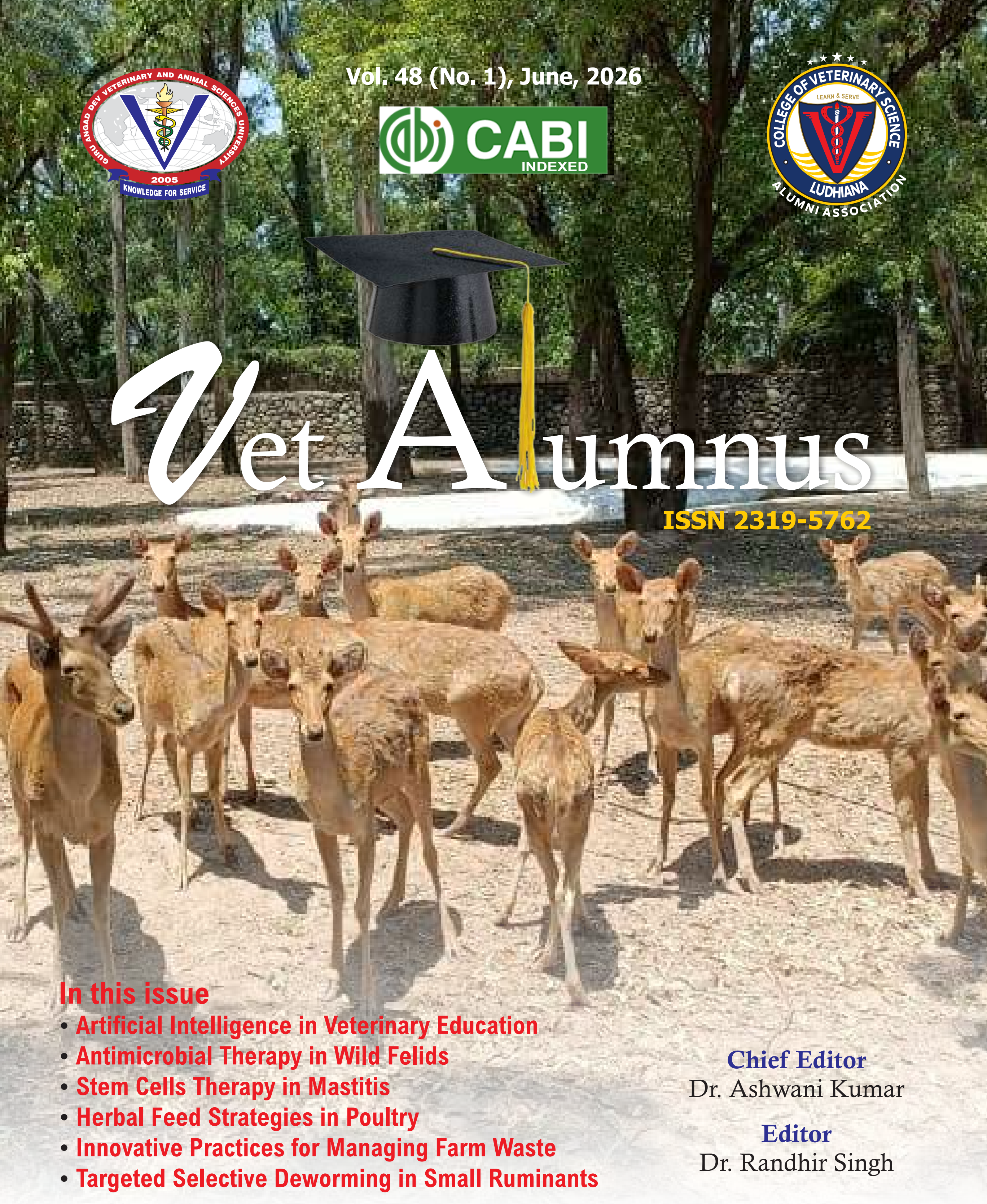

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Mini Review

Current Concepts in Nutritional Management of Canine Chronic Kidney Disease

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Abstract

Chronic kidney disease (CKD) is a major health issue that typically affects older dogs. It leads to considerable suffering in aged animals and has a negative impact on their quality of life. A highly effective long-term treatment and management strategy is proper nutrition. Dietary changes can slow the progression of the disease and may enhance survival rates. Renal-specific diets aim to lessen phosphorus consumption, manage protein metabolism, improve acid-base balance, and reduce oxidative stress. These diets are rich in omega-3 fatty acids, antioxidants, and often include nutraceuticals, as well as prebiotics and probiotics to aid in the management of CKD. Hence nutritionally, inclusion of therapeutic renal diets has the potential to optimize kidney health leading to better management of CKD.

Keywords: *Antioxidants, Chronic kidney disease, Nutraceuticals, Renal diet*

Chronic kidney disease (CKD) is one of the most commonly identified chronic conditions in dogs. It is marked by a gradual loss of functional nephrons and an irreversible reduction in kidney function (Perini-Perera et al., 2021). The severity of CKD is classified into different stages (1–4) according to the International Renal Interest Society (IRIS) guidelines based on kidney function and other markers. Affected dogs are unable to excrete metabolic waste leading to buildup of urea, ammonia,imbalances in electrolytes and metabolic acidosis ultimately leading to dehydration and systemic inflammation (Martello et al., 2021). Nutritional therapy is regarded as a crucial component in the management of CKD. Adjusting the diet directly impacts the workload on the kidneys. Diet controls uremic toxin production, phosphorus retention, and progression of renal injury (Martello et al., 2021). Studies documented that the dogs suffering with CKD receiving therapeutic renal diets usually have decreased hospital stays,improved survival rates along with fewer uremic episodes compared with dogs consuming maintenance diets (Pedrinelli et al., 2020). Therefore, targeted nutritional intervention through incorporation of functional ingredients, which deliver bioactive compounds intended to improve pet health is an effective method in modulating kidney injury and promoting early and uneventful recovery.

Pathophysiology of CKD

The CKD leads to a gradual loss of nephrons within the kidney. It results in a decrease in the glomerular filtration rate (GFR) and hampers the kidney's ability to eliminate waste (Perini-Perera et al., 2021; Mangat, 2024). As the condition advances and kidney function deteriorate, there is an accumulation of nitrogenous waste products such as urea and creatinine in the blood, which contributes to uremic syndrome (Martello et al., 2021). CKD also disrupts calcium and phosphorus metabolism leading to elevated phosphorus levels in the body. This disturbance affects the optimal calcium-to-phosphorus (Ca:P) ratio and contributes to renal secondary hyperparathyroidism (Pedrinelli et al., 2020; Mangat, 2024). High phosphorus levels can accelerate nephron damage, further exacerbating the progression of kidney disease (Perini-Perera et al., 2021). Dogs suffering from CKD commonly experience protein-energy malnutrition due to factors such as loss of appetite, nausea, vomiting, inflammation, and metabolic changes. Oxidative stress is also an important mechanism contributing to progression of renal fibrosis and nephron injury in CKD (Chen et al., 2024). Chronic inflammation occurs due to increased production of reactive oxygen species (ROS) that damage renal tissues (Chen et al., 2024). Blood Urea Nitrogen and Creatinine have been used to assess kidney function since long ago, they give a good clinical picture. But nowadays instead of serum creatinine, symmetric dimethylarginine (SDMA) and cystatin C are considered to be more sensitive tools for early detection of CKD (Hall et al., 2016; Biswas et al., 2026). International Renal Interest Society classifies (IRIS) CKD into 4 stages in dogs according to the severity of the disease with 4 being the most severe. They are summarized in following table 1

Table 1. IRIS classification / Stages of CKD in dogs

SDMA (μg/dl)	Blood creatinine(mg/dl)	Stage of CKD
<18	<1.4	1
18-35	1.4-2.8	2
36-54	2.9-5.0	3
>54	>5.0	4

(IRIS, 2023)

Renal Diets

The primary goal of managing CKD is to slow down the progression of illness by managing the key risk factors and clinical symptoms. Renal diets incorporate high quality easily digestible protein, restricted phosphorus content, regulated sodium levels, supplementation of omega-3s such as fish oil, lipoic acid, soluble fibres (Hall et al., 2014; Martello et al., 2021). Renal diets may improve appetite, body condition and if initiated early have the potential to reduce disease progression.

The primary nutritional intervention for canine CKD is protein management. Proteins are metabolized into amino acids and excess is removed as nitrogenous waste. When kidneys are not functioning properly it lead to accumulation of nitrogenous waste and may cause uremic syndrome (Mafra et al., 2025). For this reason, moderate protein restriction is advised for CKD affected dogs. It minimizes the buildup of toxins and decreases the stress on the kidneys (Perini-Perera et al., 2021). Severe protein restriction however, is also not recommended because it might lead to malnutrition and muscular atrophy (Martello et al., 2021).

The ideal strategy is to use moderate amounts of higher-quality protein at 12-14% DM (Beynen et al., 2021) with sufficient biological value and should be easily digestible. High quality protein sources provide all essential amino acids while producing fewer metabolic waste products (Pedrinelli et al., 2020). Maintenance of lean body mass is particularly important because muscle wasting is associated with inferior clinical outcomes and reduced survival in dogs with CKD (Martello et al., 2021). Various protein sources recommended for CKD affected animals along with their properties and limitations have been discussed in Table 2.

Table 2. Various Protein sources recommended for CKD patients

Protein Source	Properties	Limitations
Egg white	Highest biological value, excellent amino acid profile, very low phosphorus compared with whole egg	Avoid excessive yolk due to phosphorus
Chicken breast	Highly digestible, high-quality protein, relatively lower phosphorus than many red meats	Must be balanced with phosphorus control
White fish (cod, pollock, tilapia)	Excellent digestibility, provides essential amino acids and omega-3 fatty acids	Phosphorus moderate
Salmon	High-quality protein plus EPA and DHA	Higher phosphorus than egg white
Turkey breast	Lean, highly digestible protein source	Moderate phosphorus content

(Villaverde, 2020; Parker & Freeman et al., 2021)

Phosphorus Restriction

Phosphorus restriction has been shown to be an effective dietary intervention for reducing the course of CKD in dogs (Perini-Perera et al., 2021). Hyperphosphatemia and renal secondary hyperparathyroidism result from the decrease in phosphorus excretion by the kidneys (Pedrinelli et al., 2020).

In dogs, therapeutic renal diets have much lower phosphorus concentrations than maintenance meals (Martello et al., 2021). High dietary phosphorus intake has been associated with renal calcification, tubular injury, impaired renal function and proteinuria (Dobenecker et al., 2021). Renal mineral imbalance is reduced when dietary phosphorus intake is reduced. It helps slow the progression of renal failure and stabilizes the Ca:P ratio of 1.4-1.8 to 1 (Brunetto et al., 2021, Beynen et al.,2021). Because canine CKD patients experience faster disease progression, persistent hyperphosphatemia is linked to higher mortality (Perini-Perera et al., 2021). In advanced cases, phosphate binders such as calcium carbonate, aluminum hydroxide and chitosan may be required in addition to dietary phosphorus restriction (Martello et al., 2021).

Regular monitoring of serum phosphorus concentrations is necessary to assess the effectiveness of nutritional therapy anddietary adjustments can be made accordingly (Pedrinelli et al., 2020).

Table 3. IRIS stage of CKD and Recommendations of phosphorus levels in diet

Diet Type	Phosphorus (% DM)	Source
Adult Maintenance Diet	0.4-1.0%DM	AAFCO, 2014
Early CKD Renal Diet (Stage 1&2)	0.3–0.6% DM	Villaverde, 2020
Advanced CKD Renal Diet (Stage 3&4)	0.2–0.5% DM	Villaverde, 2020 ; Parker,2021
Therapeutic Renal Diet	0.16 g/MJ ME (~0.45% DM)	Jacob et al. (2002)

Table 4. IRIS Stage of CKD and Target Serum Phosphorus Levels

IRIS Stage	Target Serum Phosphorus
1	2.7–4.6 mg/dL
2	< 4.6 mg/dL
3	< 5.0 mg/dL
4	< 6.0 mg/dL

Omega-3 Fatty Acids

Omega-3 fatty acids provide anti-inflammatory effects (Brunetto et al., 2021). It improves clinical stability in affected dogs as well (Martello et al., 2021). Fish oil supplementation (1.5%) may reduce glomerular hypertension and oxidative stress in dogs with CKD (Hall et al., 2014).

Dietary omega-3 fatty acids may also reduce proteinuria and improve renal perfusion in the dog (Perini-Perera et al., 2021). Major anti-inflammatory benefits are seen with the use of 80-100mg eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Brunetto et al., 2021; IRIS, 2023).

Antioxidant Supplementation

Increased concentrations of ROS damage the tubular cells of kidney, promote fibrosis and causes stress and chronic inflammation in the body (Chen et al., 2024). Therefore, supplementation of antioxidants is increasingly used as part of supportive nutritional therapy in dogs with CKD (Martello et al., 2020). Vitamins E 400-600 IU/Kg and selenium 0.3-0.5mg/kg supplementation reduce oxidative damage. Lipoic acid, L- carnitine may also help in reduction of oxidative damage and improvement of cellular defense mechanisms (Hand, 2005; NRC, 2006; Hall et al., 2014; Chen et al., 2024).

Gut Microbiota and Intestinal Health

Gastrointestinal health is crucial to control symptoms such as nausea, vomiting which develop frequently. Prebiotics and probiotics may help restore intestinal microbial balance. It reduces the population of toxic metabolites in the intestine and restore intestinal barrier (Brunetto et al., 2021). Fecal nitrogen excretion is increased by fermentable fibers. It also reduces circulating nitrogenous waste products in the body (Perini-Perera et al., 2021).

Clinical Nutritional Recommendations

Individualized nutritional therapy according to disease condition, stage of disease progression is necessary. Dogs in advanced stage of disease may require assisted feeding strategies. It is introduced to prevent protein energy malnutrition and maintenance of adequate caloric intake (Martello et al., 2021). Frequent monitoring of body parameters such as body weight, body condition score or nowadays muscle mass and hydration status along with clinical laboratory tests is essential during long term management (Vendramini et al., 2026).

Nutritional management should be focused on phosphorus restriction, moderate high quality protein intake and antioxidant support (Brunetto et al., 2021). Supplementation with omega-3 fatty acids and selected nutraceuticals may provide additional clinical benefits but there is lack of adequate research. Recommendations on the nutrients restriction according to the IRIS Stage of CKD has been described in Table 5

Table 5. Recommendations on Nutrient Restriction as per IRIS Stage of CKD

Nutrient	IRIS Stage 1	IRIS Stage 2	IRIS Stage 3	IRIS Stage 4
Protein	Normal, high-quality protein	Moderate restriction	Moderate restriction	Moderate restriction while preventing muscle loss
Phosphorus	Control intake	Restricted	Strictly restricted	Strictly restricted + binders often needed
EPA + DHA	Beneficial	Recommended	Recommended	Recommended
Sodium	Normal to mildly restricted	Mild restriction	Restricted	Restricted
Potassium	Monitor	Supplement if low	Often needed	Frequently needed
Energy	Maintain BCS 4–5/9	Maintain weight	Prevent catabolism	Prevent cachexia

Future Perspectives

It is crucial to emphasize the importance of precise nutrition in managing CKD because a "one-size-fits-all" approach may not be suitable in context of protein requirement (Mafra et al., 2025). It combines antioxidant supplementation along with newer nutraceuticals to improve metabolic stability and quality of life in dogs with CKD (Brunetto et al., 2021. But still there is so much unknown about the metabolomic and microbiome research. It may further improve the understanding of disease mechanisms. It may also help in identifying new therapeutics and management strategies.

Nutraceutical supplementation has no standardized guidelines. There should be further research on antioxidant therapy and microbiome modulation in dogs suffering from CKD. Continued research may contribute to development of more effective nutritional interventions and improved survival outcomes.

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Mini Review

Targeted Selective Treatment for Mitigation of Anthelmintic Resistance in Small Ruminants

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Abstract

Targeted selective treatment (TST) is a sustainable approach for gastrointestinal parasite control in small ruminants as it reduces unnecessary anthelmintic use and conserves refugia. Integration of TST into integrated parasite management (IPM) strategies helps delay the development of anthelmintic resistance. Combined TST approaches using methods such as FAMACHA, body condition score, dag score, diarrhoea score and faecal egg count can be used to select animals for treatment and thereby minimise the need for blanket anthelmintic treatment. Although more demanding than conventional approaches, TST-IPM integration promotes sustainable parasite control while maintaining animal productivity and health. Despite its advantages, adoption of TST at the farm level remains confined due to a lack of awareness, labour-intensive monitoring, economic constraints and limited access to veterinary support. Therefore, continuous farmer education, region-specific recommendations, and improved extension services are essential for the successful implementation of sustainable parasite control strategies in small ruminants.

Keywords: *Anthelmintic resistance, Integrated Parasite Management, Small Ruminants, Targeted Selective Treatment.*

Gastrointestinal nematode (GIN) infections impose a substantial constraint on the health and productivity of small ruminants by impairing growth and reproductive efficiency. These are naturally occurring mixed GIN infections with *Haemonchus contortus* being the predominant and most pathogenic species. Severe GIN infection in small ruminants has been associated with economic losses of up to ₹1674 per animal due to poor body weight gain, decreased productivity and treatment costs (Ilangopathy et al., 2019). Since the introduction of the first anthelmintic in the 1940s, control of GINs has largely been chemocentric, relying predominantly on synthetic chemicals (Kalkal et al., 2020). However, this approach has proven inherently unsustainable as indicated by the widespread emergence of anthelmintic resistance (AR) across the globe. The AR is the outcome of interaction between parasite biology and managemental practices comprising mainly of frequent mass treatments, incorrect dosing and a single drug regimen approach.

The persistence of such approaches at the field level accelerates the establishment of resistant strains of parasites, compromising the treatment efficiency and emphasising the need for sustainable parasite management strategies (Jyoti et al., 2020). Amid the rising challenge of AR, where available anthelmintic drugs are limited and the development of new compounds in the near future remains unlikely, the primary approach is to delay the resistance progression through the strategic use of existing drugs. Targeted selective treatment (TST) is one such method that enables more judicious and effective use of anthelmintics (Edith et al., 2018).

Current Scenario of Anthelmintic Resistance

Anthelmintic resistance has become a global concern for the livestock industry particularly for small ruminants. AR is no longer limited to sporadic cases but is now distributed across all major classes of anthelmintics v*iz*. benzimidazoles, imidazothiazoles and macrocyclic lactones that are most commonly used for the control of GINs in small ruminants. The emergence of multi-anthelmintic resistance has been recorded worldwide, including India (Jabbar et al., 2006; Kenyon et al., 2009; Kalkal et al., 2020). Recent studies from Punjab have also documented this overriding issue of the emergence of multi-anthelmintic resistant GIN in sheep and goat populations, where drugs of major anthelmintic classes failed to confer curative effect despite appropriate anthelmintic treatment. (Kaur et al., 2025; Chauhan et al., 2026). Apart from the most studied *Haemonchus contortus*, AR have also been detected in several trichostrongylid genera including *Teladorsagia*, *Trichostrongylus*, *Cooperia* and *Nematodirus* species (Jabbar et al., 2006).

Determinants and Risk Factors

Anthelmintic resistance refers to the genetically inherited reduction in a parasite population's susceptibility to a previously effective drug. It occurs when higher proportion of parasites within population can survive the recommended doses of an anthelmintic. This reduced sensitivity is inheritable and passed down to successive generations, ultimately leading to the emergence of resistant parasitic strain. The genes associated with resistance are initially rare within the parasitic population or arise though spontaneous mutations but with sustained selection pressure, their proportion in the population continuously increase across subsequent generations and ultimately results in therapeutic failure. Multiple factors contribute to the development of AR, but the gradual depletion of refugia remains one of the most overlooked causes of the rapid resistance emergence. Refugia refers to the proportion of parasite population that has not been exposed to anthelmintic drug, thus escaping selection of resistance (VanWyk, 2001). After effective anthelmintic treatment, surviving resistant parasites mate with susceptible population of refugia, leading to dilution of resistant alleles and consequently delaying the emergence of AR. Several host, parasite and environmental factors affect the maintenance of parasite refugia.

Host Associated Factors: Host resilience and immunity significantly influence parasite refugia. Resilient and immunocompetent host require fewer anthelmintic treatments and thus contribute to refugia population (Kenyon et al., 2009).

Parasite Associated Factors: The frequency of resistant alleles prior to drug use, number of genes involved and their dominance or recessiveness, along with biotic potential and generation interval, are important parasitic factors that determines AR development (Jabbar et al., 2006). Parasites with high fecundity such as *H. contortus* can maintain refugia with fewer untreated animals compared to less prolific species like *Teladorsagia*, which require larger number of untreated population to sustain refugia (Keynon et al., 2009). The frequent blanket deworming inadvertently removes susceptible allele from the parasite population and as a result, resistant gene variants become dominant.

Environmental Factors: Environmental conditions influence the survival of parasitic eggs, larvae, as extreme heat and drought in tropical and subtropical regions can rapidly reduce these free-living non-parasite stages and refugia population. Deworming under such conditions exerts stronger selection pressure for resistance (Kenyon et al., 2009). Among the several management practices that contribute to the rapid AR development, routine blanket deworming, regardless of the animal's health status and seasonal variations in parasite prevalence, is the major predisposing factor (Hayward & Kenyon, 2026). Dosing inaccuracies further exacerbates this problem. Sub-therapeutic dose administration often resulting from approximate weight estimation in large flocks, allows heterozygous resistant parasites to survive treatment. Also, a common issue in mixed flocks, is drenching of both sheep and goats with same dose rate, despite goats requiring higher drug concentration due low bioavailability anthelmintic drugs like benzimidazole and levamisole. In addition, "Drench and Move" practice especially by pastoral livestock owners, involving moving the flock to clean pastures immediately after mass deworming, allowing surviving tolerant parasite to shed eggs and contaminate the pasture with resistant variants. Failure of conventional control policies in field conditions has also been attributed to the easy availability of anthelmintics as over-the-counter dewormers along with the presence of poor quality formulations in general markets in developing countries (Jabbar et al., 2006).

Targeted Selective Treatment (TST)

The TST approach refers to a system where animals to be treated are selected on an individual basis using specific criteria. In this approach, rather than treating entire flocks at regular intervals as a prophylactic measure, irrespective of their health status and seasonal prevalence of parasites, only suspected animals are selected and treated accordingly. Thus, by restricting treatment only to animals exhibiting measurable indicators of parasitic infections, TST reduces overall drug exposure while preserving a proportion of parasite population unexposed to treatment (Edith et al., 2018). Conventional approaches, including suppressive, strategic and seasonal anthelmintic treatments, have traditionally prioritized short term reductions in parasitic burdens and often at the expense of sustainability (Hayward & Kenyon, 2026). The TST relies solely on preserving refugia, which plays a critical role in moderating resistance dynamics. From a population genetics perspective, the persistence of susceptible alleles within refugia results in genetic recombination with resistant strains and hence reduces the rate at which the resistant parasite population becomes predominant (Van Wyk et al., 2006).

Implementation of TST depends upon the identification of animals that are most likely to benefit from the intervention. Animals requiring treatment can be identified through laboratory and field assessment criteria. Faecal egg count (FEC) is considered the most accurate laboratory method; however, its requirement of technical expertise and instruments makes it rather impractical for pastoralists and farmers having large flocks. So instead, at the field level, practical indicators of GIN infections including pathophysiologic (anaemia, body condition, dag score, diarrhoea) and performance parameters (growth rate, milk production) can be used. The effectiveness of TST lies in its ability to balance productivity and resistance management.

Potential Indicators for Selecting an Individual Animal for Treatment

Faecal Egg Count (FEC): In coprological examination, FEC, expressed as eggs per gram of faeces (EPG), is an important parameter routinely used to determine the intensity of GI parasitic infections. It helps in estimation of worm burden by quantifying the number of eggs shed in the faeces. The interpretation of EPG values are may vary depending on parasitic species. For strongyle infections EPG crossing a threshold of ≥750 are selected for deworming (Mancilla-Montelongo et al., 2023). Although helpful, the number of parasitic eggs detected in faeces can be influenced by various factors such as the number of adult parasites established in the GI tract, level of host immunity, species of parasite, stage of infection, parturition and consistency of faeces.

FAMACHA System: The acronym FAMACHA is derived from the name of its originator Faffa Malan, is a system designed for the sustainable control of haemonchosis in small ruminants. This system evaluates the severity of anaemia by comparing the colour of conjunctival mucous membrane of sheep and goat with standardized colour chart ranging from 1 (deep red, healthy) to 5 (almost white, severely anaemic) (Malan et al., 2001). Since *H. contortus* is highly pathogenic hematophagous parasite, progressive blood loss results in characteristic pallor of mucous membranes, making anaemia a practical clinical indicator of infection intensity. Primarily developed for control of haemonchosis, this method is practically useful during summer rainfall and warmer climates when *H. Contortus* is predominant and has proven effective under field conditions for resource-limited farmers with small to moderate sized flocks (Hayward & Kenyon, 2026). Unlike conventional mass deworming approaches, FAMACHA recommends treatment only for those animals incapable of tolerating prevailing parasitic challenge, usually animals with score 3 or above, thereby substantially reducing the anthelmintic use, treatment cost and selection pressure for anthelmintic resistance while preserving refugia. Studies have shown that upto 75 to 85% of animals in a flock can be left untreated without compromising flock productivity with only 10% of the cost incurred with conventional mass treatment (Singh & Swarankar, 2012). However, for this system to be effective, it requires mandatory inspection of animals during the warmer season every 7 to 14 days and a fair amount of training for taking treatment decision for individual animal (Van Wyk et al., 2006)

Dag Score: Dag score is a non-invasive indicator used for assessing faecal soiling in sheep and goats as an indirect measure of GIN infection (Edith et al., 2018). “Dags” refer to the accumulation of dried or semi dried faecal material attached to wool or hair around the perineal region and hind limbs, primarily resulting from altered faecal consistency associated with trichostrongylid infection (Larsen et al., 1994). Higher dag scores have been associated with increased faecal egg counts, reduced weight gains, poor body condition and increased susceptibility to fly strike due to persistent breech soiling. Severe faecal soiling has been positively associated with increased worm burden during summer. However, scouring may not serve as a sufficiently early indicator in older sheep, as production losses can occur even before diarrhoea becomes evident.

DISCO (Diarrhoea Score): This scoring system is a practical tool in TST to assess the severity of diarrhoea associated with GIN infections in small ruminants. The scoring is based on faecal consistency, where score 0 indicates normal pelleted faeces, score 1 soft faeces, score 2 loose, similar to cow pat and score 3 for diarrhoea (Cabaret et al., 2006). A strong correlation between DISCO and EPG has been reported, indicating its utility as an effective TST indicator capable of reducing anthelmintic treatments up to 85% compared to conventional deworming practices (Ouzir et al., 2011).

Body Condition Score (BCS): It is a feasible and low cost method used to assess the nutritional status, energy reserve and general health conditions of animals and originally developed for sheep and dairy cattle (Torres-Chable et al., 2025). Proposed by Jefferies, this method is based on scale ranging from 0 to 5 and involves palpation of the spinous and transverse process of lumber vertebrae along with assessment of muscles and fat cover (Edith et al., 2018). Within TST approach, animals with BCS less than 3 are treated. Reduced body weight gains and poor BCS are often associated with lower resilience to GI parasitism; however, these are non-specific as the body condition of animals can also be affected by feed availability and other environmental factors (Van Wyk & Bath, 2002). This scoring method is mainly applicable in infections predominant with *Teladorsagia* spp and *Trichostrongylus* spp. and requires virtually no additional cost other than labour (Burke et al., 2007).

Performance Indicators: In TST, milk production and body weight are commonly used as selection criteria. GIN infections are associated with significant economic losses, mainly due to reduced body weight gain (Ilangopathy et al., 2019). Also, selective treatment of high risk goats, particularly first lactation and high milk yield animals, has been observed to maintain milk production and prevent clinical parasitic infection while only treating 50 to 66% flock (Van Wyk et al., 2006).

Five Point Check: This system, developed by Bath and Van Wyk in 2009, is an integrated field based approach used for selecting small ruminants requiring targeted selective treatment against GIN infections. Using multiple selection criteria, it involves examination of five body regions: eyes (FAMACHA for anaemia caused by *H. Contortus*), nose (nasal discharge for nasal bots), jaw (submandibular oedema indicating fluke infection), back (BCS and weight loss) and tail (dag score/DISCO for scour-associated parasitism). A common disadvantage of individual methods farm level is their limited specificity and sensitivity, as their reliability can be influenced by non-parasitic factors like nutrition, environmental condition, physiological status and concurrent diseases. Therefore, instead of relying on a single indicator, multi-criteria approach has been observed to improve the accuracy and efficiency of TST, although such approach is comparatively more tedious and time consuming. A combined approach for making individual treatment decisions using FAMACHA/BCS with ≥750 EPG threshold have been reported to enable selective treatment of only half of the flock, thereby minimizing the need for mass treatment (Mancilla-Montelongo et al., 2023).

Incorporation of TST into Integrated Parasite Management (IPM)

The incorporation of TST within the IPM program is increasingly recognized as a powerful sustainable approach for conserving the efficacy of limited available anthelmintic drugs in small ruminant production system (Hayward & Kenyon, 2026). By restricting anthelmintic use to animals exhibiting clinical, production-associated indicators of GIN infection, TST helps reduce treatment costs while preserving refugia. The adoption of TST alongside complementary non-chemical strategies including rotational grazing, mixed species grazing, improved nutrition and genetic selection for host resilience enhances a long term sustainable control while reducing sole dependence on routine blanket deworming practices.

Farmer Constraints in the Implementation of TST

Despite the proven benefits of sustainable parasite management and TST, adoption by livestock owners remains limited due to several practical and economic constraints. One of the major constraint is the limited awareness and understanding of refugia based concept, as farmers still rely on the routine blanket anthelmintic treatment for immediate parasite control. The complexity of TST programs requires close monitoring of the individual animal. In addition, in many resource-poor farming systems, inadequate access to veterinary services, diagnostic facilities and trained advisors further restricts the successful application of these approaches. Frequently labour intensive and time consuming protocols may also reduce owner's willingness to adopt these sustainable strategies. Furthermore, farmers may hesitate to leave a proportion of animals untreated due to fear of production losses or mortality, especially when the benefits of refugia are not immediately visible. However, once AR reaches advanced levels, it will often be too late to implement any other remedial measure (Van Wyk et al., 2006).

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Review Article

Antibiotic Use in Wild Felids: Current Practices, Clinical Challenges and Antimicrobial Resistance

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Abstract

Wild felids including lions, tigers, leopards, cheetahs, and other nondomestic cats are susceptible to a number of bacterial infections in captive and free-ranging situations. Effective antimicrobial therapy has therefore become an essential component of wildlife conservation, zoo medicine, and rehabilitation programs. However, knowledge on the use of antibiotics in wild felids is inconsistent and estimated from domestic feline medicine, despite major physiological and pharmacokinetic differences between species. Among the major classes of antimicrobials used in nondomestic felids, third-generation cephalosporins, have emerged as valuable agents because of their prolonged duration of action. Increasing reports of multidrug-resistant bacterial isolates in large felids highlight the urgent need for evidence-based antimicrobial stewardship. Challenges associated with repeated drug administration, species-specific dosing, stress during handling, and ecological consequences of antimicrobial misuse emphasize the importance of integrated veterinary and conservation approaches. This review summarizes the major bacterial diseases affecting wild felids and evaluates current antibiotic therapies, pharmacokinetic considerations, administration strategies, and antimicrobial resistance trends within a One Health framework.

Keywords: *Administration methods Antibiotics, Antimicrobial resistance, Felids.*

Wild felids are apex predators and are key to the ecological integrity of their native environments. In captivity, including zoos, sanctuaries and rehabilitation centers, the ability to provide effective antimicrobial therapy is essential for disease control and their survival. Historically, the management of nondomestic felids was largely anecdotal and based on estimated dosages from the domestic cat (*Felis catus*). Differences in allometric scaling, fluctuations in metabolic rate, and the logistical impracticality of daily administration of oral or injectable drugs to animals necessitate a paradigm shift. The use of long-acting antibiotic formulations and evidence-based dosing regimens based on species-specific pharmacokinetic/pharmacodynamic studies is currently emphasized in zoo medicine (Cushing et al., 2017). This review gives a comprehensive overview of antibiotics used in wild felids, including common bacterial pathogens, pharmacokinetics of major classes of drugs, practical challenges of administration, and the growing worldwide threat of antimicrobial resistance in a One Health framework.

Common Bacterial Infections in Wild Felids

Usually felids are vulnerable to the same infectious diseases as domestic cats. They are also susceptible to diseases transmitted by other animals, including viral infections. Many infections are zoonotic so strict hygiene procedures are essential while handling felids. It is essential to limit the exposure of captive felids to feral and domestic cats and dogs, free ranging carnivores, bats, rodents and other small mammals. Large felids constantly face diverse bacterial pathogens that complicate systemic infections such as pyelonephritis in jaguars (Wronski et al., 2020), remaining population including small felids always remain vulnerable to undiagnosed vector borne agents, including Bartonella and Mycoplasma species (Cerreta et al., 2022). These vector-borne, Gram-negative bacteria establish persistent bacteremia within their reservoir hosts, often surviving in the feline bloodstream for extended periods without inducing overt clinical symptoms. Smaller felids harbouring Bartonella species too often remain asymptomatic; however have been reported to develop severe clinical symptoms including endocarditis, uveitis and necrotic lesions in naturally or experimentally infected cats (Chomel et al., 2006).

In free ranging animals, Bovine Tuberculosis is the most severe threat in lions (*Panthera leo)* and leopards (*Panthera pardus)* when these felids hunt infected wild ungulates like African buffaloes that leading to severe chronic emaciation, pulmonary granulomas and systemic decline can manifest (Gumbo et al., 2023). Mycobacterium orygis is found in free ranging ecosystems where livestock and wildlife overlap creating complex multi host transmission cycle (Thapa et al. 2017). In opinion of Szacawa (2025) human caretakers, shared enclosures and artificial diets acts as a catalyst for Helicobacter gastritis infections (Junginger et al., 2015), whereas rehabilitated felids due to isolation, physical traumas, bite wounds and severe immunosuppresion affects upper respiratory system caused by pneumonic pasteurellosis. Rapid switching from wild prey to commercial or domestic meats can cause gut dysbiosis and also give rise to Clostridium enterotoxemia, if the feed is not maintained. High density rehabilitation pens can increase the risk of feco-oral contamination causing Salmonellosis and Camphylobacteriosis. The key to successful antimicrobial therapy is to understand the real cause of these diseases.

***Respiratory System**:* Respiratory disease is a significant source of morbidity in captive felid populations. Respiratory symptoms are common in snow leopards. A 25-year retrospective research of snow leopards in North American zoos showed that 45% of the assessed individuals experienced at least one respiratory episode, with 61% of these cases involving Mycoplasma species and 39% cases isolated were Bordetella bronchiseptica mainly manifested in neonates and juveniles. Coinfections in 91% of the cases demonstrate the challenges of respiratory disease management in this species. *Pseudomonas aeruginosa* was frequently found in fatal cases and usually showed resistance against many common antibiotics (Wunderlich et al.*,* 2025). Mycobacterial infections including Bovine tuberculosis and human tuberculosis have been reported in lynxes. Conspecific lynxes are usually infected from prey species viz wild boar or deer, as they rarely interact with each other except the breeding season (Briones et al., 2000). The treatment of tuberculosis requires 6-12 months long multi drug therapy with drugs such as isoniazid, rifampicin and ethambutol (Chanove et al.*,* 2024). It is logistically very difficult to extrapolate such intense daily oral regimens to wild or semi captive lynxes and there is a risk of developing further resistance. Non tuberculous mycobacteria, such as *M. avium* may also potentially cause chronic respiratory symptoms. Avium subsp. *hominissuis* was isolated from *tapirs* and it was treated with isoniazid (5mg/kg), rifampicin (10-15mg/kg) and clarithromycin (10mg/kg) for 11 months (Marcordes et al., 2021).

Reproductive System*:* Among reproductive system disorders, pyometra is a life-threatening emergency in intact female big cats (Steeil et al., 2012)). The disease progresses insidiously until the animal has systemic septicemia. Management of pyometra is usually done with surgical ovariohysterectomy and abdominal lavage (McCain et al.*,* 2009).

Digestive System*:* Digestive tract diseases are also commonly observed in wild felids. Enteric infections are one of the major concerns for zoo vets with regard to the gastrointestinal health of captive wild felids, which can lead to chronic wasting, acute dehydration and increased mortality rates in neonates.

Salmonella enterica is an important gastrointestinal infection in wild felids. Some species may be commensals of *Salmonella*, but specific serovars are highly pathogenic. *Salmonella* spp can cause enteritis but affected animals often become asymptomatic carriers. A notable clinical report described the isolation of a multidrug resistant strain of *Salmonella enterica* serovar Newport from exotic felids at two different regional zoos that caused death of two felids. The genetic profile of this strain was very similar to those frequently linked to human travel and beef products, raising the possiblity of interspecies transmission (Stenger et al., 2025). In another study, *Salmonella enterica* was found in 100% of fecal samples from wild felids under veterinary care (Rojas-Sanchez et al., 2024). All the isolates were resistant to cefazoline and 71% were resistant to ciprofloxacin, a fluoroquinolone used in human medicine (Rojas-Sanchez et al., 2024). *Salmonella* serovars Bovis morbificans and southampton were isolated in yet another study from wild cats with complete resistance to second generation cephalosporins such as cefaclor and cefoxitin (Salah-Eldein et al., 2022). Enterocolitis is usually attributed to *Camphylobacter* spp and clinical form of disease has been reported mainly in cheetahs.

Another important bacterial infection of digestive tract in wild felids is *Helicobacter* infection which causes gastritis manifested as regurgitation, vomiting, weight loss and poor condition. *Helicobacter* species are regularly identified in the gastric mucosa of cheetahs and lions, usually associated with lymphphoplasmacytic gastritis. *Helicobacter felis* was isolated from an African lion with a clinical history of vomiting (Salah-Eldein et al., 2022). Research on cheetahs suggested that *Helicobacter* is common but doesn't always correlate with clinical signs or abnormal fecal scores (Peel et al., 2023). *Helicobacter* has been documented as common isolate but it is not always associated with clinical signs or abnormal fecal scores with this pathogen, the host immune responses and dietary parameters viz. carcass fed or processed diets acts as determining factors if there are clinical manifestation.

Singh et al. (2022) isolated *E. Coli* as the most common single bacterial species cultured from the cardiac blood samples from forty large cats (26 tigers, nine lions and five leopards) found dead in zoos and wildlife sanctuaries in northern India. They also emphasized that 73.1% of the bacterial isolates from these big cats were multidrug resistant suggesting environmental contamination with resistant bacteria.

Ram et al. (2020) identified Clostridium perfringens as a cause for fatal enterotoxemia with motality in sub adult male Bengal tigers. Case studies detected anthrax and parasitic diseases in the areas of Periyar Tiger Reserve and hence, established the standard for detecting and managing emerging bacterial threats like Clostridium and E.coli in free ranging Indian felids (Mahanti 2021). There is a need to conduct more focused studies on the bacterial diseases of free-ranging Indian felids.

Integumentary System: This system is affected by intraspecific violence or injuries from environmental trauma, especially in group-housed lions and tigers. *Staphylococcus species* are frequently isolated from these wounds. The most common isolates in captive felids have been documented as *Staphylococcus pseudointermedius* and *Staphylococcus felis*. Most of the strains of the isolates were sensitive to common antibiotics but some isolates such as *Staphylococcus nepalensis* had the gene mecA suggestive possible methicillin resistance (Alcantara et al*.,* 2023).

Another important infection which is commonly detected at necropsy examination of large felids is bacterial septicemia. A study on blood samples collected from 40 large felines (26 tigers, 9 lions, 5 leopards) revealed 145 bacterial strains belonging to 44 species (Singh et al., 2022). *E. coli* was the predominant bacteria followed by *Alcaligenes denitrificans*. Around 73% isolates were multi drug resistant whereas 40 per cent were categorized as carbapenam resistant strains. Absence of species specific data pertaining to use of antibiotics for various conditions poses a major challenge in wild and zoo medicine.

Common Antibiotics used in Wild Felids

Commonly used antibiotics in wild felid medicine have been discussed as under also in Table 1.

Table 1: Common Bacterial Diseases in Wild Felids

Pathogen	Clinical Syndrome	Species Affected	Reported Antibiotics	Reference(s)
Mycobacterium bovis	Chronic wasting, pulmonary granulomas, respiratory dyspnea, and generalized systemic tuberculosis.	African lion (Panthera leo), Leopard (Panthera pardus), Cheetah (Acinonyx jubatus).	Rifampicin combinations (with Isoniazid, Ethambutol, or Enrofloxacin). .	(Gumbo et al., 2023; Thapa et al., 2017)
Helicobacter acinonychis (formerly H. acinonyx)	Chronic gastritis, recurrent vomiting, gastric ulceration, weight loss, and secondary systemic amyloidosis.	Cheetah (Acinonyx jubatus), Lion (Panthera leo), Tiger (Panthera tigris).	Clarithromycin or Azithromycin combined with Amoxicillin (Triple therapy).	(Dailidiene et al., 2004; Junginger et al., 2015)
Pasteurella multocida	Acute hemorrhagic septicemia, severe purulent bite-wound cellulitis, and fibrinonecrotic pneumonia.	Amur tiger (Panthera tigris altaica), Cougar (Puma concolor), Iberian lynx (Lynx pardinus).	Penicillin G, Ampicillin, Amoxicillin-clavulanate, and Enrofloxacin.	(Junginger et al., 2015; Flaminio et al., 2019; Caramujo et al., 2026)
Clostridium perfringens	Acute necrotizing enteritis, hemorrhagic diarrhea, gas gangrene, and sudden death via alpha-toxemia.	Serval (Leptailurus serval), Cheetah, and various captive Pantheraspecies.	High-dose Penicillin G, Clindamycin, and Metronidazole.	(Junginger et al., 2015)
Salmonella enterica	Severe gastroenteritis, projectile diarrhea, dehydration, and endotoxic shock (often diet-associated).	Lion, Tiger, Jaguar (Panthera onca), and wild-caught small felids.	Sulfamethoxazole-trimethoprim, Enrofloxacin, and Chloramphenicol.	(Garcês & Pires, 2023; Caramujo et al., 2026)
Staphylococcus pseudintermedius / S. aureus	Pyoderma, deep wound abscesses, otitis externa, osteomyelitis, and dental root infections.	Wild cat (Felis silvestris), Iberian lynx, Lion, and Leopard.	Amoxicillin-clavulanate, Clindamycin, Ceftazidime, or Carbapenems (Meropenem/ Imipenem for MRSA/MRSP).	(Stevens et al., 2005; Junginger et al., 2015; Garcês & Pires, 2023)

Cephalosporins have become a mainstay in wild felid medicine, especially third generation formulations, because of their broad spectrum and the availability of long-acting injectable formulations. Cephalexin is a first-generation cephalosporin antibiotic used in wild felids at dosages of 12.5–25 mg/kg orally every 12 hours, although regimens of less than 35 mg/kg every 8 hours have also been reported. It is primarily employed for the treatment of susceptible skin, soft tissue, and urinary tract infections in captive lions and tigers. Systemic antibiotic therapy has been documented in clinical management protocols for infectious conditions such as pyometra in captive large felids (McCain et al., 2009).

Cefovecin is commonly used antibiotic in modern zoo felid medicine, though not available in India. The advantage of this antibiotic is strong plasma protein binding. Dose rate is 8mg/kg SC, IM, once only (Carpenter & Marion 2017). The duration of action of cefovecin is directly proportional to the plasma protein binding. The protein binding of cefovecin in cheetahs was found to be 99.9%, which is almost the same as in domestic cats (Yu et al., 2021). Concentrations of cefovecin >1 µg/ml have been detected in tiger plasma for up to 56 days after injection (Cushing et al., 2017). However extrapolating therapeutic efficacy to wild felids further don't gurantee therapeutic efficacy when related to detectable concentration in blood due to conjugation deficiencies and allometric scaling illusion (Lautz et al., 2021; Court, 2023).

Fluoroquinolones are used for their bactericidal activity against Gram-negative aerobes and some Gram-positive bacteria. Their use is indicated for deep tissue infections, osteomyelitis and severe respiratory tract infections. Enrofloxacin can be used in wild felids @ 5mg/kg PO, IM, SC q 24 hrs. It is highly effective against a wide range of bacteria, though it is sometimes restricted due to resistance concerns. Snow leopards have been treated effectively for most of infections by enrofloxacin. In domestic cats, enrofloxacin can induce retinal toxicity characterized by acute retinal degeneration, blindness, and dilated pupils (Newkirk et al., 2017). This is linked to abnormalities in the ABCG2 gene that prevent effective removal of the drug from the blood–retinal barrier. Retrospective histological studies and post-mortem evaluations have demonstrated that standard oral doses (5 mg/kg daily) of enrofloxacin do not cause retinal thinning or clinical blindness in lions and tigers. This is likely due to a lack of the same ABCG2 polymorphisms found in domestic cats. However, Felids are known to be at risk for retinal degeneration (Papich, 2026). Thus, in wild felids, dosing should be meticulously calculated based on lean body mass to minimize the risk of toxicity (Miller et al., 2023).

Another fluoroquinolone viz. marbofloxacin is broad-spectrum antibiotic commonly used in wild felids, including lions and tigers, at a dosage of 2 mg/kg administered orally, IM, or SC every 24 hours for the treatment of susceptible bacterial infections, particularly those involving the respiratory, urinary, and soft tissue systems

(Fowler & Miller, 2015). Marbofloxacin is preferred in zoo and wildlife medicine because of its broad-spectrum activity, good tissue penetration, and convenient once-daily dosing in large carnivores (McCain et al., 2009). Recent studies suggest that the current breakpoints for enrofloxacin and marbofloxacin, two drugs commonly used for respiratory diseases and septicemia in felids, could be lowered for use in felines. The breakpoints have also been revised for feline isolates of *Pseudomonas aeruginosa* and *Staphylococcus species*. Two dilutions lower than the current norms are recommended to prevent the clinical failure (Papich et al., 2026). This has important implications for treatment of big cats, as under dosing of these concentration-dependent drugs is a major contributor to resistance.

Beta-lactam Antibiotics: Amoxicillin and amoxicillin-clavulanate are most commonly used as first-line empirical treatment for cutaneous infections and bite injuries in wild animals. Commonly used at dose rates Amoxicillin @ 15mg/kg PO/IM/SC every 12 hrs, Amoxicillin/Clavulanic acid @ 12.5 - 25 mg/kg PO/IM/IV/SC every 12 hrs and Ampicillin @ 22mg/kg PO/IM/IV/SC. Long-acting preparation (2-3 days) of amoxicillin trihydrate, 150mg/ml can be tried at the dose rate equivalent to 1ml per 10kgs. There are limited reports of use of ampicillin in wild felids (Hahn, 2019). Amoxicillin and clavulanic acid (dosage 20 mg/kg, q 8/h) in association with marbofloxacin (2.5 mg/kg q 24/h) were administered to treat a 15-day-old, female, captive *Panthera tigris* cub, but the patient succumbed to septic shock and multiple organ dysfunction syndrome (Mazzotta et al., 2022). McCain et al. (2009) reported 11 cases of pyometra in a captive exotic felid collection over 3 years in seven African lions (*Panthera leo*), two tigers (*P. tigris*), one liger (lion-tiger crossbreed), and one leopard (*P. pardus*). Postsurgical treatment was done with broad-spectrum oral antimicrobials: amoxicillin (n=4; 15 mg/kg b.i.d), amoxicillin/ clavulanic acid (n=2; 20 mg/kg b.i.d), cephalexin (n=1; 35 mg/kg t.i.d), enrofloxacin (n=2; 5 mg/kg s.i.d), or trimethoprim/sulfamethoxazole (n =1; 30 mg/kg b.i.d). One of the animals that grew *E. coli* was switched from amoxicillin to marbofloxacin (5 mg/kg s.i.d) after antibiotic susceptibility results were obtained. The highest resistance rates against beta-lactams (penicillin and amoxicillin) were reported in *Staphylococcus aureus* isolates from felids in Ecuador. But the major challenge with this antibiotic is reported antibiotic resistance. The synthetic penicillins are not ideal for large felids as they require repeated daily oral administrations unless the animal has a high food motivation or is conditioned for medical interventions (Miller et al., 2023).

Tetracyclines can be used for respiratory infections, tick-borne diseases, and chlamydiosis in various wildlife species. The dose rates are doxycycline @ 10mg/kg PO q 24hrs and oxytetracycline @ 10mg/kg IM, SC q 24 hrs. In wild felids such as lions and tigers, oxytetracycline is commonly administered at 10 mg/kg via intramuscular (IM) or subcutaneous (SC) injection every 24 hours for the treatment of susceptible bacterial infections. Because oral absorption is often unreliable in large carnivores, parenteral administration is preferred. Long-acting oxytetracycline formulations (300 mg/ml) are particularly useful in captive and free-ranging wildlife medicine, where repeated handling is impractical. These preparations may be administered as a deep IM injection at 20 mg/kg every 72 hours, providing therapeutic coverage for approximately 3–4 days, or at 30 mg/kg every 5 days, with activity lasting up to 5–6 days after a single dose. Extended-duration regimens help reduce animal stress associated with repeated immobilization and handling in large wild cats (Fowler & Miller, 2015). Doxycycline is the drug of choice for vector borne diseases in wild animals. Lions and tigers are reported to harbor vector borne pathogens, specifically tick borne diseases such as species of *Ehrlichia* and *Mycoplasmas*. Doxycycline is highly lipid soluble, allowing it to penetrate where these infections occur in tissue and cells. Doxycycline is also effective in the management of respiratory diseases caused by mycoplasma and chlamydia infections (Wunderlich et al., 2025). Clinical Leptospirosis in a 12-year-old Sumatran tiger was successfully treated with amoxicillin–clavulanic acid (10 mg/kg, PO, q 12 h for 5 days), and doxycycline (5 mg/kg, PO, q 12 h for 21 days; began on day 6) (Webb et al., 2022).

Aminoglycosides are mainly useful for treating serious Gram negative infections. Gentamicin is the most frequently used aminoglycoside antibiotic in zoological and wildlife medicine for the treatment of severe systemic and focal bacterial infections in large wild felines. Antibiogram studies of bacterial isolates obtained from captive lions and tigers have shown that, although multidrug-resistant strains may be present, pathogens such as *Escherichia coli* and *Salmonella* spp. frequently retain susceptibility to gentamicin. The isolated bacteria from snow leopard infections were reported to be sensitive for aminoglycosides (Wunderlich et al., 2025). Hossain et al. (2020) isolated and characterized bacterial pathogens from fecal samples of captive tigers and lions and found that *E. coli*, *Salmonella* spp., and *Staphylococcus* spp. showed resistance to several commonly used antibiotics. However, most isolates remained susceptible to gentamicin, neomycin, and colistin sulphate, suggesting these antimicrobials may be effective for clinical management of such infections.

In captive large cats, gentamicin is frequently used in clinical treatment protocols for a range of documented bacterial infections, particularly wound infections and abscess formation. Large felids commonly experience bite-related injuries and opportunistic bacterial skin infections. Gentamicin offers strong broad-spectrum activity against Gram-negative bacteria, including *Pasteurella multocida* and *Aeromonas hydrophila*, which are commonly associated with oral and bite-wound microflora. Successful conservative treatment of pyometra using intrauterine lavage with diluted gentamicin in combination with systemic antimicrobial therapy in lionesses and tigresses has been reported. As in domestic cats, gentamicin administration in wild felids carries a significant risk of nephrotoxicity and ototoxicity (Miller et al., 2023). Consequently, its use is contraindicated in animals with pre-existing renal impairment, and aggressive fluid therapy should be maintained throughout treatment to support renal function. As wild felids, especially lions and tigers, are highly sensitive to renal stress, extended-interval dosing regimens (every 24–48 hours based on body weight) are recommended to enhance bactericidal efficacy while minimizing renal drug accumulation.

Apart from this, metronidazole is commonly used in wild felids, including lions and tigers, for the treatment of anaerobic bacterial infections at dosages of 7.5–10 mg/kg orally every 12 hours or 15–20 mg/kg orally every 24 hours. In addition to its antibacterial activity, it is also employed in the management of protozoal infections such as giardiasis in captive large cats due to its efficacy against *Giardia* spp (Fowler & Miller, 2015). Lane et al. (2004) treated six captive cheetahs (*Acinonyx jubatus*) with severe gastritis diagnosed by gastric endoscopy and mucosal histopathology with omeprazole, metronidazole, and amoxicillin for 3 weeks. Furthermore, potentiated Sulfonamides viz. Trimethoprim-sulfamethoxazole @ 30 mg/kg PO, q 12 hrs can be used for systemic or gastrointestinal infections (Carpenter & Marion 2017).

Toxoplasma gondi in large felids is primarily treated by clindamycin (Girling et al. 2020). Rifampicin is used in combination with macrolides (such as clarithromycin or azithromycin) to treat mycobacterial infections, which are often refractory to standard therapy (Ghielmetti & Giger, 2020). Carbapenems such as meropenam are usually saved for severe life threatening infections caused by MDR gram negative bacteria in wild felids (Ramirez-Castillo et al. 2023). The choice of oral or injectable antibiotic administration in wild felids is primarily based on species temperment, the need for consistent therapeutic levels and pharmacokinetics of the drug. In *Panthera tigris,* Cushing et al. (2017) demonstrated that a single 8 mg/kg intramuscular dose of cefovecin in captive tigers provides an exceptionally long duration of action. In Cheetahs, Yu et al. (2021) reported a single 8mg/kg intramuscular injection maintained plasma concentrations above 7 microgram/milli litre.

Administration Methods

The antibiotics can be administered in injured or captured animals parentrally, topically or orally in rehabilitation centres. In field conservation, antibiotics are delivered remotely by medicated darts, which requires precise darting and is limited to high value and endangered species. In addition, the antibiotics can be given embedded in baits to control bacterial outbreaks in wild carnivores.

Key Constraints and Challenges

Antibiotic Resistance: Inappropriate antimicrobial dosing in wild felids may contribute to the emergence of AMR, posing risks to both public health and environmental systems.

***Gut Flora Disruption**:* Broad-spectrum antibiotics can disrupt the beneficial gut microbiota of wild felines, potentially causing serious digestive and nutritional problems.

***Drug Withdrawal**:* In species under telemetry or active monitoring, veterinarians must ensure that antibiotic administered to wild felids do not persist long enough to cause toxic accumulation or disrupt reproductive behaviours.

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Mini Review

Application of Stem Cells in the Prevention and Treatment of Mastitis in Domestic Animals: Current Advances and Future Perspectives

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Abstract

Mastitis remains one of the most problematic conditions affecting dairy farming efficiency due to its negative impact on cattle health and welfare. Conventional management relies heavily on antibiotics; however, escalating antimicrobial resistance and treatment failures have driven a substantial shift toward regenerative medicine approaches. Stem cell therapy has recently emerged as a promising alternative for mastitis management, harnessing the tissue regeneration and immune-modulation capacities of various stem cell populations, particularly mesenchymal stromal cells and adipose-derived stem cells, as tools for preventing mammary gland infections. This review provides a comprehensive update on recent breakthroughs in this field, elucidating the underlying mechanisms of immunomodulation and antimicrobial action. Current preclinical and clinical evidence from multiple domestic animal species is critically evaluated, key knowledge gaps are identified, and the necessity for standardized therapeutic protocols is underscored. Overall, stem cell-based therapy represents a compelling avenue for boosting udder health and ensuring the long-term sustainability of dairy farming systems.

Keywords: *Antimicrobials, Domestic Animals, Mastitis, Pathophysiology, Stem Cells*

Mastitis, an inflammation of the mammary glands, constitutes one of the most economically devastating conditions in the livestock sector. It severely impairs animal health, milk yield and quality, and the overall profitability of dairy operations. The condition is predominantly caused by bacterial pathogens, most notably *Staphylococcus aureus* and *Escherichia coli*, which present in both clinical and subclinical forms (Seegers et al., 2003; Sordillo, 2018). Direct losses arise through reduced milk production and elevated treatment expenditure, while indirect impacts include compromised animal welfare and diminished farm productivity.

The cornerstone of traditional mastitis management has been antimicrobial therapy; however, the rising prevalence of antimicrobial-resistant (AMR) strains has critically undermined this approach and necessitates the exploration of sustainable alternatives (Sharun et al., 2021). Mesenchymal stromal cells (MSCs) have gained particular attention due to their intrinsic multipotency, immunomodulatory functions, and regenerative capacity (Singh & Choudhary, 2023). Experimental evidence highlights the ability of MSCs to modulate cytokine networks, suppress pro-inflammatory pathways, and facilitate mammary gland regeneration, making them an attractive candidate for mastitis therapy (El-Sayed et al., 2024; Liu et al., 2024).

Despite these promising attributes, the published literature on MSC applications in mastitis remains comparatively sparse in both *in vitro* and *in vivo* settings. The current trajectory in regenerative medicine represents a meaningful departure from conventional treatment in veterinary medicine, and a critical appraisal of available evidence is timely. This review aims to synthesize current knowledge, highlight translational gaps, and propose directions for future research in MSC-based mastitis therapy.

Pathophysiology of Mastitis in Domestic Animals

Mastitis arises from microbial infections and poses a major challenge to the dairy and livestock industries. Its multifaceted nature involves diverse pathogens, complex host-pathogen dynamics, and intricate immune responses, which together influence the development of effective management strategies.

***Etiology and Mechanisms*:** Mastitis manifests in two principal forms: clinical and subclinical. Clinical mastitis presents with overt signs such as udder swelling, redness, and markedly elevated somatic cell counts (SCC), accompanied by reduced milk yield and quality. Subclinical mastitis, in contrast, is asymptomatic and requires diagnostic testing for detection (Ruegg, 2017). Causative agents include both environmental and contagious pathogens, with *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* being predominant (Cheng et al., 2021). Bacterial attachment and colonization of mammary epithelial cells initiate a cascade of inflammatory responses leading to tissue destruction and impaired milk synthesis (Zhang et al., 2023).

The entry of bacterial pathogens triggers recognition of Pathogen-Associated Molecular Patterns (PAMPs) by toll-like receptors on mammary epithelial and immune cells, activating downstream signaling pathways and driving pro-inflammatory cytokine production (Khan et al., 2023). This recruits neutrophils and other immune effectors to the infection site as illustrated in Fig. 1.

***Immunological Responses*:** The host immune response in mastitis involves coordinated innate and adaptive components. Neutrophils constitute the primary first-line defence, migrating rapidly to the infection site where they engulf bacteria and generate reactive oxygen species (ROS) to enhance pathogen elimination. Although ROS production is essential for bactericidal activity, excessive generation leads to oxidative damage within mammary gland tissue (Sordillo, 2018). Notably, MSCs can mitigate this collateral injury by scavenging surplus ROS through paracrine antioxidant mechanisms, thereby limiting the tissue damage described above (El-Sayed et al., 2024).

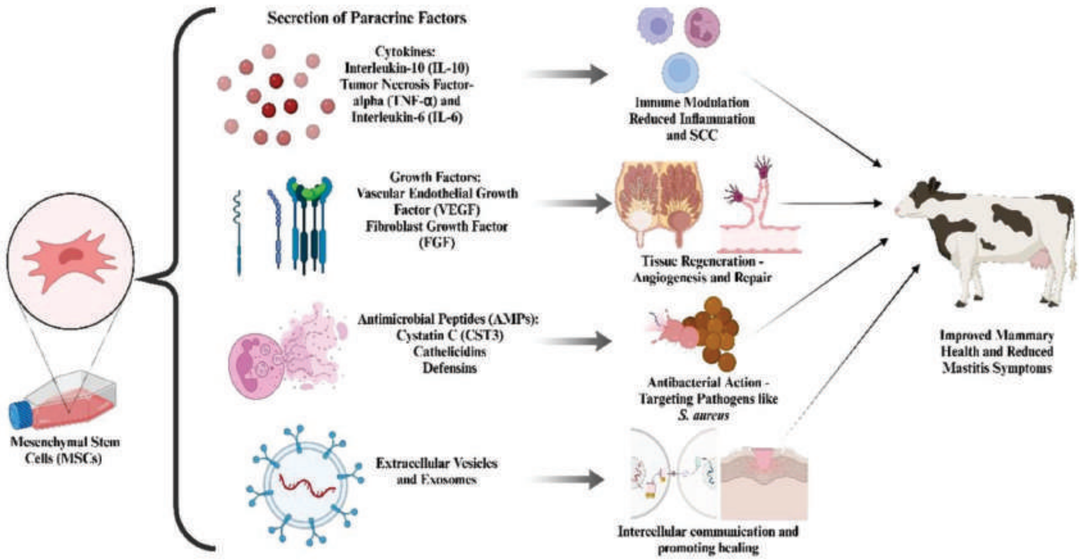

Fig. 1: Mechanisms of Mesenchymal Stem Cells (MSCs) in Modulating Immune Response, Tissue Repair, and Pathogen Suppression during Mastitis Therapy. MSCs release paracrine factors including anti-inflammatory cytokines (IL-10, TGF-β), growth factors, antimicrobial peptides, and extracellular vesicles. Collectively, these mediators suppress pro-inflammatory signaling, enhance neutrophil and macrophage effector functions, scavenge excess ROS to limit oxidative tissue damage, and promote mammary gland epithelial regeneration. This figure is an original artwork created by the authors using BioRender software (BioRender.com).

Challenges in Conventional Treatment of Mastitis

Traditional mastitis therapy relies on antimicrobials, which, despite their efficacy against susceptible bacteria, face a critical challenge in the form of antimicrobial resistance (AMR), particularly among multidrug-resistant *Staphylococcus aureus* strains (Barkema et al., 2006). Antibiotic use in dairy cattle also raises consumer safety concerns due to potential drug residues in milk. Conventional treatments do not address the underlying tissue damage caused by persistent inflammation, predisposing glands to recurrent infection and chronic milk production losses (Pokorska et al., 2024). These limitations collectively underscore the urgent need for innovative, regenerative alternatives such as stem cell therapy.

Stem Cell Biology and Types Relevant to Mastitis Treatment

Stem cell therapy is attracting growing interest in veterinary science for treating inflammatory diseases such as mastitis. The self-renewal capacity and therapeutic plasticity of stem cells offer substantial promise for immune modulation and tissue repair. Principal stem cell types under investigation are summarized in Table 1.

Table 1. Types and Properties of Stem Cells Investigated for Mastitis Therapy

Cell Type	Source	Key Properties	Advantages in Mastitis	Challenges	Reference
Mesenchymal Stem Cells (MSCs)	Umbilical cord, Bone marrow, Adipose tissue	Multilineage differentiation potential; immunomodulatory; regenerative; antimicrobial	Reduces inflammation; promotes tissue repair; potential antimicrobial activity	Sourcing complexity; batch variability; high production cost	Sharun et al. (2021)
Adipose-Derived Stem Cells (ASCs)	Subcutaneous adipose tissue	Strong immunomodulation; tissue repair; low invasiveness of harvest	Reduces fibrosis; suppresses pro-inflammatory cytokines	Scalability challenges; limited data in small ruminants	Kim et al. (2023)
Induced Pluripotent Stem Cells (iPSCs)	Reprogrammed somatic cells	High regenerative capacity; patient-specific; pluripotent	Tailored immune modulation; adaptable to host needs	Theoretical malignant transformation risk; not yet validated in vivo in cattle	Mondal et al. (2021)
Umbilical Cord–Derived MSCs	Umbilical cord blood/tissue	Low immunogenicity; high immunoregulatory potential	Outperforms antibiotics in subclinical mastitis; allogenic use feasible	Sourcing logistics; regulatory considerations	Ghai et al. (2022)
Amniotic Fluid–Derived Stem Cells	Amniotic fluid of pregnant animals	High proliferative capacity; multilineage differentiation	Promotes robust mammary epithelial regeneration	Sourcing limitations; ethical concerns; consistency challenges	Williams et al. (2024)

***Mesenchymal Stem Cells (MSCs)*:** MSCs are multipotent stromal cells with well-characterized multilineage differentiation potential, including adipogenic, osteogenic, and chondrogenic lineages. Their combined immunoregulatory, antimicrobial, and regenerative properties make them the most extensively studied stem cell type for mastitis therapy (Williams et al., 2024). In bovine mastitis, MSCs migrate to infected mammary tissue and secrete a diverse array of bioactive molecules that orchestrate local immune responses and tissue repair.

Key therapeutic mechanisms include secretion of anti-inflammatory mediators (IL-10, TGF-β), production of antimicrobial peptides, modulation of macrophage and neutrophil function, and release of extracellular vesicles (EVs) that facilitate intercellular communication and healing. MSC-derived EVs have attracted particular interest as a cell-free therapeutic modality, offering equivalent immunomodulatory benefits with lower immunogenicity and simpler handling compared to whole-cell transplantation (Xia et al., 2024).

Adipose-Derived Stem Cells (ASCs)**:** ASCs represent an abundant, readily accessible population harvested from subcutaneous adipose tissue with minimal invasiveness, making them especially practicable in veterinary settings (Kim et al., 2023). Their immunomodulatory and regenerative properties closely parallel those of bone marrow-derived MSCs, and they have demonstrated efficacy in suppressing pro-inflammatory cytokine production in experimental mastitis models (Costa et al., 2019).

Induced Pluripotent Stem Cells (iPSCs)**:** iPSCs are somatic cells reprogrammed to a pluripotent state via defined transcription factors, enabling differentiation into virtually any specialized cell type. Their patient-specific derivation eliminates immune rejection concerns and circumvents the ethical issues associated with embryonic stem cells (Williams et al., 2024). Application in bovine mastitis therapy remains investigational but holds promise for generating mammary epithelial and immunoregulatory cells. Novel scaffold-based platforms employing functionalized carbon nanotube thin films have been explored to support iPSC maintenance and directed differentiation in canine models (Mondal et al., 2021); however, it should be noted that this approach has not yet been validated *in vivo* in cattle, and regulatory concerns regarding nanomaterial residues in milk warrant careful evaluation before clinical translation. While iPSCs carry a theoretical risk of malignant transformation, this risk is considerably lower with adult MSCs and ASCs compared to pluripotent cell populations.

Other Relevant Stem Cell Types**:** Umbilical cord–derived MSCs exhibit low immunogenicity coupled with robust immunoregulatory activity, positioning them as viable candidates for mastitis treatment; allogeneic umbilical cord blood–MSC therapy has been reported to outperform antibiotic therapy in alleviating subclinical mastitis in dairy cows (Ghai et al., 2022). Amniotic fluid-derived stem cells also show promise in preclinical settings owing to their high proliferative capacity and multilineage differentiation into mammary epithelial cells.

Experimental Trials on Stem Cell Therapy for Mastitis Prevention and Treatment

Emerging preclinical and clinical evidence supports the therapeutic potential of stem cells across multiple domestic animal species.

Cattle Models**:** Preclinical studies in cattle demonstrate that MSCs, particularly those derived from adipose tissue, significantly attenuate mammary inflammation and restore epithelial integrity by downregulating inflammatory gene expression and promoting epithelial cell proliferation (Zhang et al., 2023). ASCs have similarly shown efficacy in reducing pro-inflammatory cytokine release in lipopolysaccharide-induced mastitis models, corroborating their immunomodulatory potential (Kim et al., 2023).

Goat and Sheep Models**:** In goat studies, MSC therapy produced notable reductions in somatic cell counts alongside enhanced mammary tissue regeneration in both clinical and subclinical mastitis (Costa et al., 2019). In sheep, MSCs augmented anti-inflammatory cytokine profiles and promoted angiogenesis, accelerating healing of damaged mammary tissue (Li et al., 2023).

Clinical Trials and Real-World Applications

Bovine Clinical Trials**:** Intramammary administration of MSCs has shown clinically meaningful results in dairy cows with mastitis. Studies in Holstein cattle demonstrate that MSC therapy achieves faster resolution of udder swelling and reduction in SCC relative to standard antibiotic regimens (Sharun et al., 2021). Crucially, MSC-treated animals exhibited lower mastitis recurrence rates, suggesting that stem cells not only resolve acute infection but also enhance long-term immune competence of mammary tissue.

Challenges in Clinical Applications**:** Despite encouraging results, clinical translation of stem cell therapy faces several obstacles. Variability attributable to genetic and environmental factors across individual animals' limits reproducibility (Williams et al., 2024). The high cost of cell isolation, expansion, and administration currently restricts accessibility, particularly in low-margin dairy operations (Liu et al., 2024). Standardization of cell preparation protocols represents a critical gap; the utility of advanced kinetic stem cell counting-based potency assays should be considered as a means of reducing batch-to-batch variability and establishing reproducible dosing regimens. Biosafety concerns relating to immune reactions and the theoretical risk of malignant transformation, substantially more relevant to iPSC-based preparations than to adult MSCs or ASCs, must also be addressed through rigorous preclinical evaluation before widespread adoption.

Mechanisms of Stem Cell Function in Mastitis

The therapeutic efficacy of stem cells in mastitis is underpinned by three interacting mechanisms: immunomodulation, tissue repair, and direct antimicrobial activity. MSC-derived extracellular vesicles (EVs), notably exosomes, are emerging as a particularly attractive cell-free therapeutic modality. EVs orchestrate immunomodulation and tissue healing through bioactive cargo, including microRNAs, proteins, and lipids, that suppresses pro-inflammatory cytokine expression and promotes resolution of chronic mastitis-associated inflammation. EVs also enhance pathogen clearance by modulating macrophage polarization and disrupting biofilm formation, a key driver of chronic intramammary infections. The cell-free nature of EV therapy confers practical advantages over whole-cell administration, including reduced immunogenicity, easier storage, and simplified administration protocols (Xia et al., 2024).

Challenges and Limitations in Stem Cell Therapy for Mastitis

Technical and Practical Limitations**:** Effective isolation and expansion of MSCs and ASCs while maintaining viability and functional consistency remains technically demanding. Donor-related and tissue-source variability can compromise cell quality, and the absence of universally accepted standardized protocols leads to inconsistent therapeutic outcomes (Pokorska et al., 2024). Cryopreservation, while widely employed for cell banking, introduces additional variability through freeze-thaw-induced reductions in cell viability, further complicating large-scale clinical deployment (Li et al., 2023).

***Safety and Ethical Considerations*:** MSCs and ASCs are generally regarded as safe owing to their immunomodulatory properties; however, allogeneic preparations carry a residual risk of immune-mediated adverse reactions. The risk of uncontrolled cell differentiation and malignant transformation is primarily associated with pluripotent iPSC populations and is considerably lower for adult-derived MSCs (El-Sayed et al., 2024). Application of stem cell therapy in food-producing animals necessitates thorough ethical review and robust consumer safety assessments. Regulatory harmonization across jurisdictions for stem cell-based veterinary therapeutics remains an ongoing challenge (Wang et al., 2023).

***Economic Constraints*:** The economic feasibility of stem cell therapy is a central barrier in the dairy sector, where profit margins are characteristically narrow. Costs associated with cell isolation, culture, quality control, and administration substantially exceed those of conventional antibiotic regimens (Zhou et al., 2024). While the long-term benefits, including reduced recurrence and improved udder health, may offset initial expenditures, the high upfront investment limits farmer uptake. Innovations reducing production costs, such as off-the-shelf allogeneic cell banks and simplified delivery systems, will be essential for broad commercial viability.

Future Perspectives and Research Directions

The field of stem cell therapy for mastitis is advancing rapidly, with current research addressing known limitations through several converging strategies. Genetic engineering of MSCs and ASCs to overexpress anti-inflammatory or antimicrobial genes represents a promising approach to enhance therapeutic potency (Pokorska et al., 2024). Innovations in delivery systems—including biodegradable scaffolds, hydrogels, and nanocarriers for controlled intramammary release—are poised to improve targeting efficiency (Liu et al., 2024). Combination strategies co-delivering stem cells with antimicrobial or anti-inflammatory agents may synergistically address both infection and inflammatory components of mastitis (Wang et al., 2023).

Stem cell therapy, particularly involving mesenchymal and adipose-derived stem cells, holds substantial promise for the treatment and management of mastitis in farm animals. Their immunoregulatory, regenerative, and antimicrobial properties collectively offer a compelling therapeutic option, with paracrine anti-inflammatory signaling contributing to reduced local tissue swelling and vascular permeability at infection sites, thereby promoting healing and reducing dependence on antibiotic treatment. Several challenges associated with cell-based interventions must be systematically addressed to ensure treatment success. Standardization of isolation, preparation, and administration protocols is essential to make stem cell therapy a consistent, feasible, and affordable option for farmers and breeders, ultimately establishing it as an effective and reliable strategy for the prevention and management of mastitis in farm animals.

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Mini Review

Teaching and Learning Veterinary Anatomy with Artificial Intelligence: Promises and Perils

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Abstract

The integration of artificial intelligence (AI) into anatomy teaching has the potential to transform student learning. AI offers resources that facilitate the understanding of complex topics through immersive three-dimensional virtual reality. AI-powered platforms engage students through games, simulations, and virtual reality experiences. Additionally, AI accelerates research by automating the analysis of large datasets, thereby advancing animal health and medicine. Educators can leverage AI-driven platforms to design interactive and engaging lessons that foster curiosity, promote collaboration, and support the training of future veterinary professionals. However, challenges persist, including server costs, tool development, and the need for technical support. Ongoing innovation is necessary to ensure continued excellence in veterinary anatomy education.

Keywords: *Anatomy, Artificial Intelligence, Education, 3D models, Virtual Reality*

Anatomy, often described as the "mother of veterinary education," provides essential foundational knowledge for clinical and surgical practices. It is typically introduced during the first year of undergraduate veterinary curricula (Henssen et al., 2024). The subject encompasses extensive and complex material, reflecting the intricate structure of animal bodies. Students commonly encounter learning challenges early in their studies, which may negatively affect academic performance. Traditional anatomy instruction has relied on cadaver dissections, static photographs, and textbooks, all of which are crucial for developing spatial understanding of morphology. While substantial literature addresses artificial intelligence (AI) applications in clinical settings, there is limited research on the use of AI for teaching and learning in anatomy. This mini-review examines the potential benefits of integrating AI into veterinary anatomy education, such as increased lesson interactivity and personalised learning experiences.

Artificial Intelligence in Teaching Veterinary Anatomy

AI is transforming the way veterinary anatomy is taught by introducing new learning methods, improving diagnostic accuracy, and making scientific research faster.

When students use AI to practice real-life scenarios together, they develop key skills such as critical thinking, teamwork, and decision-making. These abilities are essential for anyone planning to become a veterinarian.

Various Modes of AI

AI tools make it easier to identify different anatomical structures and help keep teaching materials consistent. With 3D reconstructions and AI-generated models, students can move and study organs and body parts much like in a real dissection, but with fewer differences between specimens (Gupta et al., 2023). AI also helps create databases of anatomical variations, giving students more resources to learn from (Henssen et al., 2024). Augmented reality apps allow teachers to show small structures that are otherwise hard to see (Ehlers et al., 2024).

Virtual and augmented reality offer significant opportunities to enhance anatomy instruction by increasing interactivity and enabling dynamic exploration of anatomical structures. AR and VR technologies have transformed veterinary anatomy education. The integration of AI into AR/VR digital models enables real-time control and exploration, thereby improving students' attention and comprehension (Atanasova & Petrov, 2021). Gamification elements, such as point systems, badges, and competitions, further promote student engagement. The use of interactive 3D models and VR applications enables practical virtual dissection and detailed examination of complex topographic regions (Dekker et al., 2024).

AR and VR technologies facilitate the teaching of complex anatomical structures by enabling manipulation and multi-angle visualisation (Singh et al., 2024). Virtual platforms support coordinated dissection exercises and collaborative problem-solving activities. For example, Li et al. (2026) evaluated the Imaging Anatomy Virtual Simulation Experiment course and suggested that virtual simulation bridges traditional human anatomy and medical imaging. This approach improved students' spatial reasoning, diagnostic accuracy, and overall knowledge retention. Telecan et al. (2025) compared Virtual Dissection Tables (VDTs) with traditional cadaveric dissection in medical curricula. Their study finds that VDTs significantly improve academic performance and student engagement, but are most effective as a supplement to, rather than a replacement for, human cadaver.Wiki-based anatomical atlases also help students work together and share their knowledge as a group.

ChatGPT represents a significant advancement in artificial intelligence technology. Since its release in November 2022, ChatGPT has rapidly gained one million users (Sedaghat, 2023). This advanced language model utilises deep learning techniques to generate human-like responses and is trained on extensive internet-derived datasets, enabling it to address a wide range of queries. In veterinary anatomy education, AI-based adaptive learning programs have improved student performance by tracking progress and adjusting lesson difficulty to facilitate efficient learning (Pandurangam et al., 2024).

Integrating artificial intelligence into the feedback process during learning enhances educational outcomes by enabling rapid corrections and supplementary guidance. Machine learning programs, combined with student interactions, identify areas requiring improvement and adjust content accordingly. Abinaya et al. (2024) noted that incorporating games with real-time assessment and feedback in educational programs supports independent, targeted learning. These systems also facilitate ongoing evaluation of teaching effectiveness and provide educators with valuable insights into student performance and development.

Research in veterinary anatomy is advancing rapidly through automated analysis of large datasets, enabling new discoveries. AI-powered tools contribute to understanding evolutionary trends and targeting disease mechanisms, thereby enriching knowledge of animal anatomy. AI algorithms analyze genomic data and identify genetic variations in domestic animals. These advancements not only deepen understanding of veterinary anatomy but also inform breeding programs.

Benefits

Artificial intelligence makes veterinary anatomy education more accessible, scalable, and effective. Virtual dissection tools and 3D models let students access anatomical resources no matter where they are or which school they attend. These tools can support more students without needing extra specimens or dissection equipment (Allsop & Sfhea, 2023). AI applications have also proven useful in creating personalised learning paths that fit each student's needs. Gamified AI learning environments, which are engaging and interactive, are associated with higher retention rates and greater course participation (Choudhary et al., 2023).

Challenges in Adopting AI

Significant financial investment is required to implement AI technologies, which represents a major challenge (Banzato et al., 2024). Additionally, educators must acquire technical training to effectively integrate AI systems into classroom instruction, a process that can be demanding (Wilson et al., 2022). Ethical concerns include the potential loss of experiential learning associated with cadaver-based instruction and the risk of over-reliance on AI for critical-thinking tasks (Busch et al., 2024). Persistent challenges also include the need for transparent AI systems to ensure that educators and students can trust the accuracy of results.

Prospects of AI in Anatomy Teaching

- Virtual reality will provide a deeper understanding of complex anatomical fields to explore intricate anatomical structures.
- AI would track and visualise the migration and differentiation of structures during development. AI methods will enhance visual learning through 3D images and 3D printing. It would provide accurate, physical models of complex anatomical structures that are difficult to dissect.
- AI systems provide detailed demonstrations of intricate anatomical regions, i.e., head, face, and neck, functioning as valuable teaching assistants.

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Mini Review

Sustainable and Controlled Use of Growth-Promoting Agents in Meat-Producing Animals

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Abstract

Growth promoters are widely used in livestock production to enhance weight gain, improve feed efficiency, and optimize carcass quality of meat-producing animals. Chemical growth promoters have been extensively incorporated into animal feed at sub-therapeutic levels to improve productivity by modulating the gut microbiota and reducing subclinical infections, thereby increasing growth rates by 4-8% and improving feed conversion efficiency. Widespread and prolonged use has raised serious concerns regarding antimicrobial resistance (AMR), drug residues in animal-derived foods, and associated public health risks. Regulatory authorities worldwide have recommended phasing out medically important antibiotics for growth promotion and strengthening surveillance and stewardship programs. Natural and chemical growth promoters have been used to enhance lean muscle deposition, although their use is restricted or banned in several regions due to food safety and ethical concerns. Growing regulatory pressure and consumer awareness have accelerated the shift toward natural growth promoters, including probiotics, prebiotics, organic acids, phytobiotics, and exogenous enzymes. These alternatives aim to improve gut health, immunity, and productivity without significantly contributing to AMR. A balanced approach integrating safer alternatives, responsible antimicrobial stewardship, and sound farm management is essential to ensure sustainable livestock production while protecting animal health, public health, and environmental integrity.

Key word: *Growth promotors, Meat Animals, Phytobiotics, Prebiotics, Probiotics*

Growth promoters are substances administered to accelerate weight gain and improve the muscle-to-fat ratio in meat-producing animals. Their primary objective is to enhance feed efficiency, optimize carcass characteristics, and increase overall productivity. Major meat-producing species include cattle, buffaloes, pigs, sheep, goats, and poultry. The use of growth-promoting agents in these animals is largely driven by the need to meet the rising demand for animal protein from a rapidly growing human population and to support global food security. To maintain a stable demand–supply balance within shorter production cycles, livestock producers have increasingly adopted various growth-promoting strategies (Peng et al., 2024; Parr et al., 2026).

Growth-promoting agents used in livestock production are predominantly antimicrobial in nature. Their widespread and uncontrolled use has raised significant concerns regarding the development of antimicrobial resistance (AMR). When administered chronically as growth promoters, residues may persist in meat and other animal-derived products, posing potential risks to consumers through continuous low-level exposure (Hosain et al., 2021; Rodrigues et al., 2021).

These concerns have prompted a paradigm shift toward the more judicious and regulated use of antimicrobials in food-producing animals. Consequently, there is increasing emphasis on identifying and adopting safer alternatives to chemical growth promoters to ensure consumer safety while safeguarding animal health, public health, food safety, and environmental sustainability (Dibner and Richards, 2005; Valenzuela-Grijalva et al., 2017).

The growth-promoting effects of feed additives were first recognized in the 1940s, when chickens fed tetracycline fermentation by-products exhibited significantly higher growth rates compared to untreated birds (Gonzalez & Vargas-Bello-Perez, 2022). Following this discovery, the use of synthetic growth promoters, including antibiotics and β-agonists, expanded widely across different livestock species.

In recent years, attention has increasingly shifted toward natural growth promoters as safer alternatives. These include plant extracts, essential oils, fungal-derived and exogenous enzymes, direct-fed microbials, prebiotics, phytobiotics, guanidinoacetic acid, spirulina, algae-derived polysaccharides, and synbiotics**.** This transition reflects a broader movement toward safer, more sustainable, and consumer-acceptable livestock production strategies.

Economic Status of Animal Growth Promoters

The global animal growth promoters and performance enhancers market was valued at approximately USD 16.59 billion in 2025 and is projected to reach about USD 27.63 billion by 2034, registering a compound annual growth rate (CAGR) of 5.83% (Precedence Research, 2025). The Asia–Pacific region is expected to witness the fastest growth during this period.

In India, the antibiotic growth promoters market for food-producing animals was valued at nearly USD 158.7 million in 2024 and is projected to reach around USD 210 million by 2032, growing at a CAGR of approximately 4.1%. However, increasing public health concerns regarding antimicrobial resistance have accelerated efforts to reduce the use of antibiotics as growth promoters, supported by evolving regulatory frameworks that encourage safer and effective alternatives.

The Indian feed probiotics market, representing a key non-antibiotic growth promoter segment, is anticipated to expand from about USD 104 million in 2026 to USD 137 million by 2031, at a CAGR of roughly 5.6% (Mordor Intelligence, 2026). Furthermore, the broader Indian livestock animal health market, including pharmaceuticals, vaccines, and nutritional products, was estimated at USD 1.73 billion in 2024 and is forecast to reach USD 4.17 billion by 2033.

Rising human population, increasing income levels, and growing demand for meat and eggs have collectively driven the need for enhanced livestock productivity within shorter production cycles, thereby promoting the continued use of growth enhancers (Grand View Research, 2025).

Growth Promoters for Food Animals

Natural and Synthetic Growth Promoters: The use of antibiotics as growth promoters (AGPs) in agricultural and animal production began in the 1950s in countries such as the United States, Australia, and several European nations (Dibner & Richards, 2005). Antibiotics are natural and commonly obtained bacterial and fungi. They are administered at sub-therapeutic doses, such as monensin, bacitracin, virginiamycin, and tylosin, are incorporated into feed to enhance growth performance and feed efficiency by modulating gut microbiota and reducing subclinical infections. These compounds are collectively referred to as AGPs.

In the U.S. pig production industry, commonly used AGPs have included β-lactam antibiotics (penicillins and lincosamides), macrolides (e.g., erythromycin), and tetracyclines (Fig. 1). To a lesser extent, compounds such as arsenicals, bacitracin, flavophospholipol, pleuromutilins, quinoxalines, and virginiamycin have also been utilized (Peter & John, 2004). In European pig production systems, approved growth promoters have included avilamycin, flavophospholipol, and ionophores, particularly monensin and salinomycin (Angulo, 2004).

In the cattle industry, commonly used AGPs in the United States include flavophospholipol, monensin, and virginiamycin (Peter & John, 2004). In Australia, frequently used agents include flavophospholipol, lasalocid, and monensin (JETACAR, 1999), with limited use of narasin, oleandomycin, and salinomycin. The glycopeptide avoparcin was previously used but was banned in 2000. In the European Union, ionophores such as monensin and salinomycin were used with restrictions, while agents such as pristinamycin and quinupristin were banned in 2000 (Dibner & Richards, 2005).

In poultry production systems for both meat and egg production, growth promoters such as flavophospholipol and virginiamycin have been used in the United States (Peter & John, 2004). In Australia, poultry producers have used arsenical compounds, flavophospholipol, bacitracin, and virginiamycin (JETACAR, 1999). In Europe, major

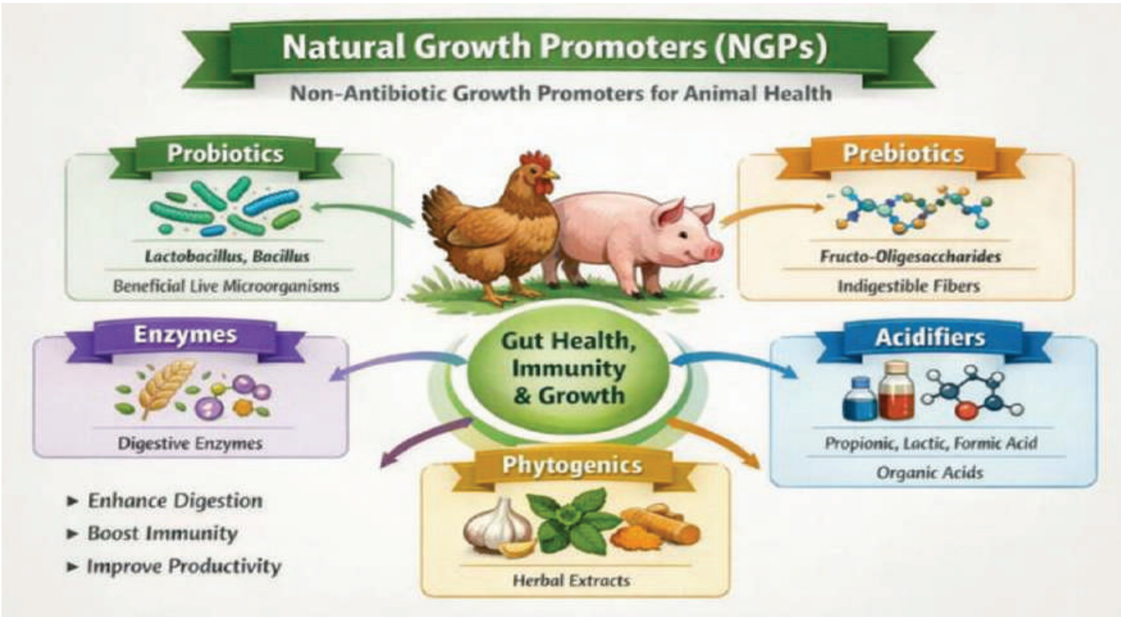

Fig. 1. Natural growth promoters, as an alternative to the antibiotic growth promoters used in livestock production (Source: https://chatgpt.com:OpenAI

approved growth promoters historically included avilamycin, avoparcin, bacitracin, and virginiamycin.

In India, antibiotic growth promoters are widely used in poultry, cattle, and pig production to enhance feed efficiency, accelerate growth, and prevent disease. Commonly used agents include tetracyclines, colistin, ciprofloxacin, tylosin, neomycin, sulfonamides, and, in some cases, critically important antibiotics for human medicine such as amoxicillin, particularly in poultry feeds. Ionophores (e.g., monensin, flavomycin, and virginiamycin) are also frequently incorporated, along with other additives aimed at reducing early chick mortality (Modi et al., 2011; Islam et al., 2024).

Hormonal implants such as estradiol, progesterone, testosterone, trenbolone acetate, and zeranol are primarily used in beef cattle to enhance growth performance and improve carcass yield. These implants are typically placed subcutaneously in the ear, where they gradually release hormones that stimulate anabolic activity, increase protein synthesis, and reduce fat deposition (Preston, 1999). The use of anabolic steroids or steroidal growth promoters in food-producing animals is completely prohibited.

β-agonists such as ractopamine and zilpaterol promote lean muscle growth by altering nutrient partitioning during the finishing phase of livestock production. When used under strict regulatory control, they can improve feed efficiency and enhance carcass composition (Johnson et al., 2014). In India, there is no explicit regulatory approval for the use of ractopamine or zilpaterol in livestock feeds. Similarly, neither compound is permitted for growth promotion in the European Union, which enforces a zero-tolerance policy prohibiting their presence in animal feed and meat products.

Alternative to Antibiotic Growth Promoters

In view of increasing regulatory restrictions and growing concerns regarding human health, alternatives to synthetic chemical growth promoters, commonly referred to as Natural Growth Promoters or non-antibiotic growth promoters, are gaining widespread acceptance. These alternatives include probiotics, prebiotics, organic acids, enzymes, and plant-derived compounds. They enhance gut health, improve immune function, and support productivity without significantly contributing to antimicrobial resistance (Valenzuela-Grijalva et al., 2017).

Probiotics are live microorganisms (e.g., *Lactobacillus*, *Bacillus*) that help maintain a balanced and healthy gut microbiota. Prebiotics are indigestible carbohydrates (e.g., fructo-oligosaccharides) that selectively stimulate the growth and activity of beneficial bacteria. Acidifiers, such as organic acids (e.g., propionic, lactic, and formic acids), help maintain a lower intestinal pH, thereby enhancing nutrient digestion and inhibiting pathogenic microbes. Phytogenics (or phytobiotics) are bioactive plant-derived compounds (e.g., garlic, oregano, turmeric) known for their antimicrobial, anti-inflammatory, and antioxidant properties. Enzymes are incorporated into feed to improve nutrient digestibility and optimize feed utilization (Fig. 1).

Strategy and Steps Towards Judicious use of Growth Promoters in Food Animals

In India, the Department of Animal Husbandry issues advisories promoting the judicious use of antibiotics and mandates withdrawal periods for Schedule H drugs. However, enforcement remains challenging due to the largely unorganized, fragmented, and under-regulated nature of the livestock sector (DAHD, 2024).

In April 2025, the Food Safety and Standards Authority of India (FSSAI) officially prohibited the use of certain antibiotics, including glycopeptides, nitrofurans, nitroimidazoles, carbadox, chloramphenicol, colistin, streptomycin, and sulphamethoxazole, at any stage of production, from rearing to processing, in meat, milk, poultry, eggs, and aquaculture. Colistin had already been banned in 2019 for use in food-producing animals and feed supplements. Additionally, chloramphenicol and nitrofurans were prohibited **(**Fig. 2**)** in March 2025 across all animal rearing systems (FSSAI, 2024).

In May 2025, the Ministry of Health and Family Welfare (MoHFW) notified a ban on 34 antimicrobials, including 15 classes of antibiotics and 18 antivirals. The restricted antibiotic classes, such as ureidopenicillins, ceftobiprole, ceftaroline,

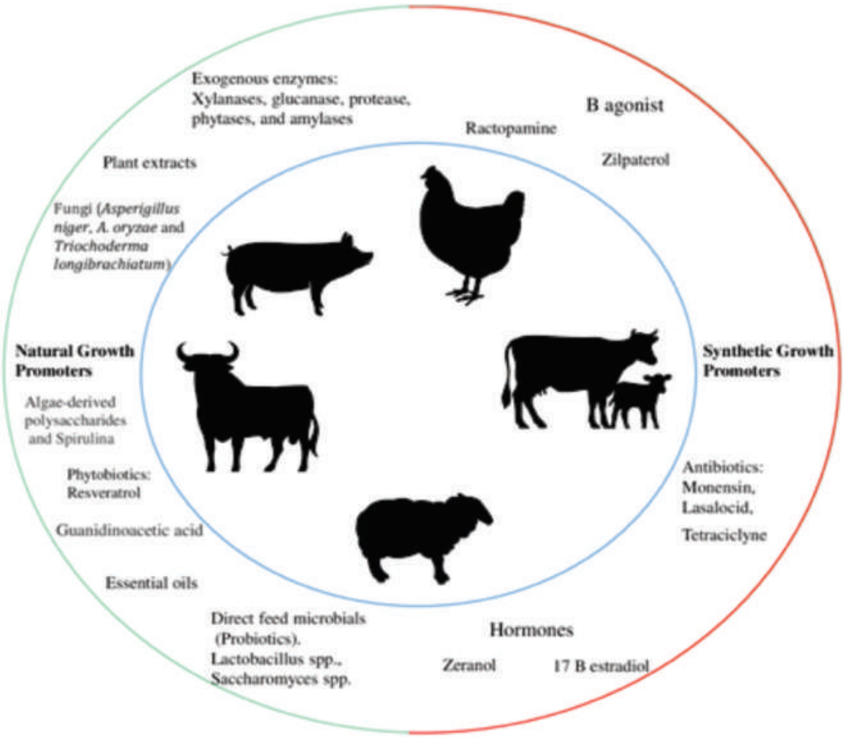

Fig. 2: Common synthetic and natural growth promoters used in livestock production. Red line indicates agents banned in some countries due to antimicrobial resistance or harm to human and animal health (Source: FAO, 2022).

siderophore cephalosporins, carbapenems, penems, monobactams, glycopeptides, lipopeptides, oxazolidinones, fidaxomicin, plazomicin, glycylcyclines, eravacycline, and omadacycline, had previously been reserved exclusively for human use (GIMHF, 2025). Furthermore, to comply with European Union standards, the Drugs Technical Advisory Board, India, has imposed restrictions on numerous antimicrobials to prevent residual contamination in meat intended for export and to ensure international trade compliance.

In 2000, the World Health Organization (WHO) recommended a set of global principles, including the rapid phase-out of all antibiotic growth promoters used in agricultural animal production, particularly those that are also employed in human medicine (WHO, 2000). Four years later, WHO further emphasized the need for risk assessment and systematic surveillance of antibiotic growth promoters used in food animals, as well as monitoring patterns of antimicrobial resistance in both animal and human bacterial pathogens (WHO, 2004).

Antibiotics used for the prevention and treatment of infectious diseases in large groups of farm animals, such as cattle, swine, and poultry, are commonly administered orally through drinking water or feed, and in some cases via intramammary infusion. Such practices may lead to the presence of antibiotic residues in edible products, including meat, milk, and eggs.

Exposure to multiple antibiotic residues may adversely affect human health, particularly during critical stages of organ development, including the nervous and reproductive systems. Infants and young children are especially vulnerable due to their relatively immature physiological defenses. However, the risk of harmful residues can be minimized or eliminated by implementing appropriate pre-slaughter withdrawal periods, restricting the use of certain antibiotics in laying hens, and discarding milk obtained from lactating animals following intramammary antibiotic treatment (Page and Gautier, 2012).

Growth promoters should not be used to compensate for poor management practices, and their routine use without adequate knowledge should be avoided. Instead, emphasis should be placed on correcting and optimizing nutrition, housing, hygiene, and biosecurity systems (Brown et al., 2017). Implementing strict biosecurity measures helps control infections and reduce stress, thereby minimizing the need for prophylactic antibiotics. Improving feed quality and ensuring balanced nutrition inherently enhances animal health and reduces dependence on growth-promoting additives. Additionally, reducing overcrowding prevents disease transmission and supports natural, healthy growth.

A strategic shift from antibiotic or synthetic growth promoters to safer alternatives is strongly recommended. Probiotics, such as Lactobacillus, Bacillus, and Bifidobacterium, help restore and maintain a balanced gut microbiota. Prebiotics, including indigestible components such as fructo-oligosaccharides and mannan-oligosaccharides, selectively stimulate the growth of beneficial intestinal bacteria. Phytobiotics, plant-derived compounds such as essential oils and herbs (e.g., oregano, garlic, and cinnamon), possess antimicrobial, anti-inflammatory, and digestive-stimulant properties. Organic acids (e.g., formic and lactic acids) act as acidifiers, lowering gut pH, suppressing pathogenic microbes, and enhancing nutrient absorption. Exogenous enzyme supplements, such as amylases, xylanases, and phytases, improve nutrient digestibility and feed conversion efficiency (Al-Dobaib & Mousa, 2009; Herago & Agonafir, 2017). Furthermore, vaccination plays a critical role in strengthening the immune system and improving overall animal health, thereby reducing susceptibility to microbial infections.

Judicious use of antimicrobial agents requires strict adherence to established safety standards set by international and national regulatory bodies such as the WHO, FDA, and relevant local authorities. Antimicrobials should be administered only under the direct supervision of a qualified veterinarian. Strict compliance with prescribed withdrawal periods prior to slaughter is essential to minimize drug residues in meat and other animal-derived food products. The use of antimicrobial agents that are critically important for human medicine should be actively avoided or prohibited. Similarly, substances with known mutagenic or carcinogenic potential, such as carbadox and olaquindox, should not be used. Proper labeling, record-keeping, and documentation of all feed additives must be maintained to ensure traceability and regulatory compliance.

Risks and Environmental Impact of Growth-Promoting Agent

Antimicrobial Resistance: The most significant concern is the emergence of antimicrobial-resistant bacteria due to prolonged exposure to low-dose antibiotics. Resistant pathogens may spread through the food chain or environment, posing serious public health threats to humans and related species of animals (WHO, 2017).

Drug Residues in Meat: Improper use and not following the actual drug withdrawal periods may result in chemical residues in meat and meat products. These residual traces may raise food safety and consumer health concerns (EFSA, 2012).

Animal Welfare and Ethical Concerns: The overuse or misuse of growth promoters harms animal health and affects animal welfare, leading to metabolic disorders and stress in intensive production systems.

Regulatory and Consumer Pressure: The significant increase in awareness amongst the consumer related to healthy food and strict follow of national and international regulations imposes restrictions on indiscriminate use of certain growth promoters, particularly antibiotics and hormones.

Judicious Use of Growth Promoters

"Judicious use" refers to the responsible, evidence-based application of growth-promoting agents in food-producing animals to maximize productivity and minimizing risks to public health, animal welfare, and environmental sustainability (WHO, 2017; FAO, 2019).

Restriction of Medically Important Antibiotics: Many countries have restricted or banned the use of medically important antibiotics as growth promoters to mitigate the development of antimicrobial resistance (AMR). Drugs like glycopeptides (e.g. avoparcin) were banned in several regions due to concerns about cross-resistance with human therapeutic agents (Dibner & Richards, 2005; WHO, 2017).

Veterinary Supervision and Oversight: Growth promoters should be administered under the supervision of a professional veterinarian. Veterinary oversight ensures appropriate drug selection, dosage, and duration, reducing misuse and promoting antimicrobial stewardship (FDA, 2015; OIE, 2019).

Strict Adherence to Withdrawal Periods: The drug withdrawal period is the time between the last administration of growth promoters and the time of slaughter. It is essential to ensure that drug residues remain below established maximum residue limits (MRLs), thereby safeguarding food safety and consumer health (EFSA, 2012; Codex Alimentarius Commission, 2018).

Prioritization of Non-antibiotic Growth Promoters**:** There is a need to increase the use and promotion of alternatives such as probiotics, prebiotics, organic acids, phytogenic additives, and enzymes offers opportunities to maintain animal performance while minimizing reliance on antimicrobial agents and reducing the risk of AMR (Valenzuela-Grijalva et al., 2017).

Improved Husbandry and Biosecurity Practices: Enhanced management strategies at the farm level, which include improved sanitation, optimized nutrition, vaccination programs, and "all-in-all-out" production systems, will reduce disease burden and the need for routine antimicrobial use in food animals (FAO, 2019; OIE, 2019).

Regulatory Compliance and Monitoring: Compliance with national and international standards, let down by the FDA, European Union legislation, and Codex Alimentarius guidelines concerning approved substances, dosage limits, and monitoring systems, is essential for ensuring responsible, appropriate use and maintaining consumer confidence in animal-derived foods (EFSA, 2012; Codex Alimentarius Commission, 2018).

Future Research Directions

Antimicrobial agents, particularly antibiotics, have long played a critical role in improving livestock health, productivity, and farm profitability by preventing disease and reducing agricultural losses. However, the subtherapeutic use of antibiotics and synthetic hormones as growth promoters, has raised serious concerns regarding AMR and the presence of drug residues in meat and meat products. Prolonged and indirect exposure to these residues may contribute to the development of resistant human pathogens, posing significant public health risks. Therefore, there is an urgent need to promote responsible and judicious use of antimicrobials in livestock production. Strict adherence to national and international regulatory guidelines, including compliance with recommended drug withdrawal periods, is essential to ensure food safety. Strengthening farm biosecurity measures, improving hygiene, and adopting strategic management practices can substantially reduce microbial load and minimize dependence on antibiotic growth promoters. Simultaneously, the development and application of safer alternatives such as plant-derived antimicrobial agents, probiotics, and bio-preservatives offer promising strategies to sustain animal productivity while mitigating AMR risks. A balanced approach integrating regulatory compliance, alternative growth promoters, and improved farm management will ultimately support sustainable livestock production and ensure the availability of safe, residue-free meat products, thereby safeguarding public health.

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Mini Review

Advances in Post-Ruminal Delivery of Micronutrients and Nutraceuticals in Ruminants

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Abstract

The complex symbiotic microecology of the ruminant forestomach presents a physiological challenge for the targeted delivery of dietary additives. Essential amino acids, vitamins, and bioactive nutraceuticals undergo extensive microbial degradation or hydrogenation within the rumen, drastically reducing their bioavailability at the targeted site of absorption, and thus compromising metabolic efficiency. To bypass these microbial processes, advanced rumen-protection technologies have been developed. These technologies utilize precise physical encapsulation or chemical modifications engineered to make the formulation resistant in the ruminal environment and release within the acidic abomasum or enzymatically in small intestine. The physicochemical principles governing rumen bypass system, includes the critical roles of particle size and specific gravity. Delivering protected amino acids, choline, fat and water soluble vitamins, L-carnitine, gamma-aminobutyric acid (GABA), glutathione, and functional oils have proven positive impact on health and production of dairy animals. By optimizing nutrient partitioning, reducing hepatic lipid accumulation, improving nitrogen utilization, and mitigating oxidative and thermal stress, post-ruminal micronutrients and nutraceutical delivery represents a fundamental component of modern high-yielding dairy herd nutrition. This mini review discusses advances in the rumen bypass technologies enabling efficient post-ruminal delivery of various micronutrients and nutraceuticals.

Keywords: *Microencapsulation, Micronutrient, Nutraceutical, Rumen bypass technology*

Decades of selective breeding and genetic advancement have dramatically increased milk yield and metabolic demand in dairy cows. As production pressure increased, rumen degradation emerged as a major bottleneck, leading to nutrient losses, inefficient nitrogen and energy use and reduced return on supplementation (Singh & Kasrija, 2019). The concept of protecting nutrients from ruminal degradation dates back to the late 1960s, with early experiments focusing on formaldehyde-treated proteins (McDonald et al., 2011). Innovation progressed from simple heat treatments and lipid coatings in the 80s and 90s to today's sophisticated pH-sensitive polymers and microencapsulation technologies, allowing for the pinpoint delivery of micronutrients and nutraceuticals. In ruminants, such formulations are used to manipulate production potential or prevent metabolic diseases. This mini review discusses advances in the technology of post-ruminal delivery of micronutrients and nutraceuticals and their beneficial effects on dairy animals.

Rumen Bypass Mechanism

Rumen protection of micronutrients and nutraceuticals is achieved through two primary methods: (1) physical encapsulation, which utilises coatings such as lipids (including waxes, stearic acid, paraffin, or hydrogenated vegetable oil) and polymers (such as ethyl cellulose, zein, gelatin, alginate, gum arabic, or shellac) (Zabot et al., 2022), and (2) chemical treatments that create amino acid derivatives by blocking the α-amino group (e.g., N-stearoyl-L-methionine, isopropyl-L-methionine, or ε-acetyl-L-lysine) and amino acid analogs by replacing α-amino group with a non-amino group (e.g., Methionine Hydroxy Analog) (Kaur et al., 2024). These protected substances withstand the rumen's neutral pH and microbial breakdown. Key factors influencing the release of encapsulated compounds include granule size, density, and shape, which reduce rumen retention. Smooth particle surfaces minimize microbial adhesion, allowing faster transit through the rumen. Microcapsules under 1mm pass easily, while particle densities of 1.2–1.7 g/cm^3 prevent trapping in the rumen fiber mat (Gowda et al., 2024). The coating dissolves in the abomasum's acidic environment and intestines through enzymatic action, enhancing nutrient bioavailability at the targeted site.

The technique of encasing micron-sized particles (1–1,000 µm) in a covering substance (Reservoir or core-shell system) or embedding them in a homogeneous or heterogeneous matrix (Matrix system) to form tiny capsules with numerous beneficial characteristics is known as microencapsulation.

Matrix system includes techniques like (1) Spray Drying in which a liquid emulsion of the nutrient and coating material is sprayed into a heated drying chamber where the solvent evaporates leaving dry particles, and (2) Spray Freezing / Congealing in which the emulsion is atomised in a cryogenic or cold chamber, suitable for heat sensitive core compounds.

Reservoir system includes techniques like (1) Coacervation, which is a chemical phase separation process that produces a polymer-rich liquid phase (the coacervate) that separates from a solution and forms a homogeneous coating around core particles. These are formed by changing factors such as pH, temperature, molecular weight, total polymer concentration, ionic strength and ratios of acid to base. Also, (2) Fluidized-Bed Coating method in which solid core particles are suspended in an upward flowing stream of air and spray coated with a thin film of protective polymer or lipid.

The matrix-type techniques of microencapsulation are more commonly used for commercial animal feed products because they are less costly and facilitate rapid production. However, advanced core-shell type technology has demonstrated improved rumen protection compared to matrix systems. Chauhan & Kumar (2020) found that core-shell fluid-bed coating provides superior rumen protection compared to matrix-type systems using methionine and lysine products. Similar findings were reported by Chauhan & Gautam (2020) with rumen-protected choline. These technologies may lead to more effective formulations that address specific dietary needs and improve feed efficiency.

Evidence-Based Benefits of Bypass Micronutrients and Nutraceuticals

Amino Acids: Increasing total crude protein intake to meet the demands of high-producing cows yields diminishing returns. As nitrogen intake increases from 450 to 750 g/day in lactating cows, nitrogen partitioning shifts. Milk nitrogen excretion reached a plateau, while faecal nitrogen output increased slightly but urinary nitrogen excretion had a steep increase (from 90 to 280 g/day), indicating inefficient amino acid utilization, where excess amino acids are deaminated, causing elevated blood urea nitrogen (BUN) and higher urinary urea excretion (Van Amburgh et al. 2015). Balancing the amino acid profile delivered to the small intestine is critical for optimizing nitrogen efficiency and maximizing milk protein synthesis. Optimizing Lysine:Methionine ratio at about 2.7:1 in high yielding cow diet by supplementing rumen protected lysine and methionine results in improved lactation performance and better nitrogen utilization (Junior et al., 2021). The bypass amino acids can be added to the ration to provide balanced amino acids for optimum milk production and reduce nitrogen loss in urine, thus offering both economic and environmental benefits.

Choline: Choline is an essential nutrient that plays a central role in lipid transport, structural membrane integrity, and methyl group metabolism in dairy cattle. Choline is converted into phosphatidylcholine which is a structural component of cellular membranes and bile lipids. Phosphatidylcholine is required to synthesize Very-Low-Density Lipoproteins (VLDL) in the liver (Lipotropic activity) . Choline is the precursor for acetylcholine, a neurotransmitter that regulates parasympathetic nervous pathways. Choline can be oxidized to betaine, sparing methionine for milk production. Unprotected dietary choline is highly unstable in the rumen, with over 80% degraded by microflora into trimethylamine, acetate, and microbial biomass. Supplementing dairy cows with rumen-protected choline chloride improves hepatic energy metabolism by facilitating VLDL synthesis and lipid export (Zom et al., 2011). Protected choline chloride supplementation also increases performance in terms of milk protein yield, milk fat content, total solids and circulating plasma insulin concentrations (Leiva et al., 2015). Inclusion of rumen-protected choline in diets could become a cost-effective strategy for high-producing herds, especially those at risk for metabolic disorders such as fatty liver or ketosis.

Vitamins: Fat-soluble vitamins are sensitive to ruminal destruction through microbial oxidation and hydrogenation. The ruminal bypass of fat-soluble vitamins results in an improved bioavailability, as shown by Baldi et al. (1997) in comparative evaluations of vitamin E (5,000 IU DL-α-tocopheryl acetate), where microencapsulated formulation in a lipid matrix (stearic + palmitic acid) resulted in higher plasma α-tocopherol concentrations in Holstein-Friesian cows compared to unprotected form.

While ruminal microflora synthesizes B-vitamins, they are extensively degraded in the rumen. High-producing cows have higher post-ruminal demand for water soluble vitamins. Juchem et al. (2012) supplemented dairy diet with 4 g/day of a rumen-protected B-vitamin complex. The complex was produced by using a protective lipid matrix applied by spray freezing and contained biotin, folic acid, pantothenic acid, pyridoxine, and vitamin B12. B-vitamins improved reproductive efficiency and reduced culling rate of cow with no effect on milk yield, suggesting bypass B-vitamins impact metabolic and reproductive health of dairy cows. This was further determined by Kaur et al. (2019) by supplementing 4 g/day rumen-protected B-vitamin blend (folic acid, B12, biotin, pyridoxine, pantothenic acid), protected with a fat-based (palmitic fat) coating to cows at 15 days in milk until endometrial biopsy at day 14 of gestation. There was significant upregulation of endometrial genes related to immune modulation, adhesion and nutrient transport, indicating improved uterine functional preparedness for pregnancy, without any adverse effect on production performance.

Niacin (vitamin B3) is an important metabolic regulator especially for high producing cows, reducing the incidence of ketosis in herd due to its anti-lipolytic action, lowering circulating plasma non-esterified fatty acids (NEFA) during early lactation. Yuan et al. (2012) supplemented 12 g/day rumen protected niacin (supplying 8 g/day niacin) to early lactating cows to find lowered circulating NEFA in plasma. This anti-lipolytic effect stabilizes postpartum energy balance and limits hepatic triglyceride accumulation. Niacin can be synthesized by rumen microbes as well endogenously from tryptophan, still the exogenous supply is required for better health and performance. Supplementation of both bypass fat-soluble and water-soluble vitamins is essential for improving the metabolic and reproductive health of dairy cows.

L-Carnitine: L-Carnitine is a quaternary amine synthesized endogenously from lysine and methionine. Because 70% to 80% of unprotected dietary carnitine is degraded by ruminal microbes, it can become conditionally limiting during periods of high metabolic demand, such as early lactation. L-Carnitine regulates the transport of long-chain fatty acids across the inner mitochondrial membrane for β-oxidation of fatty acids. Supplementing transition cows with 20 g/day of rumen-protected L-carnitine (spray freezing emulsion containing palm oil), supplying 4 g/day of L-carnitine, lowered plasma NEFA concentrations, reduced hepatic lipid accumulation, and decreased milk somatic cell counts (SCC) while increasing fat-corrected milk (FCM) yield and improving energy utilization (Danesh et al., 2021). Protected L-carnitine can serve as a nutritional intervention to support liver health, bridge the negative energy balance and maximize production potential in dairy cows.

Gamma-Aminobutyric Acid (GABA): GABA is a non-protein amino acid in its chemical structure but functions as a bioactive amine in mammalian nervous system. Rumen bypass GABA in dairy cows increases feed intake especially during early lactation and heat stress conditions by inhibiting satiety signals like cholecystokinin and increasing Neuropeptide Y in serum (hunger signal). Supplementing transition cows with 1.2 g/day of rumen-protected GABA (50% GABA coated with palm oil) by spray freezing, improved postpartum dry matter intake, milk protein yield, and total antioxidant capacity (Wang et al., 2013). Combining GABA with vitamin C, niacin, and potassium sulfate into a rumen protected blend and given at 20g/day maintained dry matter intake and milk yield in heat-stressed dairy cows (Guo et al., 2017). Rumen protected GABA can be added in diet to prevent decline in productivity during periods of physiological or environmental stress.

Glutathione: Glutathione (GSH) is a tripeptide antioxidant (glutamate + cysteine + glycine). It is the main intracellular antioxidant in animal cells, including liver, immune cells, and mammary tissue. Supplementing transition cows with 2.0 g/day of lipid-encapsulated glutathione (30% GSH) increased FCM yield and improved total antioxidant capacity (Hao et al., 2025). Supplementing glutathione can help alleviate the systemic oxidative stress typical of the periparturient period.

Functional Oils: Rumen-protected functional oils include protected fats (prilled fat, lecithin blends, calcium soaps) and microencapsulated essential oils or plant oils designed to bypass or be stabilized in the rumen. Post-ruminally, rumen-protected functional oils act mainly as high-density energy sources and targeted fatty acids for absorption, enriching milk and meat with omega-3 and conjugated linoleic acid, improving plasma fatty acid status, and supporting reproduction and overall health, generally without compromising milk yield or rumen function. Microencapsulating linseed oil, vitamin E, and rosemary extract blend and supplementing at 2% in diet of lactating HF cows, nearly doubled the omega-3 fatty acid content of milk and increased intestinal absorption of α-linolenic acid while maintaining total milk yield, compared to unprotected linseed oil (Kim et al., 2020). Rumen-protected functional oils have significant potential to enhance the nutritional quality of dairy products and animal health, without negatively affecting milk production. Further research can help optimize formulations and maximize their benefits in commercial dairy systems.

Future Prospects

At present, there is limited research on the cost-effectiveness, scalability, and integration of rumen-protected efficient post-ruminal delivery of various micronutrients and nutraceuticals into diverse feeding systems such as pasture-based vs. stall feeding, smallholder vs. commercial. Future studies should focus on:

- Finding nutraceutical blends that target specific metabolic pathways for enhanced productivity, immunity and stress resilience.
- Development of pH or enzyme-responsive polymers that adapt to gut conditions for more precise nutrient release.
- Large-scale, on-farm trials to validate efficacy, cost-benefit ratios and inform regulatory frameworks and feeding guidelines.
- Tailoring post-ruminal supplementation according to individual animal needs, physiological stage and production goals.

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Mini Review

Fibrolytic Enzymes as Feed Additives to Improve Nutrient Availability in Animals

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Abstract

Increasing feed costs and declining availability of quality fodder resources have created major challenges for sustainable livestock production. Fibrous feed resources such as crop residues, agro-industrial by-products, cereal brans, and oilseed cakes are increasingly used in animal feeding; however, their utilization is limited because of poor digestibility of structural carbohydrates and non-starch polysaccharides. Fibrolytic enzymes have emerged as important feed additives for improving the utilization of such fibrous feeds in ruminants and non-ruminants. These enzymes degrade cellulose, hemicellulose, β-glucans, mannans, pectin, and other complex carbohydrates into simpler compounds, thereby improving nutrient availability and feed efficiency. In ruminants, fibrolytic enzymes enhance ruminal fiber degradation, volatile fatty acid production, nutrient digestibility, milk yield, and growth performance. In non-ruminants, they reduce intestinal viscosity, improve gut health, nutrient absorption, and feed conversion efficiency. Enzyme supplementation also promotes beneficial gut microflora and supports intestinal integrity through increased production of short-chain fatty acids. Thus, fibrolytic enzymes represent a promising and sustainable nutritional strategy to enhance fiber utilization, improve animal productivity and feed efficiency, and support environmentally sustainable livestock production through the efficient use of low-quality fibrous feed resources.

Key words: *Fiber, Fibrolytic enzymes, Non-Starch Polysaccharides, Ruminants*

With the rapid increase in human population and rising demand for milk, meat, eggs, and fish, the pressure on animal production systems is continuously increasing. The global demand for food and animal-derived products is expected to rise sharply by 2050 due to rapid population growth and changing dietary patterns (Searchinger et al. 2019). However, livestock production systems are facing major challenges because the availability of quality feed and fodder resources is continuously declining as a result of shrinking cultivable land, climate change, and increasing competition for feed grains between human consumption and animal feeding. Feed cost alone contributes approximately 60–70% of the total cost of livestock and fish production. Therefore, improving feed efficiency and utilizing low-cost alternative feed resources have become essential for sustainable and profitable farming.

In recent years, livestock feeding systems have increasingly relied on crop residues, agro-industrial residues, cereal milling by-products, oilseed cakes and other unconventional feed resources to reduce feeding costs and overcome feed shortages (Tariq et al., 2018). However, these feed ingredients are usually high in fiber and contain complex structural carbohydrates that are not efficiently digested by animals. Poor digestibility of fibrous feeds leads to lower nutrient availability, reduced productivity, and greater feed wastage (Li et al. 2021). To overcome these challenges, fibrolytic enzymes have been increasingly used as an important nutritional intervention for improving the utilization of fibrous feeds. These enzymes break down complex fibres present in plant feeds into simpler substances that animals can digest more easily. Their use has become important in ruminants, poultry, pigs, and fish because they help improve feed digestion, animal performance, gut health, and feed efficiency. This mini-review provides an updated synthesis of current research on the application of fibrolytic enzymes as feed additives in both ruminant and non-ruminant animal production systems. It critically examines the mechanisms through which different fibrolytic enzymes enhance the degradation of fibrous feed components, improve nutrient digestibility and bioavailability, and optimize feed utilization. Furthermore, the review highlights their effects on animal performance, gut health, production efficiency, and environmental sustainability, while discussing recent advances, practical applications, and future prospects for maximizing the nutritional value of conventional and unconventional feed resources.

Composition of Dietary Fibers

Fiber is a major constituent of plant-derived feeds and is primarily composed of structural polysaccharides present in plant cell walls. In animal feeding, fiber is mainly categorized into Neutral Detergent Fiber (NDF), containing cellulose, hemicellulose, and lignin, and Acid Detergent Fiber (ADF), which primarily contains cellulose and lignin. These fibrous fractions provide structural strength to plants but are difficult to digest because of their complex arrangement and association with lignin. Ruminants utilize specialized rumen microorganisms to break down fibrous feed materials, resulting in the production of volatile fatty acids (VFA) that provide a major source of energy to the animal. Despite this unique capability, ruminants are able to utilize only about 50% or less of the energy present in lignocellulosic feed materials (Li et al. 2021). The inefficient utilization of fibrous feed resources is largely due to the highly resistant nature of lignin and the intricate association of cellulose and hemicellulose within the plant cell wall matrix. This limitation is especially evident in low-quality roughages, mature and over-mature forages, cereal straws, crop residues, and other lignocellulosic feed resources, which contain higher concentrations of structural carbohydrates and lignin. Increased lignification during plant maturation forms physical and chemical barriers that limit microbial colonization and enzymatic hydrolysis of cell wall polysaccharides, thereby reducing fiber degradation and nutrient release. Consequently, the digestibility and feeding value of these resources are diminished, resulting in lower nutrient bioavailability, reduced animal productivity, and greater feed wastage.

The situation is even more challenging in non-ruminants such as poultry, pigs, and fish because these animals possess a simple digestive system and lack sufficient endogenous enzymes to digest fiber efficiently. Their diets often contain non-starch polysaccharides (NSP), which interfere with nutrient digestion and absorption. NSP include cellulose, arabinoxylans, β-glucans, pectins, mannans, and other polysaccharides present in cereals and plant protein sources. The NSP are classified into soluble and insoluble fractions. Soluble NSP are particularly problematic because they increase intestinal viscosity, slow nutrient movement, reduce digestive enzyme activity, and promote the growth of harmful microorganisms. Insoluble NSP, although less harmful, may still reduce nutrient digestibility when present in excess. Different cereal grains vary in their NSP composition. Wheat, barley, rye, oats, and triticale are categorized as viscous cereals because they contain higher levels of soluble arabinoxylans and β-glucans capable of forming viscous solutions in the intestine (Bedford et al., 2024). In contrast, maize, sorghum, rice, and millets are considered non-viscous cereals because they contain lower levels of soluble NSP. Wheat bran may contain around 40% NSP, whereas soybean meal contains approximately 21% NSP, of which about 5–7% is soluble. These NSP negatively affect nutrient utilization and animal performance.

Fibrolytic Enzymes

Fibrolytic enzymes are specialized enzymes capable of degrading fibrous components of plant materials. These enzymes hydrolyze the bonds present in cellulose, hemicellulose, pectin, and other NSP, thereby releasing nutrients trapped within the plant cell wall matrix. Most commercial fibrolytic enzymes are produced from beneficial fungi and bacteria through controlled fermentation processes such as solid-state fermentation and submerged fermentation (Mahesh et al., 2025).

The major fibrolytic enzymes used in animal feeding include

- **Cellulase** – breaks down cellulose into glucose units
- **Xylanase** – degrades arabinoxylans and hemicellulose
- **β-glucanase** – hydrolyzes β-glucans present in barley and oats
- **β-mannanase** – breaks down mannans present in soybean meal and other ingredients
- **Pectinase** – degrades pectin substances

- **Ferulic acid esterase** – helps break lignin-carbohydrate linkages
- **Endoglucanase** – acts on internal cellulose bonds

Application of Fibrolytic Enzymes in different Livestock species

Fibrolytic enzymes function differently in ruminants and non-ruminants because of differences in their digestive systems. In ruminants, these enzymes mainly enhance the breakdown of structural carbohydrates such as cellulose and hemicellulose in the rumen by acting synergistically with rumen microorganisms, thereby improving fiber digestion and VFA production (Mahesh et al., 2025). In non-ruminants such as poultry and pigs, fibrolytic enzymes primarily degrade NSP present in cereal grains and plant-based feeds, reducing intestinal viscosity and improving nutrient digestion and absorption. Thus, in ruminants the main role of fibrolytic enzymes is to improve ruminal fiber fermentation, whereas in non-ruminants they mainly improve gut environment and nutrient utilization. In ruminants fibrolytic enzymes improve feed utilization through multiple mechanisms occurring before, during, and after digestion (Fig 1).

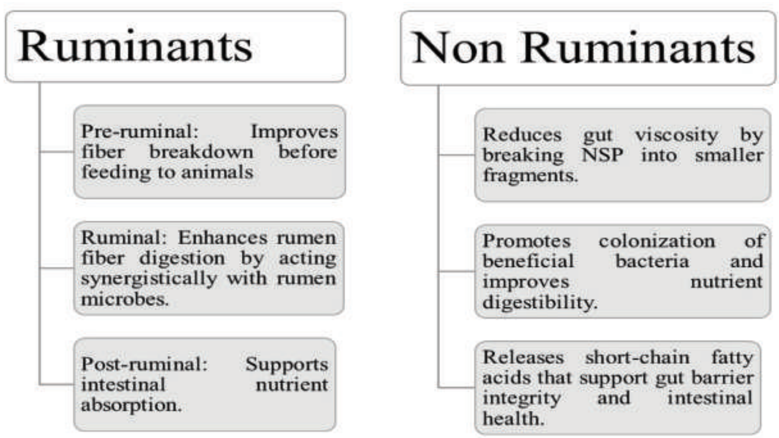

Fig 1. Application of Fibrolytic Enzymes in Livestock

Application of Fibrolytic Enzymes in Ruminants

In Vitro/ Pre-Ruminal Application of Fibrolytic Enzymes: Fibrolytic enzymes are widely used as feed additives to improve the digestibility and utilization of fibrous feed resources before they are offered to animals. These enzymes mainly act on cellulose, hemicellulose, and other structural carbohydrates present in crop residues and roughages such as wheat straw, paddy straw, maize stover, sorghum stover, sugarcane bagasse, and poor-quality hays. When fibrolytic enzymes are applied to feeds prior to feeding, partial hydrolysis of plant fiber begins outside the animal body itself, thereby enhancing nutrient availability and improving subsequent microbial fermentation in the rumen. When fibrolytic enzymes are sprayed onto dry fodders, partial breakdown of fiber occurs before feed consumption. This process softens the lignocellulosic structure of plant cell walls and releases soluble sugars that serve as an immediate energy source for rumen microorganisms. Such pre-treatment improves attachment and colonization of rumen microbes on feed particles and reduces the lag time required for microbial digestion. The increased accessibility of feed particles to microbial enzymes ultimately enhances dry matter and fiber digestibility.

Intra-Ruminal Application: Inside the rumen, non-rumen degrading fibrolytic enzymes may work synergistically with rumen microorganisms to improve fiber degradation. They increase the rate of digestion of both fibrous and non-fibrous feed fractions. Improved degradation of cellulose and hemicellulose results in greater production of VFA and increased nutrient availability. Studies have shown that increased digestibility of NDF can improve dry matter intake, milk production, growth performance and fatty acid profile of meats in ruminants (Arumbaka et al., 2025, Kadam et al., 2025, Lago et al., 2025, Dong et al., 2023; Prajapati et al., 2022). Studies have indicated that improved NDF digestibility can positively influence feed intake and milk yield, with each 1% increase in NDF digestibility potentially increasing dry matter intake by about 170 g and milk production by nearly 250 g in dairy cows (Oba and Allen, 1999).

Post-Ruminal Application: Some fibrolytic enzymes escape extensive degradation in the rumen and remain enzymatically active in the lower gastrointestinal tract, particularly in the abomasum and small intestine. Although the rumen is the primary site of fiber digestion in ruminants, a proportion of exogenously supplemented enzymes—especially those with high thermal stability and resistance to proteolytic degradation—can survive passage through the rumen and continue hydrolyzing residual plant cell wall polysaccharides in the post-ruminal digestive tract (Morsy et al., 2016).

Among the different fibrolytic enzymes, xylanases are more frequently detected beyond the rumen than cellulases because xylanases generally exhibit greater resistance to ruminal microbial degradation and retain activity over a broader range of pH values (approximately 3.0–7.0), enabling them to remain functional in the acidic environment of the abomasum and subsequently in the small intestine. In contrast, cellulases are generally more susceptible to microbial degradation and proteolysis within the rumen, resulting in lower residual activity in the lower digestive tract (Beauchemin et al., 2003).

Application of Fibrolytic Enzymes in Non-Ruminants

In poultry and pigs, soluble NSP present in feed ingredients increase digesta viscosity and hinder nutrient absorption. Fibrolytic enzymes such as xylanase and β-glucanase reduce viscosity by breaking down NSP into smaller fragments. This improves nutrient movement and enhances digestion and absorption. Breakdown products of NSP, such as xylo-oligosaccharides and mannan-oligosaccharides, act as prebiotics that support beneficial gut bacteria. Higher production of short-chain fatty acids, particularly butyrate, supports gut health by strengthening intestinal barrier function, enhancing immunity, and improving nutrient absorption. Enzyme supplementation also reduces colonization of pathogenic bacteria and lowers the risk of digestive disorders (Bautil et al., 2020). NSP-degrading enzymes can improve nutrient availability by releasing approximately 50–150 kcal/kg of metabolizable or digestible energy and increasing digestible amino acid availability by 1–3%, particularly in diets containing wheat, barley, oats, rye, triticale, corn, or sorghum (Bedford et al., 2024).

Factors Affecting the Efficiency of Fibrolytic Enzymes

The response of animals to fibrolytic enzyme supplementation is highly variable and depends on several enzyme-, feed-, animal-, and environment-related factors as discussed below.

Type and Activity of Enzyme: The efficacy of fibrolytic enzymes is largely determined by the type of enzyme and its catalytic activity. Different enzymes target specific components of the plant cell wall. For example, cellulases hydrolyze cellulose into soluble sugars, whereas xylanases degrade arabinoxylans and other hemicellulosic fractions. β-glucanases, pectinases, and ferulic acid esterases further assist in breaking down complex polysaccharide networks and enhancing the accessibility of structural carbohydrates to microbial fermentation. Enzyme preparations containing multiple activities generally produce greater improvements in nutrient digestibility than single-enzyme products because of their synergistic action on diverse fiber fractions (Beauchemin et al., 2020). Moreover, enzyme activity expressed as units per gram of product is often more important than the quantity of enzyme added, as products with higher catalytic efficiency can achieve better fiber degradation at lower inclusion rates.

Dose and Method of Application: The response to fibrolytic enzymes is dose-dependent, and both under- and over-supplementation may reduce their effectiveness. Insufficient enzyme levels may fail to adequately hydrolyze fiber, whereas excessive supplementation can be economically inefficient and may not result in additional benefits. Fibrolytic enzymes can be applied directly to feeds, incorporated into total mixed rations (TMR), sprayed onto forages before feeding, or added during silage preparation. Pre-treatment of feeds often enhances enzyme-substrate interaction before ingestion, resulting in greater improvements in fiber digestibility and animal performance (Yu et al., 2024). The optimum dosage varies according to enzyme activity, feed type, and animal species.

Feed Composition and Substrate Specificity: The effectiveness of fibrolytic enzymes depends greatly on the chemical composition and structural characteristics of the feed. Enzymes are most effective when their substrate specificity matches the predominant fiber fractions present in the diet. For example, xylanase supplementation is particularly beneficial in feeds rich in hemicellulose such as wheat bran and cereal by-products, whereas cellulases are more effective for cellulose-rich roughages and crop residues (Bedford et al., 2024). Diets containing highly lignified, mature forages may show limited responses because lignin restricts enzyme access to structural carbohydrates. Therefore, enzyme selection should be tailored to the fiber composition and degradability characteristics of the target feed resource.

Animal Species and Physiological Stage: Animal species differ considerably in their digestive anatomy and capacity to utilize fibrous feeds. Ruminants generally benefit from fibrolytic enzymes through enhanced ruminal fiber degradation and microbial fermentation, while in non-ruminants such as poultry, pigs, and fish, these enzymes reduce digesta viscosity, improve nutrient release, and increase nutrient absorption. Physiological stage also influences the response to enzyme supplementation. High-producing dairy cows, rapidly growing animals, and animals under nutritional stress often exhibit greater benefits due to their increased nutrient requirements and feed intake. In contrast, responses may be less pronounced in mature animals with lower nutritional demands (Beauchemin et al., 2003).

Feeding Duration: The duration of enzyme supplementation can influence the magnitude of the response. Short-term supplementation may improve feed digestibility, but longer feeding periods often allow greater adaptation of rumen microbial populations and stabilization of digestive processes. Sustained enzyme supplementation may therefore result in cumulative improvements in nutrient utilization, feed efficiency, growth performance, and milk production. However, responses may vary depending on diet composition and management conditions.

Environmental Conditions (Temperature and pH): The activity and stability of fibrolytic enzymes are strongly affected by environmental conditions, particularly temperature and pH. Each enzyme exhibits an optimum temperature and pH range at which catalytic activity is maximized. Extreme temperatures during feed processing or storage can denature enzymes and reduce their effectiveness. Similarly, deviations from the optimal pH range may alter enzyme conformation and decrease substrate binding and hydrolysis. In ruminants, rumen pH fluctuations associated with dietary changes can influence enzyme performance, whereas in non-ruminants enzyme efficacy depends on conditions prevailing within different segments of the gastrointestinal tract. Therefore, enzyme preparations should be selected based on their stability under the intended feeding and digestive conditions.

Research Gaps and Future Directions

Fibrolytic enzyme supplementation has considerable potential in Indian animal production systems; however, important research gaps remain regarding the optimization of enzyme type, dose, application method, and substrate specificity for diverse indigenous feed resources and animal species. Future studies should focus on developing cost-effective, thermostable enzymes from native microbial sources, elucidating enzyme–rumen microbiome interactions using advanced omics tools, and evaluating long-term effects on animal health, productivity, product quality, and methane mitigation under tropical conditions. Additionally, large-scale field validation and economic analyses are essential to establish practical, precision-based enzyme supplementation strategies for sustainable and profitable livestock production in India.

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Mini Review

Transforming Poultry Production through Phytogenic and Herbal Feed Strategies

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Abstract

The poultry industry on a world level is currently in the middle of a major overhaul. As the people are becoming more health conscious and the growing fear of antibiotic resistance and the fact that people are keen to know their diet is chemical-free, producers are progressively replacing antibiotic growth promoters. Modern poultry production systems are increasingly recognizing herbs as essential components of sustainable farming rather than optional supplements. This shift is driven by the growing need for natural growth promoters, as herbal supplements have the potential to act as viable alternatives to synthetic antibiotics. Because herbs contain a diverse array of photochemical bioactive compounds—possessing unique antimicrobial and antioxidant properties—they offer significant benefits for poultry production. Consequently, this article explores the practical possibilities of utilizing herbs and evaluates the specific effects of various plant-based compounds within poultry feeds. Using herbal supplements is no longer just an 'organic' trend. It has become a scientific necessity in today's poultry industry.

Keywords: *Feed supplement, Health, Herbs, Poultry, Production*

For more than half a century, sub-therapeutic levels of antibiotics were being used and considered as the "gold standard" for ensuring flock health and growth. These weren't used to treat active sickness, but rather as a safety net to prevent disease and unnaturally boost growth rates. However, the legal bans in Europe and the tightening regulations across Asia and the America have left a void. The real difficulty for modern poultry science is to maintain a consistent high-intensity production without compromising bird's welfare or health of general public.

Botanical additives represent a transformative shift in feeding management, moving away from synthetic chemicals toward a more holistic, nature-based approach. Rather than relying on lab-created drugs, this movement harnesses the diverse power of plant-derived compounds to support livestock health and performance. Unlike synthetic chemical drugs, these botanical additives are complex mixtures of bioactive secondary metabolites—such as alkaloids, flavonoids, and essential oils—that offer a multi-targeted approach to both, the bird and consumer's health.

The Biological Core: Mechanisms of Action

To understand the importance of herbs in poultry production, understanding the anatomy and physiology of the avian gastrointestinal tract is essential. The "Botanical Advantage" acts through different mechanism of actions as per the presence of photoactive compounds like microbiome modulation, gut barrier integrity, immunomodulation pathways, cytokine regulation, antioxidant enzyme activity (SOD, CAT, GPx) but majorly three primary pathways were identified and these were as follows:

Intestinal Histomorphology: The gut is considered as the engine of the bird. Some of the phytogenic compounds, particularly those found in Oregano and Cinnamon, have resulted to increase villus height and decrease crypt depth in parts of small intestine like the duodenum and jejunum (Alagawany et al. 2018; Ali et al., 2021). This resulted in a higher villus-to-crypt ratio that significantly increased the surface area available for absorption of nutrients, directly translating to better growth and performance.

Enzymatic Stimulation: Herbs act as digestive stimulants. Compounds like piperine found in black pepper and gingerol found in ginger stimulates the pancreas to secrete higher levels of amylase, lipase, and protease (Siregar et al., 2024). This resulted in more efficient breakdown of high-protein diets of broilers, ultimately reducing the amount of undigested nutrients that reach the hindgut, where they could otherwise act as a fuel for pathogenic bacterial growth.

Antimicrobial and Antioxidant Defense: Herbal bioactive compounds were also found to disrupt the cell membranes of harmful bacteria like *Salmonella* and *E. coli*. Simultaneously, herbs which are rich source of polyphenols (like Rosemary and Amla) neutralize free radicals produced during heat stress, protecting the bird's cellular integrity and reducing "oxidative stress" (Sisodiya et al., 2026).

Functional Effects and Performance Impacts of Herbal additives Best approach of utilizing herbs is by combining them as per their mechanism of action and amplifying their power. A polyherbal approach is considered essential for achieving synergistic therapeutic effects. Many herbs are currently driving the botanical industry and few important ones along with their active ingredients and effect on birds were enlisted in Table 1.

Economic Feasibility of Herbal Additives

For herbs implementation in diet, the transition must be economically viable for a commercial farmer. Data gathered from numerous trials showed that herbal integration provides a significant return on investment (Japitana, 2019). Some of the positive impacts of herbal supplementation directly influencing the economics of production are as follows:

Table 1: Commonly used herbal additives in poultry production

Phytogenic and Herbal compounds	Active Compound	Effect on Poultry Birds	Reference
Turmeric	Curcumin	Powerful anti-inflammatory; supports liver detoxification.	Hernández-García et al. (2025)
Garlic	Allicin	Natural "bio-active" antibiotic; enhances innate immunity.	Rahayu et al. (2023)
Oregano	Carvacrol	Potent antifungal and antibacterial; prevents necrotic enteritis.	Alagawany et al. (2018)
Ginger	Gingerol	Improves palatability and stimulates digestive juices.	Siregar et al. (2024)
Ashwagandha	Withanolides	Adaptogen; reduces cortisol levels during environmental stress	Vooha et al. (2024)
Moringa	Isothiocyanates & Quercetin	High protein (25%+); lowers meat cholesterol; pigments yolk.	Mahfuz et al. (2019); Lakhani et al. (2024)
Neem	Azadirachtin	Natural dewormer and antiviral protection.	Tufail et al. (2025)
Aloe Vera	Acemannan	Repairs intestinal lining; boosts vaccine response.	Jalal et al. (2019); Lakhani et al. (2024)
Tulsi	Eugenol	Anti-Stress and adaptogenic properties	Sisodiya et al. (2026)
Cinnamon	Cinnamaldehyde	Specific inhibition of Clostridium (Necrotic Enteritis)	Ali et al. (2021)
Amla	Vitamin C	High antioxidant capacity; critical for stress recovery	Sisodiya et al. (2026)
Thyme	Thymol, Carvacrol, Linalool	Antifungal and antibacterial action, Optimizes the Feed Conversion Ratio (FCR) in broilers	Hashemipour et al. (2013)
Rosemary	Carnosic acid, Rosmarinic acid, Carnosol	Potent antioxidant, Retards post-slaughter lipid peroxidation in broiler breast meat. Extends meat shelf-life and maintains color stability.	Wang et al. (2024)

Peppermint	Menthol, Menthone, Menthyl acetate	Relaxing the gut during enteric stress. Provides a systemic cooling effect during peak summer months.	Arab Ameri et al. (2016)
Black Pepper	Piperine, Chavicine, Piperidine	Functions as a bio-enhancer; increases the systemic absorption rate of other phytocompounds.	Abou-Elkhair et al. (2014)
Chili	Capsaicin, Capsanthin, Capsorubin	Promotes bile acid secretion, optimizing crude fat digestion.	Puvača et al. (2015)
Clove	Eugenol, β-Caryophyllene, Acetyeugenol	Antiseptic, appetite and digestion stimulant, anti-inflammatory, and antioxidant activity	Ertas et al. (2005)
Marigold	Carotene, Carotenoid, Alkalonoids, Terpenes, Flavonoids.	Antioxidant effects, enhance the health of digestive system reducing the exposure of birds to microbiological toxins.	Lakhani et al. (2024)

Feed Conversion Ratio (FCR): On average, integration of herbal supplements improved FCR by 3% to 5% (Gerzilov et al., 2015). In a large flock, the little percentage change represents a massive reduction in total feed costs.

Mortality and Immunity: By enhancing the bird's natural immunity, herbal supplements have been shown to reduce early embryonic mortality rates. When immune-modulators (like *Andrographis paniculata*) are supplemented to birds, they show higher antibody titers following standard vaccinations for Newcastle Disease and IBD.

Carcass and Egg Quality: In layers, herbal supplements like Moringa have resulted in providing a natural, deep-yellow yolk color without the use of synthetic dyes. In broilers, they resulted in an increased shelf life of the meat due to the antioxidant properties of herbs that reduced lipid oxidation (Mahfuz & Piao, 2019).

Challenges and Future Perspectives

Despite the clear advantages, the industry faces the challenges like variability in herbal composition, dosage inconsistency, palatability concerns, cost of standardization, interactions among phytochemicals and bio-availability. The environment, soil quality and harvest time can affect the concentration of active compounds in a plant. Standardized extracts and encapsulation technologies will likely shape the future of this field.

Encapsulation will help protect the volatile essential oils from evaporating during the pelleting process of feed, ensuring the bioactive compounds reach the lower gut of the bird where they are most needed. By leveraging and understanding the botanical advantage, producers can achieve high performance, good quality, ensure animal welfare, and provide the global market with safe, high-quality protein. The use of herbal supplements can provide significant benefits to both producers and consumers. Integrating these plant-based solutions into standard nutrition is no longer just an alternative; it is the essential strategy for maintaining healthy flocks and productive farms in the modern era.

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Mini Review

Association of Gout and Hydronephrosis in Poultry Birds: A Brief Review

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Abstract

Hydronephrosis and gout are important manifestations of renal dysfunction in poultry and a major cause of morbidity, mortality, and economic loss in the commercial poultry industry. In avian species, the kidneys play a central role in uric acid excretion because birds are uricotelic and lack uricase, making them particularly susceptible to hyperuricemia and urate deposition. Hydronephrosis in poultry develops secondary to ureteral obstruction or compression, leading to dilation of renal collecting structures, renal ischemia, and progressive nephron degeneration. Renal impairment subsequently disrupts uric acid excretion, culminating in visceral or articular gout. Although gout has been extensively studied in poultry, the association between hydronephrosis and gout remains underexplored despite their frequent coexistence in nephropathic conditions. Besides, infectious (nephropathogenic viruses) as well as non-infectious (dietary imbalances, dehydration, nephrotoxins and environmental stress) factors, emerging evidence suggests that oxidative stress, inflammasome activation, and gut–kidney axis dysfunction further contribute to renal injury. This review comprehensively discusses the anatomical and physiological basis of avian renal disease, the etiopathogenesis of hydronephrosis-associated gout, clinicopathological findings, diagnostic approaches, prevention strategies, and emerging molecular insights relevant to poultry medicine.

Key words: *Gout, Hydronephrosis, Kidney, Poultry*

Renal disorders are among the most significant health problems affecting poultry production and gout is the most widely recognized common metabolic consequence of impaired kidney function in birds. Gout develops when elevated blood uric acid levels lead to urate crystal deposition in the kidneys, serosal surfaces and joints (Sandhyarani et al., 2022). Although less frequently reported in birds, hydronephrosis is an important obstructive renal disorder characterized by impaired urinary flow, increased intrarenal pressure, and progressive renal tissue damage. Since birds excrete nitrogen primarily as uric acid, reduced renal function readily results in hyperuricemia and gout. The association between hydronephrosis and gout is closely interconnected and involves multiple pathological mechanisms. Obstruction-induced hydronephrosis decreases the elimination of uric acid, while accumulated urate crystals may further block renal tubules and ureters, thereby worsening urinary obstruction and renal injury. Various factors such as nephropathogenic viruses, nutritional imbalances, dehydration, nephrotoxic agents, and environmental stress are involved in gout affecting poultry (Li et al., 2022).

Avian Renal Anatomy and Physiology

Avian kidneys are paired, elongated, trilobular organs situated within the renal fossae of the synsacrum. Each kidney is anatomically differentiated into cranial, middle, and caudal lobes and receives blood supply through an extensive renal portal vascular system. In contrast to mammals, birds do not possess a urinary bladder; therefore, urine is transported directly from the kidneys via the ureters into the cloaca for excretion (Al-Agele, 2024).

Birds are physiologically uricotelic, eliminating nitrogenous waste predominantly in the form of uric acid rather than urea. Owing to its poor aqueous solubility, uric acid readily precipitates when renal excretory function is compromised. In addition, avian species lack hepatic uricase, the enzyme responsible for oxidizing uric acid into the more soluble compound allantoin, thereby increasing their susceptibility to hyperuricemia and urate deposition disorders, including gout The avian kidney contains two distinct nephron types: reptilian-type nephrons, located in the cortex and lacking loops of Henle, and mammalian-type nephrons, which possess relatively short loops of Henle extending into the medulla. This unique nephron arrangement limits the urine-concentrating ability of birds, making them more susceptible to dehydration, impaired water conservation, and subsequent renal dysfunction (Al-Agele, 2024).

Hydronephrosis in Poultry

Hydronephrosis is characterized by dilation of the renal tubular and ureteral structures secondary to impaired urinary outflow. In avian species, marked pelvic enlargement is generally absent because birds do not possess a well-developed renal pelvis comparable to that of mammals. However, obstruction of the ureters results in increased intraluminal pressure, leading to tubular dilation, compression of adjacent nephrons, ischemic injury, and progressive renal atrophy (Thotakura & Anjum 2023). The pathogenesis of hydronephrosis in poultry involves a sequence of pathological events, including:

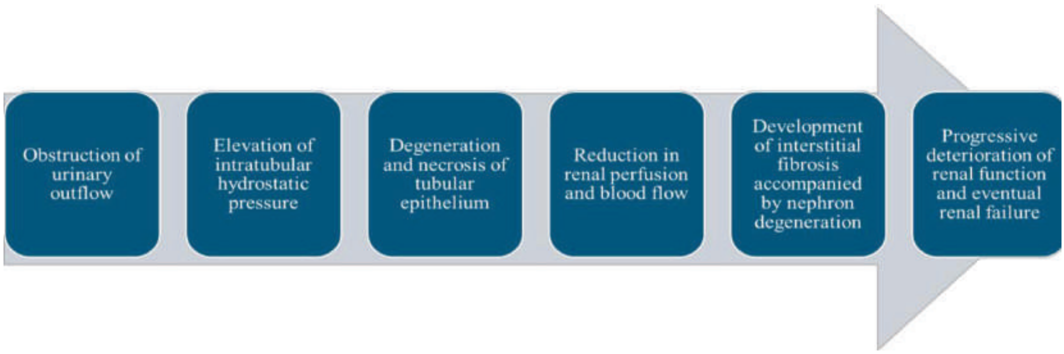
As renal excretory capacity declines, the clearance of uric acid becomes impaired, resulting in persistent hyperuricemia and subsequent development of gout.

Etiological Factors

Hydronephrosis and gout in poultry arise from multiple obstructive, infectious, nutritional, toxic, and drug-related factors that impair renal function and uric acid excretion.

Obstructive and Compressive Causes include urolithiasis, urate plugs, inflammatory debris, cloacal obstruction, congenital ureteral defects, granulomatous lesions, and neoplastic conditions such as Marek's disease and lymphoid leukosis. These conditions interfere with urinary outflow, resulting in increased renal pressure, tubular damage, and obstructive nephropathy.

Infectious Agents such as nephropathogenic infectious bronchitis virus (IBV), avian nephritis virus (ANV), and chicken astrovirus (CAstV) induce nephritis, tubular degeneration, ureteral obstruction, and visceral gout through progressive renal injury (Bulbule et al., 2013; Mitchell, 2015; Li et al., 2022).



Nutritional and Toxic Factors including excess dietary calcium, high-protein diets, vitamin A deficiency, excessive vitamin D3 supplementation, high sodium intake, mycotoxins, and copper sulfate toxicity contribute to nephrotoxicity, mineral deposition, epithelial damage, dehydration, and urate precipitation within renal tubules and ureters (Guo et al., 2005; Mir et al., 2005; Mitchell, 2015).

Drug-induced Nephropathy may result from prolonged or excessive use of sulfonamides, aminoglycosides, and certain anticoccidial drugs, which can cause tubular degeneration, crystal precipitation, and obstructive renal damage (Yadav et al., 2020).

Gout in Poultry

Gout is a metabolic disorder characterized by hyperuricemia and deposition of monosodium urate (MSU) crystals, or needle-shaped *tophi*, within tissues and organs. In poultry, gout occurs in two principal forms based on the site of urate deposition: visceral gout and articular gout.

Visceral gout is the acute and more common form, characterized by extensive deposition of urate crystals on serosal surfaces and within visceral organs, particularly the kidneys, liver, heart, pericardium, air sacs, and peritoneum, often resulting in high mortality. The condition is frequently observed in broiler chicks as young as 2-3 days of age and in layer pullets older than 14 weeks. Renal injury impairs uric acid excretion, leading to hyperuricemia and subsequent urate accumulation in tissues.

Articular gout, in contrast, is a chronic and comparatively less common form associated with urate deposition in joints, tendon sheaths, ligaments, synovial membranes, and periarticular tissues, especially in the toes, legs, and wing joints. These deposits induce chronic granulomatous inflammation, leading to swollen, warm, and painful joints, lameness, reluctance to perch, and reduced mobility. The carpal joint is commonly affected, and birds may additionally exhibit depression, dehydration, greenish diarrhea, ruffled feathers, and a moist vent.

The pathogenesis of gout involves excessive uric acid production and/or impaired renal excretion, leading to persistent hyperuricemia, tubular obstruction, crystal precipitation, and inflammatory injury. In hydronephrosis-associated gout, obstructive renal lesions further compromise uric acid clearance, promoting urate deposition within renal tubules and visceral tissues and exacerbating nephropathy.

Studies indicate that urate crystals activate inflammatory pathways, including NLRP3 inflammasome signaling, oxidative stress responses, and profibrotic mediators involved in the progression of gouty nephropathy (Martinon et al., 2006).

Pathophysiological Link Between Hydronephrosis and Gout

Hydronephrosis and gout are linked through several interconnected pathogenic mechanisms (Fig. 1). Ureteral obstruction impairs normal urine flow, resulting in elevated intrarenal hydrostatic pressure and subsequent tubular epithelial injury. Persistent pressure and reduced renal perfusion led to ischemia, nephron degeneration, and progressive decline in glomerular filtration rate. As renal function deteriorates, uric acid excretion becomes impaired, causing hyperuricemia and precipitation of urate crystals within renal tubules. Accumulation of these crystals further obstructs the urinary passages, exacerbating renal damage and promoting progressive nephropathy that may culminate in visceral gout. Consequently, a self-perpetuating cycle develops in which hydronephrosis facilitates urate deposition, while urate accumulation intensifies obstruction and renal injury (Thotakura et al., 2023).

Molecular Mechanisms in Uric Acid Nephropathy

Recent studies suggest that uric acid nephropathy involves both crystal-dependent and crystal-independent mechanisms (Wang et al., 2022) (Fig. 2)

Clinicopathological Findings

Affected birds are commonly present with non-specific clinical signs including depression, anorexia or reduced feed intake, dehydration, weight loss, poor growth performance, ruffled feathers, and diarrhea. In cases involving articular urate deposition, lameness and impaired mobility may also be observed. Severe or progressive renal involvement is frequently associated with increased mortality.

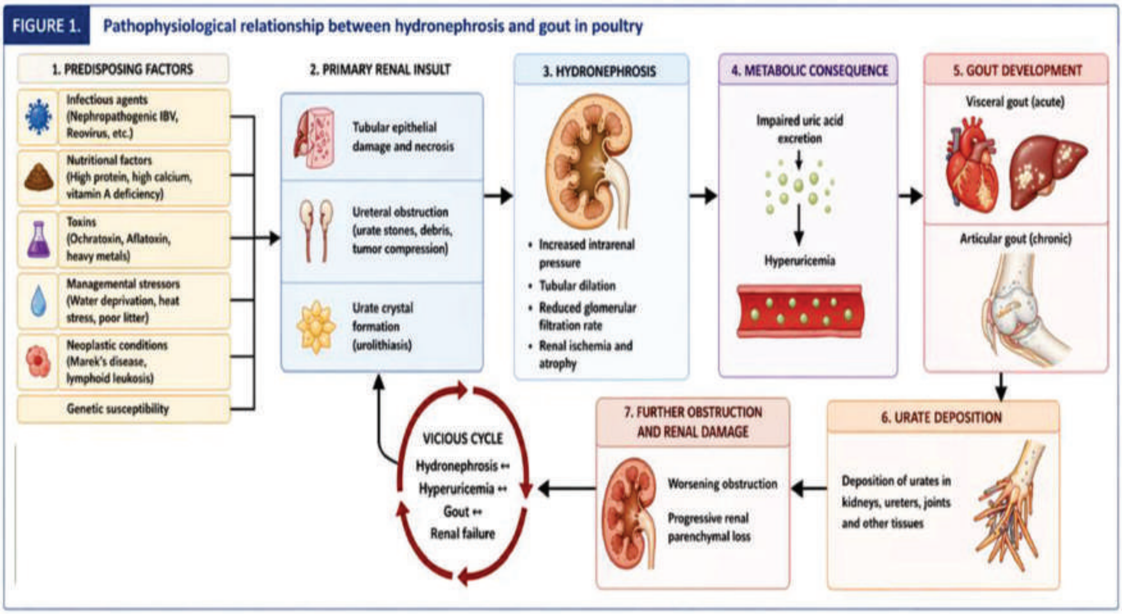


FIGURE 1. Pathophysiological relationship between hydronephrosis and gout in poultry

Oxidative Stress:	**Inflammatory Signaling:**	**Gut–Kidney Axis**
Hyperuricemia induces reactive oxygen species (ROS) production, leading to: • Lipid peroxidation, • Mitochondrial dysfunction, • Cellular apoptosis, • Fibrosis	Monosodium urate crystals activate: • NLRP3 inflammasome • NF-κB pathway • IL-1β production • TNF-α release These pathways contribute to chronic renal inflammation and fibrosis.	Emerging evidence highlights the role of intestinal dysbiosis in uric acid nephropathy. Altered gut microbiota may influence: • Uric acid metabolism • Immune activation • Oxidative stress • Renal inflammation Gut-derived endotoxins may amplify renal injury through systemic inflammatory pathways.

Lesions observed in visceral gout is chalky white urate deposits on visceral organs such as heart, liver, air sacs, and kidneys. In articular gout, there is swollen joints and white pasty urates within synovial cavities. In gout-affected kidneys, the kidneys become enlarged, pale, and hard in consistency with deposition of urate crystals (Fig. 3A); the ureters are dilated, with obstructive urate plugs. In hydronephrosis, the kidney is transformed into a fluid filled pouch like structure (Fig. 3B). Microscopically, affected kidneys commonly exhibit tubular degeneration and necrosis, interstitial nephritis, varying degrees of fibrosis, and ureteral obstruction. Deposition of urate tophi within renal tissues and associated granulomatous inflammatory reactions are also frequently observed (Fig. 3C). Laboratory findings include mild to marked increased in serum uric

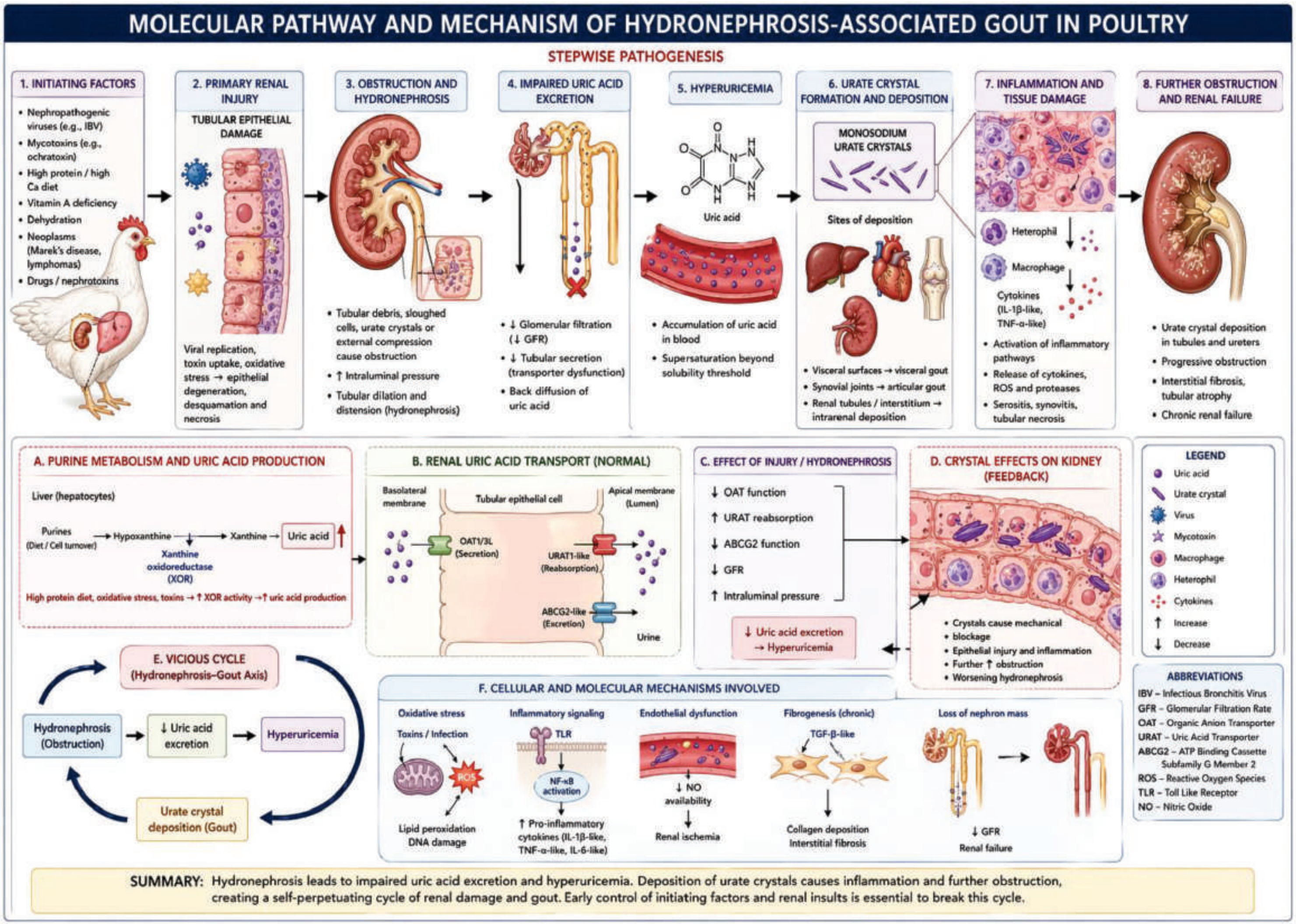


Fig. 2: Molecular pathways and mechanism of hydronephrosis-associated gout in poultry

Fig. 3A: Enlarged, pale and hard kidneys with deposition of the chalky white uric acid

Fig. 3B: Hard, pale and swollen right kidneys with deposition of the uric acid and left kidneys showing hydronephrosis (arrow)

acid, blood urea nitrogen and creatinine along with electrolytes and acid-base imbalance and presence of uric acid crystal in urine samples.

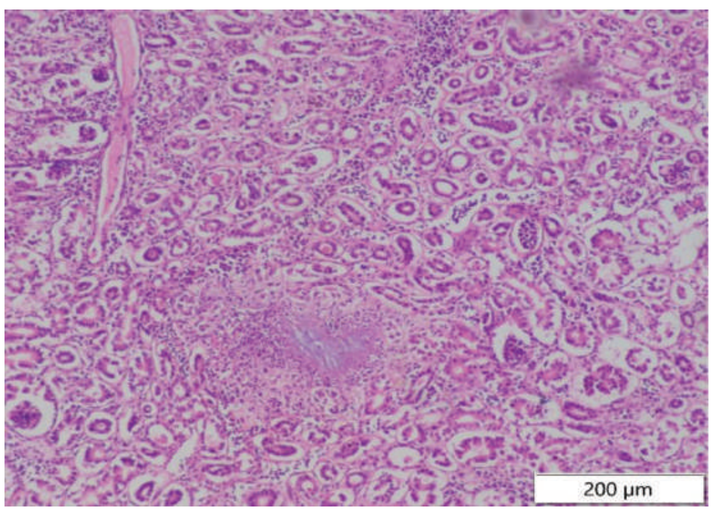


Fig. 3C: Microscopy of gout affected kidney exhibiting tubular degeneration and necrosis, interstitial nephritis, varying degrees of fibrosis and urate deposition within renal tissues.

Diagnosis

Diagnosis of hydronephrosis-associated gout in poultry is based on a comprehensive evaluation of clinical history, dietary and managemental factors, necropsy findings, histopathological examination of sample collected in absolute ethanol, serum biochemical analysis, and molecular diagnostic techniques. Polymerase chain reaction (PCR) assays are particularly valuable for the detection of nephropathogenic viruses such as infectious bronchitis virus (IBV) and avian nephritis virus (ANV).

Important biochemical alterations include elevated serum uric acid and creatinine concentrations, along with electrolyte imbalances indicative of renal dysfunction. Birds affected with gout commonly show marked hyperuricemia, with serum uric acid levels frequently exceeding 5-7 mg/100 mL. Increased uric acid concentrations are primarily associated with impaired renal excretory function and enhanced nucleic acid catabolism resulting from tissue degeneration (Chandra et al., 1985). Hyperuricemia has also been associated with prolonged administration of diets containing excessive calcium or protein (Guo et al., 2005).

Serum creatinine, a byproduct of muscle metabolism excreted through glomerular filtration, serves as an indirect indicator of renal filtration efficiency (Sandhyarani et al., 2022). Elevated serum creatinine levels (>0.7-22mg/dl) therefore reflect compromised kidney function and renal injury associated with monosodium urate crystal deposition.

Hematological findings in affected birds often include increased packed cell volume (PCV), total erythrocyte count (TEC), and hemoglobin concentration, likely resulting from dehydration secondary to diarrhea (Yadav et al., 2020). Additionally, elevated hepatic enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been reported in gout induced by high-protein diets and diclofenac toxicity, indicating concurrent hepatic injury (Hong et al., 2020; Sandhyarani et al., 2022). Oxidative stress markers further reveal decreased glutathione (GSH) levels and increased thiobarbituric acid reactive substances (TBARS) in renal and hepatic tissues, supporting the role of oxidative damage in disease progression (Sandhyarani et al., 2022)

Differential Diagnosis

Differential diagnoses for hydronephrosis and gout in poultry include infectious nephritis, dehydration-associated nephropathy, mycotoxicosis, nephrotoxicity induced by drugs or chemicals, urolithiasis, vitamin A deficiency, and heavy metal poisoning, all of which may produce renal dysfunction and urate deposition similar to gout.

Prevention and Control

Effective prevention and control of hydronephrosis and gout in poultry requires a comprehensive multifactorial approach involving nutritional, environmental, infectious disease, and managemental interventions.

Proper nutritional management is essential and includes maintaining a balanced calcium-to-phosphorus ratio, avoiding excessive dietary protein levels, ensuring adequate vitamin A supplementation, and preventing contamination of feed with mycotoxins. Appropriate water management is equally important, with continuous access to clean drinking water, prevention of dehydration, and maintenance of optimal brooding temperatures to minimize renal stress.

Control of infectious diseases, particularly nephropathogenic infectious bronchitis virus (IBV), is critical in endemic areas, and vaccination programs should be implemented where necessary. Careful drug administration is also required to avoid indiscriminate or prolonged use of nephrotoxic medications, especially during periods of restricted water intake.

In addition, strict biosecurity and hygiene measures play a major role in reducing exposure to infectious agents associated with viral nephropathies and renal damage.

Therapeutic Approaches

At present, no definitive curative treatment is available for avian gout or hydronephrosis; therefore, management is primarily supportive and aimed at reducing renal stress, correcting metabolic disturbances, and minimizing further urate accumulation. Supportive therapy includes fluid administration, correction of electrolyte imbalances, urine acidification or alkalization as indicated, reduction of dietary protein levels, and immediate withdrawal of nephrotoxic agents.

Adequate water availability is essential, and birds should be provided with unrestricted access to clean drinking water with sufficient drinker space to prevent dehydration. Temporary feeding of low-protein diets for 3–5 days may help reduce uric acid production during acute outbreaks. Nutritional management should also ensure adequate supplementation of vitamins A, D3, K, and B-complex to support epithelial integrity and renal function.

In endemic areas, revision of infectious bronchitis (IB) vaccination programs is recommended, including the use of nephropathogenic vaccine strains where appropriate. Early vaccination strategies, such as day-old beak-dip vaccination in broilers, may help reduce nephropathic viral infections.

Various urinary acidifiers and supportive additives have been used to reduce mortality and improve renal function, including vinegar, potassium chloride, ammonium chloride, and ammonium sulfate administered through feed or drinking water. Electrolyte supplementation may further assist in correcting dehydration and metabolic imbalance. Supportive feeding practices such as the provision of broken maize and jaggery during severe outbreaks have also been suggested.

Pharmacological intervention with allopurinol, a xanthine oxidase inhibitor, has been reported to reduce uric acid synthesis and may be administered through drinking water under controlled conditions (Seth et al., 2014). Dietary supplementation with methionine hydroxy analogue free acid in combination with controlled calcium levels has likewise been recommended to support metabolic balance and reduce gout severity.

Emerging therapeutic approaches targeting oxidative stress, inflammatory pathways, and modulation of the gut microbiota may provide future prospects for the management of gouty nephropathy in poultry.

Research Gaps and Future Directions

Despite the economic and clinical importance of avian gout and hydronephrosis, several aspects of their pathogenesis remain poorly understood. Most current studies focus on uric acid metabolism and renal pathology independently, while the mechanistic relationship between urinary obstruction, hydronephrosis, and urate crystal deposition has received limited attention**.** There is a lack of reliable early biomarkers for the detection of subclinical renal injury before the onset of overt gout. Future research emphasizing the role of the gut–kidney axis, genetic susceptibility, and nephropathogenic infectious agents in disease progression may improve understanding of disease mechanisms and facilitate the development of effective preventive and therapeutic strategies for gout-associated hydronephrosis in poultry.

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Mini Review

Naegleria fowleri and Primary Amoebic Meningoencephalitis: An Emerging Public Health Concern

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Abstract

Naegleria fowleri, commonly known as "Brain-Eating Amoeba" is a free-living protozoan found in warm freshwater bodies such as lakes, rivers, ponds, and hot springs. It causes rare but a highly fatal infection of the central nervous system in humans known as Primary Amoebic Meningoencephalitis. Infection occurs when water contaminated trophozoite stage of the parasite with enters the nasal cavity especially during recreational activities such as swimming, allowing the amoeba to migrate to the brain through the olfactory nerve. The disease progresses rapidly and is manifested as headache, vomition, neck stiffness, confusion, and seizures. Diagnosis involves microscopic examination of cerebrospinal fluid for parasitic stages and molecular techniques such as PCR assays. Currently, the treatment options are limited, and no vaccine is commercially available. Increased awareness, improved surveillance mechanisms, and preventive measures are hence essential to reduce the risk of infection and associated fatalities.

Keywords: *Brain-eating amoeba, Naegleria fowleri, Primary Amoebic Meningoencephalitis,*

Naegleria fowleri is a free-living amoeba in warm fresh water such as lakes, ponds, rivers and springs (Alanzi et al., 2025). The organism is commonly called as "Brain-Eating Amoeba". The amoeba does not have any animal reservoir. It is responsible for a rare but fatal brain infection known as Primary Amoebic Meningoencephalitis (PAM) in immunocompetent children and young and adults (Mahajan, 2024)

Recently, the state of Kerala in India was in news due to PAM, a disease caused by *Naegleria fowleri* commonly referred to as "Brain-Eating Amoeba". According to information shared during a parliamentary session, the state reported 170 cases and 42 deaths by November 2025. The number of cases increased steadily, from 02 cases and 02 deaths in the year 2023 to 39 cases and 09 deaths in 2024, and 170 cases with 42 deaths by November 2025 (The Tribune, 2025).

This situation created fear among the people living in Kerala and neighbouring states because the disease has a very high case fatality rate of more than 95%. The infection is commonly associated with water sources used for recreational activities, such as swimming.

Apart from Kerala, few isolated cases have also been reported from other parts of India. One case was reported in an elderly man from Himachal Pradesh who recently travelled to Goa and had contact with pond water (Angrup et al., 2020) while another case was reported in a new born infant from Uttar Pradesh who was bathed with pond water (Yadav et al., 2013). In addition, one case was also treated at the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, and another from Madhya Pradesh in a man with a history of exposure to pond water (Jain et al., 2002; Gupta et al., 2015).

Many cases in various parts of India might have gone undetected due to lack of awareness and inadequate diagnosis for the disease. The protozoa has been reported from ponds in Haryana and West Bengal, a swimming pool in Mumbai, and sewage samples from Kolkata, suggesting that the organism is present in many other regions of the country (Gogate & Deodhar, 1985; Bose et al., 1990; Gupta, 1992).

The Parasite

Naegleria fowleri, a single-celled protozoan parasite, lives freely in natural water bodies such as lakes, rivers, hot springs and can also be found in the soil. The organism feeds on bacteria present in water or sediments and can survive in temperatures as high as 45°C (De Jonckheere, 2011; Yoder et al., 2012).

The parasite has three stages in its life cycle:

1. **Trophozoite (10-20 µm)** is the infective stage responsible for causing disease in humans. It is the feeding and reproducing stage of the amoeba and multiplies by mitosis.
2. **Flagellated stage (10-16 µm)** develops when the amoeba experiences food scarcity. It has flagella and does not feed.
3. **Cyst (8-20 µm)** is formed under unfavourable environmental conditions, such as low temperature or lack of food. During this stage, the organism becomes dormant and metabolically inactive.

The protozoa become dangerous when water contaminated with the amoeba (trophozoite) enters the human body through the nose/nasal cavity. From there, it travels to the olfactory bulb of the brain through olfactory nerves and causes severe infection (PAM) in the affected individuals. The disease is believed to occur more commonly in children and young adults under 20 years of age (Iyer et al., 2024).

Primary Amoebic Meningoencephalitis (PAM)

The infection in brain caused by *N. fowleri* is technically known as Primary

Amoebic Meningoencephalitis (PAM), which affects the central nervous system of humans. The infection was first recorded in Florida, USA, in the year 1962. Later, in 1965, the disease was reported in southern Australia by Malcolm Fowler and Carter, and the organism was named after Fowler (Mahajan, 2024)

So far, infections caused by *N. fowleri* have been reported from 39 countries with India, Pakistan, United States, Australia, Mexico, and Czech Republic reporting the highest number of cases due to the presence of warm freshwater and favourable climatic conditions for the survival and multiplication of the parasite (Güémez & García, 2021).

Since 1965, more than 500 cases of the disease have been reported worldwide, with an annual incidence ranging from 0–8 cases. Most cases are associated with ponds, swimming pools, freshwater bodies, and poor water management practices (Anser & Hasan, 2018; Iyer et al., 2024). Further, Karachi in Pakistan has emerged as a hotspot for the infection due to high temperatures, heavy rainfall, and poorly managed municipal water supplies (De Jonckheere, 2011; Nadeem et al., 2023).

Life Cycle

Naegleria fowleri has three distinct stages in its life cycle (Fig 1): the cyst, the trophozoite, and the flagellate stage. Among these, the trophozoite is the only stage infective to humans. It serves as the sole feeding stage and reproduces through a process called mitosis, occurring either in the environment or within the host's brain tissue. In environmental habitats, the trophozoite feeds on bacteria found in warm fresh water and soil; however, once inside the human brain, it utilizes specialized sucker-like structures called food cups (amoebastomes) to ingest brain tissue. When environmental conditions change rapidly, the trophozoite transforms into the flagellate stage, which possesses two anterior flagella. This temporary stage is non-feeding and non-replicating, uses its flagella to swim toward more favourable conditions before reverting to the trophozoite form. Conversely, when faced with extreme environmental stressors—such as cold temperatures, desiccation, or food scarcity—the parasite encysts into a spherical, dormant form. It remains in this protective cyst stage until favourable conditions return (CDC, 2023).

Pathogenesis

The parasite enters human body through nasal passages while the person is swimming or diving in warm contaminated water primarily with trophozoites of the parasite. The infective trophozoites attach to the nasal mucous membrane and migrate to the brain through the olfactory nerve (Fig. 1). Once inside the brain, organism releases cytolytic molecules such as phospholipases, neuraminidases, acid hydrolases and phospholipolytic enzymes that destroy the brain tissue. This leads to the development of PAM, a rapidly progressing and often fatal disease.

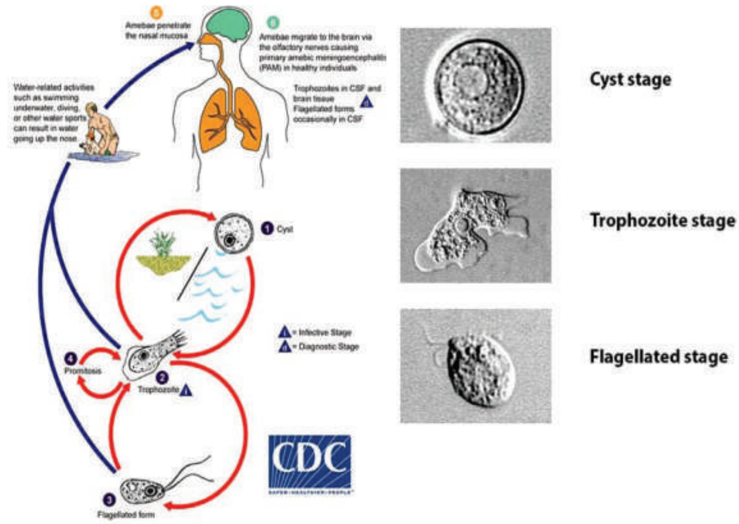

Fig. 1. Life cycle and disease (Source: CDC, 2023)

Symptoms

The symptoms usually appear 5–7 days after exposure, although in some cases the disease may develop as early as within 24 hours.The symptoms are non-specific and resemble those of other brain infections like headache, vomition, stiff neck, confusion and seizures.In severe cases, the infection causes inflammation of the central nervous system, known as pyogenic meningoencephalitis. This may lead to coma and death within about one week after the onset of symptoms (Iyer et al., 2024).

Diagnosis

In suspected cases, a history of contact with freshwater within the previous 10–12 weeks can provide an important clue. As early detection of disease is critical for saving the patient's life, so PAM must be included in the differential diagnosis panel of meningitis.

The trophozoites can be detected in cerebrospinal fluid (CSF) after staining with Giemsa and trichrome (Siddiqui et al., 2016; CDC, 2023). Traditionally, a flagellation test has been used to identify the organism. In this test suspected CSF is centrifuge at 5000 x g for 5 min followed by resuspension of pellet in sterile distilled water. The suspension is then incubated at 37 C for 4-24 hrs followed by wet mount evaluation for the flagellates (Martinez, 1985)

More advanced techniques such as polymerase chain reaction (PCR), enzyme linked immunosorbent assay (ELISA), real-time PCR, and PCR-restriction fragment length polymorphism (RFLP) can improve diagnostic accuracy (Behets et al., 2003). These molecular methods are more sensitive and rapid than the traditional methods and do not require culturing the organism. In PCR 18s RNA gene is considered best for the detection of the organism to genus and species level (Qvarnstrom et al., 2006)

Another promising technique is loop-mediated isothermal amplification (LAMP), which is rapid and does not require expensive equipment. This makes it suitable for use in resource-limited countries.

Treatment

Currently, no specific treatment is available for the infection. Developing treatment is difficult because the disease is rare, rapidly progressing, and highly fatal, which makes clinical testing of drugs challenging. Most available treatment information comes from laboratory studies on animals (Alanazi et al., 2025).

The most commonly used drug is amphotericin B, which is administered systemically. Other drugs such as miconazole, sulfisoxazole, and rifampicin may be used in combination with this therapy (Mahajan, 2024). Azithromycin has also shown some promise in the treatment of infection.

Prevention

Prevention is extremely important because effective treatment and vaccines are currently unavailable. The organism cannot survive in chlorinated or salt water, so recreational water bodies should be regularly disinfected with chlorine. Public awareness about the disease is currently low because most cases in India have been reported from Kerala. Therefore, awareness campaigns about the disease, its fatal nature, and preventive measures should be conducted to encourage people to exercise caution while using natural water bodies. State and central governments can also initiate regular surveillance of natural water sources by testing water samples, which can help to prevent future outbreaks and fatalities.

Future Outlook

At present, no vaccine is available for *Naegleria fowleri* infection. This provides an opportunity for researchers to develop preventive strategies against this fatal infection. A One Health approach involving microbiologists, physicians, public health experts, infectious disease specialists, and environmental microbiologists may help accelerate vaccine development.

It is believed that the amoeba can bypass the host's innate immune response, causing little or no inflammation in the nasal cavity but leading to severe inflammation in the brain. This often results in delayed diagnosis, which contributes to the high fatality rate.

Therefore, there is significant potential for developing early diagnostic tools that can detect the pathogen quickly and help identify effective treatment strategies before reaching a disease stage where return to normal conditions become difficult.

Punjab, India Perspectives on *Naegleria fowleri*

Punjab has several rivers, canals, and village ponds, which may serve as potential habitats for this amoeba. Therefore, the possibility of the organism being present in these water bodies cannot be ruled out outrightly.

It is important to raise awareness among communities about *Naegleria fowleri* disease, its symptoms, and preventive measures. Healthcare professionals should also consider this disease in their diagnostic protocols when patients present with similar symptoms and have a history of exposure to natural water bodies or recreational water activities. Researchers should conduct regular surveillance of water bodies to detect the presence of *Naegleria fowleri* pathogen.

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Short Communication

Integrated Livestock Farming System for the Kandi Region of Punjab, India

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Abstract

The present article highlights the importance, suitability, and various models of integrated livestock farming systems for the Kandi region of Punjab. Integration of enterprises such as dairy farming, goat and sheep rearing, poultry farming, fisheries, crop cultivation, horticulture, agroforestry, and vermicomposting helps in efficient recycling of farm resources, reduction of external input costs, and generation of multiple income sources throughout the year. Livestock components contribute nearly 40–60% of the total farm income and also improve soil fertility through recycling of organic wastes and farmyard manure. Poultry litter, livestock dung, crop residues, and pond nutrients are effectively utilized within the system, enhancing nutrient-use efficiency and environmental sustainability. Different integrated farming models suitable for Kandi conditions, including livestock–crop systems, poultry–fish farming, backyard poultry integration, goat–crop systems, and silvipastoral models, have been discussed along with their economic and ecological benefits. Scientific management practices related to breed selection, feeding, housing, vaccination, fodder production, and environmental management are also emphasized. Integrated systems significantly increase farm profitability, employment generation, nutrient recycling, and productivity compared to conventional crop farming alone.

Keywords*: Agroforesty, Fishery, Horticulture, Integrated farming, Kandi area, Livestock, Poultry, Silvipastoral,.*

Rationale and Importance of Integrated Farming Systems in Kandi area

Integrated Farming Systems are highly suitable for the Kandi region because of its undulating terrain, limited irrigation facilities, and seasonal shortage of fodder. Under these conditions, farmers cannot depend on a single enterprise for their livelihood; therefore, combining enterprises such as dairy farming, goat farming, poultry, fish farming, pig farming, and crop production helps in better utilization of available farm resources and provides multiple sources of income throughout the year. In this system, the waste and by-products of one enterprise are efficiently utilized by another, making the farm more productive, economical, and sustainable. Poultry, pig, and goat wastes can be recycled through fish ponds, composting units, and fodder production, while crop residues such as straw and stubble can be used as animal feed and bedding material.

Similarly, nutrient-rich pond water can be reused for irrigation, improving soil fertility and reducing the dependence on chemical fertilizers and external feed inputs. Such recycling improves nutrient-use efficiency, reduces production costs, and increases resilience against climatic uncertainties and market fluctuations.

Livestock farming contributes nearly 40–60% of the total income in integrated farming systems of the Kandi region. Apart from milk and meat production, livestock also support other farm activities through the production of farmyard manure and organic waste. One ton of farmyard manure contains approximately 8 kg nitrogen, 4 kg phosphorus, and 16 kg potassium, which improve soil fertility and water-holding capacity when applied to fields (Prabhakar et al., 2025). Thus, integrated farming follows the principle of "waste to wealth," where every farm resource is utilized efficiently. Fish and pig farming further strengthen this system by improving nutrient recycling and generating additional income. Composite fish farming with species such as Catla, Rohu, Mrigal, and Common carp increases pond productivity, while pig farming provides quick returns because of the fast growth and efficient feed utilization of breeds such as Large White Yorkshire and Landrace. Pig manure can also be used as an organic fertilizer for fish ponds and crop fields, thereby reducing input costs and improving farm sustainability.

Selection of suitable livestock breeds is very important under Kandi conditions. Crossbred cattle and Sahiwal cows are recommended for dairy farming because they can produce 8–15 litres of milk per day under proper management. Murrah buffaloes are preferred due to their high milk fat content of around 6–8%. For goat farming, the Beetal breed is highly suitable because of its adaptability and dual-purpose use for milk and meat production, along with its higher twinning ability. Poultry farming is also an important component of the integrated system due to its low investment and quick returns through eggs and meat. Dual-purpose birds such as Punjab Brown and Rhode Island Red (RIR) crosses perform well under Kandi conditions because of their adaptability, good egg production, disease resistance, and efficient use of locally available feed resources. Integration of poultry with goats, pigs, and fish further improves nutrient recycling and overall farm productivity.

Proper feeding and management are essential for successful livestock farming in the Kandi region, where green fodder availability remains limited for most of the year. Animals should receive dry matter equivalent to about 2.5–3% of their body weight, while urea-treated straw can help improve the protein content of poor-quality roughages. Area-specific mineral mixtures and urominlicks should also be provided regularly to maintain animal health, reproduction, and productivity. Proper housing is equally important, and animal sheds should preferably be constructed in the east–west direction with adequate ventilation and sufficient floor space. Dairy animals require about 3.5–4 square metres floor space per animal, while goats require 1–1.5 square metres. Regular vaccination against diseases such as Foot and Mouth Disease (FMD), Haemorrhagic Septicaemia (HS), Enterotoxaemia, and PPR, along with deworming every 3–4 months, is necessary to reduce disease incidence and production losses. Overall, integrated farming systems provide a practical and sustainable approach for improving income, employment, soil fertility, and nutritional security of farmers in the Kandi region.

Different Integrated Farming System Models

1. Livestock + Crop-Based Farming System

This is one of the most common systems in the Kandi region. Farmers with small landholdings can increase their income by integrating crop farming with livestock. Crop residues such as straw is utilized as livestock feed, while manure from livestock is recycled back to the fields for improving soil fertility. Grains produced on farms serve as supplementary feed for animals, and crop residues are used as fodder. Animal manure improves soil fertility because it contains essential macro- and micronutrients. Biogas can also be generated from cattle dung and urine(Godi et al., 2013).

Integrated farming not only increases income but also creates additional employment opportunities. For example, an integrated farm with six buffaloes generated about 904 man-days of employment compared to only 400 man-days from crop farming alone. One ton of dung-based manure contains about 8 kg nitrogen, 4 kg phosphorus, and 16 kg potassium, which enhance soil productivity. (Prabhakar et al., 2025)

Due to seasonal fodder scarcity in the Kandi region, scientific feeding strategies are essential:

- Dry matter requirement: 2.5–3% of body weight/day
- Green fodder to concentrate ratio: 60:40 for milking animals
- Urea treatment of straw improves crude protein from 3% to 8–10%
- Area-specific mineral mixture and uromin lick should also be provided regularly to improve milk yield and reproductive efficiency.

Housing and Environmental Management

Animal sheds should be east-west oriented. Dairy animals require 3.5–4 square metres floor space, while goats require 1–1.5 square metres. Loose housing systems with proper ventilation are most suitable.

2. Livestock + Crop + Fish-Based Farming System

Villages located near the Beas River often have sufficient water resources, making fish farming feasible along with crop cultivation and livestock farming. Farm by-products from agriculture and livestock are effectively utilized in fish farming, providing an additional source of income and reducing economic risk.

Fish farming requires comparatively less labour, and livestock wastes such as dung, leftover fodder, and feed residues help support pond productivity. Nutrients present in dung promote the growth of phytoplankton and zooplankton, which serve as natural fish feed.A large animal produces about 4,000–5,000 kg dung and 3,500–4,000 litres urine annually. About 5–6 large animals are sufficient to fertilize one hectare fish pond. Under such systems, 6,000 kg milk and 2,000–3,000 kg fish production per hectare per year can be achieved. This system also reduces labour required for manure transportation.Each large animal requires approximately 6–8 tons green fodder and 1.5–2 tons dry fodder annually, which can be supplied through integrated crop production (Kochewad et al., 2017). Mahajan et al. (2012) observed that integrating dairy and poultry enterprises into the Integrated Farming System significantly enhanced farm profitability. The inclusion of these allied components increased the annual net income to ₹37,343 compared to only ₹26,511 obtained from cultivation of field crops alone.

3. Poultry + Fish Farming System

Poultry can be integrated with fish farming to reduce feed and fertilizer costs. Poultry houses may be constructed near or above fish ponds so that droppings directly enter the pond and are recycled. Birds are generally reared under deep litter systems. Poultry litter contains about 3% nitrogen, 2% phosphate, and 2% potash, making it an excellent fertilizer source that promotes plankton growth in ponds(Kochewad et al., 2017). Approximately 1,000 birds can be integrated with one hectare fish pond, resulting in fish production of 4,000–5,000 kg/ha/year.

According to Gangwar et al. (2013), an integrated poultry–fish farming system generated approximately 45–50 man-days of employment annually and provided an additional income of about ₹4,000–5,000 per year. Sahoo and Singh (2015) reported that a poultry–fish integrated farming model achieved a benefit–cost ratio of 1.09 and yielded a maximum profit of ₹33,664.06 per 0.025 hectare per year. Bezbaruah, (2012) observed that farmers adopting an integrated system of poultry, fisheries, and horticulture earned nearly ₹40,000 from poultry, ₹25,000 from fish production, and around ₹6,000 from vegetables cultivated on pond bunds. Similarly, Kalita et al. (2016) reported that integration of fish and poultry farming in a 1.0-hectare pond, without the use of supplementary feed, produced about 4,500–5,000 kg of fish, 70,000 eggs, and nearly 1,000 kg live-weight chicken meat annually.

4. Livestock + Crop + Backyard Poultry System

Backyard poultry plays a vital role in improving income and nutritional security of rural families in the Kandi region. Integration of poultry with livestock and crop farming enhances farm productivity and increases household income. Birds survive largely on farm residues and insects available around the farm, thereby reducing feed costs. They also help control ticks and insects present in animal sheds, providing a biological method of pest control and reducing dependence on chemicals. Eggs and poultry meat serve as valuable protein sources and provide regular daily income.

Devendra & Pezo (2002) reported that backyard livestock enterprises involving sheep, goats, pigs, and poultry serve as an important emergency income source for rural households. Nirmala et al. (2012) observed that the adoption of improved scientific backyard poultry practices significantly enhanced household earnings. Their study revealed that about 51% of rural women obtained nearly 6–10% of their total household income from backyard poultry farming, while 28% earned around 11–20%. Only a small proportion of women (7%) derived as much as 21–30% of their household income from this enterprise.

5. Goat/Sheep + Crop + Backyard Poultry System

Goat farming is rapidly emerging as a livelihood enterprise in the Kandi region. However, goat farming alone does not provide regular daily income. Therefore, farmers are encouraged to integrate goat farming with crop cultivation and backyard poultry. This combination increases overall farm income while ensuring regular earnings through sale of eggs and poultry products.

Integrated systems improve productivity, maintain ecological balance, and enhance economic stability. Goats help utilize weeds and shrubs present in fields, thereby reducing disease incidence in crops. Farmers can also cultivate fodder crops and plant horticultural trees along field bunds, which serve as browsing material for goats while reducing labour costs. Fruit trees further provide an additional source of income.

6. Small Ruminants (Goat + Sheep) with Silvipastoral Farming System

This system integrates goats and sheep with trees and fodder grasses and is particularly suitable for the Kandi region where rainfall is low and soil erosion is common.

Ramana et al. (2000) reported that lambs and kids grazing on silvipasture recorded average daily weight gains of 54.8 g and 36.8 g per head, respectively, whereas animals grazing on natural grasslands gained only 41.2 g and 26.4 g per head per day over a grazing period of 478 days. The study further revealed that animals maintained continuous body weight gain under both grazing systems even without supplementation of concentrate feed.

Ramasamy et al. (2007) observed that an integrated farming system comprising sorghum + cowpea, *Leucaena leucocephala* + *Cenchrus ciliaris*, and *Acacia senegal* + *Cenchrus ciliaris* integrated with goat rearing generated an additional employment opportunity of about 113 man-days per hectare annually in dryland regions. Grazing animals also contributed positively to soil fertility through the addition of dung and urine, which enriched the soil with essential nutrients. Moreover, animal movement during grazing disturbed insect and pest habitats present in shrubs and foliage, thereby helping reduce disease incidence. Trees in the silvipastoral system additionally served as an important source of timber and fodder during feed-scarce periods.

Proposed Integrated Livestock Farming System for Kandi Farmers (1–2 Acre Model)

This model is especially suitable for small and medium farmers. Its primary objective is efficient resource utilization and nutrient recycling.

Land Use Pattern (2 Acre Model)

1. Crop cultivation (fodder + cereals + pulses) – 0.80 acre (40%)
2. Horticulture (guava, aonla, kinnow) – 0.40 acre (20%)
3. Fish pond – 0.30 acre (15%)
4. Agroforestry (bunds/boundaries) – 0.20 acre (10%)
5. Livestock unit – 0.15 acre (7.5%)
6. Poultry unit – 0.05 acre (2.5%)
7. Vermicompost and other facilities – 0.10 acre (5%)

For a 1-acre model, all components can be reduced proportionally.

2. Integrated Components

- 2 dairy animals
- 10–15 goats + 10 sheep
- 100–150 poultry birds
- 0.25–0.30 acre fish pond
- Cereals + fodder + pulse crops
- Guava, Amla and kinnow orchard
- Agroforestry trees (Subabul, Babul, Bamboo)
- 2–3 vermicompost units

3. Nutrient Flow and Recycling

- Crop residues → animal feed
- Tree leaves → goat fodder
- Animal dung (8–10 tons/year) → vermicompost + fish pond
- Poultry litter (1–1.5 tons/year) → pond + compost
- Pond water → irrigation
- Vermicompost (5–6 tons/year) → crops + horticulture

Overall, 70–80% nutrient recycling is achieved within the system.

4. Production Potential (2 Acre Model)

- Milk: 8–12 litres/animal/day
- Meat: 10–15 kids/lambs annually
- Eggs: 10,000–12,000 eggs/year
- Fish: 800–1,200 kg/year
- Field crops: wheat, maize, fodder
- Fruits: 6–8 tons/year

5. Scientific Benefits

- 8–10 tons green fodder/acre/year can be produced
- External feed costs reduce by 40–60%
- Soil fertility improves significantly
- Greater sustainability under rain fed conditions
- Overall farm productivity increases by 150–250%

Gaps and Future Directions

Although integrated farming systems offer significant potential for improving farm productivity and sustainability in the Kandi region of Punjab, their adoption remains constrained by fragmented landholdings, dependence on rainfed agriculture, seasonal water scarcity, limited availability of quality inputs, inadequate technical knowledge, weak extension and veterinary support, poor access to institutional credit, and high initial investment costs. Inadequate market linkages, lack of value addition and storage facilities, and price fluctuations further limit the profitability of diversified enterprises. Future efforts should focus on developing location-specific integrated farming models, promoting efficient water management, climate-resilient crop and fodder production, improved livestock management, and resource recycling through enterprise integration. Strengthening farmer training, extension services, digital advisory platforms, farmer producer organizations, and market infrastructure, along with long-term research on the economic, environmental, and climate resilience of integrated farming systems, will facilitate their wider adoption and enhance the sustainability of agriculture in the Kandi region.

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Mini Review

Enhancing Livestock Farm Sustainability through Innovative Waste Management Practices

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Abstract

Livestock production systems face increasing pressure to manage substantial organic waste while minimizing environmental impact and meeting rising demands. This article explores innovative waste management practices that transition conventional livestock system to circular systems, emphasizing resource efficiency and regeneration. Key technologies include anaerobic digestion, which converts organic matter into biogas and nutrient rich digestate, significantly reducing costs and emissions. Nutrient recovery technologies, such as struvite precipitation and membran based separation, further extract valuable nitrogen and phosphorus from liquid waste streams. Thermal conversion methods, including pyrolysis and hydrothermal carbonization, transform organic waste into biochar, bio-oil, syngas and hydrochar, offering diversified revenue streams and soil enhancement. Finally, biological conversion approaches like composting, vermicomposting, green manure and insect based bioconversion demonstrate effective nutrient recycling, soil improvement and creation of value-added products. These integrated strategies enhance farm sustainability by reducing environmental losses, improving soil health and strengthening overall resilience within farm operations.

Keywords*: Bioeconomy, Digestate, Nutrients, Sustainability*

Animal production systems generate substantial quantities of organic waste including manure, feed residues and processing byproducts. For decades, these materials have been managed primarily through direct land application, open storage or basic disposal methods. While common, such practices are increasingly linked to environmental concerns such as nutrient runoff, water pollution, greenhouse gas emissions and the underuse of potentially valuable resources. As global demand for food continues to rise, farming systems are facing growing pressure to increase productivity while simultaneously reducing their environmental footprint. In response to these challenges, innovative waste management practices are gaining attention as effective tools for improving animal farm sustainability. Rather than viewing organic by-products as materials to be discarded, modern approaches seek to recover their value through treatment, conversion and reuse. This shift reflects a broader transition from conventional livestock systems where resources are used and disposed of to circular systems that prioritize resource efficiency and regeneration. Within circular system, manure, feed residues, and other organic wastes are recognized as inputs for new production cycles. Through recovery and processing technologies, these materials can be transformed into renewable energy, nutrient-rich fertilizers, soil amendments, and other value-added products (Duque-Acevedo et al., 2021). By reintegrating organic resources back into farming operations, circular systems reduce environmental losses, enhance soil health, and strengthen farm resilience. This transformation is grounded in several complementary concepts. Industrial ecology emphasizes closing material loops and designing integrated systems that minimize waste. Nutrient cycling science provides the understanding necessary to capture and return essential elements such as nitrogen and phosphorus to agricultural soils. At the same time, the bioeconomy framework positions organic waste as a feedstock for producing renewable energy, biomaterials, and biochemicals alongside traditional agricultural outputs (Duque-Acevedo et al., 2021). This mini review highlights recent advancement in waste management technologies.

Anaerobic Digestion System

Anaerobic digestion system operates under oxygen free conditions in which microbial organisms convert organic matter into biogas primarily methane (CH_4) and carbon dioxide (CO_2) and digestate, a nutrient rich byproduct suitable for fertilizer applications. Anaerobic digestion system produced biogas and digestate has lower climate impacts and reduced costs compared to conventional reliance on purchased energy and synthetic mineral fertilizers (Vikki et al., 2025). In pig manure management, anaerobic digestion exhibited significant resource recovery potential. Processing one cubic meter of pig manure per hour generate approximately 0.97 m^3 of recovered water, 49.40 kg of biofertilizer, and 5.33 m^3 of biogas (Molina-Moreno et al., 2017). Such systems reduce water consumption by 47.01%, natural gas consumption by 5.33% and CO_2 emissions by 171.98 kg/hour (Molina-Moreno et al., 2017). Although digestate retains most of the nitrogen and phosphorus present in the original feedstock, these nutrients are often in dissolved forms that may require additional treatment for optimal agronomic utilization. Therefore, integrated anaerobic digestion system combined with downstream nutrient recovery technologies are increasingly conceptualized as multi product biorefineries that maximize value extraction from organic waste streams (Mousavi et al., 2024).

Nutrient Recovery Technologies

Nutrient recovery technologies are designed to concentrate nitrogen and phosphorus from liquid waste streams into transportable, plant available products. These systems are particularly effective when applied to anaerobic digestion digestate, which contains elevated concentrations of dissolved nutrients.

Struvite Precipitation: Struvite (magnesium ammonium phosphate) precipitation is among the most mature nutrient recovery technologies. Study on chicken manure digestate have reported ammonia removal efficiencies of 86% in control samples and 88% in filtered samples at pH 11.0, while nitrogen recovery efficiency reached 86.3% at pH 10.0 in raw digestate (Mousavi et al., 2025). In addition to nutrient recovery, the process reduced heavy metal concentrations and improved the microbial composition of treated digestate. When integrated with anaerobic digestion, struvite precipitation establishes a two-stage recovery system: anaerobic digestion stabilizes organic matter and generates renewable energy, while struvite crystallization produces a slow-release fertilizer suitable for agricultural application (Mousavi et al*.,* 2025).

Membrane Based Separation: Gas permeable membrane systems recover nitrogen as ammonium sulfate with efficiencies of 53% for swine manure and 92% for digestate. Electrodialytic processes achieve phosphorus recovery efficiencies of 100% for swine manure and 74% for digestate (Gonzalez-Garcia et al*.,* 2022).

Chemical Precipitation from Septage: On-farm septage treatment systems employing acid hydrolysis followed by chemical precipitation have demonstrated substantial phosphorus recovery. Acid hydrolysis release 77% of phosphate from solids and subsequent precipitation recover 98.5% of the released phosphorus as fertilizer. The system produces an average of 290 g of phosphorus fertilizer per 100 L of raw septage. Field trials confirm that the recovered fertilizer achieved vegetable crop yields comparable to commercial products, validating its agronomic effectiveness (Pausta et al*.,* 2023).

Thermal Conversion Methods

Thermal conversion technologies transform organic waste into carbon rich materials and energy products through controlled heating processes. These approaches complement biological treatments by processing materials unsuitable for anaerobic digestion or by upgrading digestate into higher value products.

Pyrolysis**:** Pyrolysis involves thermal decomposition of biomass in the absence of oxygen, producing biochar, bio-oil and syngas. A multicriteria assessment system found that co-application of anaerobic digestion with pyrolysis reduced climate impacts compared to implementing either technology independently (Marttila et al*.,* 2024). Biochar derived from pyrolysis has demonstrated soil enhancement potential.

Hydrothermal Carbonization**:** Hydrothermal carbonization treats wet biomass in hot, pressurized water to produce hydrochar. Unlike pyrolysis, hydrothermal carbonization is particularly suited for high moisture feedstocks that would otherwise require energy intensive drying.

Thermal Conversion Method	Feedstock suitability	Main products	Product	Reference
Pyrolysis	Dry manures, litter, bones	Biochar, biooil, gas/ syngas	Produces soilamendment biochar and combustible oils; energy up to ~0.3 MWe/t at higher temperature	(Gómez et al., 2023)
Hydrothermal carbonization (HTC)	Wet manures (e.g., pig, dairy)	Hydrochar, biocrude	Handles high moisture; HTC chars from manure have high energy yield and lower ash	(Kiehbadroudinezhad et al., 2025)

Biological Conversion Approaches

Biological conversion methods utilize living organisms to transform agricultural residues into value added products with comparatively low energy inputs.

Composting and Vermicomposting: Composting aerobically stabilizes manure, conserving nutrients but emitting greenhouse gases and requiring long times (Shakya et al., 2022). Vermicomposting uses earthworms to convert manure into nutrientrich vermicompost with higher available N, P, K, and Ca than traditional compost; it also lowers C:N and can accelerate stabilization 2–5 times (Sorathiya et al*.,* 2014). Combined composting + vermicomposting improve shortterm stabilization and nutrient retention compared with either alone (Sorathiya et al., 2014).

***Green Manure and Crop Residue Recycling*:** Combining early season rice straw recycling with green manure may significantly reduce annual methane emissions compared to alternative straw management practices. Daba et al. (2026) reported thatit improves soil fertility and crop productivity while lowering greenhouse gas emissions

Insect Based Bioconversion: Black Soldier Fly larvae use is an emerging biological pathway for converting organic waste into high protein feed and soil amendments. An integrated Black Soldier Fly and quail farming model demonstrate effective waste reduction, decreased feed costs and improved soil quality within a closed loop agricultural system (Sinaga et al., 2024).

Challenges and Future Prospects

Despite the promising potential of innovative waste management technologies, their adoption is constrained by high initial investment, technical complexity, maintenance requirements and limited awareness among farmers. Variability in waste composition, regulatory barriers and insufficient infrastructure for nutrient recovery and bioenergy utilization further limit large scale implementation

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Mini Review

Stereotypic Behaviours in Domestic Animals as Indicators of Compromised Welfare

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Abstract

Animal welfare is the cornerstone of the veterinary practice. However, for the purposes of meeting increased consumer demands, animals are often reared under restricted housing conditions. These conditions, although, are aimed at improving the economics of animal rearing but they often neglect the holistic welfare of the animal which encompasses physical and psychological well-being of the animal. Animals under sub-optimal housing conditions suffer from stress due to deprivation of their expression of natural behaviour. Under chronic exposure to restricted conditions of feeding and housing, animals exhibit repetitive patterns of movements in a desperate attempt to cope up with the stress of unfulfilled natural behaviour. These movements serve no function and are consistent. Such behaviour is known as stereotypical behaviour. Stereotypical behaviour can either have oral or locomotor basis. Such chronic conditions of stress impact the physiology of the animal. This mini review discusses underlying neurophysiological causes of stereotypical behaviour in farm animals along with their therapeutic and preventive strategies to optimise stress level and productivity.

Keywords: *Animal welfare, Dopaminergic signaling, Environmental enrichment, Stereotypy, Stress*

A good welfare state of the animal should ensure five freedoms: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury or disease; freedom to express normal behaviour; freedom from fear and distress (Mellor, 2016). The concept of the welfare state of the animal is not just limited to the physical aspects of survival but also has mental underpinnings. The five domain model recognises the mental state, positive as well as negative, of the animal as the fifth domain for assessing the welfare of the animal. As, animals, too, are sentient species the cost of neglecting the animal welfare is formidable.

An optimum level of sensory stimulation coupled with adequate space for environmental exploration is essential for animal welfare. Under deprived conditions, animals become distressed and start exhibiting coping behaviours known as stereotypical behaviour (Radkowska et al., 2020).

Stereotypy or stereotypical behaviour is defined as the occurrence of repetitive fixed behaviours with no obvious purpose and such behaviours are generally seen in domesticated animals reared under intensive farming systems where spatial restriction causes distress.

Zoo animals also exhibit these stereotypical patterns, termed as zoochosis, which increase with the increase in the frequency of visitors (Sekar et al., 2008). Farm animals which exhibit stereotypies under sub optimal housing conditions often demonstrate poor growth and productivity (Priya et al., 2025). As the demands for animal products has increased over time, intensive farming systems with high volume production often neglect animal welfare. But, with increasing awareness of the consumers towards the rearing conditions of the animals, prioritizing animal welfare has become increasingly important for commercial purposes as well. Therefore, optimizing animal welfare and reducing stereotypy in animals becomes paramount in current scenario. This mini review discusses underlying neurophysiological causes of stereotypical behaviour in domestic animals along with their therapeutic and preventive strategies to optimise stress level and productivity.

Prevalence of Stereotypic Behaviours in Domestic Animals

Stereotypies are classified into 2 major types: oral and locomotor stereotypies (Table 1).

Table 1: Common stereotypic behaviours in domestic animals

Species	**Oral stereotypies**	**Locomotor stereotypies**
Horses	Crib biting, Wind Sucking, Stomping, Teeth rubbing, Wall licking, Flank biting	Weaving, Box walking, Fence Pacing, Pawing, Kicking
Ruminants	Tongue rolling, Bar biting, Sham Chewing, Inter-sucking, Self-sucking, Wool biting (sheep)	Weaving, Bar kicking, Pacing
Pigs	Nosing, Rooting, Sham chewing, Teeth grinding, Tail biting, Rooting	Head swaying , Pawing
Dogs	Flank sucking, Fly biting, Chewing on toys	Tail chasing, Circling, Shadow chasing
Poultry	Feather pecking, Feeder/wall Pecking, Beak wiping, Sham dust bathing	Pacing, Route chasing, Sham dustbathing

Crib biting is the most prevalent form of stereotypy in horses (Seabra et al., 2021). In an observational study, prevalence of tongue rolling was seen to occur in 29% of the cows with a higher incidence in Jersey cows as compared to the Jersey-Holstein crosses. Feed tossing behaviour in dairy cows was observed to be 37.8% (Sun et al., 2022). The combined prevalence of tongue rolling and licking of barn equipment in Jersey cows was observed to be 14.3% with less observable stereotypies in cows which had access to pasture (Demba & Rose, 2023). Under tethered conditions, 10% of the sows were found to possess sham chewing behaviour.

Determinants of Stereotypic Behaviours

The multifactorial etiology behind the causation of oral and locomotor stereotypic behaviours in animals is presented as insights into the stereotypic behaviour in animals for improved welfare in Fig. 1.

Determinants of Oral Stereotypic Behaviours

1. ***Restricted Feeding*****:** Restricted feeding is marked by reduction in feeding time and reduction in feeding frequency. With exposure of the animal to feed for a limited duration in restricted housing, animals experience a lower sense of satiety as compared to animals under loose housing system. The timing of feed also acts a determinant of expression of stereotypic behaviour. Generally these behaviours occur as a pre-feeding ritual or conditioned response in horses anticipating feed. The consumption of concentrate meal in portions spread throughout the day was found to decrease stereotypic behaviours in stabled horses (Cooper et al, 2005). Pigs fed ad libitum diet were found to be show less stereotypical behaviour as compared to those fed restricted diet.

2. ***Roughage Deficient Diets*****:** Research studies have also highlighted the role of roughage deficient diet in the development of oral stereotypic behaviours in animals. Rumination in ruminants is seen with high roughage containing forages while diets rich in concentrates decrease rumination. In natural settings, animals tend to graze for longer periods of time consuming enough roughage while under stall fed conditions, animals do not have freedom to explore their forage and are fed high concentrate diets. Consumption of concentrate feed also acts as a major factor in developing stereotypies as concentrate diets require less time for consumption and satiety associated with a sensation of gut fill is not realized. Lack of sufficient dietary fibre in the diet has been associated with increased levels of stereotypic behaviours in cattle, horses and pigs (Radkowska et al., 2020). With decrease in rumination, animals experience frustration and to relieve their stress, they resort to oral stereotypic behaviours. As such, feeding of roughage deficient diets in animals promotes the anomalous behaviours. This change in feeding pattern under restricted conditions deprives the animal of their natural behaviours and encourages the development of oral stereotypic behaviours.

3. ***Physiological State of the Pregnant Dam***: The physiological state of the pregnant dam also impacts the neurodevelopment of the foetus. Under stressful conditions, the

Fig. 1. Insights into stereotypic behaviour in animals for improved welfare (Open AI, 2026)

high level of cortisol in the pregnant animal leads to epigenetic changes in the limbic system of the foetus which influences the temperament of the offspring and predisposes the offspring to develop stereotypies (Tatemoto et al., 2020). Environmental enrichment of sows during gestation curbs physiological responses of stress and prevents aggressive stereotypic behaviours in the offspring later on.

4. ***Maternal Separation***: Early maternal separation leads to severe levels of stress in the young animals which start exhibiting certain stereotypic behaviours in order to self-soothe themselves. Self-sucking and self-clasping are common stereotypic behaviours expressed by young animals prematurely weaned by improper methods. These behaviours are strategies adopted by the animals to cope up with the stress resulting from separation. Thwarting the intrinsic calf motivation to suckle from their mother by premature or improper weaning becomes a cause of stereotypical tongue playing and sucking of pen objects. Early weaned kittens were found to have a higher stereotypical behaviour than their timely weaned counterparts (Ahola et al., 2017).

5. ***Genetic Predisposition***: Certain horse breeds have been observed to elicit a higher stereotypical behaviour as seen in thoroughbred horses which increases the likelihood of a genetic predisposition as a factor in the development of stereotypy in horses (Wickens & Brooks, 2020).

Determinants of Locomotor Stereotypic Behaviours

1. Confinement: Animals facing limitation of space in captivity show increased incidence of locomotor stereotypic behaviour. Under prolonged restricted housing conditions, movements of the animals are constrained. This lack of adequate space compromises the natural instincts of movements of animals and forces them to express atypical movements like box walking, pacing, wall kicking in horses, head bobbing, tail swishing to ease their sense of discomfort. Such movements turn into impulsive habits under chronic exposure to sub optimal environmental conditions. Lack of enrichment in captive house sparrows resulted in stereotypical beak wiping in a windshield like manner which was triggered due to anxiety to novel object and food exposure. Promiscuous and grazing species was found to have a higher prevalence of stereotypic behaviour in captivity.

2. Social Isolation: In addition to the confinement and spatial constraints of captive animals, another determinant of stereotypy is social isolation of animals. Typically, gregarious animals which reside in herds in nature, when isolated, show locomotor stereotypic behaviour. Isolation of animals from their companion animals leads to the decrease in visual and tactile stimulus. The lack of stimulation under deprived housing conditions exacerbates the frustration of the animal. This deprivation of companionship in animals affects their psychological well-being and causes them to show stereotypic behaviours. Self-mutilation in non-human primates is seen when isolation is undertaken for prolonged periods.

Physiological and Neurobiological basis of Stereotypic Behaviours in Animals

The displacement of the animal from its natural foraging tracts to restricted stall feeding enclosures with limited avenues for exploration and sensory stimulation is the key driver behind the development of oral and locomotor stereotypies. These unfulfilled natural proclivities in stereotypical animals are manifested at the physiological level in the animal body also as the concentrations of a variety of stress related metabolites and neurotransmitters are impacted. Isolation and restriction result in a sense of fear, stress and anxiety in animals. A biomarker of stress in animals is cortisol. Different studies have reported inconsistent associations between plasma cortisol levels and stereotypic behaviour across animal species with the recommendation of incorporating additional hormones (testosterone, oxytocin etc.) for addressing the role of cortisol in the development of stereotypical behaviour (Hildebrand & Zaleśny, 2025). ACTH challenge study has revealed that stereotypy acts a coping mechanism against stress stimuli as plasma cortisol levels were higher before stereotypy while lower levels were observed after the expression of stereotypy in crib biting horses (Freymond et al., 2015). Salivary cortisol levels were higher in non-stereotypic high competition thoroughbred horses than stereotypic horses (Bazzano et al., 2024). No difference in cortisol secretion and Hypothalamo-pituitary-adrenal reactivity was found in stereotypical and non-stereotypical calves (Webb et al., 2017).

Activation of dopaminergic signaling also plays a pivotal role in eliciting stereotypic behaviour by providing a sensation of reward and relief to the animal upon engaging in stereotypic behaviour. This sensation of reward compels the animal to repeat the stereotypical behavioural patterns. Dopamine levels in animals with stereotypies are often elevated while serotonin and brain derived neurotropic factor are found to be decreased in the affected animals. Endorphin production increases in animals with the expression of stereotypic behaviour which compels the animals to perform the stereotypies repeatedly in an attempt to decrease pain perception and physiological impact of stress experienced by the animal. As reported by many studies, increased dopamine levels activate the basal ganglia of the animal brain responsible for motor movements which leads to these increased and repetitive locomotor behaviours (Zhang et al, 2026). Cortico-striatal connectivity regulation is indispensable for fine tuning the locomotor movements (Simonyan, 2019) however, under stress, the presence of increased glutamatergic signaling prevents the inhibition of automatic habitual behaviours by the prefrontal cortex and leads to the excessive engagement of the sensorimotor striatal loop.

This loss of regulation by the pre-frontal cortex (Fig. 2) represents itself as a stereotyped phenotype in chronically stressed animals.

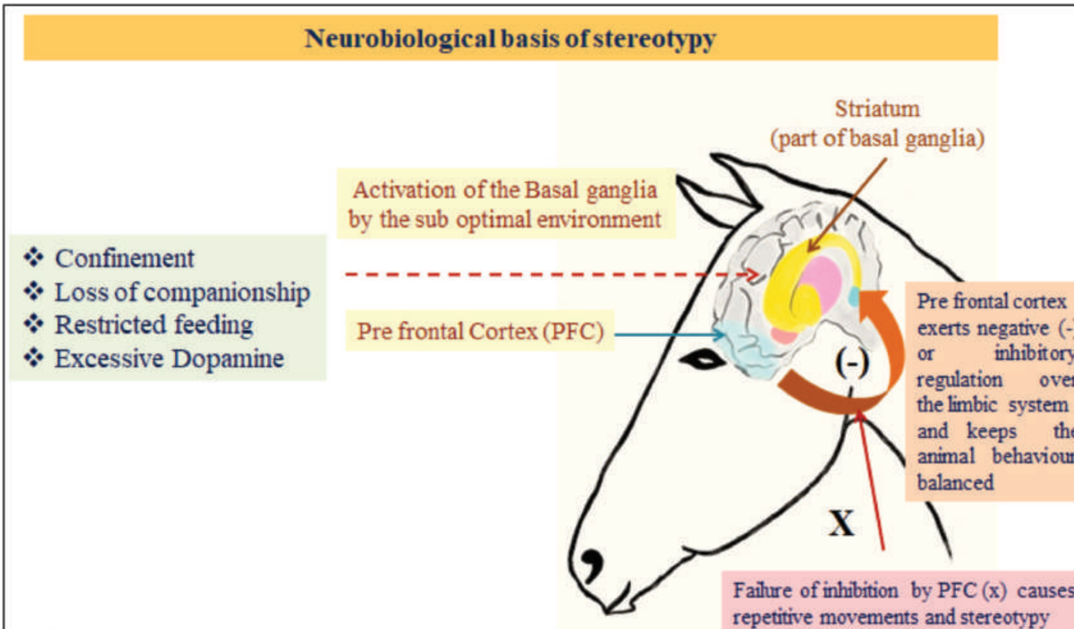

Figure 2: Loss of inhibitory control by the cortex of the animal due to action of excess dopamine leads to increased activity in animals and stereotypic behaviour

Impact of Stereotypic Behaviours on Animal Welfare

Depending upon the period for which the animal has been exhibiting stereotypies, the implications range from mildly concerning to severely detrimental. Oral stereotypies such as crib biting lead to the wear of the incisors of the horses and in severe cases, increase the risk of epiploic foramen entrapment and recurrent colic (Escalona et al., 2014). Crib biters were also observed to be more sensitive to tactile stimuli than the non-stereotypic horses (Freymond et al., 2019). Self-biting results in skin lesions, loss of animal aesthetics, increased frequency of infections and maggot wounds. Reports of head banging against the walls are present in cases of isolated animals which directly result in injury to the animals. Wall licking leads to ingestion of toxic paints causing gastrointestinal dysfunction. Kicking in horses thwarts their handling by the handlers thereby affecting their trainability and as such subsequent performance in sports. Tail biting in pigs causes lesions on tail and decreases their commercial value for consumption purposes (Taylor et al., 2010). Poor growth rate, decreased productivity and decreased reproductive rates have also been observed in stereotypical animals.

These behaviours, if left unchecked during initial phases of development, can transform into impulsive habits which fail to respond to positive changes in the environment or other treatment modalities later on, thereby, significantly, compromising the welfare of the animal in the long term.

Prevention and Treatment Strategies

Considering the neurological basis of the development of stereotypies and lack of sufficient neural (cortical) regulation to prevent them, stereotypies are surprisingly hard to treat once hard wired in the animal brain. So, efforts to prevent them from being ingrained in the animal brain should be undertaken. Also, as consumers are preferring products of animal origin with animal welfare certifications, from a commercial viewpoint as well, animal welfare has to be prioritized during animal rearing and steps for prevention of stereotypies must be followed.

Prevention is aimed at reducing the frequency of stereotypical behaviours as complete cessation is often hard to realize with their implementation. Physical, pharmacological and environmental methods have been adopted worldwide to curb stereotypical behaviours in animals with variable outcomes.

1. Environmental Enrichment: The most promising method for reducing stereotypical behaviour in animals is environmental enrichment (Mason et al., 2007). Animals with enriched environmental exposure rarely exhibit stereotypical behaviour and it has been observed that exposure to free range with increase in feeding time and company of companion animals drastically reduces the expression of such abnormal behaviours. The tactile, olfactory, visual and kinetic sensory needs are sufficiently fulfilled in such environments and animals noticeably abandon such stereotypical activities when housed loosely over pastures. Provision of rooting substrates such as straw inside pig enclosures helps in keeping the stereotypy under check. High stocking density as well as complete isolation of the animal, unless warranted, should be avoided. For poultry, dustbathing substrates should be provided to help them perform this vital ritual for their feather health. As the outlook of the captive environment gets closer to the natural environment with increased space for sufficient locomotor activities of the animal, the animal welfare is optimized and maladaptive coping behaviours are reduced.

2. Dietary Management: Efforts to increase the time budget of the animals towards feeding like incorporation of roughage in diets, use of small holed hay nets in stables also improve the outcomes significantly and animals appear less stressed with reduced exhibition of oral stereotypical behaviour. Fibre rich diet should be fed to increase the time budget of feeding to atleast 12 -18 hours per day for horses. Incorporation of tryptophan rich protein should be undertaken in the horse feed to potentiate the production of serotonin which acts to reduce the stereotypic behaviour (Marsden, 2002). In many studies, supplementation of the concentrate diet with hay reduced the incidence of non-nutritive oral behaviour in cattle (Li & Gu, 2024).

3. Physical Methods: Physical methods reduce the behavioural expression of stereotypies by discouraging the opportunities for indulging in purposeless acitivities. Use of V-shaped anti-weaving grills in horses restricts the horse from exhibiting the swaying motion of its head. Installation of stall mirrors in stables helps in reducing stereotypies in horses by providing the illusion of companionship. Use of neck collars for crib biters and E-collars in small animals to prevent self-mutilation helps in reducing the incidence of the behaviour for a limited time period but as long as the causal factor behind the stress is not treated, the animals often relapse into the stereotypical behaviour once the collar is removed. As these methods do not address the underlying cause of the behaviour as such their usage for the treatment is debated.

4. Pharmacological Treatment: Pharmacological treatment of stereotypy is based on targeting the dysfunctional neurotransmitters in the limbic system and improving the signal transmission in the cortico- striatal-thalamo-cortical loop in the brain to optimize motor movements and prevent them from becoming automatic. As dopamine increases the stereotypical behaviour in animals, injection of dopamine neurotoxin (5-hydroxydopamine) into the striatum region of basal ganglia helped in alleviating stereotypical behaviours in rats. Opioid antagonists like naloxone (McBride & Cuddeford, 2001) also have been documented to offer relief to stereotypical animals with moderate success rates. Administration of serotonergic drugs was found to decrease the stereotypical activities in animals. Also certain studies in ruminants have shown the role of low ruminal pH in the development of oral stereotypies and hence, antacids have also been used to decrease such behaviours (Li & Gu, 2024).

For a significant improvement of the animal welfare, it is imperative that the understanding of the root cause of the stereotypy should be prioritized and a holistic strategy for stereotypy for promoting the animal relief should be undertaken rather than the adoption of symptomatic treatment strategies which suffer from severe rebound effect. The internationally recognized “Five Freedoms” framework (freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury or disease; freedom to express normal behaviour; freedom from fear and distress) must be adhered to ensure complete animal welfare (Mellor et al., 2016; Priya et al., 2025).

Research Gaps and Future Directions

The current research on stereotypy in animals stresses upon the environmental enrichment of the captive animals to reduce the stereotypic behaviour. Although, enrichment reduces the severity of stereotypy in animals, it does not abolish it altogether. This point at a gaping research gap in this area and requires the scientific community to investigate the causes behind failure of the animals to successfully recover from such behaviour even when the underlying stressor or the sub optimal condition has been corrected. As such, whether a particular type of stereotypy is amenable to response and whether stereotypical behaviour can be permanently reversed will also warrant further research. The neurobiology behind the refractory cases of stereotypy which fail to respond to pharmacological intervention and to environmental stimulation needs to be explored further. The feedback reward system in such persistent and self-reinforcing cases of stereotypy must be targeted for future research as it can open the gateway to understand the lasting impact of neglect on the limbic system of the animal brain.

Another research aspect which demands scientific attention is the time of onset of stereotypy in animals and the critical window of prevention. Current studies fail to provide an actionable timeline for the prevention of stereotypy in animals. The development of hardwired neurological circuits in animals occurs gradually and the tipping point past which the reversion is deemed less probable, generally seen with ongoing chronic stress, is ill defined. As such, therapies often fail because neuronal wiring becomes inflexible. So research into the neurophysiology of stereotypy development can help in establishing these preventive timeframes.

The lack of a behavioural diagnostic manual for the recognition of stereotypy in animals acts as a deterrent to its effective prevention. With different animals showing stereotypy under different conditions, the scientific consensus on recognizing the condition is hard to establish and researchers must team up to formulate a behavioural manual to elucidate the severity of the stereotypies as a guide for the breeders for undertaking timely preventive measures. Thrust can also be provided to the identification of specific biochemical markers to help in distinguishing between responsive and non-responsive cases of stereotypy. A standard baseline for certain biomarkers should also be agreed upon to facilitate the treatment decisions, either pharmacological or environmental enrichment, to improve the animal welfare. Additionally, whether these stereotypies in animals mirror “addictions” or “tics” as seen in humans holds promise in drawing an interesting parallel with widespread implications in human medicine as well.

Lastly, the quintessential debate whether the stereotypical behaviours are coping in nature or whether they are the explicit signs and signals of distressed animals which serve no coping function still stands unanswered and needs to be examined with a fine scientific lens for arriving at the perfect perspective to approach this behavioural malfunction.

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Mini Review

Horse Parentage: A Boon to Stud Farmers

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Abstract

Accurate parentage verification is essential for effective equine breeding and conservation of indigenous genetic resources. In India, traditional reliance on phenotypic traits for parentage identification often leads to errors, compromising pedigree accuracy and herd quality. This article highlights the role of DNA based parentage testing as a reliable tool for confirming lineage in horses. Modern molecular approaches, including microsatellite markers and single nucleotide polymorphism platforms, enable highly accurate parentage assignment and support scientific breeding decisions. The need for such approaches is particularly important in indigenous horse populations of Punjab, such as Nukra and Majhuke, where indiscriminate crossbreeding and poor record keeping have contributed to genetic dilution and loss of adaptive traits. Implementation of DNA based verification helps reduce inbreeding, improve selection of superior animals, and enhance market value through verified pedigrees. Institutional support from research organizations and government programs further facilitates adoption of these technologies at the field level. However, challenges such as limited awareness, high testing costs, and lack of local facilities restrict widespread use. Addressing these constraints through training, infrastructure development, and policy support will be critical for integrating molecular tools into routine breeding practices and ensuring long term conservation and improvement of indigenous equine populations in India.

Keywords: *Equine parentage verification, Genetic conservation, Indigenous horse breeds, Microsatellite markers, SNP genotyping*

Horses have played an important role in human society for centuries, particularly in agriculture, transport, and cultural traditions. In India, horses are not just animals but are considered family members. Owning a healthy and well-bred horse in states like Punjab, Haryana, Rajasthan, and Gujarat is considered both a source of pride and an important source of income to the owner. Traditionally, breeders relied on phenotypic traits viz. coat color, gait, and body conformation, to select breeding stock. However, these visual assessments are prone to error and can result in incorrect parentage assignments. Such inaccuracies often lead to legal or administrative disputes among owners (International Society for Animal Genetics, 2021).

In recent times, DNA-based parentage verification is a widely accepted technique to confirm the potential parents of the offspring as it offers a practical and reliable solution. By using samples such as hair follicles, blood, or semen samples, the true sire and dam of an offspring can be easily determined with high accuracy when appropriate genetic markers are used (Kim et al., 2025). This helps farmers plan matings more carefully and preserve indigenous breeds. Adopting DNA based parentage verification improves the market value of equine herds. It ensures accurate pedigree records, which is important for indigenous lineages such as the Nukra and Majhuke. In Punjab, the Nukra horse is known for its white coat and ceremonial importance. Its population is estimated to be around 5000 to 7000 animals, mainly in the Malwa region, including Sri Muktsar Sahib, Bathinda, and Mansa. In contrast, the Majhuke line is rarer, with fewer than 1000 pure line individuals, mostly found in Faridkot and Ferozepur (Nolte et al., 2022).

Why Parentage Verification Matters

Horses inherit their unique characteristics such as stamina, strength, temperament, and overall performance from their parents (Petersen et al., 2013). When the true biological parents of a foal are known, it ensures that these valuable traits are preserved and not lost through random or unplanned breeding. Parentage determination also avoids ownership disputes and helps to build strong trust between buyers and the farmers. For native breeds such as Majuke and Nukra it is very important to maintain accurate parentage records to protect the identity of the breed. A scientific breeding plan can be figured out once the parentage of the breeding animals is verified. Consequently, traits like fertility, performance, and appearance could be improved over generations (Nolte et al., 2022). Moreover, a certified parentage document is useful for registering horses in studbooks, participating in local fairs, and accessing government schemes that recognize and support genuine breeders.

Preserving Indigenous Breeds

The ICAR-National Bureau of Animal Genetic Resources has registered eight indigenous horse and pony breeds in India as of 2026, including the recently recognized Bhimthadi. These breeds are adapted to a wide range of agro-climatic conditions, from the high-altitude Himalayan regions to the arid plains of western India. Major breeds include the Marwari and Kathiawari, known for their endurance and distinctive physical traits, and the Kachchhi-Sindhi, which is well suited for desert conditions. Himalayan breeds such as Spiti, Zanskari, and Bhutia are adapted to cold, rugged terrains, while the Manipuri pony is historically associated with polo and valued for its stamina. The Bhimthadi breed, recently registered, is traditionally used for transport by pastoral communities in Maharashtra. Together, these breeds represent important genetic resources with region-specific adaptations and cultural significance (ICAR-National Bureau of Animal Genetic Resources, 2026)

The horse population in Punjab has shown notable changes over time. According to the 20th Livestock Census, the total horse and pony population in the state was approximately 17,046 in 2019, including 16,683 horses and 363 ponies (Department of Animal Husbandry and Dairying, 2019). Recent findings from the 21st Livestock Census (2024–2025) indicate a recovery in population, with an increase of approximately 5,720 horses compared to previous estimates (Department of Animal Husbandry and Dairying, 2024). Indigenous breeds such as the Marwari and Nukra are commonly found in the region. District-level data indicate that areas such as Mohali and Amritsar have relatively higher horse populations. Earlier reports suggested a decline in horse numbers between 2012 and 2019; however, recent trends indicate renewed interest in horse rearing for cultural, recreational, and economic purposes.

However, the population of Nukra and Majhuke breeds are declining due to indiscriminate crossbreeding and poor record keeping, as these native lineages are often crossed with exotic breeds. The English Thoroughbred is commonly used to increase height, while the Arabian horse is used for aesthetic traits. These practices have reduced important characteristics such as disease resistance and traditional temperament (Tozaki et al., 2021). DNA based parentage testing offers a reliable solution. It helps confirm lineage and supports the registration of purebred animals. This allows breeders to select superior individuals more accurately (Lee et al., 2023). As a result, it can help improve population quality and preserve the genetic resources of Punjab's indigenous horses.

Practical Benefits for Farmers

DNA based parentage verification has clear benefits for equine management. It helps reduce the risk of inbreeding. This is especially important in small or closed populations such as the Nukra and Majhuke. In such populations, mating between closely related animals often goes unnoticed. This can create genetic bottlenecks (Asti et al., 2025). As a result, fertility may decrease, and the risk of congenital defects can increase. This leads to an overall decline in genetic fitness (Hill et al., 2022). By confirming parentage, breeders can plan mating more carefully. They can avoid close inbreeding and maintain genetic diversity (Kim et al., 2025). This helps preserve important traits such as heat tolerance and disease resistance. Verified pedigrees also improve the market value of horses. This is particularly true when the sire has a strong performance record (Hill et al., 2022). Proper documentation supports participation in horse shows and access to government schemes. It also replaces unreliable oral records with accurate genetic data (International Society for Animal Genetics, 2021). In the long term, this approach can reduce veterinary costs and improve herd management. It supports a more stable and economically viable horse population for farmers in Punjab.

How DNA-based Parentage Testing Works

Equine parentage verification has evolved from traditional blood typing to modern molecular methods. These approaches use Mendelian inheritance of genetic markers to confirm or exclude parentage with high accuracy, often exceeding a probability of exclusion of 99.99% (Van de Goor et al., 2021). Early methods included cytogenetic techniques such as karyotyping, which examined the full set of chromosomes to detect large abnormalities. Later, fluorescence in situ hybridization improved resolution by using labeled DNA probes to identify specific chromosomal regions (Raudsepp & Chowdhary, 2016). However, the current standard method is microsatellite analysis using short tandem repeats, as recommended by the International Society for Animal Genetics (ISAG). In this approach, DNA is extracted from hair roots or blood samples, and selected STR loci are amplified using PCR. The amplified fragments are then separated by capillary electrophoresis, and allele patterns are compared to confirm or exclude parentage (Nowak et al., 2021).

More recently, there has been a shift toward SNP based methods and high throughput genomic platforms. SNP arrays can analyze thousands of markers at once, while next generation sequencing methods such as genotyping by sequencing and whole genome sequencing provide more detailed information on genetic variation. These approaches offer higher resolution but require advanced data analysis. In addition, mitochondrial DNA analysis is used to trace maternal lineages, as it is inherited only from the mother (Ahlawat et al., 2025). This is particularly useful in indigenous horse populations where maternal lineage plays an important role.

The International Society for Animal Genetics has standardized a core panel of microsatellite (STR) markers for equine parentage verification to ensure uniformity and comparability of results across laboratories worldwide. These markers are selected based on high polymorphism, reproducibility, and strong power of exclusion. The commonly used ISAG panel includes a set of loci distributed across different chromosomes, minimizing linkage and maximizing discrimination power. Details of these loci, including chromosomal location, repeat structure, and repeat sequence, are presented in Table 1.

Steps for Proper Parentage Management

Parentage testing works even better when farmers keep small, basic records and follow simple breeding steps. It is recommended for every stud farmer to maintain detailed records for each horse, including breed, colour, identification marks, mating, foaling dates, and parentage information, etc. These simple yet effective practices help make breeding more transparent and systematic. Controlled matings are essential as they avoid accidental matings and, if done, are supervised with proper documentation, which further prevents disputes and ensures that each foal's parentage is clear.

Table 1. ISAG-recommended microsatellite (STR) markers used for equine parentage verification

SN	Locus	Chrom. Location	Repeat structure	Repeat sequence
1	AHT4	24q14	Compound	(AC)nAT(AC)n
2	AHT5	8	Simple	(GT)n
3	ASB2	15q21.3-q23	Simple	(GT)n
4	ASB17	2p14-p15	Simple	(AC)n
5	ASB23	3q22.1-q22.3	Simple and Compound	(TG)n and (TG)nTT(TG)4
6	CA425	28q18	Simple	(GT)n
7	HMS1	15	Simple	(TG)n
8	HMS2	10	Compound	(CA)n(TC)2
9	HMS3	9	Compound	(TG)2(CA)2TC(CA)n and (TG)2(CA)2TC(CA)nGA(CA)5
10	HMS6	4	Simple	(GT)n
11	HMS7	1q25	Compound	(AC)2(CA)n
12	HTG4	9	Complex	(TG)nAT(AG)5AAG(GA)5ACAG (AGGG)3
13	HTG6	15q26-q27	Simple	(TG)n
14	HTG7	4	Simple	(GT)n
15	HTG10	21	Simple and Compound	(TG)n and TATC(TG)n
16	LEX3	Xq	Simple	(TG)n
17	VHL20	30	Simple	(TG)n

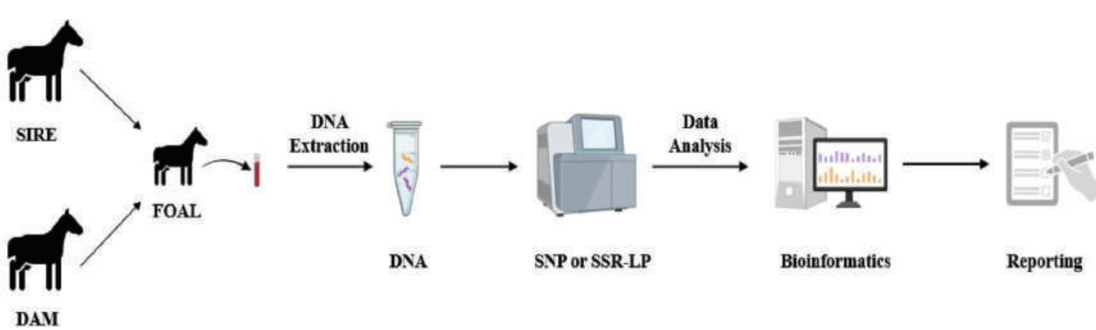

Fig. 1: Workflow of DNA-based parentage verification in horses

To connect genomic research with field use, horse breeders need proper training and support. In India, institutions such as Guru Angad Dev Veterinary and Animal Sciences University and ICAR-National Research Centre on Equines conduct training programs for this purpose. These programs teach practical skills such as sample collection from hair follicles for DNA analysis, proper pedigree recording, and basic disease management practices. Several organizations also work to increase awareness about molecular parentage verification. The International Society for Animal Genetics provides standard genetic marker panels that are used worldwide for parentage testing. In India, groups such as Indigenous Horse Society of India and the Marwari Horse Society promote breed standards and proper registration systems. They encourage breeders to move from traditional oral records to DNA based verification. Verified pedigree records are often required for participation in horse shows and government conservation programs. Combining these scientific methods with traditional management practices can help maintain the genetic quality and uniqueness of indigenous horse populations in Punjab (Leroy et al., 2018).

Support from Government and Institutions

Equine breeding in India is supported by structured research programs and institutions. One major initiative is the Network Project on Equine Genetic Resources, coordinated by the ICAR-National Research Centre on Equines. This project works with State Agricultural Universities such as Guru Angad Dev Veterinary and Animal Sciences University. Together, they focus on conserving indigenous breeds like the Nukra, Marwari, and Spiti, both in field conditions and in controlled settings. These programs include molecular characterisation and the development of nucleus breeding herds. The aim is to produce genetically superior stallions for use by farmers. At the global level, organizations such as the World Breeding Federation for Sport Horses and the Haras Nationaux use centralized data systems to evaluate breeding animals. They rank sires based on estimated breeding values, which helps in selecting better animals. In India, government schemes such as the National Livestock Mission support this approach. These programs provide incentives such as subsidized DNA testing. This encourages breeders to adopt objective and data based selection methods. Participation in these programs helps farmers obtain certified animals and access conservation support. It also supports the long term genetic improvement and economic sustainability of indigenous horse populations in Punjab.

Challenges and the Way Forward

There are numerous benefits of verified lineage, but there is still a need for widespread adoption of parentage verification. Many farmers are unaware of this process and its associated advantages. Also, the laboratories conducting horse parentage are not available locally and the test itself is expensive. Addressing these issues needs a combination of awareness campaigns, hands-on training, and affordable testing. With proper guidance, support, and farmer participation, DNA-based parentage verification can help preserve indigenous breeds. This will also enhance the herd performance and modernize stud farming into a profitable and sustainable business.

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Clinical Article

Successful Management of Dystocia due to Breech Presentation in a Marwari Mare

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Abstract

Dystocia is one of the major reproductive challenges in equine practice. This report describes a case of dystocia due to breech presentation in a nulliparous Marwari mare aged 4 years and weighing 450 Kg near complete gestation (320 days). The mare was presented with a history of prolonged straining, vaginal discharge with foul smell, and mild systemic compromise. Laboratory analyses revealed mild leukopenia. Per-vaginal examination revealed the fetus in posterior presentation with lumbo-sacral position and bilateral hip flexion. The condition was corrected manually following epidural anaesthesia and enough lubrication and thereafter controlled traction to deliver the dead foal. Postpartum management included antimicrobial therapy, fluid therapy, oxytocin administration, and supportive care with good quality forage. The case highlights the importance of prompt obstetrical intervention and appropriate post-delivery management to minimize potential life risk of mare.

Keywords: *Breech-presentation, Dystocia, Mare, Obstetrics*

Dystocia in mares is rare but serious obstetrical emergency that frequently results in fetal and maternal morbidity and mortality (Lu et al., 2006). Irrespective of the breed, the incidence of dystocia ranges between 2-13% in mares (Ellerbrock & Wehrend, 2023). Foaling in mares is generally a rapid process, with the second stage of labor (expulsion of the fetus) typically completed within 20-30 minutes after rupture of the chorioallantoic membrane (Frazer, 2011). Dystocia in mare due to fetal origin is mainly caused by abnormal fetal posture, such as improper alignment of limbs, head, or neck (Thangamani et al., 2018) rather than maternal factors. One of the uncommon and difficult types of fetal maldisposition in mares is breech presentation, which is defined by the presentation of the posterior portion of the fetus, lumbo-sacral position with both hind limbs flexed beneath the body.

The indigenous Marwari breed is valued for its adaptability and endurance. Due to its high economic value, timely and appropriate obstetrical intervention is essential to ensure the safety of both mare and foal (Ruthrakumar et al., 2023). The present case report presents the successful management of dystocia due to a breech presentation in a Marwari mare through controlled obstetrical manoeuvring to deliver dead fetus.

Case History and Observations

A nulliparous Marwari mare aged 4 years and weighing 450 Kg, near complete gestation period (320 days) was presented with a 24-hour history of continuous straining, restlessness, and the presence of bloody, yellowish, foul-smelling vaginal discharge. Feed and water intake were markedly reduced. Clinical examination revealed congested mucous membranes, profuse sweating in the abdominal and hindquarter regions, and mild dehydration. The rectal temperature, heart and respiratory rates were 100.2°F, 60 beats/min, and 24 breaths/min, respectively.

Hematological analysis revealed mild leukopenia (TLC 5,420 cells/µL) with neutropenia (38%) and relative lymphocytosis (62%) indicating an ongoing systemic inflammatory response along with normal hemoglobin 12.3 g% and PCV 33.6% (MSD Vet Manual, 2016). Following aseptic preparation and restraint using hobbles, the tail was wrapped and the perineal area cleaned using chlorohexidine solution. Per-vaginal examination revealed adequately dilated cervix (full-hand), foul-smelling discharge (reddish brown), with the tail and buttocks of the fetus palpable in the pelvic inlet, while both hind limbs were flexed beneath the fetal body, confirming a breech presentation (posterior presentation, lumbo-sacral position, both hind limbs retained under body). The fetal reflexes were absent, indicating fetal death. Based on these findings, the case was diagnosed as dystocia due to postural defect.

Treatment and Discussion

The mare was restrained in standing position in equine travis with administration of epidural anesthesia (3 ml of 2% lignocaine hydrogen chloride) at the first inter-coccygeal space under aseptic preparations. The uterus was adequately lubricated with 2% sterile sodium carboxymethyl cellulose gel (Kumar et al., 2022) to facilitate manipulation. Manual correction was attempted by repulsion of the fetal body cranially into the uterus followed by sequential extension of the hind limbs. The hock and fetlock joints were subjected to careful repulsion and extension to bring one limb at a time into the birth canal. After correction, both limbs were secured with Moore's obstetrical chains, and gentle, coordinated traction was applied to deliver the underdeveloped dead female fetus per vaginum (Fig. 1) without any further complication. The placenta was expelled within 30 minutes after administration of ecbolics (Fig 2).

The mare was administered therapeutic protocol consisting of, oxytocin @ 50 IU intravenously in 1 L of normal saline over 45–60 minutes, tetanus toxoid @ 5 mL intramuscularly, ceftiofur sodium @ 1.1 mg/kg body weight intramuscularly (o.d. for 5 days), flunixin meglumine @ 1.1 mg/kg body weight intramuscularly (o.d. for 3 days),

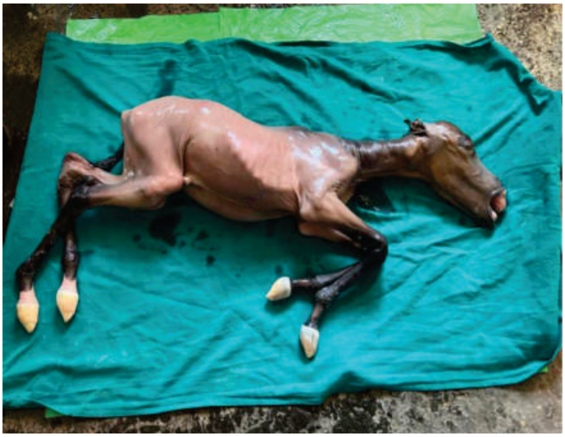

Fig. 1. Underdeveloped dead foal

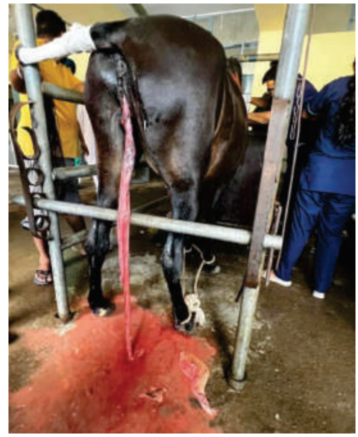

Fig. 2. Placental membranes and vaginal discharge after handling of dystocia. Note the hobbles and wrapped tail.

normal saline infusion @ 40 mL/kg body weight intravenously, ringer's lactate infusion @ 20 mL/kg body weight intravenously, metronidazole @ 10 mL/kg body weight slow intravenous infusion to manage dehydration, prevent systemic infection, and support recovery. The mare recovered successfully and no postpartum complications were observed during follow-up period. The mare was re-examined after 30 days and no signs of metritis or systemic illness were observed and no further blood examination were performed. The owner was advised to allow the mare a total rest period of 2 months after dystocia before the next covering.

Dystocia in mares is an uncommon but potentially life-threatening condition for mare and foal survival and thus requiring immediate veterinary attention. The main predisposing factor in is incomplete rotation of the fetus before foaling, leading to retention of hind limbs beneath the fetal body (Noakes et al., 2019). Such malpostures prevent normal passage of the fetus through the birth canal and often necessitate manual correction under epidural anesthesia to minimize pain and straining. The use of epidural anaesthesia and adequate lubrication were crucial to prevent injury to the soft tissues of the reproductive tract and to facilitate manipulation (Knottenbelt, 2019). Management aimed to correct the posture through controlled repulsion and sequential extension of the hind limbs a technique supported by Noakes et al. (2019) as the most reliable approach for breech dystocia in mares.

Postpartum administration of oxytocin and antibiotics ensured uterine clearance and prevented secondary infections. Favourable factors for successful vaginal delivery in this case included adequate cervical dilation, effective relaxation of the birth canal through epidural anaesthesia, and sufficient lubrication, which facilitated safe obstetrical manipulation. Timely and skilled intervention, along with the absence of significant fetopelvic disproportion, further supported successful delivery. The likely cause of fetal death was prolonged dystocia leading to hypoxia, aggravated by umbilical cord compression in the breech presentation. In such cases, early intervention or delivery may be necessary to address fetal distress and to prevent serious maternal complications, prioritizing the survival and future reproductive health of the mare. While fetal death was unavoidable because of the late presentation, early and careful intervention ensured the complete recovery of the dam, similar to findings by Gupta et al. (2018) in equine dystocia cases.

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Clinical Article

Dystocia Associated with Feto-Maternal Disproportion in Swine

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Abstract

Dystocia is one of the rare reproductive challenges in swine practice. This report describes a case of dystocia due to feto-maternal disproportion in swine with outcome of stillborns and mummified fetus. The sow was presented after delivery of one fetus with vaginal discharge and continuous straining from last 24 hours. On per-vaginal examination, the fetal head failed to pass through the pelvic inlet despite traction. The physiological parameters were in normal ranges except with mild dehydration. After stabilization, an emergency caesarean section was performed which is the treatment of choice in the cases of prolonged dystocia due to feto-maternal disproportion. The case emphasizes the significance of timely obstetrical intervention and suitable surgical management to reduce the risk of swine mortality.

Keywords: *Dystocia, Feto-maternal disproportion, Mummification, Stillbirth, Swine*

Normal farrowing typically lasts 1-5 hours in sow, with an average inter-piglet interval of approximately 15 minutes. Dystocia in swine is rare with an estimated prevalence of about 1% (Cowart, 2007). Dystocia in swine is defined as the failure to deliver piglets within 2 hours of the onset of farrowing or when the interval between successive piglets exceeds 45 minutes (Ward et al., 2019). Reported causes of dystocia in swine include uterine inertia, obstruction, fetal malposition, feto-maternal disproportion, constipation, obesity, and iatrogenic edema due to intrapelvic manipulation (Nam & Sukon, 2021; Singh et al., 2025). Cesarean section is rarely performed in swine but may be indicated for the resolution of dystocia, particularly in cases of pelvic obstruction caused by feto-maternal disproportion (Sangwan et al., 2025). This clinical study reports the prompt obstetrical intervention and appropriate surgical care to lower the risk of swine mortality.

Case History and Observations

A Landrace sow (2 years old, second parity) was presented with a history of delivering one fetus and thereafter exhibiting signs of continuous straining, restlessness, and vaginal discharge from the past 24 hours. Feed and water intake had been markedly reduced. Clinical examination revealed congested mucous membranes and vulvar swelling. The rectal temperature, heart rate, and respiratory rate were recorded as 102.2°F, 110 bpm, and 40 breaths/min, respectively. Lateral radiographic view demonstrated the presence of atleast five fetal skeletons of variable sizes, within the uterus. With ample lubrication using sodium carboxy-methyl cellulose gel the per vaginal examination revealed that only the fetal snout had entered the pelvic cavity, while the head diameter exceeded the pelvic diameter which meant less space for passage, indicating feto-maternal disproportion with no limbs palpable in the vaginal passage. The fetal reflexes were absent, indicating fetal death.

Hematological analysis of the sow revealed PCV (36%), hemoglobin (11.9 g/dL), platelet count ($340 \times 10^3/\mu L$) and erythrocyte count ($6.0 \times 10^6/\mu L$) were within normal limits. Total leukocyte count was elevated ($18.6 \times 10^3/\mu L$) with neutrophilia (78%; $14.5 \times 10^3/\mu L$) and lymphopenia (15%; $2.8 \times 10^3/\mu L$), consistent with a stress leukogram.

Treatment and Discussion

On the basis of diagnostic evaluation, cesarean section was undertaken with the consent of the owner to deliver the fetuses. The animal was restrained in right lateral recumbancy and aseptically prepared for surgery by clipping the flank region, securing an intravenous cannula in the ear vein, and initiating fluid therapy along with broad-spectrum antibiotic coverage using ceftiofur crystalline free acid at @5 mg/kg intramuscularly. A combination of ketamine (5 mg/kg) and midazolam (0.5 mg/kg) was given intramuscularly for induction/deep sedation (Linkenhoker et al., 2010). The maintenance of anesthesia was done using inj. Propofol as a continuous rate infusion in normal saline solution @ 0.2mg/Kg/min (Sangwan et al., 2025). Following stabilization of vital parameters, a regional linear infiltration was achieved using 2% lignocaine (40 ml) at the incision site in different layers. After aseptic preparation of the surgical site, a lower left flank incision (Lozier et al., 2021) was made, and the uterus was gently exteriorized. A uterine incision was created on the uterine body on dorsal side, and the fetuses were carefully extracted, including one fetus with overall large body size causing dystocia and one mummified fetus from the right uterine horn, along with three stillborn fetuses (Fig. 1) from the left horn. The uterine incision was closed using an inverting suture pattern, followed by routine closure of the muscle layers and skin.

The sow was administered with the therapeutic protocol consisting of oxytocin @ 30 IU intravenously in 500 ml of normal saline over 45–60 minutes, ceftiofur crystalline free acid @ 5 mg/kg IM (5 days), flunixin meglumine @ 2.2 mg/kg IM (3 days), ringer's lactate infusion @ 10 mL/kg in IV infusion. The sow was monitored for feed intake. Additionally, antimicrobial and fly repellent spray with regular dressing and restricted movement in clean single pen was advised. After the incision site had fully healed for 14 days, the skin sutures were removed. The sow was re-examined after a gap of 30 days

Fig 1. One mummified fetus, one oversized fetus and three stillborn fetuses

and was found to be recovered completely.In the present case, per-vaginal examination confirmed dystocia due to feto-maternal disproportion, necessitating cesarean section, which is a rarely conducted surgery in sows but remains the treatment of choice in cases of pelvic obstruction (Lozier et al., 2021). Marked size variation among fetuses within right uterine horn may suggest competitive intrauterine conditions, where reduced nutrient supply to one fetus (mummified) may have favoured the continual growth of the co-twin (larger fetus causing dystocia). Fetal deaths likely due to uterine contractions, overcrowding and compromised placental perfusion resulted in stillbirths (Lefebvre, 2015). In swine, stillbirth is a major contributor to reproductive inefficiency. The reported incidence of stillbirth in pigs generally ranges between 5-10% (Leenhouwers et al., 1999*)*. Fetal mummification typically occurs after 35-40 days of gestation, coinciding with fetal ossification, beyond which fetal resorption is no longer possible and mummification ensues. A prevalence of 3.45% mummification had been reported in Brazilian swine herds. (Borges et al., 2005).

The clinical importance of early diagnosis and fast surgical intervention in controlling complication secondary to incidence of dystocia during farrowing is explained in the current case. If feto-maternal disproportion is present, there is a significant risk of death to other fetuses from hypoxia resulting into stillbirths. Hypoxic injury during parturition, caused by umbilical cord compromise and placental compression during repeated uterine contractions, is a principal mechanism leading to piglet stillbirth. (Leenhouwers et al.,1999*)*. Though the case was delayed, prompt diagnosis and immediate surgical intervention along with perioperative stabilization and rigorous postoperative care has saved the life of the sow.

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Clinical Article

Rhinoplasty and Staphylectomy for the Correction of Brachycephalic Obstructive Airway Syndrome in Dogs

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Abstract

Two brachycephalic dogs; a 3-year-old 'Pug' and a 2-year-old 'French Bulldog' were presented with the complaint of respiratory distress and stridor. On physical examination, stenotic nares and elongated soft palate was diagnosed in French bulldog; however, the Pug seem to suffer from elongated soft palate only. Surgical correction was done for the presented components of Brachycephalic Obstructive Airway Syndrome (BOAS). Stenotic nostrils were reconstructed through Rhinoplasty while staphylectomy was performed for elongated soft palate. The reported respiratory distress was relieved immediately in both the cases and they recovered uneventfully. This clinical study describes surgical management of above mentioned two components of the BOAS in dogs.

Keywords: *Airway, Brachycephalic, Elongated soft palate, Stenotic nostrils, Surgery*

Brachycephalic dog breeds such as Pugs, Shih Tzu, French Mastiff, Chow-Chow, Pekingese, Maltese, French and English bull dogs etc have been popular amongst pet lovers for a few years. These breeds have congenitally flattened facial base of skull bones. These defects inadvertently lead to poorly developed nares (stenotic nostrils) and distorted nasopharynx (Fawcett et al., 2018). Some other developmental abnormalities like elongated soft palate, everted laryngeal saccule, hypoplastic trachea, were also observed in the brachycephalic dog breeds. Hence, this developmental condition is known as 'Brachycephalic Obstructive Airway Syndrome (BOAS)' as it involves multiple components and one or more component may be present in a single case.

This syndrome majorly affects upper respiratory tract, therefore the clinical signs include varying levels of respiratory distress, stridor and regurgitation due to difficulty in swallowing which lead to reduced ventilation, exercise intolerance and in severe cases cyanosis and uncontrolled hyperthermia (Bofan et al., 2015). Diagnosis is usually made from clinical signs, physical examination in case of stenotic nostrils, everted laryngeal saccules and elongated soft palate. Radiography is warranted to diagnose hypoplastic trachea. Surgical correction can be done for stenotic nares, elongated soft palate and everted laryngeal saccules (Fossum, 2012). This case report describes the surgical correction of stenotic nostrils and elongated soft palate in the present cases.

Case History and Presentation

Case 1: A Pug dog, aged 3 years was presented with the history of stridor and respiratory distress. On physical evaluation, the external nares appeared normal; however, soft palate appeared elongated as it was covering the tip of epiglottis to quite an extent and on inspiration, was pulled caudally to cover the glottis and hence reducing the ventilation.

Case 2: A 2-year-old French Bulldog was presented with the similar clinical signs of respiratory distress and stridor as in previous case. Stridor was more prominent during sleeping. As per owner, the dog always keep his mouth open for satisfactory respiration. On physical examination, stenotic nostrils (Fig. 1a) along with elongated soft palate (Fig. 2a) were observed.

With the consent of the owners, staphylectomy was performed under general anesthesia in both the cases; additionally rhinoplasty was also done in French Bulldog. In both the cases radiography was not performed to rule out hypoplastic trachea, however, the clinical signs resolved after correcting stenotic nostrils and elongated soft palate.

Anesthesia and Surgical Procedure

Pre-medication was done with inj. atropine @0.04mg/kg and inj. xylazine @ 0.5mg per kg administered intramuscularly. Induction was done with inj. propofol @2 mg/kg, administered intravenously 'till effect'. After that dogs were intubated and maintained on isoflurane mixed in 100% oxygen. The dogs were positioned in sternal recumbency for surgery.

Rhinoplasty: A full-thickness wedge of tissue from the dorso-lateral cartilage of the external nares was resected using a scalpel blade number 11. The extent of wedge ressection should be same on both the sides. The area to be excised should be visually marked. However, there is no objective criteria as to how much tissue should be excised, but it should be enough to widen the external nares for easy flow of air with equal extent in both dorsolateral cartilage. The cut edges were then apposed with simple interrupted sutures using nylon 3-0 (Fig.1b). The bleeding during the procedure was controlled with digital pressure (Fossum, 2012).

Staphylectomy: The oral cavity was opened wide with the help of bandages and slings, with maxilla suspended from an anchor via a bandage. The elongated end of soft palate was painted with betadine with a sterile gauze piece. Stay sutures were placed on each side of the soft palate to easily exteriorize its most caudal part. The extent to which the soft palate had to be cut was estimated in a way that the most caudal part of the palate should only touch the tip of epiglottis and should not cover it more caudally (Fossum,

Fig 1: Pre -operative (a) and immediate post- operative (b) photograph of case 2.

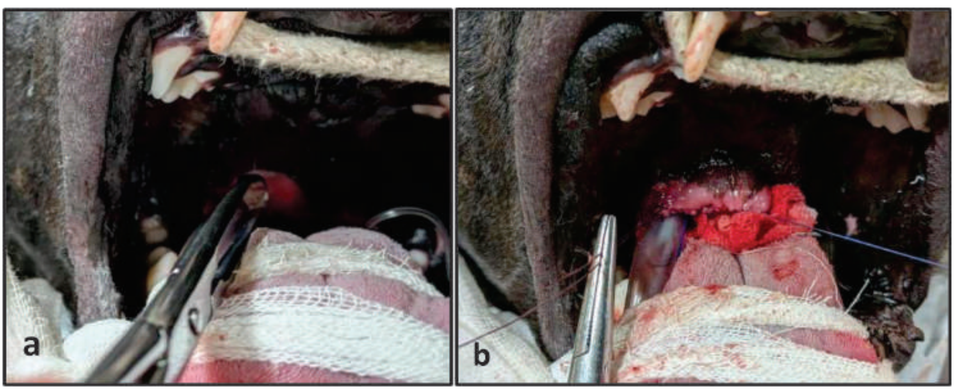

Fig. 2: Pre (a) and post-operative (b) photographs showing (a) tip of soft palate held with an Allis Tissue Forceps, (b) part of soft palate was resected and sutures being applied. The change in the tissue color of the soft palate (pink and black) was used a criteria for resection (b).

2012). The caudal tip of the soft palate was then grasped with the Allis tissue forceps (Fig. 2(a)) and the required length of soft palate was cut with the scissors in small steps and was simultaneously sutured in simple continous pattern (mucosa to mucosa) using polygalactin 910 (3-0) (Fig. 2b) (Himel et al., 2023).

Post-Operative Treatment and Follow Up

Both the dogs were advised antibiotics, inj. Cefotaxime @20mg/kg, BID for 5 days, analgesic inj, meloxicam @0.2mg/kg, OD for 3 days and inj, Rantidine@ 2.2 mg/ kg, OD, for 5 days, subcutaneously. Both the dogs recovered uneventfully with improvement seen immediately during post-operative care and further at one month follow up the French bull dog started keeping his mouth closed during normal activity (Fig. 3).

Discussion

The BOAS has been a cause of concern for veterinarians since many years as demand for such breeds has increased amongst pet parents nowadays. In early 2000s pug ruled the pet world with its ever-increasing demand and slowly with time, French bulldogs, Shih Tzu's, Chow-chow and many other brachycephalic breeds also became popular in India. However, their congenital developmental anatomy of skull bones (with normal width and reduced length, flattened bones) and upper respiratory tract make them prone to certain conditions like stenotic nares, elongated soft palate, everted laryngeal saccules and hyperplastic trachea (Lodato & Hedlund, 2012). Concurrent hyperthermia may also be noticed in many cases (Bofan et al., 2015). Initially medical management with weight reduction, corticosteroids, bronchodilators, oxygenation may be fruitful in certain cases with mild clinical signs (Siddiqi et al., 2024), but surgical management is usually warranted in severe cases.

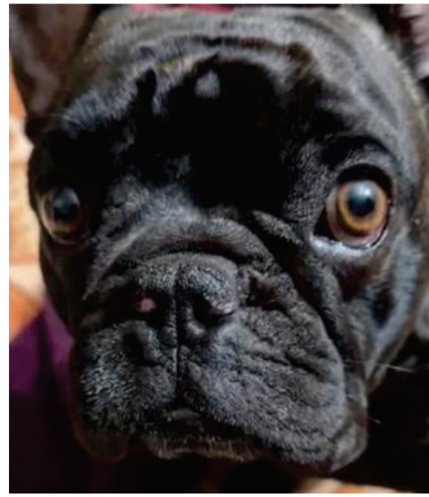

Fig. 3. One month post-operative photograph of French bull dog with mouth closed during normal activity

Stenotic nostrils are due to congenital malformation of dorso-lateral nasal cartilage, in with there is medial collapse of the cartilage and hence, there is narrowing of the external nares. This reduces the airflow and hence leads to increased inspiratory effort and dyspnea. As the time advances, it can lead to hypertrophy of nasal mucosa and further lead to upper airway obstruction. It is usually diagnosed at an early age via physical examination. Multiple surgical options are documented to treat stenotic nostrils which includes, resecting the wings of the dorsal alar cartilage to equal extent bilaterally, using an electrocautery loop probe to resect the desired part of the cartilage and wedge resection of the cartilage. Out of the documented approaches, wedge resection is the most popular and had been used to correct the condition in the case of French Bulldog mentioned in this particular report.

Elongated soft palate is the most common component of BOAS (Fasanella et al., 2010). It is usually diagnosed by physically examining the oral cavity under sedation. The caudal most tip of the soft palate in such cases covers at least 1-3mm of epiglottis caudally. During inspiration or deglutition, the soft plate obstructs the glottis and hence increases inspiratory effort leading to stridor and also leads to inefficient ventilation during deglutition and may cause aspiratory pneumonia. Macroglossia (enlarged tongue) in brachycephalic breeds is also said to contribute towards dorsal displacement of soft palate, which further worsens the clinical signs (Jones, 2020). Surgical resection of excess part is usually warranted and should be performed by the age of 4-24 months. Delayed repair may lead to laryngeal cartilage degeneration and its collapse. Staphylectomy can be performed via scissors, laser, monopolar or bipolar electrocautery. Laser and electrocautery need to be used with caution as hyperbaric oxygen is usually being supplied to the patient via endotracheal tube. The soft palate is usually resected to an extent where it reaches only the tip of epiglottis and does not extend caudally. Inefficient resection does not improve the respiratory distress, and excessive resection may lead to regurgitation or rhinitis (Fossum, 2012).

Hypoplastic trachea is another componenet of BOAS, which is congenitally present and is diagnosed via radiography. The standard procedure to diagnose the hypoplastic trachea is to calculate the tracheal diameter (TD) to thoracic inlet ration (TI), where TD is measured at the level of caudal end of seventh cervical vertebrae and TI is measured from cranial end of first thoracic vertebrae till cranial end of manubrium (Mostafa & Berry, 2022). The TD to TI ratio in brachycephalic breeds should not be less than 11% in normal scenario (Thrall, 2012). hypoplastic trachea is not always known to be associated to a clinical sign, but if there are clinical signs, there is no surgical treatment to revert its effects. Treatment for other cmponents of BOAS also does not improve the tracheal diameter (Regier, 2020)

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Clinical Article

Clinical Findings and Medical Management of Ocular and Cutaneous Habronemosis in Marwari Equines

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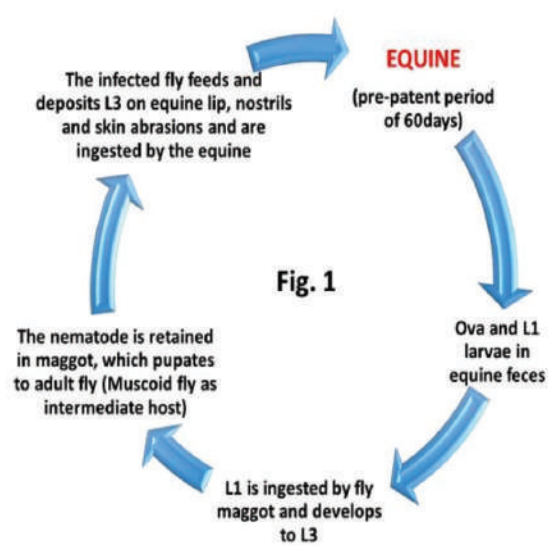
Abstract

Habronemosis is a parasitic disease of equines caused by Habronema spp. and Draschia megastoma, transmitted by muscid flies. It commonly manifests in cutaneous and ocular forms due to deposition of infective larvae in wounds or mucocutaneous junctions. This case report describes habronemosis in two Marwari mares with ocular and cutaneous forms presented with epiphora, conjunctivitis and granulomatous lesions characterized by sero-sanguinous discharge and pruritis. Diagnosis was based on clinical findings, seasonality, history of non-healing wound and response to therapy. Both mares were treated with a systemic anthelminthic (ivermectin) as prophylactic endo and ecto-parasite in combination with topical corticosteroids to reduce hypersensitivity and inflammation. Supportive wound care and fly control measures were exercised. Marked improvement was seen in both cases with no recurrence on upto 8 months follow up, though it reoccurred in the eye after 8 months.

Keywords: *Anthelminthic, Equine, Eye wound, Fly, Habronema*

Habronemosis, commonly known as Summer Sore, is a parasitic skin infection which occurs in horses and donkeys (Traversa et al., 2008). It is commonly reported in summer months, when flies are active. It is a non- healing wound that usually disappears during winters and may re-appear next summer (Traversa et al., 2007). Habronemosis is mostly reported from tropical, subtropical and temperate regions (Forouzanpour et al., 2022). It can occur in any species of equine family through stomach worms; *Habronema muscae* and *Habronema microstoma* (Barlaam et al., 2020) and are named based



on the flies which transmits them, namely *Musca domestica*, Draschia megastoma and Stomoxys calcitrans. The lifecycle of the worm is described in Fig. 1. The present report describes the clinical and cytological findings and successful medicinal treatment in two equines reported with ocular and cutaneous form of habronemosis.

Case Presentation and History

Case 1: A 2.5-year, 300Kg, Marwari female horse was presented during month of April with the history of bilateral epiphora, conjunctivitis and a pink, ulcerated growth on the medial canthus of right eye (Fig. 2a, b) for two months. Complete blood count showed leukocytosis with corresponding increase in neutrophils and eosinophils. Impression smear cytology of the ulcerated growth from medial canthus of eye revealed large number of neutrophils with many eosinophils and a lot of RBCs (Ali et al., 2015) (Fig. 2c). A detailed history revealed presence of fly activity in the region of eye in the mare. The history, clinical findings, seasonality and a history of non-healing wounds were indicative of ocular form of habronemosis. The mare was treated with oral Ivermectin (75 ml, 100ml for 400kgs) and topical gatifloxacin-prednisolone eye drops. One week telephonic follow up revealed, significant improvement in the eye condition (Fig. 2d). The horse remained fine for 8 months but the eye lesion reoccurred after 8 months or say in next summer season.

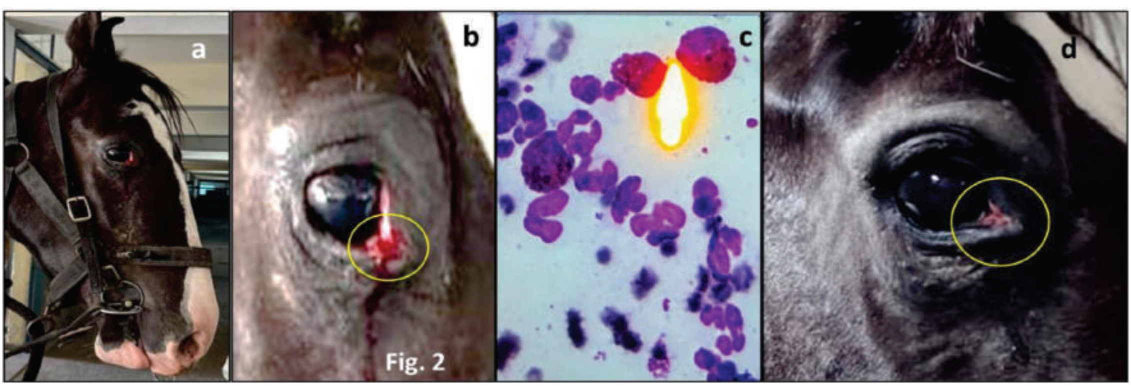
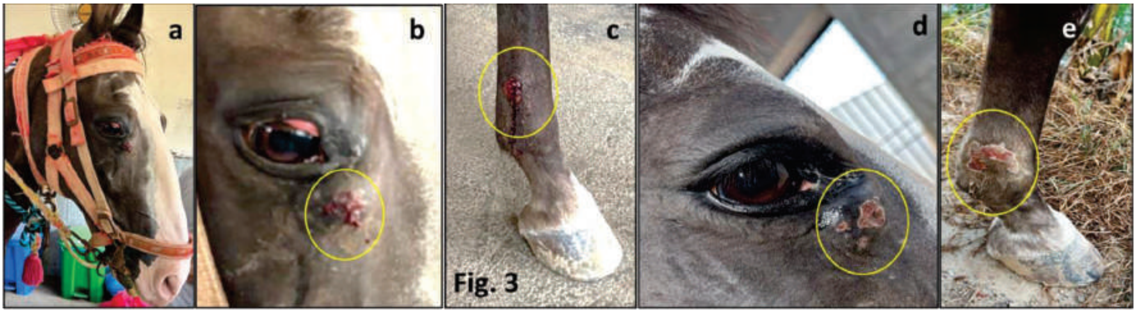


Fig. 2

Case 2: A 7 year old, 405 kg, non- pregnant, female Marwari horse was presented in the month of June, with non healing granulomatous wound near medial canthus of right eye (Fig. 3a, b) and on the left disto-medial metacarpal region (Fig. 3c). The wound has the history of occurnece from the last two summers. Diagnosis of ocular and cutaneous habronemosis was made from history and clinical appearance. Treatment included oral ivermection (100ml for 400kgs horse) solution twice at an interval of two weeks, and eyedrops gatifloxacin –prednisolone, thrice daily. The dressing of the limb wound was advised with ZIPP (40gm ZnO, 400ml liquid paraffin and 20 gm iodoform). Both the ocular (Fig. 3d) and limb wound (Fig. 3e) showed recovery with scar tissue formation at 2 month follow up.



Discussion

Habronemosis is a parasitic disease of horses, donkeys and mules occuring in tropical and subtropical regions. Indian horses have a high occurnece (4.5%) of habronema in horses, specially ponies (Devia et al., 2019), but since it is a seasonal occurnece and mostly due to poor hygiene, owners do not gets it treated much. Cutaneous and ocular form occurs when deposited larvae cannot complete their life cycle (Traversa et.al., 2007). The wound in the medial canthus of eye in equines should be differentiated from squamous cell carcinoma, foreign body granuloma and phycomycosis (Barlaam et al., 2020). Histology or PCR can confirm the diagnosis of Habronema (Traversa et al., 2007). The present study possess the limitation of cytology not confirming larvae which might be because the cytology was taken supoerficially and, possibly a deep skin scrapping would have been more reliable. The clincal signs and a history of non-healing recurring wound can also aid in diagnosis. Treatment focused on reducing the inflamation and size of the wound so that surrounding epithelium covers the wound and heals by secondary intention and to kill the parasite larvae with the use of anthelminthics (Kumar et al., 2002) and topical corticosteroids to eliminate clinical signs such as blepharitis and conjuctivitis.

In summary, the Habronemosis in equine can be diagnosed at field level based on history, and clinical signs and can be successfully treated using routinely available drugs and fly control. Though, the chances of re-occurrence are high considering the conditions in which they live.

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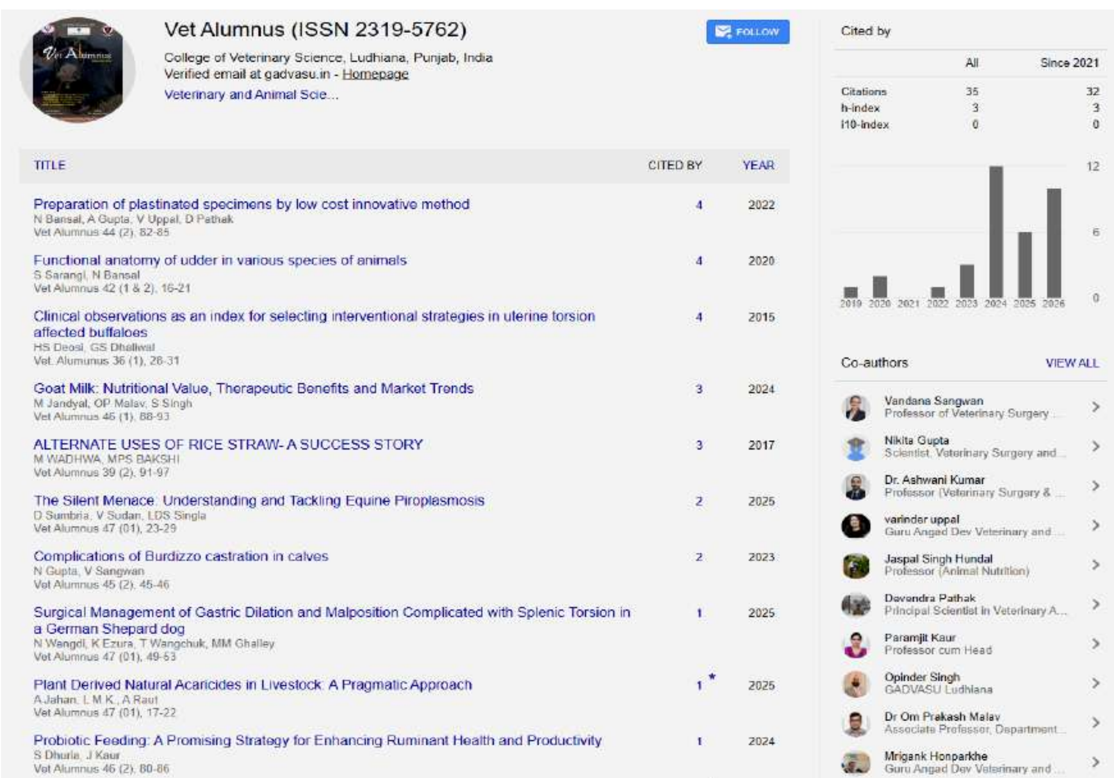

Clinical Article

Functional Recovery Following Partial and Complete Tongue Severance in Two Crossbred Cows

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Abstract

The report describes two clinical cases of tongue severance in crossbred Holstein Friesian cows, aged 2 (Case 1) and 4 years (Case 2). Case 1 had unknown history of trauma while in case 2 tongue was cut with TMR machine deployed for mixing ration. Both the cows had injury in the cranial half of the mobile portion of tongue but case 1 had one fourth portion still attached while case 2 had complete severance. In case 1, the lacerated tongue was reconstructed while in case 2, the raw wound edges were sutured together under local infiltration lignocaine anesthesia. One month follow up revealed that both cows were maintaining their prehensile function of the tongue although the sutured portion of cow 1 had fallen off due to necrosis. The study concludes that cranial half of the free tongue severed or reconstructed do not affect the final outcome of prehensile activity in cows.

Key words: *Cow, Laceration, Surgical repair, Tongue*

The tongue is a highly vascularized and the only prehensile organ in cows (Sangwan et al., 2017). Therefore, tongue conditions can lead to dysphagia and weight loss and thus are extremely harmful for health (Wilson & Anthony, 2007). Sharp items such as thorns, nails, wires, needles, or sharp teeth, sudden trauma while eating and agricultural equipment's are common causes of tongue laceration in cows (Vani, 2012). Majority of tongue lacerations occur in the anterior dorsum of the tongue and can be superficial, profound, or involve loss of part of tongue (Sangwan et al., 2017; Patil et al., 2021; Sahoo et al., 2023). The presented cases had a part of tongue hanging in one case while it was complete severed in another. Both the injuries were in the cranial half of the free portion of tongue. Both the cows successfully recovered and had regained their primary prehensile activity of the tongue.

Case History and Surgical Management

Two crossbred Holstein Friesian cows were presented with tongue laceration to the Teaching Veterinary Hospital. The detailed history and clinical presentation are summarized in Table 1. Surgical decision of tongue reconstruction or amputation in these cases was based on clinical assessment and owner's consent.

<table>
<tr><th></th><th>Cow 1</th><th>Cow 2</th></tr>
<tr><td>Breed</td><td>Holstein Friesian Crossbred</td><td>Holstein Friesian Crossbred</td></tr>
<tr><td>Age</td><td>2 year</td><td>4 year</td></tr>
<tr><td>Body Weight</td><td>300kg</td><td>400kg</td></tr>
<tr><td>History</td><td>Unknown history of trauma</td><td>Trauma with Total mixed ration machine few hours back</td></tr>
<tr><td>Presentation</td><td>Cranial half of the mobile portion of the tongue had full thickness transverse cut severing $3/4^{th}$ of tongue (Fig. 1a). Mouth closed, no bleeding, unable to eat. The tongue appeared cyanotic on first appearance.</td><td>Cranial half of the mobile portion of the tongue was completely severed with raw edges visible and mild bleeding (Fig. 2a).
Drooling of saliva, mouth open and unable to eat.</td></tr>
<tr><td>Anaesthesia</td><td colspan="2">Local 2 % lignocaine infiltration at laceration site</td></tr>
<tr><td>Position</td><td colspan="2">Right lateral recumbency</td></tr>
<tr><td rowspan="2">Surgical Findings</td><td colspan="2">The tongue was held manually caudal to the laceration without mouth gag application. The laceration was thoroughly lavaged using normal saline. Both the cows were cannulated in ear vein and 5 liters of NSS was administered, intraoperatively.</td></tr>
<tr><td>Surgical debridement of the wound edges was done and continuous suture pattern was applied in submucosa of the tongue using polydioxanone no. 0 (Fig. 1b) followed by mucosa to mucosa sutures in horizontal mattress pattern using the same thread (Fig. 1c and d).</td><td>Surgical debridement of the wound edges was done and the cut tongue edges were closed with interrupted sutures using polygalaction 910-no. 0 (Fig. 2b).</td></tr>
<tr><td>Follow up Outcome</td><td colspan="2">The cows were eating well with no apparent compromise in prehensile function.
The owner of cow 1 later revealed that the sutured portion of tongue fell off but the cow was eating well.</td></tr>
</table>

Post-Operative Treatment and Follow Up

Post-operatively, the cows were prescribed injection amoxicillin and cloxacillin (AC Vet®, Intas) at the dose rate of 10mg/kg, BID for 5 days, IM and analgesic inj. meloxicam (Injection Melonex®, Intas) at the dose rate of 0.2 mg/kg, once daily for 3 days, IM. Green unchopped fodder was advised to both the cows so that picking is easier during recovery period. Intravenous fluids (Dextrose normal saline) was advised;

however, it was not required as they started eating fodder and drinking water from the next day itself. Suture removal was advised at 14th post-operative day. But as per follow up, there were no sutures visible by day 14 and hence not removed.

One month telephonic follow up revealed both cows to be healthy with normal appetite. Both the cows were maintaining their primary prehensile activity of tongue. The owners did not share any photograph as a proof for healing despite repeated request. After repeated follow up, the owner of cow 1 informed that the cow was eating well but the sutured portion had fell off and he had sold the cow further.

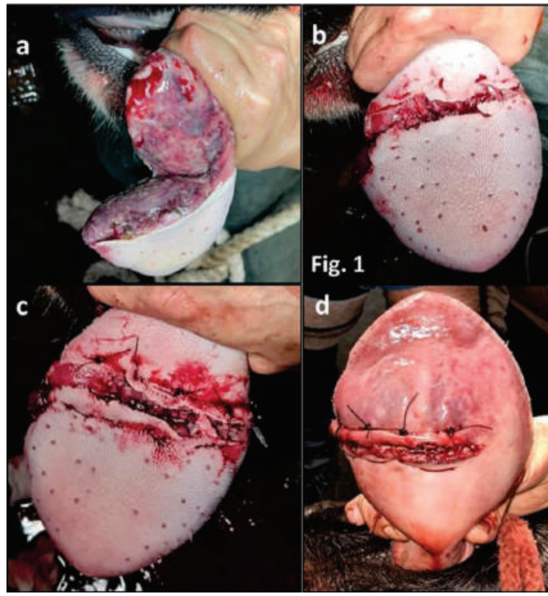

Fig. 1. Serial photographs showing the tongue laceration repair in cow 1. At presentation after debridement (a), after subcutaneous suture application from both sides (b), horizontal mattress suture application from dorsal (c) and ventral side (d).

Fig. 2. Photographs of cow 2; at presentation and after debridement (a), after suturing (b).

Discussion

Minor tongue lacerations usually heal over time without the need for surgery, while severe injuries necessitate emergency surgical intervention (Dixon & Gerard, 2006). A tongue laceration's size or gaping determines the outcome of surgery. Large flaps, active bleeding, or thorough tongue injuries are examples of complex lacerations, which are more likely repaired (Sangwan et al., 2017).

The acute clinical signs are bleeding with hanging of tongue and drooling of saliva (Sangwan et al., 2017; Patil et al., 2021). Early surgical repair of tongue laceration is advised for ideal healing by primary intention. Cranial tongue lacerations where the caudal portion can be grabbed with hand are repairable provided the transverse cut should not be involving more than 60-70% of the tongue and the cut is not old enough and necrotic. Still it is recommended that unless surgical repair is not a possibility, partial glossectomy should only be used as a last resort (Dixon & Gerard, 2006). In the present study, Cow 1 presented with a transverse tongue laceration involving more than 75% of the mobile portion. Although such extensively damaged segments rarely survive after reconstruction, surgical repair was performed at the owner's request. The reconstructed segment eventually became necrotic and detached; however, its loss did not impair the cow's prehensile function. Previous case studies (Sangwan et al., 2017; Sahoo et al., 2023) reported successful surgical repair of extensive rostral transverse/oblique tongue lacerations. The integrity of the ventral and mid region of the tongue might be the reason for successful outcome in these case studies.

The continuous suture pattern on the submucosa of the tongue followed by mucosa to mucosa sutures with horizontal pattern using the absorbable suture materials are advisable to avoid entrapment of food particles at the sutured site and consequently less suture dehiscence (Sangwan et al., 2017). Shortening or contracture of the free rostral region of the tongue in cows after laceration healing is reported in literature (Davidson *et al*., 1981) that might reshapes tip of tongue for resuming prehensile activity.

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