomic techniques such as Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH-MS) enable the simultaneous, accurate and reproducible quantification of thousands of proteins in a single run. This approach is especially effective for in-depth analysis of low-abundance protein fractions, allowing the identification of post-translational modifications and providing insight into protein interactions and dynamic expression changes. In this study, a SWATH-MS-based proteomic approach combined with chemometrics was used to explore the buffalo milk proteome and elucidate the molecular mechanisms underlying the response to heat stress. Milk samples were collected from nine buffaloes, equally distributed across three Temperature-Humidity Index (THI) classes (THI<72, THI 72–76, THI>76). Milk protein extracts (40 µg) were denatured, separated by 12% SDS-PAGE, and subjected to in-gel digestion prior to SWATH-MS analysis. The buffalo milk proteome database was processed using the MetaboAnalyst web platform, while Gene Ontology (GO) enrichment analysis was conducted with Metascape®. Comparative proteomic analysis identified 610 proteins, of which 79 were differentially abundant between THI<72 and THI 72–76, with 19 significantly enriched GO terms. Among these, the top 5 enriched terms were regulation of blood coagulation (GO:0030193), monocarboxylic acid metabolic process (GO:0032787), hexose biosynthetic process (GO:0019319), regulation of humoral immune response (GO:0002920), and regulation of immune effector process (GO:0002697). In the THI72–76 *vs* THI>76 comparison, 75 DAPs were identified, associated with 20 significantly enriched GO terms, mainly related to carboxylic acid metabolic process (GO:0019752), regulation of humoral immune response (GO:0002920), positive regulation of lipid storage (GO:0010884), and peptide metabolic process (GO:0006518). In contrast, only 29 DAPs differed between THI<72 and THI>76, with nucleotide metabolic process (GO:0009117) emerging as the most enriched pathway. Overall, the results highlighted dynamic changes in the buffalo milk proteome under heat stress conditions, affecting key metabolic and immune-related pathways and providing new insights into the molecular mechanisms underlying milk quality variations across different THI levels.

From Commodity to Premium
"Unblocking the Global Potential of Buffalo Milk"

Buffalo Milk is not a substitute for cows milk, but a superior category in its own right.
Its unlocking requires a conscious, collective strategy to reposition it globally, and its intrinsic value align perfectly with Premium Market Criteria.

• Nutritional Aspects – Naturally higher in fat, protein, solids & minerals than cow milk
• Culinary & Sensory Excellence – Buffalo Milk Products offer a distinct and richer sensory profile & creamier texture
• Health & Wellness Segment – Probiotic – rich, buffalo yogurt, lactose-reduced milk and protein for sports nutrition
• Building a Science Backed Health Narrative. Claims must be validated and communicated clearly
• Consumer Education – Demystifying buffalo milk for new audiences is crucial.
Tasting campaigns chef collaboration and clear point – of – Sale information are vital

Success Stories of Premium Buffalo Milk
1. Italy – Mozzarella di Buffalo Campana

2. India – AMUL has successfully introduced value – added products (Paneer, ghee, milk beverages) to mass market creating a trusted national brand.

3. China – Buffalo Milk is a premium product in China and is significantly more expensive than cow milk. Standard UHT Cow Milk Price Ranges from ¥ 10 - ¥20
($ 1.35 - $ 2.76) per litre whereas Buffalo Mik is sold for ¥ 30 - ¥ 50 ($ 4.14 - $ 6.90) per litre

• In USD terms, the niche premium status of Buffalo Milk in China is very clear.
Consumers are paying a significant premium for its perceived nutritional benefits

• Premium Buffalo milk in China is marketed for its richer taste, creamier texture and higher nutritional content.

• It is primarily produced in Specific Regions like Guangxi and Yunnan.

Mastitis in Mediterranean Buffaloes (Bubalus bubalis): Performance of the California Mastitis Test and Electrical Conductivity for Udder Health Assesment

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The observational prospective study assesses the clinical utility of the California Mastitis Test (CMT) and electrical conductivity (EC) for mastitis diagnosis in mediterranean buffaloes. A total of 600 quarters milk samples were aseptically collected from randomly selected, multiparous MBs. All samples were submitted for somatic cell count (SCC) and bacteriological milk culture (BMC), which served as the gold standard (GS), while the CMT and EC were recorded and performed on-farm after milking. Based on the GS results, quarters were classified as mastitis cases when an inflammatory response (SCC > 200 × 103 cells/mL) was associated with a bacteriological positivity with or without obvious clinical signs. Quarters without concurrent inflammatory response were classified as non-mastitis cases, regardless of bacteriological findings. For the validation both EC and CMT were compared with SCC as the benchmark test. Thereafter, CMT and EC were compared with the GS to assess their ability to correctly identify udder health status both independently and associated with BMC as potential alternatives to the use of SCC in field. Overall, 134 mastitis cases and 466 non-mastitis cases were detected. For the diagnostic validation (CMT and EC vs. SCC) the Cohen's Kappa revealed a significant n agreement of 0.51 and 0.27 for CMT and EC, respectively. An area under the ROC curve (AUC) of 0.97 for CMT and 0.75 for EC was calculated, with accuracy values of 0.76 for CMT and 0.73 for EC. Sensitivity (Se) was 0.97 for CMT and 0.45 for EC, while specificity (Sp) was 0.69 for CMT and 0.82 for EC. When both tests were compared with the GS (CMT and EC vs. GS), the Se was 0.97 for CMT and 0.46 for EC, while the Sp was 0.67 for CMT and 0.81 for EC, with accuracies of 0.74 for CMT and 0.73 for EC were detected. Finally, when both the tests were combined with BMC (CMT+BMC and EC+BMC vs. GS), The sensitivity was 0.97 for CMT and 0.46 for EC, while specificity was 0.74 for CMT and 0.89 for EC, with an accuracy of 0.79 for both tests were observed. The CMT demonstrated better performance as diagnostic tools for mastitis cases, with a higher AUC and sensitivity, as compared with EC. In contrast, the latter seems to be useful for confirming the absence of mastitis due to its high-er specificity. Neither test alone should be relied upon for definitive diagnosis; a combination with BMC seems to improve their diagnostic performance, but CMT consistently outperformed EC.

Semen parameters of a cloned swamp buffalo (Bubalus bubalis) bull produced from somatic cell nuclear transfer

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This study evaluated fresh and post-thaw semen parameters in a cloned swamp buffalo to validate its suitability for artificial insemination and genetic improvement programs. Following the successful cloning of a swamp buffalo from adult ear skin fibroblasts, fertility was assessed monthly over an eight-month period. A total of 8 ejaculates were collected from this cloned swamp buffalo via an artificial vagina and evaluated for volume, concentration, motility, viability (Eosin-Nigrosin), and morphology. High-quality ejaculates (n=8) were diluted in extender, cooled, and equilibrated at 4°C for 4 hours. The semen was then packaged into 0.25 mL straws, frozen in liquid nitrogen vapor, and stored at -196°C. For post-thaw analysis, straws were thawed at 37°C for 30 seconds. Spermatozoa were evaluated for motility using computer-assisted sperm analysis (CASA) and morphology. Functional integrity was further assessed through membrane integrity (Hypo-osmotic Swelling Test; HOST), viability (SYBR-14/PI staining), acrosome integrity (FITC-PNA staining), mitochondrial membrane potential (JC-1 staining), and DNA integrity (Acridine Orange staining). The cloned swamp buffalo exhibited high-quality fresh semen with a volume of 3.81 ± 1.32 mL, a concentration of 0.75 ± 0.43 × 10^9 spermatozoa/mL, 81.67 ± 0.44% motility, and 87.62 ± 4.49% viability (Eosin-Nigrosin); both normal head and tail morphology exceeded 94%. Post-thaw analysis via CASA demonstrated maintained motility at 50.66 ± 3.19%, although tail morphology and viability (SYBR-14/PI staining) decreased to 79.07 ± 18.36% and 58.57 ± 11.76%, respectively. Functional integrity assessments revealed membrane integrity (HOST) of 31.14 ± 10.71%, acrosomal integrity of 94.43 ± 3.41%, and mitochondrial membrane potential (JC-1) of 29.93 ± 10.78%. Notably, DNA integrity remained exceptionally stable at 99.93 ± 0.17%, confirming the high genomic stability of the cloned germplasm despite cryopreservation stress. In conclusion, this study demonstrates that the cloned swamp buffalo exhibits reproductive development within physiological reference ranges and produces high-quality semen. Although cryopreservation reduced motility and mitochondrial function, post-thaw parameters—specifically 50.66% motility and 99.93% DNA integrity—meet industry standards for artificial insemination. These results validate that somatic cell nuclear transfer successfully preserves the functional and genomic stability of elite germplasm, providing a viable tool for the genetic improvement of swamp buffalo.

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Advancing Buffalo Genomics: Evaluation of a Next-Gen High-Density SNP Chip for Biodiversity Studies

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The domestic buffalo (*Bubalus bubalis*) is categorized into two main types: the River Buffalo (*B. b. bubalis*, 2n = 50), mainly bred for milk production across regions extending from India to Europe, and the Swamp Buffalo (*B. b. carabanensis*, 2n = 48), traditionally used for draft work in eastern Asian countries. A new version of the Axiom Buffalo Genotyping Array (122,017 SNPs) has been developed to improve genetic gain through genomic selection in the Italian Mediterranean buffalo (IMB). This array is an advancement over the original version (~90k SNPs), initially designed and tested for biodiversity studies in buffaloes similar species. About 57% of markers of the new array came from the ~90k one due to their relevance for IMB traits. We evaluated the performance of this array across genetically divergent populations to assess its usefulness to characterize and safeguard the global buffalo genetic diversity. A biodiversity dataset (n=154) included river Buffaloes samples from Germany, Pakistan, Venezuela, Brazil, Bolivia and Philippines and swamp buffalo samples from Philippines, together with a representative IMB subset. Genotype calling for the complete set was conducted with the Axiom platform. Using only the minor allele frequency (MAF ≥ 0.05) criterion to assess polymorphism, a significantly high number of SNPs was identified: over 117,000 in river populations and more than 67,000 in swamp populations. While Affymetrix's stringent criteria selected a subset of high-quality polymorphic markers, amounting to over 107,000 for the river type and more than 21,000 for the swamp type. The complete dataset was subjected to a secondary quality control. Following standard filtering (call rate ≥95%; MAF ≥0.05) and the exclusion of heterozygosity-based outliers, the process yielded a final dataset of over 109k SNPs and 146 individuals for downstream analysis. These results confirm the overall high quality of the array also for biodiversity purpose. Principal Component analysis revealed a clear population structure: PC1 (10% explained variance) separated IMB and rivers from swamps, while PC2 (7.2% explained variance) distinguished IBM and German versus other river animals. Admixture analysis (K=6) showed homogeneous groups (IMB, swamp) and admixed populations (Bolivia and Brazil rivers). These findings highlight the enhanced robustness of the new array, confirming its value as a powerful tool for genomic characterization and conservation of global buffalo breeds.

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Microbiological and Oxidative Characterisation of Buffalo and Cow Dairy Products: correlation between Lactic acid bacteria, Lipid degradation and Electronic Nose Data

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Microbial activity, lipid stability, and aroma profile are key factors affecting dairy product quality. The aim of this study was to investigate the relationships between lactic acid bacteria activity, lipid degradation processes, and aroma profiles in dairy products from buffalo and cows fed a novel functionalized diet. In this regard, from Sep-2025 to Feb-2026, animals were alternately subjected on a monthly basis to a control (CTR) and an experimental (TRT) diet, containing high-quality protein sources and bioactive/nutritional compounds. Mozzarella, scamorza, and caciocavallo cheeses, obtained from functionalized and non-functionalized milk, were analyzed at different processing stages (milk, curd, cheese). Free fatty acids (FFA), lactic acid bacteria (LAB 30°C) and volatile profiles (E-nose, PEN3.5) were evaluated. Data were statistically analyzed using ANOVA, with significance declared at $p<0.05$. E-nose data were additionally analyzed by PCA employing a score plot model, and data interpretation focused on the most representative and responsive sensors: S1 (favorable aromatic compounds), S7 (sulfur-related compounds) and S8 (alcohols), selected for their potential association with lipid degradation (FFA) and microbial activity (LAB). The dietary treatment showed a more consistent and beneficial effect in buffalo dairy products, where it was associated with an improved lipid stability, as indicated by lower FFA levels observed in TRT samples compared to CTR, higher microbial activity, with an increased LAB populations (CFU/g) in TRT cheeses and a greater volatile profile, characterized by more elevated S1 responses and reduced S7 and S8 signals. These results may suggest improved lipid stability and cheese quality, as indicated by increased S1 responses. An opposite trend was observed in bovine products, where higher LAB counts (CFU/g) were detected in TRT curds but decreased in the final cheeses. This may indicate higher microbial activity during early processing stages, followed by a decline during cheese production. Consistently, TRT samples showed a decrease in S1 signals and an increase in S7 and S8 responses, suggesting a less favorable evolution of volatile profile, characterized by an increase in off-flavor–related compounds. Overall, the functionalized feed induced more favorable responses in buffalo than bovine dairy products, showing species-specific effects on microbial activity, lipid degradation and aroma development.

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Socioeconomic Impacts of In Situ Conservation of Thai Swamp Buffalo among Smallholder Farmers in Thailand

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The Buffalo Modern Farm (BMF) project was established in 2013 to integrate in situ conservation of Thai swamp buffalo with smallholder livelihood development. This study evaluated socioeconomic outcomes across 56 farms in eight provinces of northeastern Thailand during 2013–2025 using farm and household records, genetic evaluation data, and Social Return on Investment (SROI) analysis. In March 2025, participating farms maintained 1,097 buffaloes, averaging 18.91 head per farm. Records from 43 farms showed cumulative mean revenue of THB 494,124 from buffalo sales and THB 103,790 from manure per farm. Buffalo-related income contributed approximately 29% of household income. Locally produced forage and rice straw supported feed self-sufficiency, while pedigree and performance records enabled BLUP-based breeding value estimation and selection of five superior sires. Across six cohorts, an investment of THB 44.003 million generated assessed socioeconomic benefits of THB 143.586 million, with an overall SROI ratio of 1:3.26 (range 1:2.27–1:4.92). Benefits included diversified income, reduced input costs, improved farmer skills, stronger community networks, and conservation of indigenous genetic resources. Greenhouse gas emissions imposed environmental costs. The BMF model demonstrates how integrated breeding, farm resource management, and institutional support can link indigenous buffalo conservation with sustainable smallholder livelihoods.

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Use Of Progesterone Associated To The Ovsynch Protocol And Delay In The Application Of Pgf2α For Ovulation Synchronization And Tai In Buffaloes During The Nonbreeding Season

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The aim of this study were to compare the ovulation rate in buffaloes subjected to five variations of Ovsynch protocol and to verify the pregnancy rate between the two variations that result at the highest ovulation rates. For this purpose, 2 experiments were carried out. In Exp.1, 116 buffaloes were submitted to five variatins of Ovsynch protocol: Ovsynch (O, n=18), Ovsynch with delayed administration of PGF2α (O-PGFD9, n=19), Ovsynch plus injectable P4 (O-P4i, n=26), Ovsynch plus injectable P4 and delayed administration of PGF2α (O-P4i-PGFD9, n=26) and Ovsynch plus intravaginal P4 device (O-P4v, n=27). On Day 0 (D0), all buffaloes received intramuscular administration of GnRH (25µg im of Lecirelin), plus injectable P4 (140mg im of 7% P4) in O-P4i and O-P4i-PGFD9 or intravaginal of P4 device (0.5g of P4) in O-P4v. On D7, the buffaloes from O, O-P4i, and O-P4v received a dose of PGF2α (526µg im of Cloprostenol sodium), and the P4 was removed from that buffaloes in O-P4v. After two days (D9), all animals received GnRH (25µg im of Lecirelin) and females from O-PGFD9 and O-P4i-PGFD9 received a dose of PGF2α (526µg im of Cloprostenol sodium). Ultrasound examinations were performed on D0, D7, D9 and from D10 to D12 (12/12 hours for 60h) to verify the ovarian activity, the diameter of the ovulatory follicle and the ovulation rate. The observed ovulation rates were 44.4% to O, 31.6% to O-PGFD9, 61.5% to O-P4i, 53.8% to O-P4i-PGFD9 and 77.8% to O-P4v groups. For Exp.2, 249 buffaloes were divided according to age, ovarian status on D0, number of calvings, days postpartum and BCS into the O-P4i (n=121) and O-P4v (n=128) groups. The females were subjected to the same treatments that these two groups received in Exp.1 and additionally underwent TAI 16h after the second GnRH. Ultrasound examinations were performed as at Exp.1 and on D40 to perform the pregnancy diagnosis. The data was analyzed using the GLIMMIX procedure of SAS. There was no difference between O-P4i and O-P4v for the variables: number of follicles (18.8±0.8 and 19.5±0.9 mm; P=0.55) and diameter of the ovulatory follicle (13.0±0.3 and 13.2±0.2 mm; P=0.62). The pregnancy rate was greater in O-P4v (32.8%) than O-P4i (18.2%; P=0.006). It was concluded that the intravaginal P4 device associated to the Ovsynch protocol was more effective than the others variations to induce ovulation and better than the injectable P4 to promote pregnancy in buffaloes submitted to TAI during the nonbreeding season.

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Oxytocin Treatment Or Additional Dose Of Prostaglandin $F_{2\alpha}$ At Timed-Ai Failed To Increase Pregnancy In Buffaloes Synchronized During The Nonbreeding Season

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The aim of this study was to verify the effects of oxytocin treatment or additional dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at the moment of artificial insemination on the pregnancy of buffaloes submitted to TAI protocol. For this purpose, 3 experiments were carried out. In Exp.1, 192 lactating dairy buffaloes received an intravaginal P4 device (P4; 750 mg) plus estradiol benzoate (EB, 2.0 mg im) on Day 0 (D0). On D9, the P4 was removed, and buffaloes received D-cloprostenol ($PGF_{2\alpha}$, 150 µg im) plus FSH/LH (30 IU im). After 24h, the ovulation was induced using EB (1.0 mg im). On D12 (64h after P4 removal), buffaloes were then allocated to one of two groups: Control (C; saline solution administration; n = 96) or Oxytocin administration (OT; 50 IU im; n = 96), and all animals were submitted to TAI. In Exp.2, 374 buffalo heifers received on D0 the same treatment verified in Exp.1. On D9, the P4 was removed, and heifers received sodium cloprostenol ($PGF_{2\alpha}$, 0.53 mg im) plus eCG (400 IU im). After 24h, the ovulation was induced using EB (1.0 mg im). On D11 (56h after P4 removal), heifers were then allocated to one of two groups: Control (C; saline solution administration; n = 126), or $PGF_{2\alpha}$ administration (PGF; 0.53 mg im; n = 128). From this moment, heifers of both groups were submitted to TAI. In Exp.3, 434 lactating dairy buffaloes received on D0 the same treatment verified in Exp.1. On D9, the P4 was removed, and buffaloes received $PGF_{2\alpha}$ (0.53 mg im), eCG (400 IU im) and estradiol cypionate (1.0 mg im). On D11 (56h after P4 removal), buffaloes were then allocated to one of two groups: C (saline solution; n = 150), or PGF (0.53 mg im; n = 149), and all animals were submitted to TAI. Ultrasound examinations were performed on D0 to ascertain ovarian follicular status, on D9 to measure the largest follicle (LF), at TAI to establish the diameter of LF and verify early ovulation (animals with LF ≤ 9 mm or early ovulation were excluded from the study), and on D42 to perform the pregnancy diagnosis. The data was analyzed using the GLIMMIX procedure of SAS. No effect of treatment was observed for pregnancy/TAI in Exp.1 (C = 42.7 % and OT = 36.5 %; P = 0.36), Exp.2 (C = 37.3 % and PGF = 46.9 %; P = 0.11) and Exp.3 (C = 27.7 % and PGF = 26.2 %; P = 0.79). It was concluded that neither oxytocin treatment nor additional dose of $PGF_{2\alpha}$ at TAI were effective to increase pregnancy in buffaloes synchronized during the nonbreeding season.

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The effect of not milking at the end of lactation on weaning weight and the economy of a buffalo herd

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Economic sustainability on livestock farms can be achieved through animal management strategies that provides provides an insight into animal performance and its impact on profitability. In dual-purpose buffalo, meat production in breeding systems is assessed based on weaning weight. This study aims to evaluate the effect of suspending milking at one month before the end of lactation period on weaning weight (WW) in a buffalo herd. The study was conducted in 2024 in Puerto Boyacá, Colombia, using WW records from 71 animals of both sexes, aged between 7 and 8 months, that were divided in two groups: treatment (no milking during the last month before weaning) and control (conventional milking). WW weight and maternal milk production were recorded, and average daily gain (ADG) were calculated. Descriptive statistics were applied, comparisons between groups were made using ANOVA/t-tests, linear regression models were performed in R, and correlation analyses were conducted between milk production and growth variables. WW was 149.69 ± 26 kg in the non-milked group and 155.67 ± 23 kg in the control group, with no significant differences ($p = 0.386$). No differences were observed in ADG either ($p = 0.848$). In the non-milking group, the mother's milk production was not significantly related to WW ($p=0.15$). The difference in kilograms between the groups represents $59,800 COP, while the unsold milk represents $405,500 COP. This indicates that greater milk availability promotes growth, though not necessarily final weight. Although the differences were not significant, the additional money that can be received for increases in meat weight is considerably less than what could have been received from the sale of buffalo milk. Milk production influences ADG but does not determine WW. Continuing to milk remains a viable production strategy; suspending milking resulted in a significant drop in income for the system. Management decisions aimed at improving biological parameters do not necessarily lead to greater profitability for the production system.

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Impact of robotic milking on feeding behaviour of Italian Mediterranean dairy buffaloes

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Automatic milking systems (AMS) allow animals to be milked according to individual voluntary schedules, potentially improving efficiency, productivity, and animal welfare. While AMS are widely adopted in dairy cattle, their application in Mediterranean buffaloes remains limited, and virtually nothing is known about their effects on feeding behaviour. This study aimed to compare behavioural patterns, feed intake, and feed sorting between buffaloes managed under AMS and conventional milking systems (CMS). The study was conducted on a commercial buffalo farm in South Lazio (Italy) during 2024-2025. Two groups of multiparous lactating buffaloes were monitored: AMS (n=33, 118±68 DIM, 1.07 parity) and CMS (n=24, 152±70 DIM, 1.44 parity). Animals were housed under identical environmental conditions, fed the same total mixed ration (TMR), and differed only in milking system (MS). The CMS group was milked twice daily in a tandem milking parlour, whereas the AMS group had free access to voluntary milking with a minimum interval of 8h between milkings. All animals were equipped with ear tags sensor (DeLaval, Sweden) to record individual eating time (ET) and rumination time (RT). Group feed intake (FI) was estimated as the difference between feed offered and refusals. Feed sorting behaviour was assessed through repeated sampling of TMR and refusals across 24 monitoring days. TMR, refusal, FI and sorting data were analysed at group level using a linear mixed-effects model with MS and years as fixed effect. Sensor data were analysed at individual animal level using linear mixed-effects models with MS, years and parity as fixed effect, DIM as covariate, to control for lactation stage and parity imbalance, and animal as a random effect. Although diets were identical ($P>0.05$) and overall intake was similar, refusals differed between groups: AMS group showed greater feed sorting, tended to consume a higher proportion of fine particles and starch ($P<0.05$) and to leave coarser, more fibrous fractions. Sensor data indicated significantly higher ET in AMS compared to CMS group (377.08 vs 312.10 min/d; $P<0.01$), while RT did not differ between groups (386.03 vs 368.97 min/d; $P>0.05$).
In conclusion, AMS management appears to affect behavioural patterns in buffaloes compared with CMS, as shown by increased feed sorting and longer ET without changes in RT. The increase in ET may partly reflect repeated visits to the milking robot and associated feed-seeking behaviour.

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Genomic Selection: New Opportunities for the Italian Mediterranean Buffalo breeding

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The Italian Mediterranean Buffalo (IMB) is a breed of high economic and cultural relevance, whose genetic improvement has historically been based on production traits. Until 2022, genetic indices were estimated using BLUP methodology. Since 2023, the IMB has implemented single-step genomic BLUP (ssGBLUP), marking a turning point in the breed's selection strategy. Currently, approximately 7,300 genotypes are available and routinely used for genomic evaluation of the IMB. One of the main advantages of genomic selection is the substantial increase in prediction accuracy, rising from 0.29 (BLUP) to 0.60 (ssGBLUP) in young animals. This gain allows GEBV to be obtained before phenotypic records are available, drastically reducing generation interval and accelerating annual genetic progress. Genomic tools enabled the evaluation of innovative characters related to animal welfare and environmental sustainability. For example, feed efficiency (FA), defined as the ratio of milk yield to dry matter intake, showed a moderate-to-high heritability ($h^2 = 0.34$), with accuracy increasing from 0.55 (BLUP) to 0.80 (ssGBLUP), confirming that genomic selection for this trait is effective. Since FA is correlated with methane emissions, an indirect EBV for CH_4 was derived, revealing a favorable indirect selection trend over time. The IMB is the only breed and species in Italy to have genetic evaluations for health traits (brucellosis, tuberculosis, and paratuberculosis), a unique achievement among buffalo populations worldwide. Regarding udder health, somatic cell scores genomic accuracy increased by more than 50% compared to BLUP, with a declining genetic trend confirming effective selection for lower somatic cell counts, resulting in improved udder health and higher milk quality. Finally, the ssGWAS approach was applied to the most frequent defect in the IMB, extremely open nails (EON). The largest proportion of genetic variance was explained by SNP windows located on chromosomes 2 and 3, with 72 informative windows explaining 55.73% of the observed genetic variance. These results confirm a clear genetic component underlying EON, and the identification of these regions represents a valuable tool for the early detection of carrier animals before the defect manifests phenotypically. These results demonstrate that genomic selection is a powerful tool for the IMB, enabling simultaneous improvement of productivity, efficiency, health, and environmental sustainability.

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Education and Development of Emerging Profiles with Relevant Artificial Intelligence Skills forthe Improvement of the Buffalo Dairy Supply Chain (ESPERIA – ACADEMY)

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ESPERIA - ACADEMY (Education and Development of Emerging Profiles with Relevant Artificial Intelligence Skills for the Improvement of the Buffalo Dairy Supply Chain) is an innovative educational initiative designed to address the growing demand for digital and technological skills in the buffalo dairy sector, supporting its transition toward sustainable, data-driven, and resilient production systems. The project integrates Artificial Intelligence (AI), Internet of Things (IoT), advanced sensing technologies, Machine Learning, Computer Vision, Precision Livestock Farming (PLF), and sustainability into vocational education and professional training. The educational framework was developed through a participatory needs assessment involving farmers, veterinarians, technicians, and other stakeholders across the buffalo dairy value chain, ensuring alignment with current industry needs. All participants first attend the Elements of AI for Business programme, providing a common foundation in Artificial Intelligence before progressing to six specialized training pathways: Next Gen Farmer, Advanced Digital Veterinary Clinician, Agro-Energy & Green Innovation Manager, Smart Barn Operator, Smart Livestock Technician, and Digital Dairy Producer 5.0. The programmes combine theoretical instruction with practical activities in farms and dairy plants, enabling participants to acquire competencies directly applicable to production environments. The Academy targets professionals already employed in the sector as well as graduates, unemployed individuals, and career changers seeking opportunities in an increasingly technology-oriented agri-food industry. Implemented in the Campania region, where approximately 90% of Italy's PDO buffalo mozzarella is produced, the initiative promotes collaboration among universities, industry partners, local institutions, and professional organizations to facilitate knowledge transfer and innovation. The project aims to train 180 participants, certify their digital competencies, and strengthen the alignment between education and labour market needs through direct engagement with companies across the buffalo dairy value chain. By integrating engineering, Artificial Intelligence, veterinary medicine, and sustainable livestock management, ESPERIA represents a scalable model for workforce development, contributing to the digital transition, competitiveness, and long-term sustainability of the livestock sector.

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The Identity of the Thai Buffalo and the Design of Khid Fabric Patterns for Contemporary Product Development: A Case Study of the Ban Sa Kaeo Enterprise Group, Ban Dung Subdistrict, Udon Thani Province

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The Thai buffalo is an important cultural symbol deeply embedded in Thailand's agrarian heritage, representing local wisdom, identity, and the socio-cultural relationship between communities and their environment. This study aimed to examine the integration of Thai buffalo identity into contemporary Khid fabric pattern design and to develop culturally distinctive products that support community-based enterprise development. The research was guided by the concepts of cultural identity and the creative economy.

A qualitative case study approach was employed with the Ban Sa Kaeo Enterprise Group in Ban Dung Subdistrict,Udon Thani Province. Data were collected through field observations, in-depth interviews with 40 key informants from 20 districts across Udon Thani Province, participatory design workshops involving 40 community participants, and visual analysis of traditional Khid fabric motifs. Informants were selected through purposive sampling based on their expertise in local culture, textile production, buffalo-related traditions, and community enterprise activities. Qualitative data were analyzed using thematic analysis, while visual data were interpreted through cultural symbolism and design analysis frameworks.

The findings revealed that buffalo-inspired elements, including horn geometry, hoof patterns, body silhouettes, and representations of agricultural life, were successfully translated into contemporary Khid fabric motifs. Through the participatory design process, three prototype patterns were selected by community participants as the most suitable representations of local identity and cultural value. The selected designs were further adapted for application in community textile products and cultural souvenirs. Evaluation results showed that participants expressed the highest level of satisfaction with the developed patterns ($\bar{x}$ = 4.72/5.00),particularly regarding cultural authenticity, aesthetic quality, uniqueness, and marketability. The integration of buffalo symbolism enhanced product differentiation and strengthened cultural branding by communicating values associated with resilience, diligence, and the agricultural heritage of the Thai buffalo.

The study demonstrates that culturally grounded design innovation can effectively link heritage preservation with creative economic development. The transformation of buffalo identity into contemporary Khid fabric patterns contributes to the transmission of local knowledge across generations while increasing the cultural and commercial value of community products. The Ban Sa Kaeo case provides empirical evidence that cultural symbols can be transformed into tangible design assets that support both the sustainability of intangible cultural heritage and long-term community economic resilience.

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Milkability in Mediterranean Italian Buffalo in comparison with other Ruminants

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Milkability is an important phenotypic trait that defines an animal's ability to secrete milk rapidly, completely and regularly from the mammary gland in response to proper milking techniques. In all the Mammalians, this process is strongly influenced by the anatomy and the physiology of the mammary gland, specific to the species. In a field study, we investigated the relationship between milkability patterns and milk yield in Mediterranean Italian buffaloes. The study included 2,870 Mediterranean Italian buffaloes from 89 farms, all of which were milked using a conventional milking machine. Additionally, 24 buffaloes from one farm were milked using an automatic milking system (AMS; Lely Astronaut™, Lely, the Netherlands). Buffaloes had an average milk yield of 3.94 kg per milking, with a peak flow of 1.24 kg/min, and exhibited a long total milking time (10.25 minutes). The extreme variability in lag time (2.22 ± 2.31 minutes) and the incidence of bimodality (13.3%) indicate inadequate pre-stimulation. Automatic milking systems (AMS) are less commonly used in buffalo farming; in this experience 20% of milk emission curves exhibit incomplete milking, with an average milk yield of 2.8 kg per milking, at an average frequency of 2.7 times per day. When we compared the main milkability traits of Mediterranean Italian buffaloes with those of other dairy species, we found that dairy buffaloes had the longest total milking time (10.25 minutes), much longer than that of dairy cattle (7.2 minutes), while goats and sheep had shorter times, ranging from 1.98 to 1.38 minutes. The interspecific differences in milk flows parameters highlight the need to adapt mechanical machine settings and pre-stimulation protocols for each species to optimize milking efficiency and animal welfare.

Bridging Tradition and Welfare: Opportunities for Cow-Calf Contact (CCC) via Maternal and Foster Rearing in Italian Water Buffalo Farming

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The early separation of calves from their mothers is a long-standing conventional practice in Italian Water Buffalo farming. However, this method is increasingly being questioned as social sustainability and animal welfare become central topics of discussion among consumers. Modern consumers are increasingly questioning the 'naturalness' of systems that prevent maternal bonding and restrict innate behaviours. This is driven by a growing demand for livestock practices that prioritize animal welfare.
In response to these concerns, the TransformDairyNet (TDN) project brings together a multi-actor network of 26 European partners from 14 countries, including farmers, veterinarians, researchers and policymakers, to promote and disseminate dairy systems that allow cow-calf contact (CCC). This paper explores opportunities for the Italian buffalo livestock to implement CCC using either biological mothers or foster cows (nurse cows). While maternal rearing fosters the primary biological bond, foster cow systems offer a versatile alternative that can simplify milking parlour management while still providing calves with the essential social and physical contact they require.
Through a SWOT analysis comparing various CCC methods with traditional calf rearing techniques, we highlight the trade-offs between improved calf development and the economic sustainability of the farm. Furthermore, the study investigates stakeholder awareness and propensity to adopt these practices through targeted questionnaires. Ultimately, the paper discusses how specialized certifications could provide added value for farmers, turning the adoption of maternal or foster rearing into a competitive advantage for the Italian buffalo dairy chain.

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Functional analysis of the IGF-system in buffalo milk

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Milk contains factors that control growth and development of offspring, including insulin-like growth factors (IGF1 and IGF2). The bioactivity of these two peptide hormones is regulated by IGF-binding proteins (IGFBP-1 to -6) and IGFBP proteases, both of which are also present in milk. All compounds of this growth-regulatory IGF-system can be secreted in a time- and development-dependent manner. In buffalo milk, the IGF system has not yet been studied in detail.

A longitudinal study was conducted using milk from eight buffalo cows. Colostrum was collected at parturition, followed by two early milk samples on days 1 and 2 after birth. Four samples of mature milk were collected on days 46 and 155 days after concenption and on days 7 and 30 after birth. Caseins were removed by acidic precipitation, obtaining whey for further analysis. IGFs and IGFBPs were quantified using specific ELISA and Western ligand blotting assays, respectively. IGF-related bioactivity was determined by BIRA assays, while proteolytic activity was assessed by IGFBP-protease assays.

The concentrations of IGF1 and IGF2 in whey were highest in the colostrum samples. In addition, higher concentrations of all IGFBPs were found in colostral whey and early milk than in whey prepared from mature milk. IGFBP-2 to -5 were detected in whey, with IGFBP-3 being the dominant form among all IGFBPs. IGF-related bioactivity was lowest at birth and highest in the whey of early milk two days after parturition. Proteolysis of IGFBPs could be detected in whey samples taken 1, 2, or 7 days after birth.

Our results confirm previously published findings of higher levels of IGFs and IGFBPs in colostrum and early milk from dairy cows, now also in buffalo. However, although IGF concentrations peaked at parturition, IGF-related bioactivity was reduced in colostral whey. In the absence of IGFBP proteolysis in colostrum, we hypothesize that the reduced bioactivity is due to stabilized IGF/IGFBP complexes in buffalo colostral whey. This conclusion is supported by the observed increase in IGF-related bioactivity in response to IGFBP proteolysis shortly after birth. Our results suggest that the activation of IGF-related bioactivity in buffalo milk soon after birth is associated with the induction of IGFBP proteolysis at the same time, which may provide a maternal mechanism for the activation of the IGF-system in the buffalo calf´s intestinal tract.

Comparative analysis of thyroid regulation in Bubalus bubalis and Bos taurus

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Water buffaloes and cattle differ in growth and energy metabolism, processes regulated in part by the hypothalamic–pituitary–thyroid (HPT) axis. As buffaloes exhibit lower serum selenium concentrations and selenium is essential for thyroid hormone activation, this study aimed to compare serum selenium and thyroid hormone concentrations, as well as thyroid morphology and thyroidal expression of genes involved in thyroid hormone metabolism, between the two species.

Serum concentrations of selenium and thyroid hormones (T3, T4) were determined in 25 buffaloes (10 cows, 15 bulls; mean age 3.25 ± 0.8 years; mainly pasture-raised) and 21 cattle (15 cows, 6 bulls; mean age 3.93 ± 2.6 years; mainly housed indoors). Thyroid sections from 11 cattle and 7 buffaloes were evaluated histologically for colloid follicle diameter, and thyroidal expression of Dio1, Dio2 and Slc16a2 was analyzed by qPCR (n = 15/species). Species differences were assessed using Welch's t-tests, while species and sex effects on serum hormone concentrations were analyzed by two-way ANOVA.

Serum selenium concentrations were significantly lower in buffaloes (-35%; $p < 0.001$). T3, T4, and the T3/T4 ratio did not differ between species overall; sex-specific differences in thyroid hormone levels (T3 and T4) were observed exclusively in cattle ($p < 0.05$ and $p < 0.01$). Female cattle exhibited lower T4 concentrations ($p < 0.05$) and comparable T3 concentrations, but tended to show higher T3/T4 ratios ($p = 0.06$) than female buffaloes. Colloid follicles in buffalo thyroid glands displayed larger mean diameters (289.1 ± 196.8 µm; $p < 0.0001$) than those of cattle (181.4 ± 127.6 µm). Small follicles (diameter ≤ 100 µm), which are generally associated with higher follicular activity, were detected in all 11 cattle thyroid samples (15.5% of all measured follicles), but only in 3 of 7 buffalo thyroid samples (1.6%). Buffaloes showed higher Dio1 ($p < 0.001$) and Dio2 mRNA abundance ($p < 0.01$), whereas Slc16a2 mRNA expression was higher in cattle ($p < 0.05$).

Overall, systemic thyroid hormone concentrations were largely comparable between buffaloes and cattle despite lower serum selenium concentrations in buffaloes. However, differences in follicle morphology and thyroidal expression of Dio1, Dio2 and Slc16a2 indicate species-specific differences in thyroid hormone metabolism and regulation. Further studies are required to clarify the functional significance of these findings and their relationship to selenium status.

The use of lipid indices to assess the nutritional quality of Italian Mediterranean Buffalo and Friesian meat

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Lipids are key components of meat that shape its nutritional value by providing essential fatty acids with beneficial roles in human metabolism, inflammation regulation, and cognitive function. Although some fatty acids are beneficial to human health, excessive intake of saturated fatty acids may increase cardiovascular risk. In Italy, buffalo meat consumption remains relatively low compared with beef, mainly because it is considered a niche product and its lipid-related nutritional qualities are not widely known. This study aimed to compare the nutritional quality of lipid in meat from Italian Mediterranean Buffalo (IMB) and Friesian (F) bulls by using health-related nutritional ratios and indices: Polyunsaturated/Saturated Fatty Acids (PUFA/SFA), Linoleic Acid/α-Linolenic Acid (*LA/ALA), and* Hypocholesterolemic/Hypercholesterolemic (*h/H) ratio*, Atherogenic (AI), Thrombogenic (TI), and Nutrition Value (NVI) indices. Twenty *longissimus thoracis* samples (10 IMB, 10 F) were obtained from local farmers. The fatty acid methyl esters were quantified by GC-FID. The one-way ANOVA showed that IMB meat had a lower total fatty acid (FA) content (1408.70 *vs* 2028.28 mg/100g; $P<0.05$) and a more favourable lipid profile across most of the indices. The only exception was the LA/ALA ratio, which was higher, and thus less favourable, in IMB meat (31.46 *vs* 22.07; $P<0.05$). The IMB showed a higher h/H ratio compared to F meat (1.98 *vs* 1.72; $P<0.05$): values above 2 are preferred as they indicate a better nutritional quality, due to higher content of specific FA that helps to reduce plasma cholesterol and lower the risk of cardiovascular diseases. Moreover, IMB meat obtained a significantly higher NVI (2.39 *vs* 2.13; $P<0.05$), indicating a better FA composition. No significant differences were highlighted comparing the IMB and F meat for the PUFA/SFA ratio (0.24 *vs* 0.19), AI (0.56 *vs* 0.61) and TI (1.58 *vs* 1.54) indices. There are no recommended values for TI and AI, although lower values indicate a more beneficial FA profile in terms of human health. The results suggest that IMB meat can be a viable alternative to F meat, with a comparable or even better lipid nutritional quality. These indices are proxies based on the FA composition, but the real health impact should be considered in light of portion size, the overall dietary pattern, and meat cooking method.

Reference

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Cases of abortion related to *Chlamydia pecorum* in Mediterranean water buffalo (*Bubalus bubalis*) from central Italy

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Abortions in Mediterranean dairy buffaloes (*Bubalus bubalis*) represent a significant economic and reproductive concern in intensive farming systems. Among infectious agents, *Chlamydia pecorum* has emerged as a potential cause of reproductive disorders in ruminants; *however* its epidemiological and pathogenic role remains poorly documented, and the infection is likely underdiagnosed.
This report describes late-term abortion cases (n=7) that occurred *between October 2023 and December 2025* in Mediterranean dairy buffalo herds (n=3) located in *the Lazio region* (central Italy), in which *C. pecorum* infection was *identified* and other major abortifacient pathogens were excluded.
Affected animals did not show significant systemic clinical signs prior to abortion. In some cases, mild multifocal necrotizing placentitis and retained fetal membranes were observed. Gross examination of aborted fetuses revealed nonspecific lesions, including mild autolysis and occasional serosanguineous effusions. A Chlamydia-genus-specific PCR was performed on placental and fetal tissues, and subsequent sequencing of the PCR amplicons allowed species-level identification as *Chlamydia pecorum*. Other major abortifacient pathogens, including *Brucella* spp., *Coxiella burnetii*, *Leptospira* spp., *Neospora caninum*, *Toxoplasma gondii*, *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., and *Bovine Viral Diarrhea Virus*, were excluded through bacteriological culture and molecular testing.
These findings support the involvement of *C. pecorum* as a causative agent of abortion in Mediterranean dairy buffaloes and highlight the importance of including chlamydial infections in *the differential diagnosis* of reproductive problems in buffalo herds.
Further epidemiological investigations, including seroprevalence studies, will be important to better understand infection dynamics and potential herd-level impact. The implementation of strengthened biosecurity measures, systematic reproductive monitoring, and routine molecular diagnostic screening is recommended to reduce economic losses and improve herd health management.

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Diversification and its economic impact on dual-purpose livestock farming with Bubalus bubalis in the low tropics

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By demonstrating diversification as a differentiating factor in the economic performance of farms located in flood-prone areas but with different soil conditions, their current records were used as a basis for simulation (projection) based on their respective herd movements. To evaluate the aforementioned economic impact, Business Valuation indicators were used, employing the Discounted Cash Flow Method. For this purpose, two buffalo farms located in the tropical lowlands of Venezuela were compared: a conventional dual-purpose farm and a dual-purpose farm diversified in agroecological terms with added value to production and in the process of full diversification (including other sectors). The first is located on one of the most fertile soils in Venezuela, with a carrying capacity of 1.5 animal units per hectare (587 ha), situated at kilometer 15 of the Pan-American Highway, near El Vigía; The second farm was located on soils considered precarious, with a carrying capacity of 0.5 animal units per hectare (282 ha), along the San Fernando de Apure highway via San Juan de Payara, Venezuela. Both production units were analyzed using an econometric model (simulator) based on a United Nations (UN) methodology implemented in Excel. This model extracted various reproductive, productive, and financial variables from the farms' statistical records and analyzed their relationships to generate first-order indicators. Both farms had the same minimum investment of USD 56,077; identical financial and economic conditions, including 13% annual interest, the same payment terms, a price of USD 2 per kilogram of live weight, and identical wages and salaries. Both farms had an 80% birth rate and identical mortality rates. Both farms raised the same breed: Murrah cattle. Thanks to diversification, on the farm with low fertility soils, from the sixth projected year onwards, a net present value (NPV), an internal rate of return (IRR), a benefit-cost ratio (B/C) and investment recovery periods (IRP) similar to those of the farm located in the fertile zone were achieved.

Keywords: livestock profitability, livestock agroecosystems, sustainability in livestock farming, conservation of tropical ecosystems.

Occurrence of Aflatoxin M_1 in bulk buffalo Milk: A Retrospective Analysis of Surveillance Data

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Aflatoxins are mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* that frequently contaminate agricultural commodities such as cereals, grains and silage. When lactating animals consume feed contaminated with aflatoxin B1, the toxin is metabolized into aflatoxin M1 (AFM1), which is subsequently excreted in the milk. Due to its high thermal stability, AFM1 remains unaffected by standard dairy processing posing a persistent risk to human health through the consumption of final dairy products. In the European Union, Commission Regulation 915/2023 enforces a strict maximum level for AFM1 in milk at 0.050 µg/kg. For that, continuous surveillance is crucial for maintaining food safety standards.

The aim of this study was to assess AFM1 presence in bulk buffalo milk in the Lazio region, Italy. A retrospective study was performed on 8,744 samples collected from 300 farms during routine surveillance activities from 2021 to 2025. AFM1 levels were screened using an enzyme-linked immunosorbent assay (ELISA), with a limit of quantification (LOQ) of 0.010 µg/kg.

Results showed that AFM1 concentration in buffalo milk samples ranged from below the detection limit (<0.010 µg/kg) to 0.61 µg/kg, with the majority of samples (99.5%, n = 8,700) were compliant (< 0.05 µg/kg) and in 71 (0.81%) of these, the AFM1 concentration was within the alert range (0.03–0.05 µg/kg), representing borderline cases. Only 0.50% of the samples (n=44) from 39 farms were found to be non-compliant, exceeding the legal limit of 0.05 µg/kg.

Moreover, statistical analysis revealed a significant seasonal influence on aflatoxin occurrence ($chi2 = 11.47$, $p < 0.01$). The highest positivity rate was observed during the winter months (n=19, 0.87%) and autumn (n=13, 0.59%), which were more than three times higher than those observed in summer (n=4, 0.19%) and spring (n=8, 0.36%). AFM1levels in milk could be higher in winter and autumn, because animals are fed more stored feed instead of fresh pasture. Although *Aspergillus* grows in warm, humid conditions, contaminated silage and grains kept for long periods can increase exposure during colder seasons.

These finding results indicate a low level of AFM1 contamination in buffalo milk in the studied region compared to previously reported data in Southern Italy 7.2% and Turkey 4%. This may be due to the climatic conditions in this region, characterized by moderate temperatures and sufficient rainfall, which limit the growth of *Aspergillus* species.

Concurrent Eosinophilia, Basophilia, and Regenerative Anemia: A Characteristic Hematological Pattern in a Cohort of Thai Swamp Buffaloes

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Hematological survey data from Thai swamp buffalo (*Bubalus bubalis*, 2n=48) under traditional smallholder management are scarce, limiting understanding of subclinical disease burden in this population. This study described the complete blood count (CBC) profile and characterized hematological abnormalities in a cohort of smallholder-owned Thai swamp buffaloes enrolled in a provincial reproductive program in Northeast Thailand.

CBC was performed in 130 Thai swamp buffalo cows from Nong Han (n=75) and Thung Fon (n=55) districts using an automated hematology analyzer. Animals were maintained under traditional extensive management: communal pasture grazing, no concentrate supplementation, and irregular anthelmintic treatment. Prophylactic anthelmintic treatment was administered at enrollment. Parameters including erythrocyte indices, leukocyte differential, NRBC%, and platelet count were interpreted against published Thai swamp buffalo reference intervals.

Mean values were: WBC 7.09×10^9/L (range 3.6–11.5), HGB 10.99 g/dL (range 6.7–17.8), HCT 32.4% (range 20.3–55.1), MCV 60.8 fL, and PLT 184.9×10^9/L (range 43–463). HGB below reference was detected in 70 animals (53.8%). Anemia (HCT <30%) was present in 49 animals (37.7%): mild in 36 (27.7%) and moderate in 13 (10.0%), nadir HCT 20.3%/HGB 6.7 g/dL. Normocytic anemia predominated (87.8%), consistent with anemia of chronic disease; microcytic anemia was identified in 10.2%. Elevated NRBC% (>5%) confirmed regenerative response in 4 animals. Eosinophilia (EOS% >10%) was identified in 7 animals (5.4%), with values of 37.1%, 26.1%, and 22.0%, co-occurring with anemia and leukopenia, suggesting tissue-invasive helminthiasis (*Fasciola gigantica*, *Haemonchus contortus*). Neutrophilia (NEU% >81%) was detected in 23 animals (17.7%) and thrombocytopenia (PLT $<90\times10^9$/L) in 13 (10.0%), the most severe at 43×10^9/L, consistent with tick-borne hemoprotozoa. Overall, 37.7% exhibited simultaneous abnormalities in four or more CBC parameters.

Thai swamp buffaloes under traditional smallholder management carry a substantially higher hematological disease burden than recognized, characterized by concurrent normocytic anemia, eosinophilia, and thrombocytopenia. These findings provide the first large-scale descriptive hematological dataset for this breed under field conditions and underscore the need for systematic CBC screening prior to reproductive interventions.

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Hematological Indicators of Systemic Inflammation and Anemia in Thai Swamp Buffaloes: Insights into Leukocyte and Platelet Dysregulation

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Leukocyte differential and platelet data from Thai swamp buffalo (*Bubalus bubalis*, 2n=48) under traditional smallholder management are absent from the literature. This study characterized the leukocyte profile, identified inflammatory leukogram patterns, and described platelet dysregulation in a cohort of smallholder-owned Thai swamp buffaloes enrolled in a provincial reproductive program in Northeast Thailand.

CBC including full leukocyte differential and platelet indices was performed in 130 Thai swamp buffalo cows from Nong Han (n=75) and Thung Fon (n=55) districts. Animals were maintained under traditional extensive management: communal pasture grazing, no concentrate supplementation, irregular to absent anthelmintic treatment, and no routine vaccination. Leukocyte differential (NEU%, LYM%, MON%, EOS%, BASO%) and platelet parameters (PLT, MPV) were interpreted against published Thai swamp buffalo reference intervals.

Mean WBC was 7.09×10^9/L (range 3.6–11.5), within reference at the population level but masking individual dysregulation. Neutrophilia (NEU% >81%, maximum 88.5%) was detected in 23 animals (17.7%); in 14, it co-occurred with lymphopenia (LYM% <12%, minimum 3.2%), forming a classic inflammatory leukogram consistent with HPA axis activation or subclinical infection. Leukopenia (WBC $<5.0\times10^9$/L) was identified in 14 animals (10.8%). Lymphocytosis (LYM% >33%) was present in 17 animals (13.1%), reflecting chronic antigenic stimulation. Eosinophilia (EOS% >10%) affected 7 animals (5.4%), with extreme values of 37.1%, 26.1%, and 22.0%; the most severe co-occurred with leukopenia and normocytic anemia, a triad pathognomonic of tissue-invasive helminthiasis (*Fasciola gigantica*, *Haemonchus contortus*), persisting despite pre-enrollment anthelmintic treatment. Thrombocytopenia (PLT $<90\times10^9$/L) was identified in 13 animals (10.0%), the most severe at 43 and 50×10^9/L, consistent with tick-borne hemoprotozoa (*Anaplasma*, *Theileria* spp.). Overall, 37.7% exhibited simultaneous abnormalities in four or more parameters.

This survey provides the first large-scale leukocyte and platelet dataset for Thai swamp buffalo under smallholder conditions, revealing that neutrophilia-lymphopenia inflammatory leukogram, eosinophilia, and thrombocytopenia are prevalent and frequently concurrent, identifying a high-risk phenotype with direct implications for reproductive efficiency and pre-breeding health protocols.

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Buffalo Farming and Dairy Innovation Transfer: An Italian-Cuban Cooperation Framework for Sustainable Local Food Systems

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Food security in urban and peri-urban settings across developing nations remains one of the most pressing challenges on the international agenda. In this context, technical and scientific cooperation between advanced research institutions and local actors constitutes a privileged instrument for fostering resilient, inclusive, and sustainable food systems. This paper presents the preliminary activities of the sub-project "BUFFALO & Co. – Transfer of Technical Innovations for Improving Buffalo Farming and Dairy Processing of Buffalo and Other Species' Milk", developed within the framework of the Italian Agency for Development Cooperation (AICS) Initiative AID 12935, entitled "Productive Diversification and Strengthening of the Local Food System in the Municipality of Guanabacoa", Cuba. Organised in conjunction with the Cuban partner institution ACTAF (Asociación Cubana de Técnicos Agrícolas y Forestales) and financed by Italian cooperation, the project is structured around two overarching outcomes: the reinforcement of technical competencies in buffalo management and milking practices, and the enhancement of the professional capacities of personnel engaged in dairy processing and transformation. The intervention combines agroecological principles, efficient natural resource management, and the promotion of networks and public-private partnerships, with the pivotal goal of diversifying and increasing the availability of livestock milk-derived products, thereby contributing to the long-term stability and self-sufficiency of the local food system. Building on consolidated expertise in buffalo and small ruminant research—spanning reproductive biology, nutritional science, genetics, and dairy processing—forthcoming activities will include direct technical assistance to local operators, targeted training in milk collection and transformation, and the context-sensitive transfer of technological innovations adapted to Cuban conditions. Expected outcomes include measurable improvements in buffalo milk production, a broader diversification of dairy products, and strengthened institutional capacity for agrifood governance at the local level. The cooperation model presented here offers a replicable and scalable paradigm for knowledge transfer in support of the Sustainable Development Goals – most notably SDGs 2 (Zero Hunger), 8 (Decent Work and Economic Growth), and 17 (Partnerships for the Goals) – in contexts marked by economic fragility and acute food security pressures.

Keywords: buffalo production, milk production, international cooperation, dairy supply chain, food security, technology transfer, agroecology, sustainable development, Cuba, cheesemaking.

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Impact of different cheese-making processes on the composition and techno-functional properties of powdered buffalo whey protein concentrates

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Owing to its high solids content, buffalo milk has been extensively used for producing cheeses with high yields. During this process, more than half of the milk components remain in the whey, resulting in an effluent with a high pollutant load. However, due to its nutritional value, buffalo whey has strong potential for developing innovative products such as nutritional supplements. This study aimed to investigate the influence of whey origin on the characteristics of buffalo whey protein concentrates (BWPC). With this purpose, whey was obtained from mozzarella-type cheeses produced from raw milk (RM) and pasteurized milk (PM; 65 °C, 20 min) and from a provolone-type cheese produced from pasteurized milk with a heat-treated curd (PP). Whey samples were defatted by centrifugation and subjected to microfiltration (0.7 and 0.45 μm). Subsequently, they were concentrated by ultrafiltration followed by diafiltration, and the resulting concentrates were freeze-dried. Chemical composition was determined using AOAC methods, and protein solubility at pH 4 and 7 and hygroscopicity were evaluated. BWPC samples showed protein contents ranging from 79.07% to 81.82% on a dry basis (db), with no significant differences attributable to the cheese-making process. Lactose content ranged from 4.24% to 4.69% (db), while mineral content ranged from 1.18% to 1.69% (db). Whey from PP showed lower lactose content and higher mineral content ($p < 0.05$). All BWPC exhibited good protein solubility, ranging from 75.84% to 78.51% at pH 4, increasing to 81–82% at pH 7, with no significant differences among treatments ($p > 0.05$). In contrast, hygroscopicity was affected by whey origin, with values of 13.69 ± 0.21% (RM), 14.48 ± 0.35% (PM), and 14.82 ± 0.09% (PP). Overall, cheese-making technologies slightly influenced the composition and quality of whey protein concentrates. Despite the significant differences observed in lactose and mineral contents, all three samples exhibited favorable compositional and techno-functional properties, highlighting their potential as promising raw material for the development of high-quality protein nutritional supplements.

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TransformDairyNet project: A European effort to promote Cow-Calf and Buffalo-Calf Contact dairy production systems

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TransformDairyNet (TDN) is a European project promoting dairy systems in which calves remain with their dams for extended periods, instead of being separated shortly after birth as in conventional farms. Cow–calf contact (CCC) systems are increasingly recognized for their potential to improve animal welfare and health in dairy cattle and water buffalo.

The project involves 26 partners across Europe—farmers, veterinarians, researchers, policymakers, and industry stakeholders—and is funded by the European Union. A key objective is the establishment of 11 National Innovation Practice Hubs (NIPs) to support knowledge exchange on CCC practices, foster innovation, and develop practical guidelines, while building a European network for sustainable dairy farming.

In Italy, dam–calf contact in the buffalo sector is not widespread and is currently limited to very few farms (2), often without structured protocols or economic valorization. This represents both a knowledge gap and an opportunity for innovation.

To address this, ANASB is developing a Living Lab focused on buffalo dam–calf contact throughout the weaning period. The initiative investigates calf behavior under continuous maternal contact, with attention to growth performance, weight gain, and milk organoleptic properties. The aim is to identify advantages of CCC systems in terms of animal welfare and farm economics, providing a scientific and practical basis for wider adoption in Italy.

Within this framework, TDN enhances resilience and sustainability by fostering collaboration and knowledge co-creation. The project focuses on: (i) innovation through Living Labs within NIPs, where solutions are tested on farms; (ii) accelerating CCC adoption via a European Multi-Actor Network (EKIN); (iii) strengthening long-term capacity through collaboration and knowledge sharing; (iv) modernizing dairy systems by integrating science and practice; and (v) amplifying impact through digital tools and shared resources.

Beyond dairy, TDN supports broader agricultural transitions aligned with societal demand for sustainable, regenerative, and low-carbon systems.

Further details available on the project website [https://transformdairynet.eu]

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Thermodynamic analysis and stability of vitamin E nanocomplexes with buffalo whey proteins

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Buffalo whey is the main by-product of cheesemaking and, with the increasing demand for buffalo cheese, its production is steadily rising, posing a growing environmental challenge. Despite this, whey represents a valuable source of proteins with outstanding techno-functional properties. Among these, its encapsulation capacity is particularly relevant for the development of delivery systems for bioactive compounds. In this context, understanding the thermodynamic aspects of molecular interactions and their impact on protection efficiency is of great interest. Therefore, the aim of this study was to evaluate the thermodynamic parameters (Gibbs free energy, ΔG; enthalpy change, ΔH; and entropy change, ΔS) associated with the encapsulation of vitamin E by buffalo whey protein concentrate (BWPC), as well as to assess its effect on vitamin E stability. A lab-scale obtained BWPC was dispersed in phosphate buffer (pH 7) at 813 µM and mixed with a vitamin E solution in ethanol (212 mM) to obtain final mixtures containing 5 µM protein and 0–100 µM vitamin. Samples were analyzed by spectrofluorometry at four temperatures (283, 288, 293 and 298 K), and emission maxima were fitted to the van't Hoff and Gibbs–Helmholtz equations to determine thermodynamic parameters. For stability assessment, samples were exposed to combined thermal treatment (50 °C) and UV radiation, and vitamin concentration was monitored by HPLC over three days. Thermodynamic analysis revealed negative free energy values at all temperatures, indicating a spontaneous interaction process. ΔS and ΔH took positives values reflecting that the complexation process was entropically driven. In addition, positive entropy and enthalpy changes were associated with a high contribution of hydrophobic interactions to complexes formation. In terms of stability, vitamin degradation occurred at a significantly lower rate ($P < 0.05$) in the complexed form compared to the free form, confirming an effective protective effect. These findings demonstrate the potential of buffalo whey proteins as encapsulating agents and highlight their applicability as delivery systems for hydrophobic bioactive compounds, contributing to the development of functional food formulations with improved stability and nutritional value.

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Buffalo milk custards as functional matrices: impact on the bioaccessibility of encapsulated antioxidants during *in vitro* digestion

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Buffalo milk represents a valuable raw material for the development of novel dairy products, particularly those oriented toward the functional food market. Its distinctive composition, characterized by higher protein and fat contents, offers potential advantages for the design of food systems capable of acting as carriers of bioactive compounds. In this context, the incorporation of encapsulated antioxidants into buffalo dairy matrices may further enhance their nutritional and functional value. The aim of this study was to evaluate the effect of buffalo milk-based custards on the bioaccessibility of encapsulated antioxidants during *in vitro* gastrointestinal digestion. Antioxidant extracts rich in anthocyanins and phenolic compounds were encapsulated by ionic gelation using sodium alginate and subsequently freeze-dried or vacuum-dried. The encapsulated systems were incorporated into buffalo milk-based custards formulated with non-caloric sweetener and starches as structuring agents. Samples were subjected to simulated gastrointestinal digestion using the INFOGEST protocol. Both encapsulated and non-encapsulated antioxidant extracts were evaluated either in free form or incorporated into the dairy matrix as functional ingredients. The content of bioactive compounds and their antioxidant activity were monitored throughout the oral, gastric, and intestinal phases. Encapsulated systems exhibited a more controlled release profile, with greater stability under gastric conditions and increased availability during the intestinal phase compared to non-encapsulated counterparts. Encapsulation increased bioaccessibility by 2.5 to 5 times for anthocyanins and by 2 to 4.5 times for polyphenols. In addition, in the dairy matrix, the stability of anthocyanins and phenolic compounds was improved significantly. Anthocyanin bioavailability increased between 3 and 5 times, while phenolic compounds showed increases ranging from 2 to 5 times. These effects were attributed to interactions between milk proteins and phenolic compounds, which may protect bioactives from degradation and modulate their release, enhancing intestinal availability. In conclusion, buffalo milk-based custards act as effective functional matrices, enhancing the bioaccessibility of encapsulated antioxidants and promoting controlled release during digestion. These findings highlight the potential of buffalo milk as a promising ingredient for the development of innovative functional dairy products.

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Enhancing PUFA n-3 Content in Buffalo Meat through Linseed-Supplemented Diets

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The lipid composition of meat has long been recognized as a key factor in determining its nutritional value and its impact on human health. Current research is focused on improving the content of polyunsaturated fatty acids n-3 (PUFA n-3), due to their beneficial effects.
Diet is one of the most relevant factors to modify the fatty acid composition of animal products, particularly the proportion of n-6 and n-3 fatty acids. Linseed is widely used in ruminant nutrition as a functional lipid source because of its high content of α-linolenic acid (18:3 n-3). Its inclusion in the diet can increase the proportion of n-3 fatty acids in animal products and promote the synthesis of long-chain n-3 fatty acids.
The aim of this study was to evaluate the effect on meat quality of linseed inclusion in buffalo diet, during the finishing period. Sixteen male buffaloes at 11 months of age were randomly assigned to two dietary groups: a control diet (C) and a linseed-supplemented diet (L, control diet plus 3% linseed). At 14 months of age, buffaloes were slaughtered, and the *Longissimus thoracis* (LD) and *Biceps femoris* (BF) muscles were sampled for physical and nutritional analyses.
No significant differences were observed in physical and proximate composition between diets and muscles, expected for redness (a*), showing significant differences, in both diets and muscles. The control group reported higher a* values than the linseed group ($p<0.044$), and BF exhibited higher redness values than LD ($p<0.027$). These differences may be attributed to dietary treatment and muscle type, since linseed supplementation influences meat colour, particularly a*, as reported in literature. The main effect of linseed inclusion was observed in the fatty acid profile and nutritional indices (thrombogenic index, TI, and atherogenic index, AI). In the L group saturated fatty acids, n6/n3 ratio, TI and AI were significantly lower than in C group, mainly due to an increase in PUFA n-3 content (4.42% vs 1.79% for L and C group respectively). Differences were also observed between muscles, likely due to variations in intramuscular fat content, which was higher in LD. Accordingly, LD showed higher TI and AI values than BF ($p<0.05$). In conclusion, linseed inclusion in the diet improved the nutritional value of buffalo meat by increasing PUFA n-3 levels and reducing thrombogenic and atherogenic indices. However, further studies are needed to fully consider buffalo meat as a potential functional food.

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Developing skills for multifunctional buffalo farming: the MYFARM European training approach

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The increasing diversification of agricultural activities represents an opportunity to strengthen farm resilience, economic sustainability and the connection between farms and rural communities. However, multifunctional farming requires specific technical, managerial and entrepreneurial skills that are not always adequately addressed by conventional agricultural education. The Erasmus+ project MYFARM – Multifunctionality Farming aims to address this gap by developing an innovative European training pathway and digital tools to support farmers, students and agricultural professionals in understanding and implementing multifunctionality. The project integrates regional analyses of multifunctionality, a self-assessment tool, an interactive map of examples and best practices, and an 800-hour online training programme structured into four modules. The training combines theoretical content with practical, project-based activities and work-based learning, providing learners with competencies relevant to the development and management of multifunctional farms. Although developed for the agricultural sector as a whole, the MYFARM approach has particular potential for buffalo farming, where production activities can be integrated with complementary services such as direct sales, agri-tourism, educational activities, environmental services and valorisation of local products and cultural heritage. In this context, multifunctionality may contribute to broadening farm income sources and strengthening the territorial role of buffalo enterprises. First implementation data collected in August 2026 indicate substantial engagement with the digital training environment, with 519 registered learners and 2,839 badges awarded. Learners were registered from several countries participating in the project, including Cyprus, Greece, Italy, Portugal, Romania and Ukraine, demonstrating the transnational reach of the training initiative.
These preliminary results indicate the capacity of MYFARM to provide a scalable digita framework for developing competencies related to multifunctional agriculture. For buffalo farming, the project offers a structured pathway through which existing production systems can be complemented by diversified activities and services, supporting a broader concept of farm sustainability and territorial resilience.

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Measuring progesterone and cortisol levels during follicular wave presynchronization in fixed-time artificial insemination programs in water buffaloes (Bubalus bubalis)

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Animal welfare is a priority in production systems, fixed-time artificial insemination (FTAI) might cause discomfort in the animal due to the strength of the vaginal muscles. Alternative pharmacological approaches must be sought to reduce it. Cortisol (C) levels are a good indicator of stress. This study aims to evaluate the use of two different presentations fo P4 (injectable and vaginal) used for the induction of ovarian activity (PS) at the beginning of favorable reproductive season in a buffalo herd in Corrientes, Argentina. From February to March 2025, thirty healthy buffalo females, with a weight range from 468-768 kg, with normal reproductive tracts, were used and randomly assigned to three PS groups: group 1 (intramuscularly injected P4 -BullSynch® Agropharma); group 2 (intravaginal device -Dispocel ®, Fatro Von Franken), and group 3 (control group, saline solution). To determine P4 and C levels, blood samples were collected from the jugular vein into dry tubes on days 1, 4, 7, and 14 of treatment by chemiluminescence. The data obtained were compared using nonparametric statistics and $p < 0.05$ difference was considered statistically significant. The P4 values obtained ranged from 1.09±1.57, 1.65±1.07, 1.35±1.23, and 1.04±1.41 ng/mL on days 1, 4, 7, and 14, respectively, showing significant differences between day 1 and day 4 ($p <0.001$). The P4 range in this study was 0.1 to 6.53 ng/mL. There were significant differences in P4 levels between group 1 on days 1 and 4 ($p =0.03$). In other groups, there were no significant differences. The C values obtained were 0.80±0.21, 1.33±0.66, 1.51±0.84, and 2.42±1.23 ng/mL for days 1, 4, 7, and 14, respectively, with significant differences between days 1, 7, and 14 ($p < 0.01$). There were no significant differences between days 1 and 4. In all treatments, significant differences were observed between day 1 and day 14 (group 3 $p=0.01$, group 2 $p < 0.01$, group 1 $p< 0.01$). The difference in P4 levels between days 1 and 4 can be explained by the animals' physiology and the drug's pharmacokinetics, with a faster increase observed after P4 injection. The control did not show a significant increase in P4 levels. In addition, all treatments show differences in C levels between days 1 and 14, indicating that the animals are stressed regardless of the form of medication used, and possibly that the medications had a cumulative effect over time, as evidenced by a tendency toward an increase in levels. The insertion of vaginal implants does not increase cortisol levels.

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Dietary strategies to enhance buffalo mozzarella nutraceutical properties and endothelial resilience *in vitro*

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Rising consumer interest in functional dairy products has heightened demand for feeding strategies that improve the health-promoting properties of animal-derived foods.
This study evaluated the effect of a diet rich in simple sugars in Italian Mediterranean buffaloes (IMB), specifically analyzing the functional profile of mozzarella cheese and the *in vitro* biological activity of milk extracts.
A four-month trial was conducted on 100 multiparous IMB reared on a commercial farm and randomly allocated to two homogeneous groups balanced for days in milk (DIM), parity, and milk yield (104 ± 65 DIM, 3.70 ± 1.60 parity, 16.5 ± 4.40 kg milk/day). Both groups received isoenergetic and isoproteic total mixed rations differing in the source of simple sugars. The control diet included sucrose and a higher proportion of farm-produced feed containing biscuit meal, while the treated diet was supplemented with a commercial liquid sugar feed. At the end of the trial, mozzarella obtained separately from the pooled bulk milk of each group was analysed for betaine and carnitine derivatives by HPLC-ESI-MS/MS and for antioxidant activity. Milk from the same groups was also used to prepare extracts for experiments on human aortic endothelial cells. Following pre-treatment with milk extracts, cells were exposed to high glucose (30 mM, 48 h) to assess viability, cytotoxicity, inflammatory cytokines, cell-cycle distribution, oxidative stress, senescence, and sirtuin protein expression. Mozzarella from the treated group showed significantly higher concentrations of δ-valerobetaine (12.8±0.50 vs 11.3±0.50 mg/kg), acetyl-L-CARNITINE (27.1±0.90 VS 24.5±0.90 mg/kg), and ferric reducing antioxidant power (26.6±0.90 vs 22.6±0.90 µmol/kg; $P<0.05$), indicating a partial transfer of the beneficial effects associated with the experimental diet to the final dairy product. *In vitro*, milk extracts from the treated group increased basal endothelial cell viability after 48 h ($P<0.05$ vs control). Under high-glucose conditions, these extracts significantly improved viability ($P<0.01$ vs Hg), attenuated G1-phase arrest (63.80±2.62% vs 80.17±5.72%; $P<0.05$), reduced LDH release, inflammatory cytokine production, oxidative stress, and senescence, while upregulating SIRT1, SIRT3, and SIRT4 protein expression ($P<0.05$). Overall, dietary supplementation with liquid simple sugars appears to be an effective strategy to enhance the functional value of buffalo mozzarella, supporting the bioactivity of the milk in a model of endothelial dysfunction.

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Occurrence and molecular characterization of methicillin-resistant *Staphylococcus aureus* and ESBL-producing *Escherichia coli* in bulk tank milk from water buffalo farms in southern Italy

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Water buffaloes (*Bubalus bubalis*) represent an important dairy species in Italy, primarily used for making high-value cheeses like mozzarella. However, data on antimicrobial-resistant (AMR) bacteria in buffalo milk remains limited. This study investigated the occurrence and molecular features of *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* in bulk tank milk (BTM) from buffalo farms in the Campania region, southern Italy.

Sixty-eight BTM samples were collected from farms in the province of Caserta between June 2022 and June 2024. Bacteriological analyses and MALDI-TOF identification were followed by antimicrobial susceptibility testing, PCR detection of *mec*A, *mec*C, and β-lactamase genes (*bla*CTX-M, *bla*TEM, *bla*SHV, *bla*CMY-2), and whole-genome sequencing (WGS) of selected isolates.

S. aureus was detected on 42 out of 68 farms (61.76%), with 21 RS-PCR different genotypes identified, including one novel genotype (DB). Three isolates recovered on MRSA-selective media were initially considered presumptive MRSA; however, WGS confirmed only one isolate as MRSA (1/68; 1.47%), carrying *mec*A. This isolate belonged to ST72 and harbored a Type V (5C2&5) SCCmec variant with a multidrug-resistant resistome including *bla*Z, *mec*A, *aac*(6')-*aph*(2''), *dfrG*, and mutations in *gyr*A/*grl*A, as well as *tet*(38) and *fos*B, in the absence of plasmids. The virulome included adhesins, the *ica*ABCD biofilm operon, and toxins such as *tsst*-1, *sec*, *sell*, and *luk*ED, but lacked PVL.

ESBL-producing *E. coli* were detected in 6 out of 68 samples (8.82%), all carrying *bla*CTX-M; one also harbored *bla*TEM. WGS identified three sequence types (ST1312, ST202, ST69), all with *bla*CTX-M-15 and *qnr*S1. ST1312 isolates showed chromosomal integration of resistance genes with IncF plasmids carrying accessory traits, whereas ST202 carried all ARGs on IncY plasmids; ST69 harbored a multi-replicon IncF plasmid. Additional ARGs included *tet*(A), *sul*2, *dfr*A14, and aminoglycoside resistance genes. Virulome analysis revealed conserved colonization factors, with one ST1312 isolate displaying an expanded virulence profile.

These findings provide the first genomic characterization of MRSA and ESBL-producing *E. coli* in buffalo BTM in Italy and highlight the need for strengthened AMR monitoring, improved milking hygiene, and prudent antimicrobial use. Including buffalo dairy systems in One Health strategies could help reduce the spread of AMR.

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Pedigree and heritability in the Italian Mediterranean Buffalo: preliminary results on key milk traits and implications for breeding strategies

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The Mediterranean Italian River Buffalo (MIRB) is a dairy population of major economic importance, as its milk is the primary ingredient for high-value products such as Mozzarella di Bufala Campana PDO [1]. However, robust selection-oriented models integrating pedigree, repeated phenotypes, and molecular markers are still under development for this species. The aim of this study was to estimate variance components and genetic parameters for key milk production traits in the MIRB population using test-day records and pedigree information. A reconstructed pedigree of 1,662 animals was combined with 15,244 test-day records from 405 buffaloes. Four traits were analysed: daily milk yield (MY), fat percentage (FP), protein percentage (PP), and $\log_{10}$-transformed somatic cell count (log10SCC). Variance components and genetic parameters were estimated using a univariate animal model with a Bayesian approach, implemented through the Gibbs2f90 and postgibbsf90 programs of the BLUPF90 family [2]. The model included days in milk as a covariate; herd-test-day, age at calving, and parity as fixed effects; and permanent environmental, additive genetic, and random residual effects as random components. Heritability estimates (with posterior standard deviations) were moderate to low for MY (0.19 ± 0.05) and PP (0.17 ± 0.06), and low for FP (0.11 ± 0.03) and log10SCC (0.06 ± 0.03), suggesting substantial additive genetic variation exists within the MIRB population, although environmental and management factors remain important. These results align with [3], who reported similarly low to moderate heritability estimates for milk production traits in water buffalo. Although based on a relatively limited dataset, the present approach suggests that repeated test-day records combined with pedigree information can support the estimation of genetic parameters in geographically defined MIRB populations, as previously reported for local dairy cattle breeds analysed through Bayesian animal models [3-4]. Genomic evaluation can improve the prediction of milk traits in MIRB when pedigree and genomic information are jointly exploited. Given the moderate genetic determinism observed, integrating genomic information into future studies represents a promising approach to enhance selection accuracy and improve dairy traits in this breed, thereby facilitating more efficient, sustainable, conservation-oriented, and genetic improvement programs.

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Correlation between intra-vitam diagnostic tests and post-mortem evidence of Mycobacterium bovis infection in Mediterranean buffaloes (*Bubalus bubalis*)

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Official tests for tuberculosis (TB) in Mediterranean buffaloes (*Bubalus bubalis*) include the single and comparative tuberculin skin tests (sTST and cTST) and the interferon-γ (IFNγ) release assay (IGRA). In sTST, the increase in skinfold thickness (Δs) is measured 72h after injection of bovine tuberculin (bPPD). In cTST, the difference (Δc) between responses to bPPD and avian PPD (aPPD) is calculated to address cross-reaction with environmental mycobacteria. Both tests are positive if Δ≥4 mm. IFNγ production by blood lymphocytes stimulated with bPPD/aPPD is measured by IGRA optical density (OD); positivity is given when the ΔOD (bPPD– aPPD) and bPPD-baseline are ≥0.1. Animals positive to at least one test are considered TB suspects and confirmed by lesion detection at necropsy/slaughterhouse inspection and/or *Mycobacterium tuberculosis* complex identification by culture/molecular assays. However, not all suspects are confirmed due to limitations of ante- and post-mortem tests. This case–control study included 1183 TB suspect buffaloes, classified after slaughter as infected (n=526) or uninfected (n=657) according to the criteria above. For each test, positive predictive value (PPV) was calculated as the proportion of infected among test-positive animals. Age and immune response variables (Δs, Δc and ΔOD) were compared between groups using the Wilcoxon rank-sum test, and factors linked to TB confirmation were assessed by multivariable logistic regression (significance if p≤0.05). Overall, PPVs were low (sTST=0.44; cTST=0.46; IGRA=0.55). Infected animals were older than uninfected animals (88 vs 64 months). Median Δs and Δc were significantly higher in infected cases (19 vs 12mm for sTST; 6.9 vs 4mm for cTST), as was ΔOD in IGRA (0.97 vs 0.64). Multivariable analysis showed increased odds of TB confirmation in animals older than 7years (OR=1.53), in those with moderate Δc (7–14.9mm; OR=1.4), and in animals with ΔOD increases from moderate (0.5–0.9 OD; OR=1.82) to high (≥1.0 OD; OR=2.23). These findings support a risk-based interpretation of ante-mortem TB test results in Mediterranean buffaloes, integrating age and magnitude of immune response rather than relying only on dichotomous test outcomes.

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Predisposing Factors for Neonatal Mediterranean Buffalo Diarrhea

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Identifying critical points in animal health management of Neonatal Mediterranean Buffalo Diarrhea (NMBD) is essential for its prevention. This study aimed to apply a novel checklist designed to identify management-related predisposing factors for NCD in calves.
Thirty-three Mediterranean Buffalo farms located in Southern Italy (Campania Region) [median herd size: 352 cows; interquartile range (IQR): 176–554] were investigated between September and December 2025. The checklist included 91 questions grouped into seven domains (D, 13 question/D): mothers/close-up (D1); calving area/calving event (D2); calf care/colostrum intake (D3); colostrum, milk, and milk replacer management (D4); calf management up to 28 days of age (D5); biosecurity (D6); and health monitoring program and veterinarian involvement (D7). Each item was scored from 0 (minimal predisposition) to 2 (strong predisposition). Differences among domain scores were assessed using the Friedman test followed by Wilcoxon signed-rank tests with Bonferroni correction ($p<0.0083$). Correlations among domains were evaluated using Spearman's rank correlation, and internal consistency was assessed via Cronbach's alpha. The study population was stratified into intensive and semi-intensive farming systems, and differences between these groups were analyzed using the same statistical approach.
At the time of the survey, clinical NMBD was observed at the farm level in 23% of cases. The highest median scores were observed in D1 (Me=15.00/26.00, IQR=13.00–17.00), D6 (Me=14.00/26.00, IQR=12.50–18.00), D7 (Me=14.00/26.00, IQR=13.00–14.00) and D2 (Me=10.00/26.00, IQR=7.5.00–1300), indicating the greatest predisposing factors for NMBD. Lower median scores were observed in D5 (Me=8.00/26.00, IQR=6.00–9.00), D3 (Me=5.00/26.00, IQR=4.00–7.50) and D4 (Me=4.00/26.00, IQR=3.00–6.00). Significant differences were detected among most domains, except for D1 vs. D6, D1 vs. D7, D3 vs. D4. D3 vs. D5 and D6 vs. D7. A good positive correlation was found between D5 and D6 ($r_s=0.52$, $p<0.01$). No dominant responses emerged within domains.
Overall, the checklist proved to be a reliable tool to identify NMBD-related predisposing factors, highlighting that mothers/close-up (D1), biosecurity (D6), health monitoring program and veterinarian involvement (D7) and calving area/calving event (D2) may require the greatest attention for effective prevention of neonatal diarrhea in Buffalo farms.

Comparing welfare assessment protocols for dairy buffalo

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The Mediterranean buffalo is one of the most economically and culturally significant livestock species in Italy. Maintaining high welfare standards is essential for promoting herd health, milk quality and productivity. On-farm welfare is commonly assessed using ClassyFarm® (CF), the official system implemented by the Italian Ministry of Health. This system evaluates farm conditions through risk assessment evaluation of different areas (Management, Structures and animal-based-measures) and provides an overall score to welfare levels. In contrast, the Welfare Quality® (WQ) protocol mainly relies on animal-based indicators derived from direct observations, including: (a) avoidance distance at the feeding place, (b) qualitative behaviour assessment (QBA), (c) behavioural observations, (d) social interactions and coughing, (e) clinical scoring and (f) resource checklists. These are all integrated into a comprehensive welfare score. The aim of this study was therefore to evaluate the reliability of WQ in correctly classifying animal welfare status, using CF as the gold standard.
Four randomly selected dairy buffalo farms located in the Campania region (two in the Salerno district and two in the Caserta district, for a total of 1,736 adult buffaloes) were simultaneously scored by independent evaluators blinded to each other's assessments.
The average time required to complete the WQ protocol was 305.0 ± 15.8 minutes (mean ± SD). Overall, WQ showed good agreement with the gold standard in identifying major welfare issues. The main critical points detected included inadequate and poorly maintained drinking facilities, widespread dorsal alopecia and the presence of lameness. The protocol also highlighted low QBA scores, reflecting limitations in the expressive quality of behaviour and interactions with conspecifics and the environment, although it represents a parameter specifically developed for dairy cows.
Although WQ took longer than CF, it provided valuable additional insights, particularly regarding behavioural expression and social dynamics, which are not emphasised as much in the official system. However, some indicators may need to be adapted to better reflect the characteristics of this particular species. In addition, the protocol's accuracy and applicability can be improved by taking into account factors such as breeding and housing systems, as well as the seasonal variability that can strongly influence buffalo behaviour.

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Effects of dietary energy liquid supplementation on lactating buffaloes under hot conditions on milk production performance in dairy buffaloes

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Glucogenic energy liquids are increasingly used in dairy cows to support animals during periods of high energy demand or heat stress by providing readily available energy. This study evaluated the effects of Opti Fluid, a supplement containing glycerol, molasses, maltodextrins, and sucrose, on performance and behaviour in mid-lactating Italian Mediterranean dairy buffaloes. The experiment was conducted at the CREA buffalo experimental farm (Rome, Italy) on 40 multiparous lactating buffaloes, randomly allocated into two balanced groups (n=20). The groups were homogeneous for milk yield (MY) (9.50±2.43 *vs* 9.42±2.58 L/head/day; P=0.85), days in milk (DIM) (99.35±33.98 *vs* 93.40±32.12; P=0.51), and parity (4±1.76 *vs* 4.15±2.37; P=0.83). Animals were housed in two adjacent pens and equipped with ear tag sensors (CowManager, Netherlands), which recorded their behaviour. Temperature and humidity were recorded to calculate the temperature-humidity index (THI). A 2 × 2 crossover design was applied over 8 weeks with a 14-day washout; one group received Opti Fluid (1 kg/head/day) while the other served as control, then treatments were switched. The control diet was adjusted with barley meal (400 g/head/day) to provide equivalent energy. Supplements were administered manually after total mixed ration (TMR) distribution. Samples of TMR, individual milk, group faeces, body weight, and body condition score were collected 3 times per period. Feed intake was measured daily by recording the feed offered and the corresponding orts. Statistical analyses were performed in R (v.4.3.1) using linear mixed-effects models with Treatment, Period, and their interaction as fixed effects, and Animal as a random effect; DIM or sampling day were included as covariates, when relevant. Significance was assessed by Type III ANOVA and set at $P<0.05$. TMR analysis showed similar diets between groups, except for $aNDF_{om}$, which was higher in the control group (42.20 *vs* 37.60%, $P<0.05$). Opti Fluid supplementation increased daily MY by 3.3% without affecting milk fat or protein content; minor changes were observed in lactose and milk freezing point. Other parameters were not influenced by the treatment. Variations in behaviour and rumination were mainly associated with THI. In conclusion, low-level Opti Fluid supplementation supports milk production under hot condition without compromising milk quality, intake, or animal welfare. Further studies are needed across seasons and physiological stages.

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Genomic DNA methylation changes in the ruminal wall of dairy buffaloes versus dairy cattles

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Buffalo (*Bubalus bubalis*) and cattle (*Bos taurus*) differ in digestive physiology and metabolic adaptations, suggesting the involvement of regulatory mechanisms beyond gene expression. DNA methylation is one of the key epigenetic signatures influencing gene activity, yet comparative rumen epigenomic studies between these species are lacking. This study aims to characterize differential genomic methylation in the rumen of buffalo vs cattle (8 animals/group), using Whole-Genome Bisulfite Sequencing (WGBS). Following sequencing, reads were aligned to the respective bisulfite-converted reference genomes. DNA methylation analysis performed independently within each species revealed, in buffalo, an average of 244,983 regions containing CpG sites/sample, of which 204,346 were methylated (83%), while, in cattle, we found an average of 187,111 regions containing CpG sites/sample, of which 152,425 were methylated (81%). A differential methylation analysis between buffalo and cattle was restricted to genes showing a one-to-one orthologous relationship between the two genomes. We found 1,199 genes differentially methylated at their gene body, of which 732 were hyper-methylated (hyper-meth) and 467 hypo-methylated (hypo-meth), and 720 genes differentially methylated at their promoter, of which 401 were hyper-meth and 319 hypo-meth. Among the top hyper-meth gene bodies, we found *CAV1*, involved in lipid transport and energy metabolism, *LOX*, with roles in the cross-linking of collagen and elastin, the cholesterol transporter *NPC2*, and *RAB24*, related to autophagy and vesicular trafficking. Among the top hypo-meth gene bodies, there are *APOF*, linked to cholesterol metabolism, *BCL6*, with a role in immune and inflammatory response, and *ATP5MG* and *PET100*, both implicated in the energy metabolism. Within the top hyper-meth promoters, we found the signal transducer *MAPK1*, *LNPEP*, responsible for peptide hormone degradation, *NDUFC1*, involved in energy metabolism, and *DNMBP*, with a role in cytoskeleton organization and cell junction. The list of top hypo-meth promoters includes *PECR*, linked to fatty acid β-oxidation, *KIAT1*, implicated in metabolism of amino acids, *LIPT2*, associated with mitochondrial function and oxidative metabolism, *NDUFAF1*, related to the mitochondrial respiratory chain.
Collectively, these findings provide new insights into species-specific epigenetic mechanisms potentially underlying differences in rumen physiology, metabolism and functionality between buffalo and cattle.

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Characterizing the quality and hygienic-sanitary parameters of water buffalo bulk milk in the Lazio Region (Italy)

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In Italy, buffalo milk is primarily destined for cheese production, particularly Mozzarella di Bufala Campana PDO (Protected Designation of Origin), whose manufacturing process strongly depends on milk quality. While several studies have investigated temporal dynamics and seasonal variation in milk quality and hygienic parameters in dairy cows, much less information is available for buffalo milk. Therefore, evaluating compositional changes and hygienic conditions in buffalo milk over time represents an important and less explored research area.

A total of 13,768 buffalo bulk tank milk samples from 113 farms in the Lazio region (Italy) were collected between 2021 and 2025 during routine quality monitoring. Samples were analyzed for compositional parameters (fat %, protein %, lactose %) and hygienic-sanitary parameters, including Total Bacterial Count (TBC - CFU/mL) and Somatic Cell Count (SCC – n. * ml). Only farms monitored across all five years and with at least one sample per season were included in the dataset. Data were aggregated by farm and season using median values while seasonal effects were evaluated through linear mixed effects models, with Season as a fixed effect and Farm as a random.

Considering the entire study period, the median fat content in bulk milk across farms was 7.85%, (95% range: 7.42 and 8.35%), while the median protein content was 4.57% (95% range: 4.31 and 4.79%). Lactose was the most stable parameter, with a median of 4.61% and a narrow 95% range of 4.53–4.70%. On contrary, hygienic and sanitary indicators showed greater variability: the median TBC was 5.44 Log CFU/mL (95% range: 4.85–6.11), and the median SCC was 5.22 Log cells/mL (95% range: 4.96–5.58). Seasonal patterns consistently affected main milk quality traits, with key components such as fat and protein showing lower levels during the warmer months.

The results indicate that the milk quality and hygiene indicators observed in this study are consistent with values previously reported by other Italian studies, highlighting the high performance of the Mediterranean Italian in comparison to other buffalo breeds. Moreover, the statistical analysis revealed marked variability in milk quality both among farms and across seasons. In particular, the general reduction in major milk components during the warmer months likely reflects fluctuations in feeding conditions and the heat stress experienced by buffaloes during this period.

Buffalo "burrata" produced in the province of Salerno: a comparison of different production technologies

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Burrata di Bufala, a traditional Southern Italian cheese listed among the PATs (Traditional Agri-food Products) of the Campania Region, is a fresh stretched-curd product filled with cream and characterized by a short shelf life (5–20 days), influenced by production practices and microbiological quality.

This study evaluates the physicochemical and microbiological profiles of buffalo burrata produced by dairies in the province of Salerno, with the aim of providing a comprehensive characterization of the product.

Fifteen samples were analyzed, including 9 industrial and 6 artisanal products. Physicochemical parameters (water activity, pH, nutritional composition) and microbiological criteria (Listeria monocytogenes, Salmonella spp., Yersinia enterocolitica, Bacillus cereus, Escherichia coli, coagulase-positive staphylococci, Enterobacteriaceae, and Pseudomonas spp.) were assessed at production (T0) and at the end of shelf life (TF).

No pathogens were detected in any sample, confirming the overall microbiological safety of the product. However, hygiene indicator microorganisms (Enterobacteriaceae and Escherichia coli) were found in several samples, with significant increases observed from T0 to TF in both production systems. While water activity remained stable, pH values decreased over time, indicating ongoing biochemical changes during storage.

Nutritional composition showed variability among producers but no significant differences between artisanal and industrial products. Changes in fat and protein content between T0 and TF suggest an influence of moisture dynamics on product composition.

Overall, buffalo burrata demonstrates high microbiological safety and valuable nutritional properties, although variability in hygiene indicators highlights the need for improved process control to ensure consistent quality throughout shelf life.

Genomic regions associated with teat-related defects in Italian Mediterranean Buffalo

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Genome Wide Association Studies (GWAS) are tools that explore the relationship between genetic variants and specific phenotypes within a population. Genetic studies in livestock have gained importance in recent years to understand the genetic influence of various phenotypic traits such as udder and teat morphology. Identifying genetic variants associated with mammary system defects could provide valuable information for the development of selection strategies to reduce the incidence of these conditions in the Italian Mediterranean Buffalo (IMB). The most frequent mammary system defects in the IMB population were analyzed: supernumerary teats (SNT) and udder attachment defects (UAD), both defined as binary traits (presence/absence).

The dataset consisted of 1,121 (UAD) and 833 (SNT) records, with a pedigree including 21,929 and 21,909 animals for UAD and SNT, respectively. Regarding genotypes, information from 7,323 genotyped animals with 52,917 loci was used. SNP (single nucleotide polymorphism) effects were estimated using a single-step GWAS (ssGWAS), which back-solved the genomic estimated breeding values predicted by single-step genomic BLUP, fitting a single-trait animal model.

Regarding the SNT defect, the largest genetic variance (0.62%) was explained by a SNP window located on chromosome 19. Fourteen informative windows explaining at least 0.50% of the additive genetic variance were identified, all concentrated on chromosome 19, explaining a total of 7.80% of the observed genetic variance

In the case of UAD defect, the largest genetic variance (0.63%) was explained by a SNP window located on chromosome 1, followed by chromosome 8 (0.60%) and chromosome 6 (0.59%). Overall, a total of 33 informative windows explaining at least 0.50% of additive genetic variance were identified, explaining 18% of the observed genetic variance. These results suggest that both defects have a genetic component, although with different genomic architectures. While SNT showed a major genomic region concentrated on chromosome 19, UAD displayed a more polygenic architecture with informative windows distributed across chromosomes 1, 6, 8 and 9. The identification of these regions and their underlying candidate genes represents an important tool for breeding programs, contributing to a better understanding of the genetic architecture of udder and teat defects in IMB and enabling early selection against their manifestation.

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Long-term GreenFeed monitoring of enteric methane emissions in lactating buffaloes: practical challenges and emission patterns

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The GreenFeed system (GF; C-Lock Inc., Rapid City, USA) is widely regarded as the silver-standard for measuring individual enteric emissions *in-vivo*. This study aimed to quantify enteric emissions in Mediterranean buffaloes, a species poorly monitored with on-farm techniques, using GF during a long-term monitoring, and to identify methodological challenges associated with its application. The trial was conducted in a commercial dairy buffalo farm (Amaseno, FR) from June 2024 to January 2026. The GF unit shaped for animals with big horns was placed in the feed alley. Animals were encouraged to visit the GF by dispensing pelleted concentrate with up to 8 feed drops per visit, each delivering 38 g every 30 seconds over a total of 4 minutes. Buffaloes were allowed to visit the unit up to five times per day, receiving a total of 1.52 kg of concentrate per head. Before starting the trial, buffaloes were trained for 4-weeks to voluntarily visit the GF. Training involved individually attracting animals with a pellet bin and manually drop the pellet once they approached the unit. A total of fifty-six lactating buffaloes were ear-tagged for GF detection; however, at the end of the trial only thirty-seven visited the unit at-least once. Animals were fed TMR distributed two times per day. Individual data on milk yield, days in milk (DIM), and parity were collected through routine monthly milk recording by the Provincial Buffalo Breeders Association. Preliminary results showed that, among the 37 animals monitored between June 2024 and January 2026 that visited the GF, usage averaged 1.49 ± 0.84 visits per animal per day, with only four buffaloes reaching the maximum allowed frequency of 5 visits per day. Average daily methane (CH_4) and carbon dioxide emissions per animal were 333.8±111.3 and 9,247.6±1,502.7 g/day, respectively, while CH_4 intensity averaged 43.3±19.7 g CH_4/kg milk. Seasonal variation in average group CH_4 emissions was observed, with the highest values in spring (384.6 g/day) and summer (334.6 g/day), and lower emissions in autumn (280.6 g/day) and winter (275.5 g/day). This pattern may be linked to calving deseasonalization driven by market demand, which increases the proportion of animals in early and peak lactation and, consequently, feed intake during spring and summer. These results represent the first evidence of GF on Mediterranean buffaloes; however, broader monitoring trials are needed to confirm the emissions pattern for this species.

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Utilization of yeast fermented liquid (YFI) in concentrate on feed intake and blood biochemical parameters of swamp buffaloes

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This study was conducted to evaluate the utilization of yeast fermented liquid (YFI) in concentrate on feed intake and blood biochemical parameters of swamp buffalo. Four male swamp buffalo with an initial body weight (BW) of 270±9 kg and 25 months of age were used in this research. The experimental design was a 4×4 Latin squared design with YFI utilization at 0%, 5%, 10% and 15% in concentrate. Animals were fed concentrate at 1.0% BW, while rice straw was fed *ad libitum*. It was found that the utilization of YFI did not affect nutrient digestibility ($p > 0.05$), while dry matter intake increased with increasing levels of YFI ($p < 0.05$). Moreover, the ruminal pH was within the normal range and was similar among treatments ($p > 0.05$). In contrast, ammonia nitrogen (NH_3-N) and blood urea nitrogen (BUN) increased linearly with YFI utilization. In addition, blood biochemical parameters were not altered by YFI supplementation. Based on this study, it can be concluded that the utilization of YFI in concentrate could improve feed intake without affecting digestibility and blood biochemical parameters in swamp buffalo.

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Activation of Inactive Water Buffalo Bulls Through L-Carnitine Treatment: Effects on Libido and Testosterone Levels

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The Bull Entrustment Program deploys breeding bulls to rural communities to improve the production of upgraded calves. However, reports of bull inactivity and failure to breed estrus females have constrained program outcomes. This study evaluated 135 reported inactive riverine buffalo bulls through breeding soundness examination, libido assessment, and subsequent intervention. Of these, 59 bulls (43.7%) exhibited normal libido when presented with estrus-synchronized or naturally estrus females, 33 (24.4%) were immature or smaller than available females, 38 (28.1%) were confirmed non-performing, and 4 (3.0%) were unreported mortalities. Intrinsic constraints included continuous tying (100%), while extrinsic factors included low female population (5–8 head) and larger female size. Non-performing bulls commonly exhibited testicular abnormalities (slim, cleft >1 cm, twisted, or asymmetrical), overgrown claws, and locomotor defects. Night corralling was recommended to 37 handlers; only one complied, resulting in 26 calves sired within a year.

Thirty-six non-performing bulls underwent L-carnitine treatment (5 mL/animal, weekly, subcutaneous). Twenty-four bulls (66.7%) responded: 1 after two doses (4.17%), 22 after four doses (91.67%), and 1 after six doses (4.17%). Hormone assay showed that responsive bulls (n=9) had mean testosterone levels of 585.74 pg/mL pre-treatment and 596.80 pg/mL at Week 4, while non-responders (n=2) increased from 600.73 pg/mL to 2,221.00 pg/mL. Statistical comparison using paired t-test indicated significant differences in testosterone changes between responders and non-responders ($p<0.05$). One-year post-treatment, the 24 activated bulls served 229 females and sired 27 calves. Findings demonstrate that L-carnitine effectively improves libido and functional performance in previously inactive riverine buffalo bulls, contributing to increased calf production in village herds.

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Body condition score and liveweight automation perspective in lactating Mediterranean buffaloes

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Identifying variations in liveweight (LW) or body condition score (BCS) allows for early detection of metabolic disorders or nutritional deficiencies. Traditional methods are labor-intensive and may cause stress to animals. In previous studies on BCS and LW prediction via laser telemetry (Zoometer®), we found consistency between predicted and measured BCS (r=0.92, n=821) and LW (r=0.95, n=161) using back (BI) and side (SI) image analysis.

The present study aimed to obtain reliable weights and BCS scores from top-down image analysis (HI) using the Zoometer. This setup enables automatic daily data acquisition during milking sessions by installing a single camera above the entrance gate of the milking parlor. Data can be continuously uploaded to a dashboard alongside other environmental, behavioral, and individual sensor-based metrics (e.g., milk yield, days in milk, parity, age, feeding time/intake, rumination, activity, and temperature). This provides a timely snapshot of animal's condition, following an individual-level Precision Livestock Farming (PLF) approach.

Data from 218 HI showed high accuracy ($y = 0.9999x + 0.0002$) but low precision ($R^2 = 0.69$) between instrumental and official BCS (performed by a trained operator). Additionally, LW measured on a subset of 12 animals (with both SI and HI data) showed a correlation of r=0.86 with the HI-associated area. Despite the small sample size, this study showed a high accuracy and low precision ($y = 1.0014x + 0.001$; $R^2 = 0.7445$) between predicted and measured weight, indeed the individual data points were dispersed (range ±30 kg). This served as a pilot study to establish a monitoring procedure for growing and fattening buffalo veal, subject of a separate communication.

Reliable BCS and LW can be easily obtained using a fixed device during routine operations. The use of HI is suitable, and estimates can be improved by increasing sample sizes, fine-tuning experimental design (e.g., distance, camera angles, considered proxy angles further or different proxy angles, luminosity etc.), and the system automated. Top-down visuals provide unbiased results despite the observed dispersion.

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From Skills Gaps to Future Competences: The VITAL Approach for a Sustainable and Digital Livestock Sector

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The livestock sector is undergoing profound transformation driven by digitalisation, artificial intelligence, precision livestock farming, climate change, sustainability requirements, emerging health risks and increasing societal expectations regarding animal welfare. These changes are generating new competence requirements for farmers, veterinarians, technicians, farm managers and other livestock professionals, potentially widening the gap between existing skills and those required by future production systems. VITAL – Vocational Innovation and Training for Animal Livestock is an Erasmus+ Blueprint project involving 25 partners from nine European countries. The project aims to identify current and future skills needs in livestock production and translate them into new professional profiles, learning pathways and innovative training opportunities. A multi-stakeholder approach is adopted through national ecosystems involving higher education and VET institutions, labour-market actors and external stakeholders.During the first phase, national ecosystems were established and Academic, Industrial and Technological working groups created to integrate perspectives from education, the labour market and technological innovation. A stakeholder survey was developed to assess five dimensions of competence: current level, required level, training needs, future importance and coverage by existing education and training. The instrument was reviewed by an international expert group and pilot-tested within the consortium before deployment across participating countries. By October 2026, more than 2,000 stakeholders have been recruited in all Europe to perform the surveys. The resulting skills intelligence will support the identification of priority competence gaps and the development of future professional profiles and learning pathways. Although VITAL addresses the livestock sector as a whole, its approach is particularly relevant to buffalo production, where digital technologies, data-driven management, animal health, biosecurity, reproduction, sustainability and animal welfare are increasingly interconnected. VITAL provides a framework for connecting sectoral needs, technological innovation and education and training, supporting the development of a skilled workforce capable of driving the transition towards more sustainable, resilient and technology-enabled buffalo production systems.

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AI-Powered Application for Predicting pH from Indicator Strip Images as an Example of Biosensor Application in Buffaloes

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Colorimetric indicator strips offer a low-cost, rapid route to estimating pH and related metabolites of veterinary interest, including as markers of milk quality and reproductive status in buffaloes. Conventional visual matching of the developed strip colour to a reference chart is inherently subjective, being affected by human colour perception, ambient illumination and observer experience, limiting reproducibility and field utility. Objective, device-agnostic quantification is therefore required to translate such assays into scalable digital-phenotyping tools, providing a strong case for the development of AI-assisted biosensors that remove observer bias and enable reliable on-farm decision-making.

To demonstrate the feasibility of such a biosensor, a dataset of 540 mobile-phone images of pH test strips was used to train and evaluate two complementary approaches. The dataset was generated using pH solutions ranging from 5.0 to 10.0, with a step size of 1.0 pH unit. Each solution was calibrated using pH 700 meter from Eutech. Solutions were applied to paper test strips, which were photographed alongside a reference colour palette under varied lighting (artificial and natural shade) using three mobile camera models. A classical computer-vision pipeline combined with Ridge regression on colour features achieved strong predictive performance ($R^2 = 0.92$). An end-to-end deep-learning model based on a fine-tuned Xception backbone further improved accuracy, reaching a mean absolute error of 0.28 pH units and $R^2 = 0.95$ on the held-out test set (20% of the dataset). These results suggest that smartphone images has potential to deliver laboratory-comparable pH readings. The trained model was deployed as a Progressive Web Application (pH Predictor PWA; https://msk99.github.io/ph-predictor/) that runs directly in the browser on mobile devices. It is being tested and supports camera capture, automatic strip cropping, manual cropping, offline use and home-screen installation, delivering predictions in under two seconds and making the biosensor readily accessible under field conditions. The dataset is currently being expanded with real biological samples.

Planned extensions include dataset expansion across strip brands, users and field lighting; benchmarking of additional CNN architectures; finer-scale data collection for higher-resolution quantification; and on-farm validation, supporting generalisation to further colorimetric metabolite assays.

Keywords: Colorimetric assay, deep learning, convolutional neural network, smartphone diagnostics, precision livestock farming, digital phenotyping.

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Genetic evaluation of weaning weight in buffalo under conditions of limited information: a comparison of linear and mixed models in Colombia

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Weaning weight (WW) is a key indicator of productive performance in buffalo systems, as it integrates both genetic and environmental effects. In Colombia, the limited availability of records and low genetic connectivity due to small herd size, restrict the application of conventional statistical models for genetic evaluation. This study aims to compare the performance of linear and mixed models under different data structures and to propose a methodological approach applicable to systems with limited information. Data from of two comercial herds were analyzed: Middle Magdalena (2024: n=153; 2025: n=37) and Cordoba (2025, n=196). In all cases, linear and mixed models were fitted. In 2024, the models had low explanatory power (adjusted $R^2 < 0.18$), although the sire effect was identified as the main source of structured variation. In 2025, the dataset consisted exclusively of males, which reduced variability; the linear model identified a significant effect of season ($p<0.01$), while the mixed model detected no genetic variance ($\sigma^2 \approx 0$), highlighting the limited capacity for genetic inference under conditions of small sample size and lower data diversity. In Córdoba, both approaches identified relevant sources of variation; the mixed model revealed genetic variability among sires ($\sigma^2=96.52$), while the linear model showed environmental effects associated with lactation, where milk yields exceeding 1,700 L significantly increased the PD ($p<0.05$). Additionally, the estimation of genetic values (BLUPs) allowed for the classification of breeding stock and highlighted differences among sires. The results show that the models' ability to detect genetic variation depends critically on sample size, data structure, and data variability. Under limited conditions, the environmental effect predominates; however, as data quality improves, mixed models allow for a more robust genetic evaluation. The data obtained allow to think that it is possible to implement progressive genetic selection strategies in small herds through the combined use of linear and mixed models, adapting their application to data availability and improving their accuracy as records increase.

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Relationship between reproductive tract ultrasonographic morphometry and fertility outcomes in buffalo heifers

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This study related biometric and clinical parameters to pregnancy rates in buffalo heifers reared in extensive natural multi-sire pasture system in Paraguay. Seventy-six Murrah crossbreed buffalo heifers of similar age (average 22 months), at a random stage of the estrous cycle, were enrolled. One experienced operator performed Body condition score (BCS) evaluation and transrectal ultrasonography (TU) (Mindray Vetus E7 ultrasound scanner with 7.5/10 MHz linear array) to ensure consistency. BCS, weight, uterine diameter (UD) (average of the two diameters of the larger horn), ovarian dimensions and status were recorded. Ovarian volume was estimated by approximating the ovary to an ellipsoid by the formula: V=0.532(length x width2) where length and width represent the two measured diameters. Total volume (VOT) (sum of the two ovaries) and the volume of the largest (VOM) were recorded. Ovarian status was categorized: C0 (animals with only follicles with a diameter <1cm), C1 (animals with at least one follicle >1cm in diameter), and C2 (presence of a corpus luteum). Bulls were introduced in the herd (bull to heifer ratio 1:25) 24h after evaluation, and pregnancy was assessed after 5 months. Differences between pregnant and non-pregnant animals were analyzed by t-test. ANOVA with Bonferroni Post-Hoc was used to assess differences among ovarian classes. Statistical significance was set at $P < 0.05$. Bivariate correlations were assessed using Pearson/Spearman correlation test. ROC curves were generated and analyzed. Average parameters were (mean ± SD): BCS (4.1 ± 0.6), Weight (322.8 ± 34.8 Kg), UD (1.6 ± 0.3 cm), VOT (4.8 ± 1.8 cm3), VOM (3.1 ± 1.8 cm3). Overall pregnancy rate was 82.9%. Pregnant heifers showed higher BCS compared to non-pregnant ones (4.2 vs 3.9, respectively, $P<0.05$). Pregnancy rates were lower ($P<0.05$) in C1 (54.5%) than in C0(86.8%) and C2(91.7%). C1 and C2 had similar weight (352 kg), which was higher than C0 (324 kg, $P < 0.05$). A positive correlation was found between weight and VOM ($r=0.44$; $p<0.001$), VOT ($r=0.49$; $p=0.02$), and ovarian status ($r=0.39$; $p<0.01$). BCS showed a predictive trend for pregnancy outcome (AUC = 0.641; $P = 0.055$), with optimal cut-offs at 4.25 and 4.75 (84.6% and 92.3% specificity, respectively). These results demonstrated that ovarian parameters and BCS correlated with pregnancy outcomes in buffalo heifers, proving effective for identifying at-risk animals and optimizing reproductive management under pasture conditions

Field Implementation of GnRH/CIDR-Based Synchronization Protocols for Fixed-Time Artificial Insemination in Swamp Buffaloes: A Large-Scale Study in Northeast Thailand

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Large-scale field data on GnRH- and CIDR-based FTAI protocols under smallholder conditions in Southeast Asia are still scarce. Whether tailoring synchronization protocols to individual ovarian status can meaningfully improve pregnancy outcomes in this setting has not been rigorously tested at scale. We addressed this gap by comparing 3 protocol strategies, each assigned based on pre-synchronization ovarian assessment, across 9 districts of Udon Thani Province, Northeast Thailand. From December 2025 to April 2026, 571 swamp buffalo cows belonging to 299 smallholder farmers were enrolled. Pre-synchronization workup included B-mode transrectal ultrasonography, BCS, complete blood count and uterine evaluation. Based on these findings, animals received 1 out of 3 protocols: (1), standard Ovsynch (GnRH–$PGF_2\alpha$–GnRH–FTAI), reserved for cows with active ovaries and BCS ≥2.5; (2), CIDR-based Ovsynch, for those with inactive ovaries or postpartum anestrus; and (3), Ovsynch or CIDR-based Ovsynch with 330 IU eCG administered at $PGF_2\alpha$, for cows with BCS <2.5 or poor follicular response. Frozen-thawed semen from 9 certified sires was used. Pregnancy diagnosis was performed by ultrasonography at day 35 post-FTAI, and data were analyzed by logistic regression and Chi-square tests ($P < 0.05$). Of the 571 enrolled cows, 459 (80.4%) proceeded to FTAI; the remainder were excluded owing to concurrent pregnancy, uterine pathology, persistent ovarian inactivity, or farmer withdrawal. Mean BCS stood at 2.9 ± 0.5 and subclinical anemia (HCT <30%) was found in 37.7% (49/130) of animals tested. The overall d-35 pregnancy rate reached 81.9% (376/459), though district-level variation was considerable, ranging from 70.9% to 94.6% ($P < 0.05$). Protocol 3 returned the best outcome at 90.0% (45/50), statistically superior to both Protocol 1 (79.3%, $P = 0.038$) and Protocol 2 (80.7%, $P = 0.045$). Among the 387 non-pregnant cows with follow-up records, 12.7% returned to estrus. Nutritional deficiency, subclinical anemia and uncontrolled bull exposure emerged as the most actionable barriers to FTAI success. Matching synchronization protocols to ovarian status at the individual animal level and adding eCG in nutritionally compromised cows, produced an overall pregnancy rate of 81.9% in a true field setting. To our knowledge, this represents the first large-scale field evidence that individualized protocol selection can drive meaningful gains in buffalo reproductive efficiency in Southeast Asia.

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Prevalence of Hematological Dysregulation in a Cohort of Thai Swamp Buffaloes: Insights into Red Blood Cell Morphology, Leukocyte Distribution and Platelet Characteristics

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Comprehensive hematological surveys of erythrocyte morphology, leukocyte differential and platelet characteristics in Thai swamp buffalo (*Bubalus bubalis*, 2n=48) under traditional smallholder management are absent from the literature. This study described the prevalence and pattern of hematological dysregulation across all blood compartments in a cohort of smallholder-owned Thai swamp buffaloes in Northeast Thailand.

A cross-sectional survey was conducted in 130 Thai swamp buffalo cows from Nong Han (n=75) and Thung Fon (n=55) districts using an automated hematology analyzer. Animals were maintained under traditional extensive management: communal pasture grazing, no nutritional supplementation and irregular anthelmintic treatment. Erythrocyte parameters were used to classify anemia type and regenerative status. Leukocyte differential and platelet parameters were compared against published reference intervals.

Mean values: RBC 5.36×10^{12}/L (range 3.5–8.2), HGB 10.99 g/dL (range 6.7–17.8), HCT 32.4% (range 20.3–55.1), MCV 60.8 fL (range 45.1–74.7), PLT 184.9×10^{9}/L (range 43–463). Anemia (HCT <30%) was present in 49 animals (37.7%): mild in 36 and moderate in 13, nadir HCT 20.3%/HGB 6.7 g/dL. Morphological classification revealed normocytic anemia (87.8% of anemic animals), consistent with anemia of chronic disease; microcytic anemia (MCV <50 fL, minimum 45.1 fL) in 5 (10.2%) indicating chronic blood loss; and macrocytic anemia in 1 (2.0%). Regenerative response (NRBC% >5%) was confirmed in 4 animals; 45 anemic animals showed non-regenerative patterns. Neutrophilia (NEU% >81%) was detected in 23 animals (17.7%), lymphopenia (LYM% <12%) in 36 (27.7%), and lymphocytosis (LYM% >33%) in 17 (13.1%). Eosinophilia (EOS% >10%) was identified in 7 animals (5.4%) and leukopenia in 14 (10.8%). Thrombocytopenia (PLT $<90\times10^{9}$/L) was present in 13 animals (10.0%), lowest at 43×10^{9}/L, and thrombocytosis (maximum 463×10^{9}/L) in a minority.

Hematological dysregulation is highly prevalent across all blood compartments. Population-level means mask individual-level pathology in over half the cohort, underscoring the importance of individual CBC screening rather than herd-average assessment in field health programs. When abnormal hematological results are identified, CBC findings should always be followed up with further investigations, including metabolic profile assessment and screening for infectious diseases, to establish a definitive diagnosis and guide appropriate management.

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Taurine Content in Buffalo Milk versus Holstein Cow Milk: Compositional Superiority, Molecular Mechanisms, and Processing Stability

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This study systematically evaluated the taurine content in raw, pasteurized, and ultra-high-temperature (UHT) processed buffalo milk and in Holstein cow milk, with a total of 8 Holstein cow milk samples and 18 milk samples from each of the three major buffalo breeds (Nili-Ravi, Murrah, Mediterranean) included in the analysis. The study investigated the underlying molecular mechanisms and assessed intrinsic variation factors. The results demonstrated that buffalo milk contains a significantly higher taurine concentration (4.6 mg/100 g milk) compared to Holstein cow milk (0.6 mg/100 g milk), representing an approximately 8-fold difference ($p < 0.01$). Assessment of intrinsic factors revealed that taurine content in buffalo milk changed dynamically across lactation stages, peaking at mid-lactation, whereas no significant differences were observed among the three main buffalo breeds (Nili-Ravi, Murrah, Mediterranean). Management practices across different farms showed a modulating effect on taurine content. Pasteurization exhibited no significant effect on taurine content in either milk type. However, while UHT treatment significantly reduced taurine levels in Holstein milk, it had no significant effect on taurine content in buffalo milk, highlighting its superior processing stability. Quantitative proteomics (DIA) revealed the molecular basis for this enrichment, identifying three markedly upregulated proteins in buffalo milk: thiosulfate sulfurtransferase (TST, FC = 185.9), flavin-containing monooxygenase 2 (FMO2, FC = 48.1), and glutathione peroxidase 1 (GPX1, FC = 13.8). These proteins form a synergistic network supplying the precursor cysteine, catalyzing the final oxidation of hypotaurine, and maintaining redox homeostasis. Furthermore, differential proteins were significantly enriched in pathways related to “sulfur compound metabolism,” “taurine and hypotaurine metabolism,” and “glutathione metabolism.” Collectively, buffalo milk represents a premium raw material for functional dairy products owing to its naturally high taurine content, excellent thermal stability, and unique molecular synthetic network.

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Lignin Degradation Capability and Pathways of *Aspergillus fumigatus* Isolated from Buffalo Rumen

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Buffaloes are the only mammalian species reported to date with lignin-degrading capability, which is closely related to their rumen microbes, especially fungi. In this study, medium with sodium lignosulfonate as the sole carbon source was used to isolate fungi from buffalo rumen fluid, and their lignin-degrading capacity were investigated.

Buffalo rumen fluid was collected via rumen fistula and subjected to anaerobic screening. Based on ITS and 28S rRNA sequence analyses, the fungal strain was identified as *Aspergillus fumigatus*. Following cultivation in enzyme-producing medium and 30-day anaerobic fermentation, acetyl esterase (AE) , feruloyl esterase (FE) and lignin-degrading ability were assayed. Furthermore, whole-genome sequencing and GC–MS were employed for functional gene analysis and metabolomic profiling.

The *Aspergillus fumigatus* strain achieved a maximum degradation rate of 15.12% for sodium lignosulfonate. Enzymatic assays confirmed that the strain secreted AE and FE. The maximum AE activity was 1.59 U/mL, and FE activity peaked reaching 0.033 U/mL. Following 30 days of anaerobic incubation with corn and rice straw as substrates , the degradation rates of acid detergent fiber reached 26.66% and 17.80%, those of cellulose were 24.39% and 20.66%, and acid detergent lignin were 29.35% and 33.29%, respectively.

Whole-genome sequencing of *Aspergillus fumigatus* identified 29 genes encoding lignin-degrading enzymes, including multicopper oxidases, lignin-modifying peroxidases, oxidoreductases, monooxygenases, and feruloyl esterases. Metabolomic and degradation products analyses revealed that lignin degradation by this strain through six metabolic pathways: galactose metabolism, phenylalanine metabolism, pyruvate metabolism, benzoate degradation, toluene degradation, and aminobenzoate degradation. Fumaric acid, 3-hydroxybenzoic acid, and 3,4-dihydroxybenzoic acid were identified as the key metabolic intermediates in this process.

This study confirms that *Aspergillus fumigatus* in buffalo rumen degrade lignin via its enzymatic systems and core metabolic pathways, and further suggests that the buffalo rumen represents a rich microbial reservoir with lignocellulose-degrading potential.

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Neddylation inhibition by MLN4924 modulates cell proliferation and secretory function in buffalo mammary epithelial cells

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Neural precursor cell expressed developmentally downregulated 8 (NEDD8) is a highly conserved ubiquitin-like protein that regulates diverse cellular processes through neddylation. MLN4924, a specific neddylation inhibitor, is well-documented for inducing cell cycle arrest and apoptosis in tumor cells, as well as regulating embryonic development [1, 2]. However, the precise impact of MLN4924-mediated neddylation inhibition on the proliferation and differentiation of buffalo mammary epithelial cells (BMECs) remains unexplored.

This study aimed to evaluate the effects of MLN4924 on the proliferation, cell cycle, and secretory functions of BMECs. Results showed that NEDD8 is widely expressed in various buffalo tissues, with relatively high expression levels in the mammary gland, and is distributed in both the nucleus and cytoplasm of BMECs. After treatment with 1000 nM MLN4924 for 48 h, BMECs exhibited a distinctly hypertrophic morphology. MLN4924 treatment markedly reduced the cell number and induced cell cycle arrest at the G1/S checkpoint. Notably, MLN4924 treatment enhanced the secretory capacity of BMECs, as evidenced by increased levels of α-casein, β-casein, and κ-casein. Furthermore, MLN4924 treatment significantly elevated triglyceride secretion and intracellular lipid accumulation. These findings suggest that inhibiting neddylation prompts BMECs to exit the cell cycle and enter a highly active lactogenic state.

In conclusion, MLN4924-mediated inhibition of neddylation suppressed BMEC proliferation, while enhancing milk protein and lipid synthesis. These findings suggest that neddylation plays a crucial role in regulating mammary epithelial cell function and provide new insights into the molecular mechanisms underlying lactation in buffalo.

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Phenotypic Characterization of Greek Indigenous Buffalo

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The aim of this study was to assess the phenotypic characteristics of the Greek indigenous buffalo population (*Bubalus bubalis*) reared in the Lake Kerkini area, Greece. This population represents an important local genetic resource with limited available phenotypic data. A total of 100 primiparous female buffaloes were randomly selected from six representative farms and individually measured between May 2019 and August 2020. The evaluation included morphometric body traits, horn characteristics, and coat and limb coloration, using a measuring tape and a caliper. All animals exhibited backward-oriented horns (100%), with most presenting a sickle shape (89%) and medium size (95%). Front and hind limbs were predominantly black (88% and 99%, respectively), while hooves were mainly brown (70%) or showed black streaks (30%). The main morphometric measurements (mean ± SD) were: withers height 135.1 ± 4.92 cm, thoracic perimeter 217.93 ± 20.09 cm, body length 108.77 ± 7.56 cm, pelvis length 40.43 ± 3.51 cm, rump width 49.59 ± 4.26 cm, thigh width 56.85 ± 5.10 cm, head length 47.68 ± 1.87 cm, ear length 24.08 ± 2.74 cm, horn length 42.66 ± 7.71 cm, and horn perimeter 20.62 ± 1.29 cm. These findings provide baseline phenotypic data for the Greek indigenous buffalo population, for which limited information is currently available. The recorded morphometric traits may support future selection, breeding, and conservation strategies under the specific environmental and production conditions of the Lake Kerkini system.

Keywords: *Bubalus bubalis*, indigenous buffalo, morphometry, body measurements

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Effect of age at first calving on productive and reproductive performance in the first lactation of Murrah buffalo in an improved system in Venezuela

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The effect between age at first calving and milk production in buffaloes of the Murrah breed in an improved system in the lake basin of Zulia State towards the eastern coast of Venezuela was analyzed. Historical data from 552 buffaloes that calved between 2015 and 2024, aged between 30 and 39 months, were evaluated. The GLM procedure of the SAS statistical analysis program was used to perform the statistical analysis. The results showed that as the age at first calving increases, adjusted milk production at 270 days decreases significantly. Buffaloes that calved between 30 and 34 months produced more total milk per lactation compared to those that calved between 35 and 39 months. A significant difference ($P<0.05$) was observed in daily milk production per lactation according to age at first calving, but no significant effect ($P>0.05$) was found for the year of calving. Total daily milk production was affected by age at first calving, with buffaloes calving between 30 and 34 months showing higher production compared to those calving between 35 and 39 months. The dry period was also influenced by age at first calving, with significant differences between buffaloes that calved at 30 and 34 months, with respect to buffaloes calving between 35 and 39 months. In the reproductive variables, a significant difference ($P<0.05$) was found in the interval between calving's according to age at first calving, buffaloes given birth at an age between 30 and 34 months presented an interval of 3.52 months less compared to buffaloes that performed their first calving between 35 and 39 months of age. No significant interaction was observed between age at first calving and year of calving for the variables analyzed. This study shows that age at first calving has a significant effect on milk production in buffaloes of the Murrah breed in the area under study. Based on the results obtained, it is recommended to maintain good nutritional management for future replacement females in order to reach an early age of first calving between 30 and 34 months, which would have an impact on productive and reproductive efficiency in buffalo farms.

Keywords: Murrah, milk production, calving interval.

Comparison of two systems of buffalo production in Zulia state Venezuela

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This paper presented a technical and comparative analysis of two buffalo production systems with Murrah breed predominance. The farms shared genetics and agro-ecological conditions (1800-2000 mm of precipitation), which makes it possible to isolate the effect of technical, nutritional and administrative management on productive and reproductive yields. Farm 1 had an area of 500 hectares with 650 heads; Farm 2 had a surface area of 556 hectares and 725 heads. Farm 1 used Voisin Grazing (PRV); Farm 2 used conventional rotational grazing. Birth Rate: Farm 1 (95%) vs. Farm 2 (97.3%). The interval between calvings, farm 1: 401 days; Farm 2: 420 days. Gestation: Farm 1 (310 d); Estate 2 (308.9 d). Farm 1 performed early gynecological evaluation (25-30 days postpartum); Farm 2 performed gynecological check-ups three months postpartum. Farm 1 had Mechanical Milking without breeding/supplementing buffaloes; Farm 2 had manual milking and support with breeding. Daily milk production, Farm 1: 7.85 kg/buffalo; Farm 2: 6.30 kg/buffalo. Duration of Lactation, Farm 1: 255 days; Farm 2: 272 days. Total Production/Lactation, Farm 1: 2,001.75 kg; Farm 2: 1,713.60 kg. Milk/Cheese Ratio, Farm 1: 5.1; Farm 2: 5.4. Total Solids, Farm 1: 18.2%; Farm 2: 17.9%. Farm 1 produced 288 kg more milk per lactation/buffalo. Farm 1 required 300 ml less milk to make 1 kg of cheese; This is explained by the use of concentrate (3 kg/day total) that improves the caloric density of the diet and the contribution of solids precursors in milk. For the growth of offspring and fattening, the use of concentrate (3/4 kg) in Farm 1 showed a significant impact on weaning weight. Female weaning: Farm 1 (179 kg) vs. Farm 2 (167.1 kg), +11.9 kg of difference. Weaning males: Farm 1 (198 kg) vs. Farm 2 (185.1 kg), +12.9 kg of difference. Completion of breeding cycle, Farm 1: slaughter weight 485.5 kg at 22.8 months; Farm 2: slaughter weight 490.2 kg at 25.12 months. Farm 1 saves 2.32 months of handling to bring animals to market. Despite the fact that the same genetics are shared by the 2 farms, the constant presence of the owner, recurrent veterinary advice and the investment in animal nutrition on Farm 1 was significantly more efficient and productive than Farm 2.

Regenerative water buffalo production in the NEA region, Argentina: holistic management for productive efficiency and ecosystem restoration

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Abstract Water buffalo (*Bubalus bubalis*) production in the Argentine Republic is positioning itself as a vanguard model through the adoption of regenerative ranching and holistic management, with potential across more than 8 million hectares of wetlands. Buffalo production is key to sustainable livestock farming in the NEA region, offering high profitability, social equity, and the rehabilitation of wetland areas with historically low productivity indices. Mixed cattle-buffalo models have demonstrated superior productive and economic results for the latter, with projections to exceed 430,000 head of buffalo in Argentina over the next decade. This paper characterizes the current state of the sector, highlighting the transition from extractive systems toward management frameworks that integrate ecological, social, and economic dimensions within mixed livestock systems. The objective is to demonstrate how the buffalo, under a grazing planning focus centered on pasture recovery periods rather than stocking rates, acts as a critical tool for the restoration of degraded ecosystems. Results indicate that implementing these principles facilitates soil rehydration and the reconstruction of the "carbon sponge," promoting biodiverse and biologically active systems with fungal dominance. In the NEA region, the integration of trees through silvopastoral schemes enhances climate resilience and animal welfare. While beef production remains the primary axis—offering high potential for strategic substitution to release exportable cattle surpluses—buffalo dairying is emerging as a high-profit component for diversifying the productive matrix. The feasibility of certifying and adding value to the resulting product is emphasized, specifically as high-protein natural meat with the potential to regenerate environments by sequestering atmospheric CO2 and incorporating it into the soil. This sequestration enables access to voluntary carbon markets, mitigating greenhouse gas impacts while generating economic revenue for producers practicing this management. It is concluded that buffalo ranching under holistic management not only optimizes forage conversion efficiency in marginal environments but also drives a regenerative economy that values rural settlement and local culture. Argentina thus presents itself as a triple-impact investment scenario, where premium red protein and dairy production converge with landscape regeneration, positioning the country as an international benchmark for sustainable and resilient livestock systems in the 21st century.

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Water buffalo production and territorial development in Argentina: productive concentration and value chain limitations

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Water buffalo production in Argentina has shown sustained growth in recent years, reaching a national stock of 202,752 head. However, this expansion is characterized by a strong territorial concentration, where the provinces of Corrientes (46.82%), Formosa (21.56%), and Chaco (14.78%) account for 83.16% of the total. The herd structure shows a predominance of female categories, indicating a system primarily oriented toward reproduction and expansion.

A comparative analysis between 2016 and 2025 reveals significant stock increases across most provinces; however, this has not resulted in substantial changes in territorial distribution, as concentration levels remain virtually constant (83.48% in 2016 and 83.16% in 2025). This suggests that the system's growth has not been accompanied by a process of spatial decentralization, with the Argentine Northeast remaining the productive core.

Despite the increase in livestock inventory, slaughter levels remain low (2,315 head) and exhibit a territorial distribution distinct from that of the stock. Provinces with the highest livestock concentration, such as Corrientes, record low participation in slaughter (13%), while provinces with smaller stocks, such as Buenos Aires, concentrate a significant proportion of processing (25%). The analysis of the relationship between slaughter and inventory reveals marked territorial disparities, with values ranging from 0.31% in Corrientes to 15.30% in Buenos Aires.

These results highlight a territorial dissociation between production areas and slaughter centers, suggesting the existence of interregional flows of live animals toward zones with greater processing capacity. This dynamic reflects structural limitations in the integration of the buffalo value chain, particularly in the primary producing regions, where low local industrialization capacity may imply a loss of added value at the source. Consequently, strengthening the articulation between production and industry is key to enhancing the sector's contribution to territorial development and the future expansion of the meat market.

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Bacterial DNA in follicular fluid: a possible player in buffalo oocyte competence?

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This work investigated bacterial DNA in buffalo follicular fluid (FF) and its association with oocyte competence. In laboratory, FF was aseptically collected from abattoir-derived individual ovaries, centrifuged and stored at -80°C, while the COCs underwent *in vitro* embryo production. Blastocyst yield (BY) was assessed and FF retrospectively classified as GOOD (BY>35%) or BAD (BY<10%). Five FF samples/group and two controls (CTR) were analyzed. DNA was extracted by GeneJET Genomic DNA Purification Kit (Thermo Scientific) according to the manufacturer's instructions. The 16S rRNA V3-V4 gene region amplification and library preparation were performed using a two-step PCR approach as described in Illumina's application note. Sequencing was performed on MiSeq (Illumina). Beta diversity was computed by Bray-Curtis and RPCA methods and differences between groups were evaluated by PERMANOVA and PERMDISP tests. Significance was set at $p<0.05$. The ANCOM-BC test was used to identify specific differential abundance in bacterial taxa. Significance was set at $q<0.05$. High bacterial DNA was found in both the CTR and the FF, with a qualitative difference in both Bray-Curtis and RPCA distances (RPCA method: PERMANOVA p=0.006; PERMDISP p=0.641). A distinct profile was observed between CTR and FF ($q<0.05$) including *Gemella, Actinobacillus, Aggregatibacter, Fusobacterium, Cutibacterium* and *Staphylococcus* genera. The microbial composition differed between GOOD and BAD at the genus level using Bray-Curtis (PERMANOVA, p=0.013, PERMDISP, p=0.109) but not RPCA (PERMANOVA, p=0.478). The genus *Asinibacterium* was enriched (q=0.022) in BAD compared to GOOD group (log-fold change=1.55). These results suggest that, despite a high technical background, FF may contain a distinct microbial population. Moreover, Bray–Curtis separation suggests that differences between GOOD and BAD are driven by specific bacteria rather than the global microbial community. Indeed, the FF of non-competent oocytes was enriched in *Asinibacterium*. The unexpected abundance of bacterial genera in CTR may have masked differences in the FF microbiome. In conclusion, the results support the hypothesis of a specific FF microbiome associated with oocyte competence. The detection of signals in CTR is a technical constraint warranting cautious interpretation. Consequently, future research should focus on improved sampling protocols to minimize environmental noise and identify bacteria at the species level.

Environmental Services and Conservation Challenges of Swamp Buffalo in Diverse Agroecosystems of Indonesia

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Swamp buffalo in Indonesia play important roles in food production, rural livelihoods, and cultural systems across diverse ecosystems. However, their environmental services remain underexplored compared to their economic and social functions. This study aimed to (1) identify the environmental services of swamp buffalo and (2) analyze conservation challenges and potential community-based strategies across three agroecosystems (swamp area in South Kalimantan, mountainous area of Gayo in Aceh, and dryland area of Tapanuli in North Sumatera). This study employed a literature review, expert consultations, and validation through Focus Group Discussions (FGDs) with relevant stakeholders. SWOT and historical timeline analyses were applied during the FGDs. Results show that swamp buffalo provide distinct ecological services across regions. In South Kalimantan, grazing reduces sedimentation in swamp areas. In Gayo Lues, buffalo activities help maintain hilly landscapes and offer potential for low-impact ecotourism. In Tapanuli, buffalo contribute to nutrient cycling through manure, supporting integrated farming systems. However, buffalo systems face multiple challenges, including declining populations and productivity, limited grazing land, seasonal feed shortages, land-use change, habitat degradation, and conflicts over land use. Other constraints include weak institutions, limited access to capital and technology, and low farmer capacity. The study proposes transitioning toward semi-intensive production systems, strengthening community institutions, and promoting ecotourism-based strategies to support sustainable buffalo management.

Keywords: swamp buffalo; ecological function; community-based management; production systems transformation.

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Validation of an ear-tag accelerometer for monitoring rumination and feeding behaviour in dairy buffaloes

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Precision livestock farming (PLF) technologies are increasingly used to support individual monitoring and management of dairy animals. Despite the economic importance of dairy buffalo production in Southern Italy, the adoption of PLF tools in this species remains limited, particularly compared with cattle. Ear-tag devices are less invasive than other wearable sensors and may be better suited to the reactive temperament of buffaloes. This study evaluated the accuracy of an ear-tag accelerometer (SenseHub, Allflex/Merck Animal Health) for measuring rumination and feeding behavior in buffaloes under commercial farm conditions. Sixteen buffaloes at different physiological stages (n = 5 early lactation, DIM 19 ± 10; n = 7 late lactation, DIM 194 ± 26; n = 4 dry period) were monitored on a dairy farm in Southern Italy between spring and summer 2025. Overall, 240 h of behavioral observations were conducted in 3-h sessions from 07:00 to 19:00, excluding time spent in the milking parlor. This included 111 h in spring and 129 h in summer, for a total of 80 days of observations. Visual observations, used as the reference method for validation, were conducted at one-minute intervals by two trained observers to identify rumination and feeding behavior. The agreement between the two observers was assessed prior to data collection and showed almost perfect agreement ($\kappa = 0.81–1.00$). Agreement between observer- and sensor-derived data was assessed using Intraclass Correlation Coefficient (ICC), and Bland–Altman analysis. During the study, three devices were replaced due to identified operational malfunctions. Average rumination and feeding times were 528 and 131 min/day, respectively, likely reflecting reduced daytime feeding activity during summer. Both rumination (ICC = 0.953) and feeding (ICC = 0.917) showed excellent agreement between methods. Bland–Altman analysis indicated minimal systematic bias (rumination: +0.225 min/h; feeding: –0.296 min/h). No evidence of proportional bias was detected. Overall, the ear-tag accelerometer provided estimates of rumination and feeding time comparable to those obtained through direct visual observation in buffaloes maintained under commercial conditions. The possible influence of environmental temperature on feeding behavior warrants further investigation across a wider range of farm systems and climatic conditions, particularly regarding its potential implications for health and welfare monitoring.

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Effect of timing for GnRH administration after CIDR removal on ovulation, corpus letuem development, and pregnancy in recipient embryo transfer buffaloes

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Embryo transfer facilitates the selective breeding of buffaloes with superior genetic traits, improving milk quality and enhancing herd productivity. The objective of this study was to determine the effect of administering GnRH at 84 hours after CIDR removal in a 7-day CIDR Cosynch protocol on ovulation, serum ovarian steroids, metabolome, and pregnancy per embryo transfer (P/ET) in recipient buffaloes. Postpartum buffaloes DIM (104 ± 0.6) were selected and synchronized using a 7-day CIDR-Cosynch to become recipients for embryo transfer. The recipient buffaloes were randomly assigned to receive GnRH either at 72 (GnRH-72 n = 119) or 84 hours (GnRH-84, n = 117) after CIDR removal. Follicular dynamics, serum concentration of estradiol (E2), progesterone (P4), and metabolome were determined. Embryos were transferred on day 6, and pregnancy was diagnosed on 30 and 60 days after estrus. Animals treated with GnRH-84 had larger follicle diameter at 84, 90, and 96 hours and greater preovulatory follicle size than the GnRH-72 treatment. E2 was higher at 84 and 90 hours, whereas P4 was elevated at time points 120, 168, and 216 hours after CIDR removal in the GnRH-84 treatment. Short intervals between GnRH administration to ovulation with greater CL diameter and higher P/ET were observed in the GnRH-84 treatment. Serum collected at 78 and 90 hours after CIDR removal revealed 82 and 140 differentially expressed metabolites. Enrichment pathways analysis of metabolites time point 78, affected by the treatments, revealed an association with energy production and vitamin B6 metabolisms, and time point 90, affected by the treatments, revealed an association with energy production, vitamin B6, porphyrin, and steroidogenesis metabolisms. In conclusion, the effect of timing for GnRH administration at 84 hours after CIDR removal increases the size of the preovulatory follicle and corpus letuem, synchronized ovulation resulting in greater progesterone and pregnancy maintenance.

Keywords: Buffalo, CIDR, Corpus letuem, Embryo transfer, GnRH, Metabolome

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Serum biochemical profile of cattle and buffalo fed whole shelled corn or conventional concentrate diets

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Fluctuations in blood metabolites across a feeding cycle are well recognized in ruminants; however, the interaction between diet type and species remains poorly understood. This study investigated serum and plasma biochemical profiles in cattle (BOV, n=11) and buffalo (BUB, n=11) after 64 days on either a roughage-free whole shelled corn diet (WSC: 85% intact corn kernels + 15% protein-mineral supplement) or a conventional concentrate diet (CON: 70% ground corn concentrate + 30% corn silage). Blood samples were collected before feeding and every 4 hours over an 18-hour period at 7, 11, 15, 19 and 23 hours. Data were analyzed using mixed models (PROC MIXED, SAS), with covariance structure selected by AIC/BIC. Species differences were significant ($p < 0.05$) for eight variables: buffalo had higher creatinine (155.7 vs 120.5 µmol/L), AST (79.2 vs 45.5 U/L), magnesium (1.82 vs 1.33 mmol/L) and lactate (4.11 vs 2.59 mmol/L) than cattle, reflecting inherent interspecific metabolic divergence. Diet significantly affected urea, which was lower in WSC than CON animals (11.4 vs 17.7 mg/dL; p=0.001), consistent with the slower ruminal starch fermentation rate of intact corn kernels reducing ammonia absorption and hepatic urea synthesis. Sampling time was the main factor, affecting 12 of 18 variables, especially NEFA ($p < 0.001$), GGT ($p = 0.0002$), and phosphorus ($p = 0.0002$). Diet×Time interactions were detected for creatinine (p=0.038) and lactate (p=0.015). These findings demonstrate that postprandial metabolic status strongly influences biochemical parameters regardless of species or diet, highlighting the need to standardize blood sampling time in ruminant studies.

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Contrasting ecological drivers of *Rhipicephalus microplus* and *Amblyomma* spp. infestation in Amazon livestock

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Rhipicephalus microplus and *Amblyomma* spp. co-occur in Amazon livestock, yet their epidemiological drivers are rarely analysed separately. This study compared their prevalence and associated factors in animals slaughtered in western Pará, Brazil, to determine whether genus-level analyses reveal distinct ecological patterns. An active surveillance study with systematic weekly sampling was conducted from December 2023 to April 2026, examining 10,789 animals (10,161 cattle; 628 buffaloes) from 499 farms across 17 municipalities from western Pará state, Amazon region. *Amblyomma* spp. presence was recorded separately from *R. microplus*. Multivariable logistic regression was performed independently for each genus with season and host species as predictors. *R. microplus* prevalence was 28.29% (95% CI: 27.44–29.15%), while *Amblyomma* spp. prevalence was markedly lower at 1.68% (95% CI: 1.45–1.94%). Cattle had 25 times greater odds of *R. microplus* infestation than buffaloes (OR = 25.03; 95% CI: 13.38–46.83; $p < 0.001$), whereas host species was not a significant predictor for *Amblyomma* spp. (OR = 1.64; 95% CI: 0.67–4.03; $p = 0.278$). Seasonal effects diverged sharply: the dry season modestly increased *R. microplus* odds (OR = 1.35; 95% CI: 1.24–1.48), but increased *Amblyomma* spp. odds 4.5-fold (OR = 4.52; 95% CI: 3.27–6.24). Monthly data confirmed this contrast: *Amblyomma* spp. prevalence rose from below 1% in rainy months to 4.57% in October (peak of dry season), while *R. microplus* showed a more gradual pattern (21.5% in April to 40.1% in October). The contrasting patterns reveal distinct ecological drivers: *R. microplus* infestation is primarily determined by host species, while *Amblyomma* spp. is driven by environmental seasonality, consistent with its dependence on wildlife reservoir hosts. These findings support genus-specific tick surveillance and control strategies in mixed livestock systems of the Brazilian Amazon.

Growth Performance and Feed Intake of Lao Native Buffaloes Fed Fermented Total Mixed Ration (FTMR) Inoculated with *Lactobacillus plantarum*

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This study evaluated the effects of *Lactobacillus plantarum* as an inoculant in fermented total mixed ration (FTMR) on voluntary feed intake and growth performance of Lao native buffaloes (*Bubalus bubalis*). Eight 2-year-old males (235 ± 35.51 kg) were assigned to non-inoculated or *L. plantarum*-inoculated FTMR (5 mg/kg), fermented for 14 days at room temperature and fed ad libitum over a 120-day period. Diet composition: DM 23.13 ± 0.66%, CP 14.40 ± 1.20%, NDF 49.80 ± 3.06%, pH 3.81 ± 0.42. Voluntary DM intake and ruminal pH (6.91 ± 0.21, measured 4 h post-feeding) did not differ between treatments ($p > 0.05$). However, inoculated buffaloes showed significantly greater total body weight gain (~188 vs. ~107 kg) and average daily gain (1.56 vs. 0.89 kg/head/day) ($p < 0.05$). These findings demonstrate that *L. plantarum* inoculation substantially improves growth performance in Lao native buffaloes without altering feed intake or rumen pH, suggesting its practical value as a cost-effective additive for enhancing beef production efficiency in smallholder farming systems.

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Venous blood gas parameters and acid–base values in newborn hypoglycemic buffalo calves with added sweet sorghum

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Neonatal hypoglycemia is a serious metabolic disorder that may compromise adaptation and survival in newborn buffalo calves during the early postnatal period. Assessment of venous blood gas parameters provides valuable information on acid–base balance and metabolic disturbances associated with early hypoglycemia. The aim of this study was to monitor clinical status, blood glucose concentrations, and venous blood gas parameters in newborn buffalo calves with early-onset hypoglycemia following administration of a solution prepared from pre-hydrolyzed sweet sorghum (Sorghum bicolor) juice. The study included 10 buffalo calves with venous plasma glucose concentrations below 2.0 mmol/L, determined between 2 and 5 h after birth. Clinical status was assessed using a modified APGAR scoring system, and rectal temperature was measured during the first hour. The animals received a 5% solution of pre-hydrolyzed sweet sorghum juice at a dose of 2 mL/kg body weight twice daily for three days. At the initial sampling, the mean venous pH was 7.249, and the mean lactate concentration was 6.49 mmol/L. By day 3, nine of the ten buffalo calves had survived. In the surviving animals, clinical scores and rectal temperature increased, venous pH rose, and lactate concentrations decreased. The mean glucose concentration reached 1.97 mmol/L but remained below 2.0 mmol/L. The results indicate clinical improvement and partial metabolic recovery in surviving buffalo calves during the three-day observation period. However, in the absence of an appropriate control group, the efficacy of the administered preparation cannot be conclusively established.

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Growth rates and health of un-weaned dairy buffalo calves in partial cow-calf contact system

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Prolonged cow-calf contact (CCC) is of growing importance to the dairy sector, given the increasing societal interest in animal welfare, however, implementation of CCC in larger sized farms require significant research and technical efforts. The aim of the current research was to comparatively evaluate growth rates and health parameters in water-buffalo calves raised under partial cow-calf-contact (CCC) and conventionally. The study was conducted at the Research and Development Station for Buffalo in Sercaia – Romania, with data being collected for 6 consecutive years (2020-2025). The study herd included a total number of 542 calves, of which 136 calves were reared under partial CCC conditions (reunited with dam twice per day, during milking, and allowed to suckle 2 quarters of the udder), and 406 calves were raised conventionally (CONV, separated 2 days following birth, and bucket fed 4 litres of milk/day). Our analysis was focused on growth rates and health of calves from birth till 3.5 months of age, taking into account sub-sets based on dams age (young dams 3-5 yrs.; mid age dams 6-10 yrs. and older dams of 11+ yrs.). Mann–Whitney U test was performed to determine the relationships between the production system. Average daily gains were significantly higher ($P \leq 0.001$) in CCC calves, with an average of 396.65±1.93 g/day, compared to calves raised conventionally, which had growth rates of 329.97±1.46 g/day. Within the CCC system, the age of the dams had no influence ($P > 0.05$) on growth rates of calves. Enteritis incidence was on average 14.71% in CCC calves, and slightly lower, of 13.30% in CONV calves group, with differences being not significant ($P > 0.05$). For pneumonia incidence, the highest of 9.36% was recorded in the CONV group, with the CCC raised buffalo calves having an incidence of 7.35%, the difference not being statistically significant ($P > 0.05$). The slightly higher enteritis rate in CCC calves could be attributed to the lack of control from the farmer on the milk intake of calves suckling from their dams directly, thus, the more voracious eaters might consume higher than needed quantities or the intake rate could be the cause, with similar reports being observed in dairy cattle CCC systems. Age of the dam in CCC system had no influence on growth performance or the health parameters of un-weaned buffalo calves, thus farmers that wish to transition to CCC systems, could use including less experienced primiparous cows.

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Field Fertility Rates of Thai Swamp Buffaloes Following a Scheduled Synchronization Program: Insights from the Udon Thani Livestock Development Project, Thailand

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Field fertility data from fixed-time artificial insemination (FTAI) programs in Thai swamp buffalo (*Bubalus bubalis*, 2n=48) under smallholder conditions remain limited. This study evaluated the feasibility of a scheduled synchronization and FTAI program across 9 districts within a single breeding season, and assessed pregnancy rates confirmed by B-mode transrectal ultrasonography.

Between December 2025 and April 2026, 571 Thai swamp buffalo cows from 299 smallholder farmers underwent pre-synchronization assessment including B-mode transrectal ultrasonography, body condition scoring (BCS), and uterine evaluation. Eligible animals (BCS ≥2.5, active ovaries, no uterine pathology) were assigned to standard Ovsynch (GnRH–$PGF_2\alpha$–GnRH–FTAI), CIDR-based Ovsynch, or Ovsynch supplemented with 330 IU equine Chorionic Gonadotropin (eCG). Frozen-thawed semen from 9 certified Thai swamp buffalo sires was used. Pregnancy was confirmed by ultrasonography at day 35 post-FTAI. Non-pregnant animals identified at day 18–24 return-to-estrus check received re-insemination (AI2).

Of 611 cows examined, 459 (77.6%) received FTAI across 9 districts within five months, exceeding the initial target of 500 cows in 6 districts. Mean herd BCS was 2.87±0.52. Pregnancy confirmed at day 35 yielded 376 pregnancies from 459 inseminated animals, an overall conception rate (CR) of 81.9%, substantially exceeding the published benchmark of 33–47% for standard Ovsynch in tropical swamp buffalo. District-level CR ranged from 70.9% (Thung Fon) to 94.6% (Chaiyawan). Return-to-estrus rate across 7 districts (n=387) was 12.7%; 83 animals received AI2. The highest RTER of 45.5% (Wang Sam Mo; BCS 2.65±0.45) corresponded with the lowest single-service CR, yet AI2 effectively rescued non-pregnant animals across all districts.

A scheduled FTAI program covering 459 smallholder-owned Thai swamp buffaloes across 9 districts within five months is operationally feasible and delivers an overall pregnancy rate of 81.9% confirmed by day-35 ultrasonography. Systematic AI2 following return-to-estrus monitoring is critical to achieving high cumulative pregnancy rates under smallholder field conditions in Northeast Thailand.

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Oligothelia in two buffaloes with different effect on morphology, productive and reproductive performance – a case report

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Various mammary abnormalities have been observed in the buffalo, a study in India finding that the incidence of oligothelia (two teats) was as high as 8.9%, polythelia – 26.7%, and fused teats – 2.2%. In addition, several cases of only two mammary teats have been also reported, including a case of this abnormality observed also in the proband's daughter, implying inheritance.

The present paper is to report an unprecedented case in the Bulgarian Murrah breed of a buffalo heifer with two mammary teats only (Buffalo #1), followed by another after 5 years (Buffalo #2).

Except clinical examination by inspection and palpation, mammary ultrasound examinations were performed via SonoScape S2Vet ultrasound device (SonoScape, China) and a linear probe (5-10 MHz) set at 7 MHz. After visualizing the morphological structures in the mammary papilla and the udder, the following measurements were taken: teat canal length, teat diameter at the base, diameter of the milk cistern (at 4 cm above the teat tip), teat wall thickness (average of wall thickness measurements at two opposite points). Teat length was taken manually with a ruler.

Buffalo #1, which has 25% blood from the Italian Mediterranean breed, had cylindrical teats (unlike the usual Bulgarian Murrah teat shape). The abnormality of her udder was compensated by greater cisternal diameter (volume) and thicker papillary wall. In this way, the productivity was definitely not affected, performing like a normal udder – 8.8-9.2 kg initial daily milk in first-third lactation. First calving was at 1,205 days but the calving intervals were good – 340-430 days. Buffalo #1 has three normally developed offspring (two males and a female) with normal number of mammary buds. On the other hand, Buffalo #2 (pure Bulgarian Murrah), after calving a normally developed male, did not have any galactopoiesis and was not milked. Correspondingly, her teats had different morphology and histology – smaller cistern, thinner wall and conical exterior. If there is some genetic explanation of such udder malformation (as suggested in the literature), it is obviously not applicable to Buffalo #1 which is outbred. But as for Buffalo #2, even if it is low, the coefficient of inbreeding (Fx= 0.031) might have played an effect. To enlighten the phenomenon, proofs for the congenital predetermination and the triggering causes for such abnormal mammary conditions need to be studies.

Intestinal infections by zoonotic protozoa and associated risk factors in water buffalo farms in southern Italy

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Cryptosporidium spp. and *Giardia duodenalis* are major enteric protozoa affecting livestock worldwide, with increasing relevance in water buffalo (*Bubalus bubalis*) production systems. In water buffaloes, infections predominantly affect neonatal and young animals, causing diarrhea, dehydration, impaired growth, and consequent economic losses due to reduced productivity. Young animals represent the main source of environmental contamination through the shedding of oocysts and cysts.
Beyond their veterinary impact, these parasites represent a significant public health concern. Ruminants are recognized as key reservoirs for human infection, and although most studies have focused on cattle, growing evidence indicates that water buffaloes also contribute to environmental dissemination (de Aquino et al., 2020; Front Vet Sci 7:557967). The persistence of infective stages in water and their resistance to common disinfectants further amplify transmission risks at the human–animal–environment interface. Since 2025, a monitoring and control programme targeting these parasitic infections has been implemented in a highly endemic area of continental Southern Italy (Campania region). This programme combines innovative procedures designed to better understand and mitigate transmission dynamics. The main activities include: (i) parasitological analyses in water buffalo farms with neonatal diarrhea, including detailed analysis of risk factors associated with *Cryptosporidium* and *Giardia* infections; (ii) analysis of water sources used for animal drinking and milk reconstitution in positive farms; (iii) implementation of targeted biosecurity measures; and (iv) outreach and training activities for farmers and veterinarians. The present study provides an updated overview of the epidemiological situation in the Campania region of Southern Italy, confirming a high prevalence of these infections in buffalo farms. Overall, 68.6% of buffalo farms reporting neonatal diarrhea tested positive for at least one protozoan species. Among these, 53.1% were positive for *G. duodenalis*, 46.9% for *Cryptosporidium*, and 18.7% showed co-infections. These findings highlight the relevance of these parasites in buffalo farming systems and their potential public health impact, supporting the need for integrated control strategies within a One Health framework.

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Nutritional and technological characteristics of low-moisture buffalo meat

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Food preservation through dehydration and salting is an ancestral practice that remains relevant in modern meat processing systems. Charqui, a traditional dried meat product, has gained renewed interest due to the increasing demand for shelf-stable, high-protein foods with extended shelf life and reduced need for refrigeration. In this context, buffalo meat (*Bubalus bubalis*) represents a promising alternative due to its low fat content, high protein concentration, and favorable nutritional profile; however, its technological behavior during dehydration and smoking processes remains insufficiently documented. The aim of this study was to evaluate the nutritional and technological characteristics of low-moisture buffalo meat processed as smoked charqui under controlled conditions. Buffalo shoulder and eye round cuts were sliced (0.5 cm thickness) and subjected to two marination treatments for 15 h: a traditional formulation based on NaCl (6.0%) and sucrose (4.5%), and an industrial-style formulation containing NaCl (0.4%), brown sugar (1.5%), soy sauce (6.0%), Worcestershire sauce (3.0%), apple cider vinegar (5.5%), and spices (black pepper, onion, garlic, paprika, and chili). Samples were then dehydrated (8 h at 65 °C) and smoked (1 h at 75 °C). Moisture content was determined using the gravimetric method, cooking loss was calculated, and color parameters (L*, a*, b*) were measured with a Minolta CR400/410 colorimeter. Moisture values ranged from 26.27% to 34.07%, depending on cut and treatment, while cooking losses were considerable (44.75%–54.08%), reflecting structural modifications in the muscle matrix. Fresh samples showed typical color values (L*≈37–46; a*≈22), whereas dehydration induced marked darkening and a reduction in redness, particularly in samples treated with the industrial marinade. Smoking contributed to partial color stabilization, resulting in final values of L*≈22–25 and a*≈6–8. These findings indicate that buffalo meat exhibits suitable technological performance for charqui production, generating a stable low-moisture product with desirable preservation characteristics. The observed effects of cut type and processing conditions highlight its potential as a value-added raw material for the dehydrated meat industry.

Milk quality from Mediterranean and Murrah buffalo raised in wetlands of Corrientes, Argentina

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Buffalo milk is recognized for its high nutritional and technological value, mainly due to its elevated fat, protein, and total solids content. However, milk composition can be influenced by breed and environmental conditions, particularly in wetland production systems, where scientific information remains limited in Argentina. The aim of this study was to evaluate and compare the physicochemical quality of milk from Mediterranean and Murrah buffalo raised in wetlands of Corrientes, Argentina. Milk samples were collected in Paso Florentín (General Paz Department) from 15 Mediterranean and 15 Murrah buffalo. Animals were milked once a week after overnight calf separation. Milk yield and composition were evaluated, including protein, fat, lactose, pH, ash, density, and titratable acidity. Protein, lactose, and ash contents were determined using an ultrasonic milk analyzer (Milk-o-Test), density by lactodensimetry, titratable acidity by the Dornic method, pH using a Testo 205 pH meter, and fat content by butyrometry. Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey's test, with a significance level of 5%.

Mediterranean buffalo showed higher milk yield (7.28 ± 0.39 L) than Murrah (6.15 ± 0.39 L). Fat content was 5.49 ± 0.35% in Mediterranean and 5.07 ± 0.35% in Murrah. Protein (3.24 ± 0.04 vs. 3.23 ± 0.04%), lactose (4.84 ± 0.05 vs. 4.85 ± 0.05%), solids-not-fat (8.82 ± 0.10 vs. 8.81 ± 0.10%), density (1035.35 ± 0.27 vs. 1035.9 ± 0.27 kg/m³), acidity (17.33 ± 0.62 vs. 16.35 ± 0.62 °D), ash (0.72 ± 0.01%), and freezing point depression (−0.58 ± 0.01 °C) were similar between breeds. A significant difference ($p < 0.05$) was observed only for pH, with higher values in Murrah (6.74 ± 0.04) compared to Mediterranean (6.62 ± 0.04).

These results indicate that both breeds produce milk with comparable compositional quality under wetland conditions, with minimal breed-related differences. The overall composition confirms the high technological value of buffalo milk, while the observed pH variation could influence processing properties. These findings contribute to the characterization and valorization of buffalo production systems in the northeast of Argentina.

Total fatty acids in buffalo and goat cheeses: a comparison of some niche dairy products

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In recent years, the dairy sector has shown increasing interest in unconventional cheeses, driven by growing consumer awareness of product quality and food biodiversity. Alternative cheeses, often artisanal products linked to specific geographical areas, are gaining scientific and commercial relevance due to their distinctive compositional and sensory characteristics. Buffalo milk is mainly used for mozzarella cheese production; however, a lesser-known range of soft and semi-hard buffalo cheeses exists, showing valuable nutritional and compositional properties. Similarly, goat cheeses include numerous typologies appreciated for their high nutritional value and distinctive flavor profiles. Cheese identity is strongly influenced by its lipid fraction, which reflects the fatty acid composition of raw milk and undergoes modifications during processing and ripening, contributing to product specificity.

This study aimed to characterize and compare the total fatty acid profiles of selected buffalo and goat dairy products, highlighting interspecies differences and product-specific features. Two soft cheeses (stracchino and robiola), one semi-ripened cheese (caciotta), and a whey cheese (ricotta), produced in local dairies in Lazio and Campania (Italy), were analyzed, collecting 3 different cheeses of each type.

Significant differences were observed between species. Goat cheeses showed higher total saturated fatty acids (SFA) than buffalo cheeses (74.5 vs. 65.7%, $p<0.001$), mainly due to a greater proportion of short-chain SFA (22.3 vs. 5.1%, $p<0.001$), which are more rapidly metabolized and potentially easier to digest. In contrast, buffalo cheeses contained higher levels of monounsaturated fatty acids (MUFA) (30.1 vs. 21.3%, $p<0.001$), particularly cis-MUFA, associated with cardioprotective effects. Total PUFA content was similar between species; however, buffalo cheeses exhibited higher concentrations of conjugated linoleic acid (CLA), a bioactive fatty acid with anti-atherogenic and anti-carcinogenic properties. Instead, goat cheeses showed higher levels of n-3 cis PUFA, and consequently a lower n-6/n-3 ratio compared to buffalo cheeses (4.0 vs. 6.5, $p<0.001$). However, lipid profile with a lower n-6/n-3 ratio may also be positively influenced by animal feed. By highlighting the distinctive differences between buffalo and goat cheese, this work can contribute to the characterization and valorization of niche dairy products with high added value.

Genetic evaluation of the age of first calving in Murrah buffaloes in some states of Venezuela

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The early age at first calving (CFA) in buffalo farming is of utmost importance since it indirectly reflects the development process from birth in future replacement females, which will be reflected in the reproductive and productive behavior of the animals. The objective of the present study was to estimate the parameters and genetic values for age at first calving (CFA) with a predominance of Murrah breeds, in order to identify and select genetically superior buffaloes in the herds. Age records at first calving of 31505 buffaloes from 15 farms in several states of Venezuela between 1994 and December 2025 were considered. The effects were evaluated: farm (F); ecological zone (EZ): very dry tropical forest (BMST), humid forest (BH) and premontane humid forest (BHPM); year of birth (BY): from 1996 to December 2025; time of birth (BT): January-April, May-August and September-December. To perform the statistical calculations, the statistical analysis system SAS version 9.1 was used; for the estimation of the effects of the factors: farm, ecological zone, year of birth, time of birth, a mixed linear model was used; the components of variance and heritability were estimated using Univariate animal model and Restricted Maximum Likelihood (REML). On average, buffaloes presented the first calving at an early age (1050.08 ± 160.21 days; approximately 34,428 months of age) with a CV of 22.25%, being of great importance since the earlier the animal, the more it can produce during its reproductive life. The values of the F-tests for the factors farm, time of birth, year of birth and ecological zone were 22.12, 8.38, 11.65 and 10.14, with P values of 0.0001, 0.0006, 0.0001 and 0.0019 respectively; these results indicated that there were statistically significant differences between the levels of the fixed factors. PTA ranges ranged from -112 to +203. The heritability estimator (h^2) obtained for the age of first calving was 0.451 (45.1 %). The heritability found suggests that selection for this trait is feasible in buffaloes, however, in the farms evaluated a decrease in CFA has been observed as a result of improved general management. The fixed effects considered in the present study affected the age at first calving.

Genetic and environmental factors on birth weight in buffalo in the Lake Maracaibo basin. Venezuela

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The first parameter to evaluate in an animal is its birth weight, which can be indicative of its future performance and behavior in its productive life; which is why in every buffalo farm it is important to carry out an evaluation of the factors that could affect it. In the present work, the effects of genetic and environmental factors on birth weight (WB) in calves are evaluated, and heritability for birth weight was also calculated. A total of 41,804 birth weight records from 13,913 buffaloes and 21 breeding buffaloes were evaluated between 1996 and 2025. The factors under study were: farm of birth (BF); ecological zone (EZ) very dry tropical forest (BMST) and premontane humid forest (BHPM); year of birth (YB): from 1996 to December 2025; time of birth (TB): January-April, May-August and September-December; sex of the offspring (S): male or female; origin (O): natural mating product (NM), artificial insemination (AI); number of births of the mother (MB); race (R): Murrah predominant, Mediterranean predominant and undefined mestizo. The statistical analysis system SAS version 9.1 was used to perform the statistical calculations. The data were analyzed by: analysis of variance and least squares of Mixed Models and Maximum Likelihood Studies. For the calculation of the effects of the factors (genetic and non-genetic), the PROC GLM procedure was used, where type III square sums (SSIII) were used. The mean overall birth weight was 37.59 ± 0.56 kg with a CV of 9.69. The effect of BF, EZ, YB, TB, S and MB was highly significant ($P<0.001$) on birth weight; (O) had a significant effect ($P<0.05$), with a higher birth weight being observed in the offspring born as a result of artificial insemination compared to those born from natural mating. The results obtained express that the effect of genetic and non-genetic factors on birth weight in offspring must be taken into account and also reflect the excellent genetic potential existing in buffalo exploitation in Venezuela. Heritability for birth weight was low: 0.15.

Genetic and genomic improvement program of Murrah buffalo at Fortaleza farm, Colombia

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Buffalo production plays a key role in dairy production and food security in tropical regions, particularly in Central and Latin America, where the Murrah breed is widely used. The implementation of genetic and genomic selection programs is essential to improve productivity and sustainability. Since 1989, Hacienda Fortaleza has developed a Murrah breeding program focused on genetic improvement and the production of high-value genetic material. The objective of this study was to perform genetic evaluations and genomic association analyses for economically important traits, including milk yield (MY), calving interval (CI), age at first calving (AFC), and weaning weight (WW). Phenotypic, genealogical, and genomic data were analyzed using animal models to estimate breeding values (EBVs), and genomic associations were evaluated using SNP markers. The EBVs ranged from −206 to +302 L for MY, −40 to +64 days for CI, −2.6 to +2.8 months for AFC, and −45 to +44 kg and −26 to +27 kg for direct and maternal WW, respectively. The average inbreeding coefficient was less than 1%, indicating high genetic variability. Genomic markers associated with milk yield were also identified. These results demonstrate the effectiveness of integrating genomic tools into Murrah breeding programs under tropical conditions and highlight the potential for sustainable genetic improvement in buffalo populations in Latin America.

Immunogenicity and DIVA capability of a BoHV-1ΔgEβgal marker vaccine for the control of bovine and bubaline alphaherpesviruses in mixed herds

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The expansion of buffalo production in Argentina, driven by Law 27,076 and increasing derivative consumption, necessitates robust animal health protocols. Cohabitation in mixed herds facilitates viral circulation and interspecies transmission. Given the proven susceptibility of buffaloes to bovine herpesviruses (BoHV-1 and 5) and bovines to buffalo herpesvirus (BuHV-1), vaccination is key to mitigating pathogen spread and improving productive welfare.

This study evaluated the serological immune response in water buffaloes from Corrientes, Argentina, using a experimental marker vaccine (BoAHV1ΔgEβgal strain) designed as a DIVA (Differentiating Infected from Vaccinated Animals) strategy, compared to a conventional commercial vaccine.

Thirty seronegative buffaloes (9-15 months old) were divided into three groups: Group 1 (G1) received the inactivated marker vaccine with Montanide adjuvant; Group 2 (G2) received a commercial inactivated whole-virus vaccine. Both were revaccinated 21 days post-vaccination (dpv). Group 3 (G3) served as a non-vaccinated control. Neutralizing antibody levels were measured against BoHV-1 (LA), an Argentine BoHV-5 isolate, and two local BuHV-1 isolates. Anti-glycoprotein E (gE) antibodies were assessed via IDEXX ELISA.

Both vaccinated groups (G1 and G2) showed increased antibody levels after revaccination, peaking at 74 dpv (titers 0.9 to 1.8). Regarding bovine herpesvirus, the marker vaccine (G1) neutralized BoHV-1 more effectively, while the commercial vaccine (G2) showed higher neutralization against BoHV-5. Both vaccines induced antibodies that neutralized local buffalo herpesviruses. Crucially, G1 animals did not develop anti-gE antibodies, confirming the vaccine's marker capability, whereas G2 animals did. Negative results in G3 ruled out natural infections during the trial.

The BoAHV1ΔgEβgal marker vaccine proved immunogenic in buffaloes, inducing neutralizing antibodies and immunological memory that provide cross-protection against various ruminant alphaherpesviruses (both bovine and species-specific). IDEXX ELISA confirmed the absence of natural infection as G1 and G3 remained gE-negative. Furthermore, the DIVA capability enables the identification and segregation of infected animals, essential for health control in mixed herds. This vaccination strategy—complemented by welfare protocols—not only prevents disease and reduces pathogen shedding but also optimizes animal health, product quality, and sector profitability.

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Replacing traditional feed ingredients with by-products: implications for carbon footprint and water scarcity in dairy buffaloes

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The dairy buffalo sector represents a key component of the Mediterranean livestock economy, particularly in Italy, where it underpins the production of high-value traditional cheeses. However, this intensive sector faces growing public and regulatory pressure to reduce its ecological footprint and mitigate human-animal food-feed competition. Incorporating agro-industrial by-products into animal diets is a promising circular economy strategy to optimize resource efficiency and decrease reliance on conventional crops. This study aimed to evaluate the environmental sustainability and food-feed competition of integrating a by-product based concentrate feed into the total mixed ration of lactating dairy buffaloes compared to a conventional feeding regimen. Two iso-energetic and iso-proteic diets were formulated: a Conventional Diet (CD), utilizing standard concentrate ingredients (such as soybean meal, maize meal, and barley meal), and a Circular Diet (CiD), where traditional concentrates were replaced by an agro-industrial by-product concentrate containing wheat bran, biscuit meal, and corn gluten meal. The environmental impacts were assessed using a cradle-to-farm-gate Life Cycle Assessment (LCA) approach, modeled via openLCA 2.6 software and the Agribalyse v. 3.0.1 database. The Functional Unit (FU) was defined as 1 kg of concentrate feed. The impact categories evaluated through the EPD 2018 method included Carbon Footprint (CF, kg CO_2-eq) and Water Scarcity (WS, m^3 eq). The LCA results showed that the CiD significantly lowered the environmental burdens of feed production. Specifically, the CiD achieved a remarkable reduction of 38.6 % in CF compared to the CD, primarily driven by the avoidance of raw material cultivation, land conversion, and its associated fossil energy inputs. Furthermore, the WS indicator established a substantial reduction of 26.1 % in water resource consumption, reflecting the lower irrigation requirements of by-product loops compared to standard arable crops. In conclusion, the formulation of concentrates exclusively from agro-industrial by-products represents an effective, resilient, and sustainable dietary strategy for the dairy buffalo sector. This approach not only reduces the carbon and water footprint of high-value dairy lines but also enhances the bio-economy framework by converting manufacturing leftovers into high-quality human food, effectively minimizing human-animal food competition

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Technical Efficiency and Technology Adoption in Dairy Buffalo Milk Production in Central Luzon, Philippines

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This study analyzed the technical efficiency (TE) and the technology adoption (TA) behavior of dairy buffalo farmers in Central Luzon, Philippines using cross-sectional data of 185 farmers from the Philippine Carabao Center's (PCC) client database with complete data for production year 2024. Respondents were mostly male [92.4%], middle-aged [mean=52 y], secondary school educated [mean=9.75 y], with an average of 14 y in dairying, and 73% considering it their primary income source. Sample farms are predominantly small-scale holding an average cow-herd size of nine and a mean milk output of 3.2 liters. A log-log Cobb-Douglas Production Function (PF) was modeled and analyzed using Stochastic Frontier Analysis (SFA). Single-stage estimation procedure was used to simultaneously estimate the technical inefficiency (TI) model. The SFA revealed that the number of cows, feed use and dicalcium phosphate use significantly influenced milk output. The PF specification provides that the estimated input coefficients equal their partial elasticity vis-à-vis the output and interpreted through their marginal physical product (MPP). MPP analysis showed that adding 1 cow or 1 kg of dicalcium phosphate will increase milk output by 121–122 liters, while per kg of additional feed will yield an incremental 0.44 liters. The returns-to-scale (RTS) was 0.72 indicating decreasing RTS. This means that a 100% proportional increase in all inputs increases the output by 72%. Common literature-cited reason is managerial inefficiencies – consistent with the significance of the TI model. Mean TE was 52% indicating that milk output can increase by 48% by approaching full TE, not needing an increase in input level and technology. The TE was found increasable through wallowing pond (WP), 24-h estrus detection (ED), complete confinement management system (CCMS), and technology-specific training attendance. The logistic regression for the TA model showed that technology-specific training participation consistently increased adoption probability among the six technologies covered i.e. WP [64.7%], CCMS [60.1%], 24-hour ED [52.9%], fixed-time artificial insemination [45.2%], silage production [44.9%], and milk-test day [32.2%]. Other positive significant adoption factors are dairy farms as major income source, distance from PCC national headquarters, being male-gendered, registration to the department's registry system, access to extension and veterinary services and years in dairy farming.

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Cultural Representations of Water Buffaloes in India: Marketing and Product Development Implications

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In October 2023, the Vishwa Hindu Parishad, organization under Rashtriya Swayam Sevak Sangh, held a press conference to announce they would not allow worship of Mahisasura at several locations of coastal Karnatak, as an affront to the Goddess Durga (Soorinje, 2023). Mahisasura's name is generally interpreted as meaning "Buffalo Demon," but "Mahisa" in Sanskrit can also mean "great." Worship of this figure, and buffalo-heroes like him, dates back centuries; quite possibly to times before his role a protector and guardian would have been regarded as a counternarrative to the Goddess' slaying of a demon that had been threatening all the Gods.

The traditional bovine animal of India, also native, *Bos indicus* (the "Desi cow"), is the buffalo's counterpart in dairy production. Buffaloes and bovine cows now contribute about equally to India's milk supply, providing essential nutrition, especially for the country's many vegetarians. Widely accepted Hindu cosmology, though, allocates separate moral realms for these animals: the docile, intelligent, motherly bovine cow- almost invariably white- versus the dark-colored buffalo, with temperament imagined on a spectrum between unpredictable and malign.

This presentation examines negative treatments of buffaloes in Hindu tradition, representing them as less valuable than bovine cows. While both are appreciated for their nearly equal contributions to household and commercial dairy supply, cultural perceptions nonetheless shape reception in consumer choices. Marketing and product development need to take cultural attitudes- even the most removed from reality- into consideration to expand opportunities for promotion of conventional dairy products from buffaloes and develop new ones. A particularly effective marketing strategy for buffalo milk in the Philippines, the "Buffy's Milk" brand, will be discussed for possibilities of application to consumers in India, where bovine milk remains preferred, and mixed milk is commonly sold. Possibilities for marketing new products from buffalo milk, such as cheese, will also be considered.

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Impact of Murrah progeny testing project on the dairy farmers of Punjab

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Buffaloes play a crucial role in the Indian dairy industry and are the main contributors to the country's milk production. To expedite genetic advancement through systematic, data-informed sire selection, the Government of India introduced the National Dairy Plan-I (NDP-I) and the Murrah Progeny Testing (PT). Project was launched in Punjab in 2013 and continues under Rashtriya Gokul Mission (RGM) till date, with the primary objective of producing High Genetic Merit (HGM) Murrah bulls and developing a scalable genetic improvement model for the state and its direct socioeconomic effects on the dairy farming community of Punjab. The performance of the project was evaluated using key indicators including the number of test and nominated artificial inseminations, daughter registrations and calving's, first-lactation milk recording, type classification, parentage verification, registration and procurement of High Genetic Merit male calves, utilization of High Genetic Merit bulls by semen stations, and farmer outreach and extension activities. These parameters were used to assess the effectiveness of the progeny testing programme and its contribution to the genetic improvement of Murrah buffaloes in Punjab. The major achievements of the project were the identification and production of superior germplasm. The primary goal of the program the creation of elite male breeding assets—was accomplished with the registration of adequate number of HGM male calves. Out of these, the project acquired sufficient no. of calves, successfully lifting and transferring elite young bulls to organized semen stations throughout India. The availability of high-quality Murrah germplasm contribute to enhanced milk productivity, improved reproductive efficiency, and increased profitability of dairy farming in Punjab. In conclusion, the Murrah Progeny Testing Project, which was conducted in Punjab from 2013 to 2026, serves as a standard for scientific livestock breeding within developing dairy economics. The project fulfilled its primary objective of establishing a consistent supply chain of High Genetic Merit bulls to bolster national artificial insemination programs. At the same time, it acted as a significant economic intervention, enhancing animal productivity at the field level and promoting sustainable, long-term income growth for Punjab's dairy farmers.

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Analysis of colostral antibodies to herpesvirus 1 and 5 in murrah buffalo calves

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Familiar agriculture is predominant in the production of milk buffaloes in São Paulo southwest, Brazil. The production of buffaloes' milk is sold almost exclusively to cheese production inside and out São Paulo State. This search was done in a proprerty located in Alambari, São Paulo, specialized in the production of Murrah buffaloes, with 21 animals, born between March and May in the year 2017. Blood was collected at 24 to 48 h from the birth, every 15 days and at the completetion of weaning (91 days of age). The samples were collected in vacuum tubes of 10 mL with coagulum activator, by a puncture of the jugular vein. With the application of the virus neutralization tests, we could see 90.47% reagent animals for BoHV-5 and or BoHV-1 in the first two days of life. The geometric medium titles (GMT) of antibodies reduced significantly from the time 0 (1.4563) to the time 6 (05721) (P=0.0001), showing adequate transference of passive immunity through the ingestion of colostrum by buffalo calves, with the persistence of antibodies until 3 months old. Considering that most buffalo calves were reagents after sucking the colostrum, it is possible to conclude that there is a great occurrence of BoHV5 and or BoHV-1 once there is a crossed reaction among these and the cattle with no historic of vaccination for the agents.

Stimulating Artisanal Cheese Production in Family Farming

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Family farmers raising buffalo - many of whom transitioned from cattle farming due to low prices for cow's milk and restrictive purchasing policies imposed by large dairy companies - are now facing similar challenges in the commercialization of buffalo milk. Once highly valued, buffalo milk has recently experienced a decline in price, while processing industries increasingly require larger minimum production volumes, making it difficult for small-scale producers to remain competitive. This study aimed to identify the factors threatening the sustainability of buffalo dairy farming and to propose effective strategies for the sector's development. A diagnostic survey was conducted by the Agricultural Office of Sarapuí in partnership with the Coordenadoria de Assistência Técnica Integral (CATI), Itapetininga regional office. Semi-structured interviews were conducted with 22 (91.7%) of the 24 buffalo dairy farmers affiliated with the Cooperative of Milk and Other Agricultural Family Products Producers of Sarapuí and Region (COLAF). The survey revealed that 60% of the farmers' children were not interested in continuing the activity, mainly due to low financial returns. The establishment of the Municipal Inspection Service (SIM) for animal-origin products enabled the legalization of artisanal rural dairy-processing facilities, facilitating access to public procurement programs. Additionally, four training courses focused on dairy processing helped milk producers transition into dairy product manufacturers. The commercialization of these dairy products was made possible through the National School Feeding Program (PNAE) and the Municipal Food Acquisition Program (PAA), under the "purchase with simultaneous donation" modality. As a result of the initiatives aimed at strengthening the production chain, the number of members in the COLAF cooperative increased from 24 to 56 producers, representing a growth of 233.33%. The legalization of three rural processing kitchens enabled the purchase of buffalo milk yogurt for inclusion in school meals and distribution to vulnerable families. Consequently, the gross price paid per liter of milk increased from R$ 3.20 to R$ 13.20, representing a 306% increase achieved through value addition in yogurt production. The integration of scientific knowledge with the implementation of specific public policies for the sector proved effective in promoting the sustainable and efficient development of buffalo dairy farming in Sarapuí.

Contrasting ecosystems shape feed resource biodiversity for swamp buffalo: evidence from swamp area of South Kalimantan, dry land of Tapanuli, and mountain areas of Gayo

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Swamp buffalo are important in tropical livestock systems due to their adaptability to diverse environments and ability to utilize low-quality feed resources. However, ecological differences may influence feed resource biodiversity and availability, affecting production sustainability. This study aimed to compare feed resource biodiversity, nutritional characteristics, and carrying capacity of swamp buffalo raised in swamp areas of South Kalimantan, mountainous areas of Gayo (Aceh), and dryland areas of Tapanuli. Vegetation sampling was conducted using stratified random sampling with quadrat methods to assess species composition, including relative density and dominance. Forage samples were analysed for proximate composition, while feed resource potential and carrying capacity were estimated based on dry matter production. This explorative research did not employ any statistical approach, except for mean and deviation standard calculations. Averages among areas were compared descriptively. Results showed that swamp ecosystems had the highest forage biodiversity (14 species), followed by mountainous (6 species) and dryland areas (5 species). However, biomass production was higher in mountainous areas (0.97 ± 0.42 kg/m²) than in swamp areas (0.30 ± 0.05 kg/m²). Swamp buffalo relied mainly on natural forage without utilizing abundant agricultural by-products, whereas mountainous and dryland systems incorporated cultivated forage and crop residues. Nutritional analysis indicated generally adequate energy but low crude protein in most natural forages, except for *Neptunia oleracea*. Carrying capacity analysis revealed high potential for population expansion in swamp and dryland ecosystems, while mountainous areas showed limited capacity (ratio 1.3). In conclusion, ecosystem differences significantly shape feed resource biodiversity and utilization. Optimizing locally available feed resources is essential to improve swamp buffalo productivity and sustainability.

Keyword: Swamp buffalo; feed resource biodiversity; grazing ecosystems; carrying capacity; Buffalo production system

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Ethnosilvopasture: A Blueprint for Sustainable Buffalo Farming Where Local Tradition Meets Modern Science

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This study was conducted in Gayo Lues Regency, Aceh, Indonesia, using an ethnographic approach to examine the socio-ecological dynamics of buffalo husbandry. Data were collected through participant observation, interviews and focus group discussions. Ongoing ethnographic interpretation, combined with thematic analysis, identified relationships among local knowledge, grazing practices and resource governance. Results revealed a fundamental weakness in existing livestock development strategies, namely the limited recognition of the social and cultural dimensions of local communities. For centuries, buffaloes have been integral to Gayo civilization, possessing profound symbolic value and being managed through indigenous knowledge transmitted across generations. However, conventional top-down development programs often overlook these cultural values, threatening both cultural continuity and ecological sustainability. As nearly 70% of Gayo Lues is designated as a conservation area, integrating livestock production with forest conservation has become essential. The Gayo people have long practiced *peruweren*, a traditional free-range grazing system ecologically compatible with forest ecosystems. Building on this local wisdom, the study proposes Ethnosilvopasture as a transformative framework for sustainable livestock development. Unlike conventional silvopasture, Ethnosilvopasture incorporates ethnozoological knowledge as an essential component. The model is based on socio-ecological integration, revitalization of customary institutions such as *Pawang Uteuen*, *Petue* and *Pawang Uwer* and the application of ethnoscientific knowledge in grazing management. It also recommends establishing legally recognized grazing zones through local regulations to provide tenure security for pastoral communities within conservation landscapes. Successful implementation requires active collaboration among local communities, government agencies, forestry authorities and customary institutions throughout planning, implementation and evaluation. Modern technologies, including RFID, GPS and drones, are integrated to support livestock identification and monitoring without disrupting traditional grazing practices. By combining indigenous knowledge with science-based management, Ethnosilvopasture offers an innovative socio-ecological model that promotes forest conservation, safeguards cultural heritage, strengthens local livelihoods and supports sustainable community-based governance.

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Historical description of Mediterranean buffalo production using Test Days measures

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In Italy, buffalo population has increased by approximately 13% from 2013 to 2025, reaching a total of about 429,000 animals. To analyze the evolution of milk production and key qualitative traits, a total of 1,957,430 test-day (TD) records from 130,531 animals raised in 329 herds in Southern Italy, provided by the National Breeding Association, were utilized. Milk yield, fat and protein percentages and somatic cell count (SCC) were recorded from 2013 to 2023. The SCC was transformed into somatic cell score (SCS) to achieve a normal distribution. Linear mixed-effects models were used for milk traits (fat, protein and SCS), while a generalized linear mixed model (binomial distribution) estimated the culling rate. Year and herd were included as fixed and random effects, respectively. The analysis was carried out on RStudio. Results are expressed as estimated marginal means ± standard error. Daily milk yield showed variation over the study period, increasing from 8.43 ± 0.06 kg in 2013 to 8.99 ± 0.06 kg in 2023 with a peak of 9.18 ± 0.06 kg in 2019 ($p < 0.0001$). The fat content showed non-linear temporal pattern over the study period. From 2013 to 2015 a significant decline was registered (from 7.90 ± 0.04 to 7.77 ± 0.04 %, $p <0.001$), then values rose sharply to a peak of 8.12 ± 0.04% in 2018, followed by a consistent and significant reduction reaching its absolute minimum (7.70 ± 0.04%) in 2023. Similarly, protein percentage peaked in 2017 (4.73 ± 0.01%) and subsequently declined, settling at 4.65 ± 0.01 % in 2023 ($p < 0.0001$). The SCS increased from 2.40 ± 0.04 in 2013 to 3.42 ± 0.04 in 2023 ($p < 0.0001$). Over the study period, the percentage of primiparous showed a marked decline, decreasing from 36.00 ± 0.61% in 2013 to 32.35 ± 0.60 in 2023% ($p < 0.0001$). Overall, the increase in milk production reflected genetic progress and improved management practices. At the same time, changes in herd structure may explain the rise in SCS, since older buffaloes tend to show higher values due to mammary gland aging. Finally, the fluctuations in fat and protein percentages could be linked to shifts in feeding strategies and environmental factors, which can affect rumen function and nutrient use. In conclusion, these results highlighted long-term changes in milk traits and herd composition, offering a clear picture of the buffalo sector's evolution in Italy.

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Effect of dam age and some climatic conditions on lactation milk yield in Anatolian Buffaloes

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Milk production is effected by genotype and environmental factors. Genotype is determinant for milk production. However, since the heritability of milk yield is moderate, environmental factors must also be considered in milk production. The main environmental factors affecting milk yield can be listed as: dam age, dry period, calving season, dam live weight, nutrition, and climatic conditions. The Anatolian Buffalo breed is classed as a Mediterranean Water Buffalo among river type buffaloes. The Anatolian Water Buffalo is local dairy animal in Turkey. Lactation milk yield and lactation length in Anatolian Buffaloes are affected by environmental factors such as dam age and season of calving. However, there has been scarce information about the lactation milk yield of buffalo under the climatic conditions. One of the most prominent problems in production performance of animals is the changes in ambient temperature, relative humidity, wind velocity and precipitation. Climate conditions such as ambient temperature and relative humidity are used for identification of thermal stress. Ambient temperature, wind velocity and precipitation are used for the identification of cold stress. The common effect of ambient temperature and relative humidity is expressed in a single value which called "temperature-humidity index (THI)" on production traits in livestock. The common effect of ambient temperature, wind velocity and precipitation is expressed in a single value which called "cold stress index (CSI)" on production traits in livestock. The objective of the present study was to evaluate the effects of dam age, temperature humidity index and cold stress index on lactation milk yield in Giresun Province in Türkiye. These results show that, lactation milk yield increased with increase dam age ($P<0.001$). Additionally, lactation milk yield tends to decrease with increase the temperature-humidity index and especially, the buffaloes at first lactation period are more affected by heat stress. Also, lactation milk yield tends to increase with cold stress index increases.

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Descriptive sensory profile of firm yogurt made from buffalo *(Bubalus bubalis)* milk in combination with different gelatin concentrations

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The demand for scientifically backed functional foods is growing rapidly. Yogurt made from buffalo milk (Bubalus bubalis), due to its high protein content, is emerging as a probiotic alternative with high nutritional value and immunomodulatory properties. Based on this, a descriptive sensory evaluation was conducted on set-style natural yogurt (SNY) and the same yogurt with added fruit (strawberries in syrup), both made from whole milk from B. bubalis (protein: 4.32%; fat: 8.78%; lactose: 4.90%; solids-not-fat: 9.96%; total solids: 16.23%; pH: 6.60), incorporating unflavored gelatin at four concentrations according to the treatments (T): T_0: 0%, T_1: 0.25%, T_2: 0.50%, and T_3: 0.75%, adjusted to comply with national regulations (COVENIN 2393:2001, Venezuela) which specify a maximum addition of 1% gelatin in the finished product. The raw milk was homogenized and pasteurized at 95 °C; the temperature was immediately reduced to 43 °C to add the standard commercial starter culture (Lactobacillus delbrueckii subsp. bulgaricus, L. rhamnosus, and Streptococcus thermophilus) equivalent to 3% of the total milk volume as recommended by COVENIN. The mixture was packaged in labeled plastic containers and incubated at 42 °C for 8 hours, then refrigerated (4 °C/48 h) until consumption. For the sensory evaluation, 20 evaluators who met the criteria set forth in ISO 8586:2023 were selected; they completed a questionnaire validated by experts using a 5-point hedonic scale for the attributes of appearance, odor, texture, and flavor. The statistical analysis included descriptive statistics and one-way ANOVA with Duncan's test. The results revealed that incorporating 0.75% gelatin (T_3) into the mixture optimized the sensory attributes in both yogurts, although with varying degrees of effect. In the SNY, improvements were significant for all evaluated descriptors ($p<0.05$), with T_3 outperforming T_0 in smoothness (4.37 vs. 3.49), consistency (4.43 vs. 3.53), and acidity (4.13 vs. 3.24; $p<0.0001$); whereas the fruit yogurt showed significant differences in consistency (T_2: 4.43; $p<0.0001$), mouth coating (T_3: 4.25; $p<0.0001$), and acidity (T_3: 4.42; $p=0.0034$), while color, odor, and homogeneity did not vary significantly ($p>0.05$), possibly due to the interference of the syrup on the gel structure. It is recommended to add 0.75% gelatin as an effective technological strategy to enhance the sensory properties of yogurt manufactured from whole buffalo milk.

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Assessment of serum vitamin D (25-hydroxycolecalciferol) concentrations in lactating buffaloes adapted to the humid tropic of the southern Lake Maracaibo Region, Venezuela

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Vitamins are organic micronutrients that act at the cellular level as coenzymes in various metabolic pathways fundamental to life. Limited studies have been documented in large ruminants regarding physiological aspects linking serum vitamin D with productivity indicators through its calcium-regulating role, and its functional capacity to activate leukocyte populations during immunomodulation; a biochemical association that suggests a minimum basal concentration of 30 ng/mL of the compound 25-hydroxycholecalciferol (25-OH-D) for cattle. However, no updated scientific reports on this topic exist for *Bubalus bubalis.* For this reason, 60 crossbred Murrah x Mediterranean lactating buffaloes with an average body condition score of 3.96 ± 0.26 were randomly selected and equally distributed into four postpartum subgroups according to days (d) postpartum (5, 90, 180 and 270 d), all with a record of four lactations. To quantify serum vitamin D levels and their association with the lactation period in B. bubalis, a blood sample (6 mL) was obtained by jugular venipuncture using vacutainer needles and anticoagulant-free collection tubes; serum was then separated by centrifugation (3600 rpm/20 minutes) and analyzed by enzyme-linked immunosorbent assay (ELISA) based on the competitive binding principle. Data were organized in a Microsoft Excel® spreadsheet and subjected to analysis of variance (ANOVA) including multiple mean comparisons by Tukey test. The highest 25-OH-D concentration was observed in freshly calved buffaloes with 11.8 ± 0.44 ng/mL, followed by the subgroup of lactating females at 90 d postpartum recording 10.37 ± 0.46 ng/mL, both values being statistically similar ($p>0.05$); whereas animals at 180 and 270 d of lactation yielded the lowest mean values of 8.97 ± 0.64 ng/mL and 7.68 ± 0.35 ng/mL respectively, with statistical analysis revealing a significant difference ($p<0.05$) between serum 25-OH-D values from calving through 90 d postpartum compared to the 180 and 270 d groups, the latter being similar to each other ($p>0.05$). Results indicate very low 25-OH-D levels in lactating B. bubalis compared to those reported for cattle, suggesting that the high dermal melanin concentration in buffalo may influence the ultraviolet radiation required for 25-OH-D synthesis; furthermore, buffaloes exhibit maximum metabolic calcium demand during the first 90 d postpartum, requiring greater vitamin D availability for its absorption.

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Association of kappa casein A and B alleles with milk production in buffaloes in Brazil

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Brazil has the largest buffalo herd in the Western Hemisphere, with approximately 3 million head, and generated an income of around R$ 1 billion in 2024. The nutritional characteristics of the milk, its high commercial value and yield in the production of dairy products, especially mozzarella, contribute to the constant growth of this activity in the country.

The possibility of using genes that encode the main milk proteins as molecular markers, aiming at the selection of economically important traits (production and quality), is an important tool for genetic improvement. For kappa-casein, the A and B alleles are the most frequently reported in the literature. Some studies with these variants indicate that the B allele provides an 8 to 10% increase in milk protein content, allowing for greater added value in cheese production for the BB genotype. The database of the Brazilian Association of Buffaloes Breeders (ABCB) was used with cows (407) whose lactations (P305 - 305-day accumulated milk – 1.187)) occurred from 2009 to 2026 and was part of the dataset of the Genetic Breeding Program of ABCB.

The kappa casein genotypes of 407 females were 45,95% for the AA, 45,21% for the AB and 8,84% for the BB genotypes respectively. For the allele frequency, 68,55% for allele A was observed and 31,45% for the allele B.

In the Bayesian mixed model fitted for P305, with random intercept per animal and fixed effects of allelic substitution, effect of contemporary group (CG) to control for environmental and management variation, cow age as both linear and quadratic covariates to adjust the production curve according to the animal's maturity and number of milking (1 or 2). no significant evidence of an effect of kappa-casein allelic substitution on P305 was observed. However, there was a significant effect of parity and number of milking on the trait. The variance between animals was significant, resulting in an approximate repeatability of 0.47.

Further studies need to be conducted with a larger number of genotyped animals, and it is of great importance that fat and protein production also be measured, as they have a greater effect on the quantity and quality of cheese production.

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Effect of P21 Inhibitors on the Development of IVF Water Buffalo Embryos

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Mammalian embryo development is composed of a series of mitotic events and the cleavage development dynamics is closely related to the embryo's developmental ability. As a cyclin-dependent kinase inhibitor (CKIs), P21 participates in regulating the cell cycle and DNA damage repair of embryonic cells and plays an important role in embryo development. To explore the effect of P21 on buffalo embryo development, this study treated buffalo *in vitro* fertilized embryos with different concentrations of P21 inhibitor UC2288 and statistically analyzed the embryo development and gene expression. The results showed that treatment with 0.2 μmol/L UC2288 could significantly reduce the expression level of P21 mRNA in buffalo embryos, and increase the 2-cell, 4-cell, 8-cell and blastocyst rates of buffalo *in vitro* embryos ($P < 0.05$). In addition, UC2288 treatment could reduce the expression levels of BAX and Caspase-3 in buffalo blastocysts and increase the expression level of OCT4 ($P < 0.05$). This indicates that treatment with an appropriate concentration of UC2288 can inhibit P21 expression and promote the development of buffalo *in vitro* fertilized embryos by down-regulating apoptosis genes and up-regulating the expression of development-related genes.

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Genetic Improvement Activities of Nikookar Buffalo Farm and Buffalo Marketing Challenges in Iran

Mrs. Fatemeh Derisavi

Objective: This study presents the genetic improvement activities conducted at Nikookar Buffalo Farm (NBF) and evaluates the current status and marketing challenges of buffalo production in Iran.

Materials and Methods: NBF, established in 2000 in Gilan Province, implemented artificial insemination, embryo transfer, and cross-breeding programs using frozen semen and embryos imported from elite Italian Mediterranean buffaloes. Productive and reproductive records were collected from the farm and from a regional genetic improvement program involving 32 buffalo herds.

Results: The farm currently maintains approximately 60 productive female buffaloes, including F1 to F5 crossbred generations. Selected crossbred buffaloes achieved an average milk yield of 14 kg/day during a 270-day lactation period (3,780 kg/lactation). Age at first calving ranged from 30–34 months and the average calving interval was 13 months. A total of 300 doses of frozen semen were used for 650 breeding females. In 2015, embryo transfer was successfully performed using frozen embryos imported from Italy. Five elite F2 and F3 males were transferred to national research centers for breeding and reproductive biotechnology programs. Buffalo production in Iran is concentrated mainly in Khuzestan, Gilan, Mazandaran, East Azerbaijan, West Azerbaijan and Ardabil provinces. Despite the high economic value of buffalo milk, the sector faces challenges including limited milk supply, traditional hand-milking, inadequate marketing systems, long transportation distances and declining buffalo populations.

Conclusion: The NBF experience demonstrates that advanced reproductive technologies can effectively improve buffalo productivity and genetic merit. Strengthening breeding programs, milk collection systems and marketing infrastructure can contribute to sustainable buffalo development and rural entrepreneurship in Iran.

Keywords: Buffalo, Genetic Improvement, Artificial Insemination, Embryo Transfer, Milk Production, Iran

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Glucogenic Support with X-ENERGY® Improves Energy Status, Milk Yield, and Reduces Subclinical Ketosis in Transition Dairy Buffaloes: A Randomized Controlled Trial

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Negative energy balance during the transition period predisposes dairy buffaloes to excessive lipomobilization, reduced productive performance, and subclinical ketosis. Glucogenic precursors like glycerol and propylene glycol are frequently used in dairy cattle, but dose-response studies in buffaloes are limited. This randomized controlled trial evaluated the effects of X-ENERGY® (a glycerol–propylene glycol mixture) on intake, milk production, metabolic status, and early postpartum health outcomes of transition dairy buffaloes.

Sixty multiparous buffaloes (650 ± 38 kg) were blocked by parity and expected calving date and randomly assigned from −21 to +42 d relative to calving to Control (no supplement), T1 (150 mL/d prepartum; 300 mL/d postpartum), or T2 (300 mL/d prepartum; 500 mL/d postpartum). All animals were fed a corn-silage-based total mixed ration formulated to meet buffalo requirements (postpartum diet: 16.5% CP, 32% NDF, and 1.54 Mcal NEL/kg DM). Outcomes were dry matter intake (DMI), milk yield, energy-corrected milk (ECM), body weight (BW), body condition score (BCS), blood metabolites (NEFA, BHBA, glucose, and insulin), and early postpartum health events. Continuous outcomes were analyzed using repeated-measures mixed models, and health incidences were analyzed using generalized linear mixed models with a logit link.

Supplementation of X-ENERGY® increased DMI (Control 16.1, T1 16.8, T2 17.2 kg/d; SEM 0.40; P=0.031), milk yield (9.8, 10.8, and 11.6 kg/d; SEM 0.60; P=0.010), and ECM (10.9, 12.2, and 13.3 kg/d; SEM 0.70; P=0.008) in a dose-dependent manner. Metabolic status improved, with decreases in NEFA (0.68, 0.58, and 0.52 mmol/L; SEM 0.05; P=0.021), BHBA (1.15, 0.95, and 0.85 mmol/L; SEM 0.08; P=0.014), and increases in glucose (57, 60, and 62 mg/dL; SEM 2.0; P=0.033) and insulin (4.8, 5.4, and 5.9 µIU/mL; SEM 0.5; P=0.047). Importantly, the incidence of subclinical ketosis was reduced from 30% in Control to 18% in T1 and 12% in T2, an absolute reduction of 18 percentage points and a relative reduction of 60% in T2 vs Control (P=0.028). BW loss (−18, −12, and −8 kg; SEM 3.0; P=0.022) and BCS loss (−0.25, −0.18, and −0.12 units; SEM 0.05; P=0.038) were also attenuated. Milk composition and hepatic enzyme indicators were within normal ranges and were not adversely affected.

In conclusion, the supplementation with X-ENERGY® improved intake, lactation performance, energy-status biomarkers, and subclinical ketosis outcomes in transition dairy buffaloes; the high dose (300/500 mL/d prepartum/postpartum) provided the most consistent response. These single-trial results suggest the higher dose could be a potential transition-management approach in similar buffalo herds, but multi-herd replication across different management systems is needed before making broad regional recommendations.

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