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Volume 16 (3); May 2026

Research Paper

Nanoemulsified chive (*Allium schoenoprasum* L.) essential oil in drinking water improves growth and gut health of broilers under cold-humid stress

Son HV, Dung NM, Hai PV.

Online J. Anim. Feed Res., 16(3): 125-131, 2026; pii: S222877012600015-16

DOI: https://dx.doi.org/10.51227/ojafr.2026.15



Abstract
This study evaluated whether supplementing drinking water with a nanoemulsion of chive (*Allium schoenoprasum* L.) essential oil could improve growth performance and intestinal health of broiler chickens reared under cold and humid stress conditions. One hundred and eighty one-day-old colored Rilai male broiler chicks were brooded for four weeks and then allocated to five treatments (four replicates, nine birds/replicate) for an eight-week trial: an unsupplemented negative control, a positive control receiving tetracycline (50 ppm), and three nanoemulsion doses (25, 50, and 100 µL/L). Chicks were housed on wet litter during a cold-humid period (approximately 12-15°C; 68-75% relative humidity). Compared with the negative control, supplementation at 50 µL/L improved final body weight and weight gain and reduced feed conversion ratio ($P < 0.05$), while feed intake, water consumption, and mortality were unchanged ($P > 0.05$). Nanoemulsion supplementation increased lactic acid bacteria counts in ileocecal digesta and reduced *Salmonella* spp., with no detectable colonies in supplemented groups. Jejunum histomorphology results showed greater villus height and villus width and deeper crypts in the 50 µL/L group ($P < 0.01$). These findings indicate that nanoemulsified chive essential oil supplied via drinking water, particularly at 50 µL/L, can support performance and intestinal health in broilers under cold-humid stress and may help reduce reliance on antibiotic growth promoters. Further studies are required to evaluate safety, dose tolerance, and possible adverse effects before broad field application.
Keywords: *Allium schoenoprasum*; Broiler chickens; Cold stress; Essential oil; Gut morphology; Intestinal microbiota.

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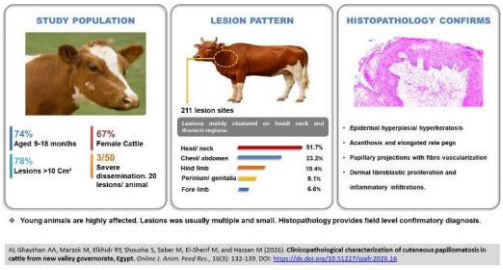
Research Paper

Clinicopathological characterization of cutaneous papillomatosis in cattle from new valley governorate, Egypt

AL Ghaythan AA, Marzok M, Elkhidr RY, Shousha S, Saber M, El-Sherif M, and Hassan M.

Online J. Anim. Feed Res., 16(3): 132-139, 2026; pii: S222877012600016-16

DOI: https://dx.doi.org/10.51227/ojafr.2026.16



Abstract
Bovine papillomatosis is a contagious proliferative skin disease of cattle caused by bovine papillomaviruses (BPVs). It is characterized by exophytic, papillary, or cauliflower-like lesions affecting the skin or mucous membranes and occurs worldwide, particularly in young animals. Histopathology remains a reliable confirmatory diagnostic tool in field settings lacking molecular techniques. This descriptive observational field study included 50 cattle with lesions clinically consistent with cutaneous papillomatosis. Animals of both sexes, aged 9 months to 4 years, were evaluated for sex, age, lesion size, multiplicity, and anatomical distribution. Representative lesions were surgically excised, fixed in 10% neutral buffered formalin, processed by routine paraffin embedding, sectioned at 4µm, and stained with hematoxylin and eosin. Data are presented as counts and percentages, and the study followed STROBE guidelines. Most affected cattle were female (76.0%) and aged 9–18 months (74.0%). Lesions smaller than 10 cm³ predominated (78.0%). Multiple lesions were more common than solitary ones, with three animals presenting up to 20 lesions each. A total of 211 lesion sites were recorded, with the highest frequency in the head and neck region (51.7%), followed by the chest, abdomen, and withers (23.2%). Histopathology revealed epidermal hyperplasia, hyperkeratosis, acanthosis, elongated rete pegs, papillary projections with fibrovascular

cores, fibroblastic proliferation, and variable inflammatory infiltration, including occasional vascular thrombosis. This study showed that bovine cutaneous papillomatosis in this setting predominantly affects young cattle and shows a marked predilection for the head and neck. Histopathological features align with classical papillomatous and fibropapillomatous lesions, supporting their value as a practical diagnostic tool in field conditions.
Keywords: Cattle, Molecular typing, Mucous membranes, Papillomatosis, Skin.

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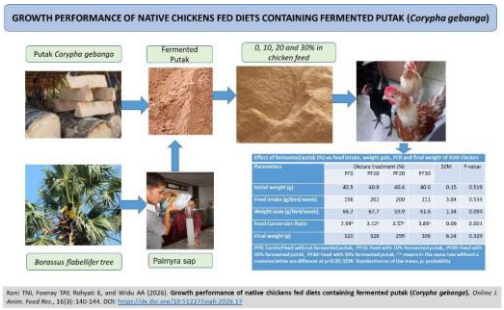
Short Communication

Growth performance of native chickens fed diets containing fermented putak (*Corypha gebanga*)

Koni TNI, Foenay TAY, Rohyati E, and Widu AA.

Online J. Anim. Feed Res., 16(3): 140-144, 2026; pii: S222877012600017-16

DOI: https://dx.doi.org/10.51227/ojafr.2026.17



Abstract
Putak, a potential feed ingredient obtained from the inner pith of the gebang palm (*Corypha gebanga*), is traditionally used on Timor Island, Indonesia. However, its low crude protein content and high fibre level restrict its inclusion in poultry diets. Previous studies have indicated that fermentation with 10% palmyra sap for six days enhances the crude protein content of putak. This study evaluated the effects of graded dietary inclusion levels of fermented putak on the growth performance of native chickens during the starter phase. A completely randomized design was applied with four dietary treatments and five replications (7 birds per replicate): 0% (P0), 10% (P1), 20% (P2), and 30% (P3) fermented putak inclusion in the diet. Increasing levels of fermented putak significantly affected feed conversion ratio (FCR; $P < 0.05$) but did not significantly influence feed intake or body weight gain ($P > 0.05$). The FCR of birds fed the control diet did not differ significantly from that of birds fed diets containing 10% fermented putak, whereas poorer FCR values were significantly observed at inclusion levels of 20%. In conclusion, fermented putak can be included at levels of up to 10% in the diets of native chickens during the starter phase without adverse effects on feed efficiency. Future studies should aim to optimize the inclusion level of fermented putak beyond 10% by improving its nutritional quality, particularly through enhanced fermentation techniques or the use of feed additives such as enzymes. Research should also investigate its long-term effects across different growth phases of native chickens, as well as its impact on carcass characteristics and overall health.
Keywords: Feed efficiency, Gebang palm, Growth performance, Native chickens, Palmyra sap.

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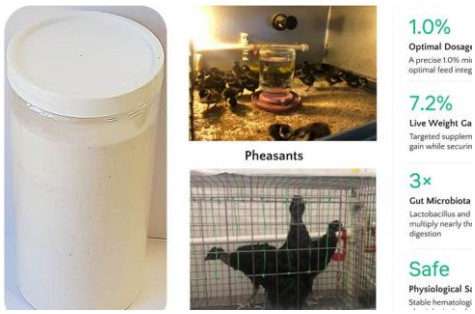
Research Paper

Influence of dry microbial composition based on lactobacilli strains on the physiological and microbiological status of Romanian pheasants

Luneva AV, Lysenko YA, Marchenko EY.

Online J. Anim. Feed Res., 16(3): 145-151, 2026; pii: S222877012600018-16

DOI: https://dx.doi.org/10.51227/ojafr.2026.18



Abstract
This study presents the results of evaluating the effects of a dry microbial composition based on lactobacilli strains (*Loigolactobacillus coryniformis* and *Lactobacillus johnsonii*), isolated from the intestinal tract of pheasants, on productivity, physiological status, and gut microbiocenosis of Romanian pheasants. The experiment was conducted on 140 birds divided into four groups: one control group and three experimental groups receiving the microbial composition at inclusion levels of 0.5–1.5% of the feed mixture. The trial duration was 91 days. Survival rate, body weight dynamics, feed intake, hematological and biochemical blood parameters were assessed, along with bacteriological analysis of the cecal contents. The results demonstrated that supplementation with the microbial composition at a dose of 1.0% yielded the most pronounced effects, including an increase in survival rate up to 100%, a statistically significant increase in body weight by 7.2%, and a reduction in feed conversion ratio by 4.2% compared to the control group. Hematological and biochemical analyses revealed no signs of toxicity or adverse effects of the microbial additive on the experimental birds. Microbiological analysis of the cecal chyme showed that administration of the microbial composition induced a restructuring of the gut microbiome, characterized by an increase in beneficial microbiota, namely *Lactobacillus* spp. (1.1–2.9-fold) and *Bifidobacterium* spp. (1.4–3.6-fold), accompanied by a

reduction in opportunistic microorganisms. The study shows the efficacy of the developed dry microbial composition in Romanian pheasant production, with the optimal effect, at a dosage of 1.0% of the compound feed.
Keywords: Body weight, Dry microbial composition, Feed conversion ratio, Gut microbiota, Lactobacillus.

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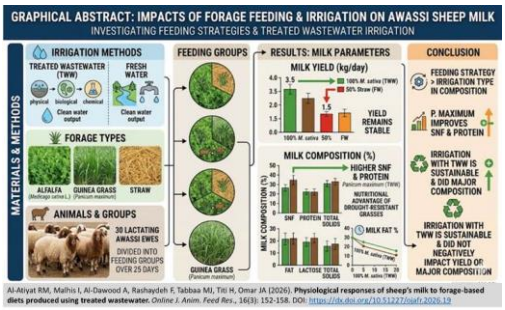
Research Paper

Physiological responses of sheep's milk to forage-based diets produced using treated wastewater

Al-Atiyat RM, Malhis I, Al-Dawood A, Rashaydeh F, Tabbaa MJ, Titi H, Omar JA.

Online J. Anim. Feed Res., 16(3): 152-158, 2026; pii: S222877012600019-16

DOI: https://dx.doi.org/10.51227/ojafr.2026.19



Abstract
The study aimed to investigate the effects of different feeding strategies and irrigation methods of forage on sheep milk yield and composition. Specifically, it evaluated the impact of alfalfa (*Medicago sativa* L.) and guinea grass (*Panicum maximum*)-based diets irrigated with either treated wastewater (TWW), after physical, biological, and chemical methods to remove contaminants or fresh water. 30 lactating ewes were assigned to different feeding groups, and milk yield and composition parameters including fat, solids-not-fat (SNF), lactose, protein, and total solids were analyzed over a 25-day period. Results indicated that while milk yield remained stable across feeding groups, significant differences ($P \leq 0.05$) were observed in milk composition. The highest milk yield (3.5 kg/day) was recorded in ewes supplemented with 100% *M. sativa* irrigated with TWW, while the lowest yield (1.5 kg/day) was observed in the 50% straw irrigated with freshwater treatment. Milk fat percentage and lactose did not significantly differ between feeding treatments, meanwhile SNF, density, total solids, and protein content were significantly affected. Ewes consuming *Panicum maximum* irrigated with TWW had higher SNF and protein levels, suggesting a nutritional advantage of drought-resistant grasses in dairy production. A significant decline ($P \leq 0.05$) in milk fat percentage was observed over time, particularly in ewes consuming 100% *M. sativa* irrigated with TWW. These findings suggest that feeding strategy plays a more decisive role in milk composition than irrigation type, with *P. maximum* improving SNF and protein levels. Irrigated with treated wastewater did not negatively impact milk yield or major composition parameters, indicating its potential as a sustainable irrigation source for forage plants.
Keywords: Feeding strategies, Medicago sativa, Panicum maximum, Sheep, Wastewater.

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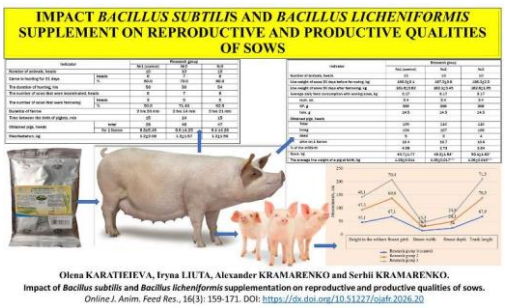
Research Paper

Impact of Bacillus subtilis and Bacillus licheniformis supplement on reproductive and productive qualities of sows

Karatieieva O, Liuta I, Kramarenko A, and Kramarenko S.

Online J. Anim. Feed Res., 16(3): 159-171, 2026; pii: S222877012600020-16

DOI: https://dx.doi.org/10.51227/ojafr.2026.20



Abstract
In modern livestock management, biotechnological products, including feed additives, are widely utilized to enhance productivity. One such agent is probiotics, which have been employed for a long time in both human food and livestock. Their effects are based on various biological mechanism–including the reduction of intestinal acidity, inhibition of pathogenic microorganisms, and stimulation of the immune response. Unlike antibiotics, which are prohibited in animal feed, probiotic supplements are regarded as safe alternatives for maintaining animal health and prophylaxis disease. In particular, in the diets of sows, probiotics contribute to the improvement of overall physiological condition and stimulate the development of beneficial intestinal microflora. Consequently, there has been increasing interest in studying effective probiotic bacteria and developing advanced biotechnological preparations. Among promising candidates are probiotics based on *Bacillus subtilis* and *Bacillus licheniformis*, which have been evaluated in various animal species for their potential beneficial effects on host physiology. The use of such probiotic feed additives has demonstrated favorable effects in sows across different ages and physiological stages. The administration of this probiotic for both young and adult animals during periods of preparation for fertilization, as well as before and after farrowing, positively affected their reproductive function. Improvements were observed in indicators such as sexual reflex expression, fertilization rates, litter size, and the number of live births. Additionally, significant

enhancements in growth, weight gain, and the survival of piglets up to two months of age were recorded. The optimal doses of probiotic use were determined to be 1.0% of the daily ration for young sows and 1.5% for adults. Therefore, the results of this study substantiate the inclusion of a probiotic supplement combining these two bacteria in the diets of sows to enhance their productivity and the quality of their offspring.
Keywords: Biotechnological products, Gastrointestinal microflora, Immune system, Probiotics, Sows, Reproductive capacity.

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Research Paper

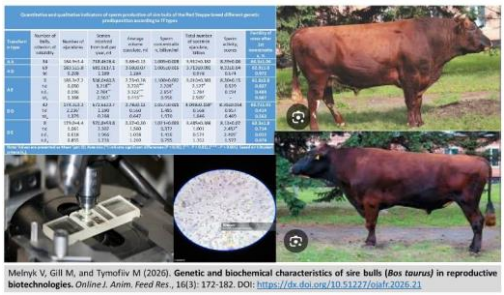
Genetic and biochemical characteristics of sire bulls (*Bos taurus*) in reproductive biotechnologies

Melnyk V, Gill M, and Tymofiiv M.

Online J. Anim. Feed Res., 16(3): 172-182, 2026; pii: S222877012600021-16

DOI: https://dx.doi.org/10.51227/ojafr.2026.21

Abstract
This study aims to investigate the relationship between polymorphic serum protein systems – transferrin *(Tf)*, amylase (*Am*), and ceruloplasmin, (*Cp*)- and sperm production parameters, as well as fertilization rates, in Red Steppe and Angeln cattle. Electrophoresis was employed to determine protein phenotypes and calculate levels of heterozygosity. The relevance of this study is justified by the necessity of establishing immunogenetic markers to enhance selection efficiency. Analysis revealed breed-specific immunogenetic markers. Regarding the Transferrin (*Tf*) locus in Red Steppe bulls, the highest sperm production indicators were observed in homozygous individuals (Tf^{AA}, Tf^{DD}), whereas in the Angeln breed, superior sperm volume was noted in heterozygous individuals (Tf^{AD}). Cows inseminated with sperm from heterozygous pairs generally exhibited significantly higher fertility rates than those inseminated with sperm from homozygous pairs. The Amylase (*Am*) system confirmed the negative influence of the Am^{CC} phenotype, which is associated with the lowest level of sperm production and fertility. In the Red Steppe breed, homozygous Am^{BB} sire bulls possessed the highest reproductive qualities. The study of the Ceruloplasmin (*Cp*) system revealed that in the Red Steppe breed, heterozygotes (Cp^{BC}) demonstrated the highest fertility, despite the superior sperm productivity of homozygotes (Cp^{AA}). Conversely, in the Angeln breed, Cp^{BB} homozygotes prevailed in both indicators. These results suggest that the optimal level of heterozygosity may depend on breed-specific genetic backgrounds. Red steppe bulls that were homozygous or possessed a 25% heterozygosity level exhibited the highest sperm production and fertilization ability, whereas the optimal level of Angeln bulls was 50%. It is recommended that homozygosity levels of *Tf*, *Am*, and *Cp* be incorporated into selection indices when purchasing sire bulls to improve the reproductive qualities of the herd.
Keywords: Amylase, *Bos taurus*, Ceruloplasmin, Heterozygosity, Semen quality, Sire bull, Transferrin.



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Research Paper

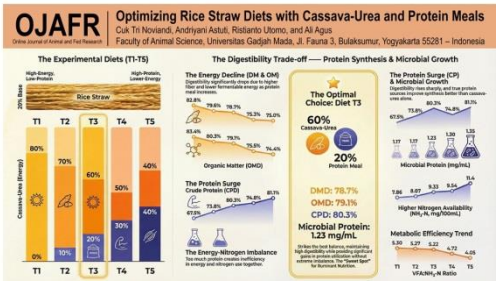
Enhancing nutrient digestibility and microbial fermentation in rice straw-based diets using cassava, copra, and palm kernel meals

Noviandi CT, Astuti A, Utomo R, and Agus A.

Online J. Anim. Feed Res., 16(3): 183-188, 2026; pii: S222877012600022-16

DOI: https://dx.doi.org/10.51227/ojafr.2026.22

Abstract
This study assessed the effects of cassava-urea and protein meal supplementation at different proportions on the *in vitro* fermentation characteristics and digestibility of rice straw-based diets. Five diets were prepared with 20% rice straw as the base, and cassava-urea to protein meal ratios of 80:0 (T1), 70:10 (T2), 60:20 (T3), 50:30 (T4), and 40:40 (T5). *In vitro* digestibility and fermentation traits were analyzed using a two-stage method. The results showed that higher levels of protein meal improved crude protein digestibility from 67.5 to 81.1%. However, this increases reduced dry matter and organic matter digestibility, dropping from 82.8 to 75.0% and from 83.4 to 74.4%, respectively ($P < 0.05$). The pH remained within the optimal range of 7.29 to 7.41, with no significant change in total volatile fatty acids (VFA) production, which ranged from 40.9 to 44.8 mM. The ammonia nitrogen (NH_3-N) levels rose significantly from 7.86 to 11.4 mg/100 mL. This



led to a decrease in the VFA:NH_3-N ratio, which changed from 5.30 to 4.05 ($P < 0.05$). This suggests an energy-to-nitrogen imbalance at higher protein meal levels. Microbial protein synthesis improved from 1.17 to 1.35 mg/mL ($P < 0.05$), likely due to increased nitrogen availability despite the imbalance. Including a moderate amount of protein meals, specifically 60% cassava-urea and 20% protein meal, provided a better balance in digestibility and fermentation efficiency. This highlights the importance of optimizing the energy-to-protein ratio in ruminant feed formulations.
Keywords: Cassava meal, *In vitro* digestibility, Microbial protein synthesis, Rice straw, Rumen fermentation.

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Research Paper

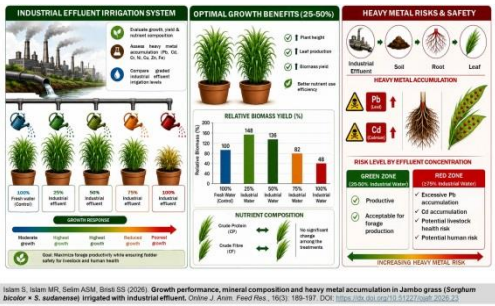
Growth performance, mineral composition and heavy metal accumulation in Jambo grass (*Sorghum bicolor* × *S. sudanense*) irrigated with industrial effluent

Islam S, Islam M.R, Selim ASM, Bristi SS.

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Abstract



The use of industrial wastewater for forage production is increasing in peri-urban livestock systems, yet its implications for fodder safety remain uncertain. This study evaluated the effects of graded industrial effluent irrigation on growth characteristics, nutrient composition, and heavy metal accumulation in Jambo grass (*Sorghum bicolor* × *Sorghum sudanense*). A pot experiment was conducted using five irrigation treatments comprising fresh water (control) and 25%, 50%, 75%, and 100% industrial effluent. Morphological traits, biomass yield, proximate composition, and concentrations of Pb, Cd, Cr, Ni, Cu, Zn, and Fe were determined in soil and plant tissues. Moderate effluent application (25–50%) improved plant height, leaf production, and total biomass yield compared with the control. However, progressive increases in effluent concentration resulted in elevated accumulation of Pb and Cd, particularly in roots and leaves. At higher effluent levels (≥75%), Pb and Cd concentrations in edible plant parts exceeded recommended limits for livestock feed, despite proximate composition remaining largely unaffected. Crude protein and fiber fractions showed no significant variation among treatments. The results indicate that diluted industrial effluent may support Jambo grass productivity, but unrestricted use poses potential risks to livestock health due to heavy metal contamination. Careful dilution and routine monitoring are therefore essential if wastewater-irrigated fodder is to be used in animal feeding systems.
Keywords: Forage quality, Jambo grass, Heavy metals, Livestock feed safety, Wastewater irrigation.

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NANOEMULSIFIED CHIVE (*Allium schoenoprasum* L.) ESSENTIAL OIL IN DRINKING WATER IMPROVES GROWTH AND GUT HEALTH OF BROILERS UNDER COLD-HUMID STRESS

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ABSTRACT: This study evaluated whether supplementing drinking water with a nanoemulsion of chive (*Allium schoenoprasum* L.) essential oil could improve growth performance and intestinal health of broiler chickens reared under cold and humid stress conditions. One hundred and eighty one-day-old colored Rilai male broiler chicks were brooded for four weeks and then allocated to five treatments (four replicates, nine birds/replicate) for an eight-week trial: an unsupplemented negative control, a positive control receiving tetracycline (50 ppm), and three nanoemulsion doses (25, 50, and 100 µL/L). Chicks were housed on wet litter during a cold-humid period (approximately 12-15°C; 68-75% relative humidity). Compared with the negative control, supplementation at 50 µL/L improved final body weight and weight gain and reduced feed conversion ratio ($P < 0.05$), while feed intake, water consumption, and mortality were unchanged ($P > 0.05$). Nanoemulsion supplementation increased lactic acid bacteria counts in ileocecal digesta and reduced *Salmonella* spp., with no detectable colonies in supplemented groups. Jejunum histomorphology results showed greater villus height and villus width and deeper crypts in the 50 µL/L group ($P < 0.01$). These findings indicate that nanoemulsified chive essential oil supplied via drinking water, particularly at 50 µL/L, can support performance and intestinal health in broilers under cold-humid stress and may help reduce reliance on antibiotic growth promoters. Further studies are required to evaluate safety, dose tolerance, and possible adverse effects before broad field application.

Keywords: *Allium schoenoprasum*; Broiler chickens; Cold stress; Essential oil; Gut morphology; Intestinal microbiota.

INTRODUCTION

In intensive broiler production, housing microclimate fluctuations represent key stress factors that directly impact productivity, health, and animal welfare. In addition to heat stress, cold stress (environmental temperatures below the thermoneutral zone) causes substantial losses through elevated thermogenesis, metabolic alterations, and immune suppression (Akinyemi and Adewole, 2021). Notably, cold stress can impair gut structure, compromise epithelial barrier integrity, and suppress antioxidant and inflammatory responses, thereby facilitating intestinal pathogen proliferation; these effects have been documented in recent broiler studies (Gao et al., 2025).

Meanwhile, high relative humidity exacerbates litter moisture and air contamination (e.g., NH_3), adversely affecting growth and feed utilization efficiency (Weaver and Meijerhof, 1991). Physiologically, high humidity at constant temperature can reduce growth of the chicks and modify mitochondrial glucose/ATP consumption in broilers (Zhou et al., 2019). The combination of low temperature and high humidity (cold-humid) serves as a realistic stress model, commonly encountered by producers during cold seasons in subtropical tropical regions, where ventilation is restricted to conserve heat, leading to moisture and gas buildup.

Amid efforts to reduce the use of growth-promoting antibiotics, phytogenic additives and essential oils are gaining attention as natural solutions to enhance digestion, immunity, and pathogen control. Chive (*Allium schoenoprasum* L.) is a spice herb from the *Allium* genus, rich in sulfur-containing compounds (sulfides, thiosulfinates), flavonoids, and antioxidants (Hai et al., 2025), with reported antibacterial and antioxidant properties (Dai et al., 2024; Hai and Khuong, 2025). Essential oils from the *Alliaceae* family generally (including chive) demonstrate antibacterial activity by interacting with bacterial cell membranes and increasing permeability; studies on local chive varieties also describe sulfide-rich essential oil compositions with antimicrobial effects (Hai et al., 2020). Broader Allium literature indicates that organosulfur compounds can interfere with microbial membranes and metabolism while also modulating host redox and inflammatory pathways (Putnik et al., 2019; Singh et al., 2025). This is particularly relevant under cold–humid stress because the proposed stress cascade involves two main processes: (i) oxidative damage and activation of inflammatory signaling in the intestinal epithelium, and (ii) disruption of the gut microbial balance (dysbiosis) that favors the expansion of opportunistic pathogens. In principle, chive essential oil could help attenuate cold–humid stress through convergent

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mechanisms: (i) exerting direct antimicrobial activity against enteric pathogens, thereby reducing pathogen-driven inflammation; (ii) supporting a more favorable gut microbiota, which indirectly strengthens colonization resistance; and (iii) modulating redox status and inflammatory responses, potentially preserving intestinal barrier integrity and improving nutrient absorption during stress exposure (Putnik et al., 2019). However, despite promising bioactivity, essential oils face practical constraints in poultry production due to volatility, poor water solubility, and instability during storage and mixing.

To address these delivery limitations, nanoemulsion technology has emerged as an enabling platform for lipophilic phytochemicals. Nanoemulsions can enhance dispersion and apparent solubility, protect actives from degradation, and improve contact with biological membranes, thereby increasing functional efficacy following oral administration (Buya et al., 2020). Reviews of nanoemulsions further highlight that nanometric droplet size increases surface area and can improve stability and bioavailability, helping to overcome the intrinsic limitations of essential oils (Barradas et al., 2021; Omidian et al., 2025). From an application standpoint, delivering phytogenic compounds through drinking water is attractive because it enables easy oral administration and may also help reduce microbial contamination in the water system, thereby complementing their gut-directed effects.

Accordingly, this study aimed to (i) characterize chive essential oil nanoemulsion (NC) and (ii) assess the effects of its supplementation in daily drinking water on growth performance, ileocecal microbiota, and intestinal morphology in broilers under cold-humid stress. It was hypothesized that NC, owing to its antibacterial and antioxidant activities, could alleviate the negative effects of cold-humid stress, enhancing feed efficiency and gut health parameters.

MATERIALS AND METHODS

Animals, diet, and experimental design

A total of 180 one-day-old male colored Rilai broilers were used. The chicks were brooded for 4 weeks, then randomly divided into five treatments to ensure uniformity in age, male-female ratio, initial body weight, care and feeding regime, and disease prevention. The coops were made of wire mesh (1 m x 1 m x 1 m), with a floor made of rice husk microbial bedding. The chicks were provided with sufficient feed and water throughout the experiment (3 months). The basic diet was managed in the form of a homemade mixed feed for chickens meeting the feed standards for broiler chickens of the Vietnam Ministry of Agriculture and Rural Development (10 TCN 661-2005). The feed composition included main ingredients such as corn, rice bran, anchovy meal, soybean meal 48%, oyster meal, vitamin premix, mineral premix, CaCO3 powder, L-lysine, and DL-methionine. The experiment commenced at 1 month of age and continued until the end of 12 weeks of age. Birds were randomly assigned to 5 treatments with 4 replicates (9 birds/replicate):

CON: negative control (unsupplemented drinking water); AB: positive control (drinking water supplemented with 50 ppm tetracycline); 25NC: drinking water supplemented with NC at 25 µL/L; 50NC: drinking water supplemented with NC at 50 µL/L; 100NC: drinking water supplemented with NC at 100 µL/L

Temperature (°C) and humidity (%) in the coop were recorded daily using a Testo thermometer/humidity meter (Model 608-H1), suspended at a height of 0.3m in each coop. This data was automatically recorded every 5 minutes throughout the experiment using a HOBO data logger (Onset Computer Corporation, Pocasset, MA, USA) (Figure 1). According to Weaver and Meijerhof (1991), weeks with relative humidity (RH) ≥ 70% were classified as high-humidity conditions (orange alert), whereas weeks featuring prolonged low environmental temperatures combined with high RH were deemed severe cold-humid episodes (red alert). Based on recorded data, broilers experienced pronounced cold-humid stress during weeks with markedly low temperatures (11.6–16.6°C) and RH ~71–73% (e.g., weeks 5–6 and 8–10). Conversely, certain periods reflected humidity-induced stress even at rising temperatures (e.g., week 11: 22.2°C; RH 71.6%).

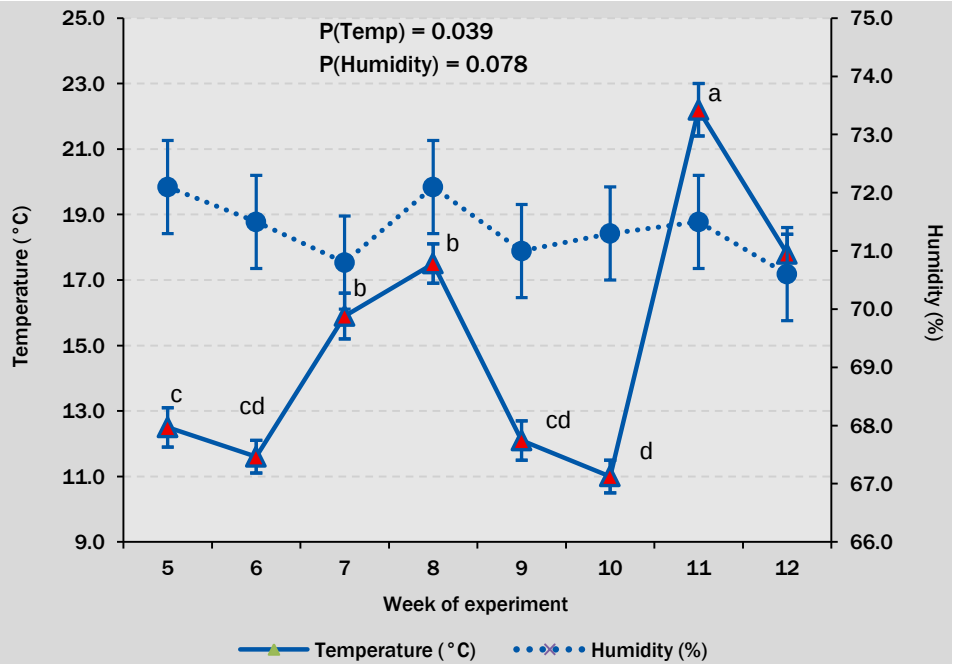


Figure 1 - Temperature and humidity throughout the experiment

Preparation of chive essential oil nanoemulsion

Chive (*Allium schoenoprasum*; GenBank ID: NC_057575.1), aged ~4.5 months and cultivated under VietGAP standards (TCVN 11892-1:2017), was used as a raw material. Essential oil was extracted from the bulbs following Hai et al. (2020) with minor modifications. Briefly, 200 g of fresh bulbs were macerated in Meizan Gold vegetable oil at a 1:5 (w/v) ratio and incubated at 60 °C with shaking (120 rpm) for 48 h. After filtration, the essential oil was separated using ethanol and recovered by vacuum evaporation under reduced pressure. The oil was dried over anhydrous Na_2SO_4 to obtain purified chive essential oil, yielding 2.04% (w/w). The nanoemulsion was prepared via magnetic stirring with an optimized ratio of 16.90% pure chive essential oil, 68.81% Tween 80, and 14.29% polyethylene glycol 400; the mixture was stirred at 300 rpm for 15 min and stabilized in a 45°C water bath for 15 min (Yani et al., 2020). Stability tests (heating–cooling cycles, freeze–thaw cycles, centrifugation), transmittance (650 nm), self-emulsification time in artificial gastric fluid, particle size/PDI/zeta potential (DLS), and morphology (TEM) followed standard nanoemulsion protocols (Vladisavljević 2019; Zhao et al., 2023).

Performance parameters

Feed and water intake were recorded daily per replicate; body weight was measured weekly. Weight gain, feed conversion ratio (FCR), and mortality rate were calculated for the experimental period (Quintana-Ospina et al., 2023).

Ileocecal microbiota

At experiment end, ileocecal digesta from representative birds per replicate were sampled to quantify LAB on MRS agar and *Salmonella* spp. on SSA agar, incubated at 37°C for 24 h, and expressed as log CFU/g, following ISO standards (ISO compilation, 2019).

Intestinal histology

Approximately 2 cm of the middle jejunum was excised, flushed with saline, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin (H&E). Villus height (VH) and crypt depth (CD) were measured using an optical microscope with image analysis software (Phan et al., 2025a).

Statistical analysis

Data were analyzed by one-way Analysis of Variance (ANOVA) using SPSS software (Version 22.0). Differences among treatment means were separated using Tukey's Honestly Significant Difference (HSD) test, with statistical significance declared at $P < 0.05$.

RESULTS AND DISCUSSION

Characteristics of chive essential oil nanoemulsion

The nanoemulsion (NC) showed high clarity (99.90 ± 0.05% transmittance) and rapid self-emulsification (48.08 ± 0.57 s), with a mean droplet size of 13.05 ± 0.07 nm (PDI = 0.257 ± 0.05) and zeta potential of −5.65 ± 0.07 mV. TEM images indicated near-spherical droplets without visible aggregation. These properties confirm a well-dispersed nanoscale system, which is expected to improve dispersion of hydrophobic essential-oil constituents in drinking water and promote intestinal exposure. This interpretation is consistent with reports that nanoemulsion/self-emulsifying systems can enhance dispersion and oral delivery of lipophilic bioactive (Buya et al., 2020).

Growth performance

Cold-humid stress reduced growth performance in the negative control (CON), reflected by the lowest ADG and poorer feed efficiency, which agrees with stress-driven shifts in nutrient partitioning from growth to maintenance and impaired gut function (Akinyemi and Adewole, 2021; Gao et al., 2025). Supplementing NC via drinking water improved performance in a dose-responsive manner. Compared with CON, ADG increased by 12.5% and 14.2% in 50NC and 100NC, respectively ($P < 0.05$), and the 100NC response was comparable to the antibiotic group (AB; 50 ppm tetracycline). Feed efficiency improved accordingly, with significantly lower FCR in 50NC and 100NC ($P < 0.05$) (Figure 2). The improvement under cold-humid challenge likely reflects gut-mediated effects, supported by microbiological and histological outcomes below.

Ileocecal microbiota

NC supplementation increased beneficial LAB populations, with 50NC and 100NC showing significantly higher LAB counts than CON and 25NC ($P < 0.05$). In parallel, *Salmonella* spp. counts were highest in CON and declined dose-dependently with NC (Figure 3). Such shifts are biologically relevant under cold-humid stress, where immune competence and gut barrier function may be compromised, increasing susceptibility to enteric pathogens (Akinyemi and Adewole, 2021; Gao et al., 2025). The antibacterial activity of Allium essential oils has been linked to sulfur-containing compounds

that disrupt bacterial membranes and key metabolic pathways (Phan et al., 2025b), and nano-dispersion may strengthen these effects by increasing contact between actives and bacterial cells. The LAB increase may also contribute to colonization resistance through competitive exclusion and local acidification, thereby limiting *Salmonella* spp. proliferation.

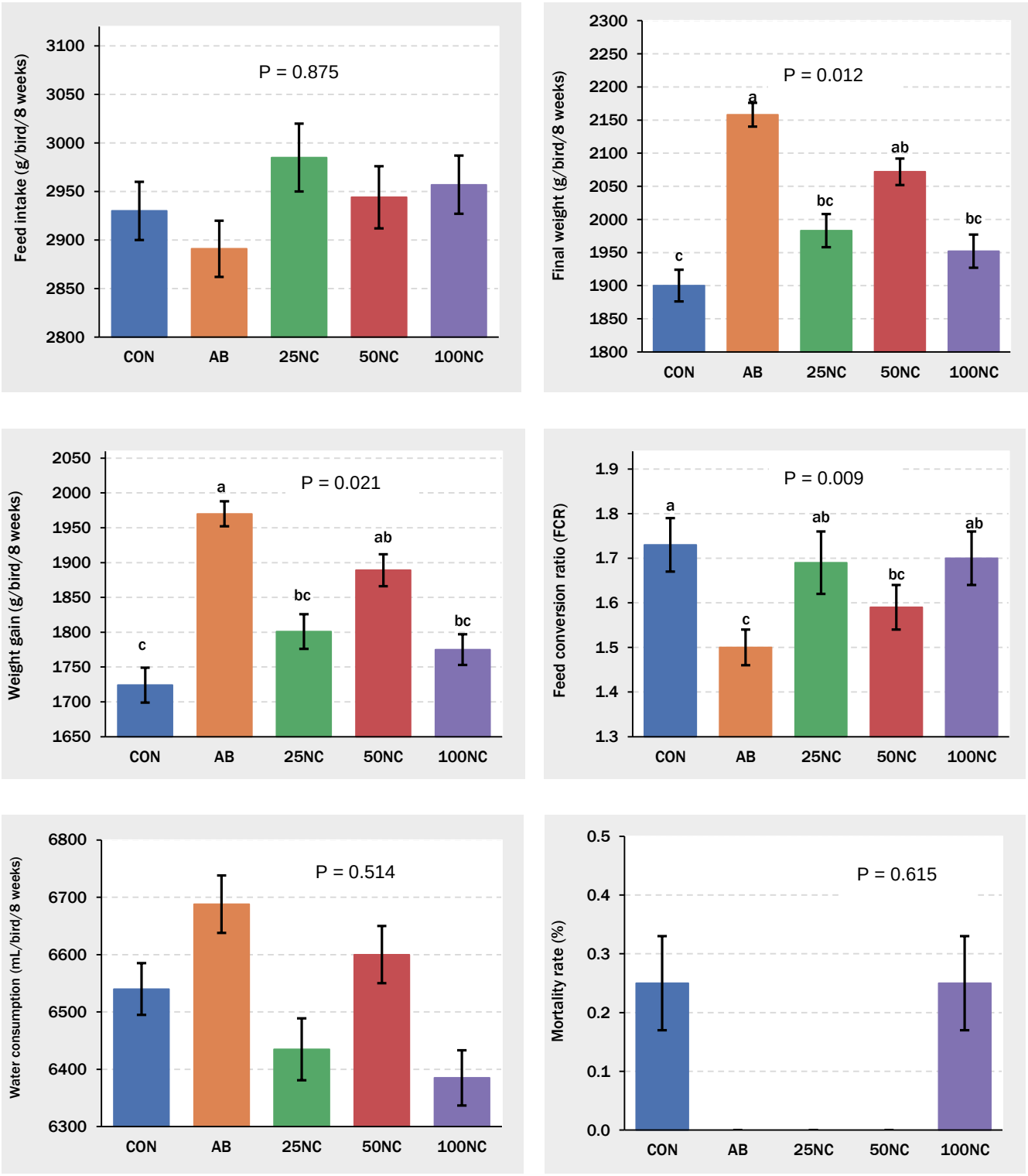


Figure 2 - Performance of broiler chickens supplemented with NC in drinking water. Note: CON = negative control; AB = 50 ppm tetracycline; 25NC = 25 µL/L NC; 50NC = 50 µL/L NC; 100NC = 100 µL/L NC. Values in the bars with different superscripts differ significantly ($P < 0.05$).

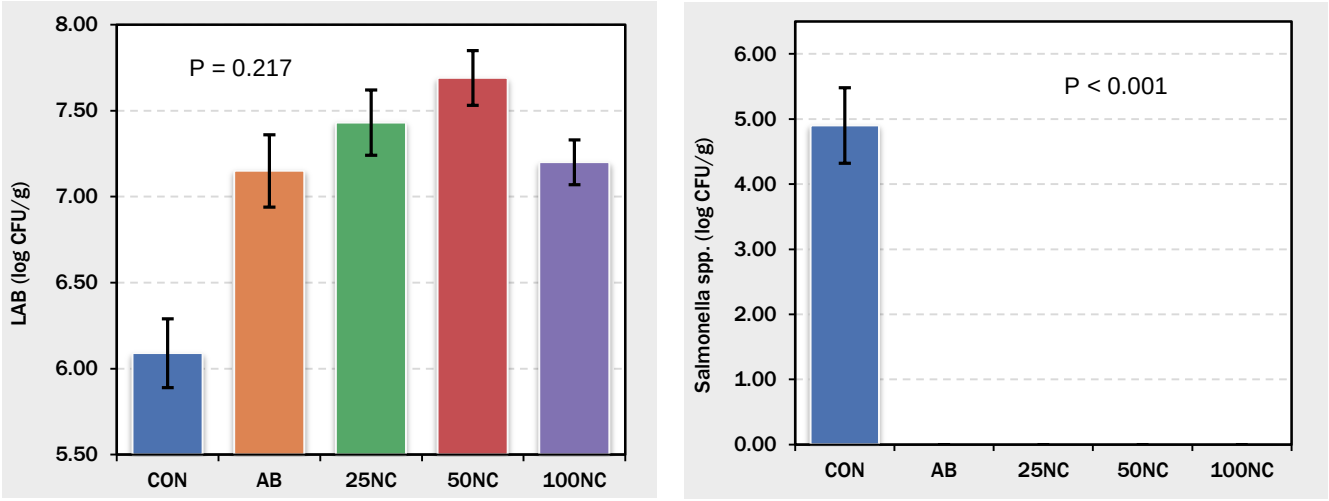


Figure 3 - Ileocecal microbiota of broiler chickens supplemented with NC in drinking water. Note: Treatment designations as in Figure 2; values in the bars with different superscripts differ significantly ($P < 0.05$).

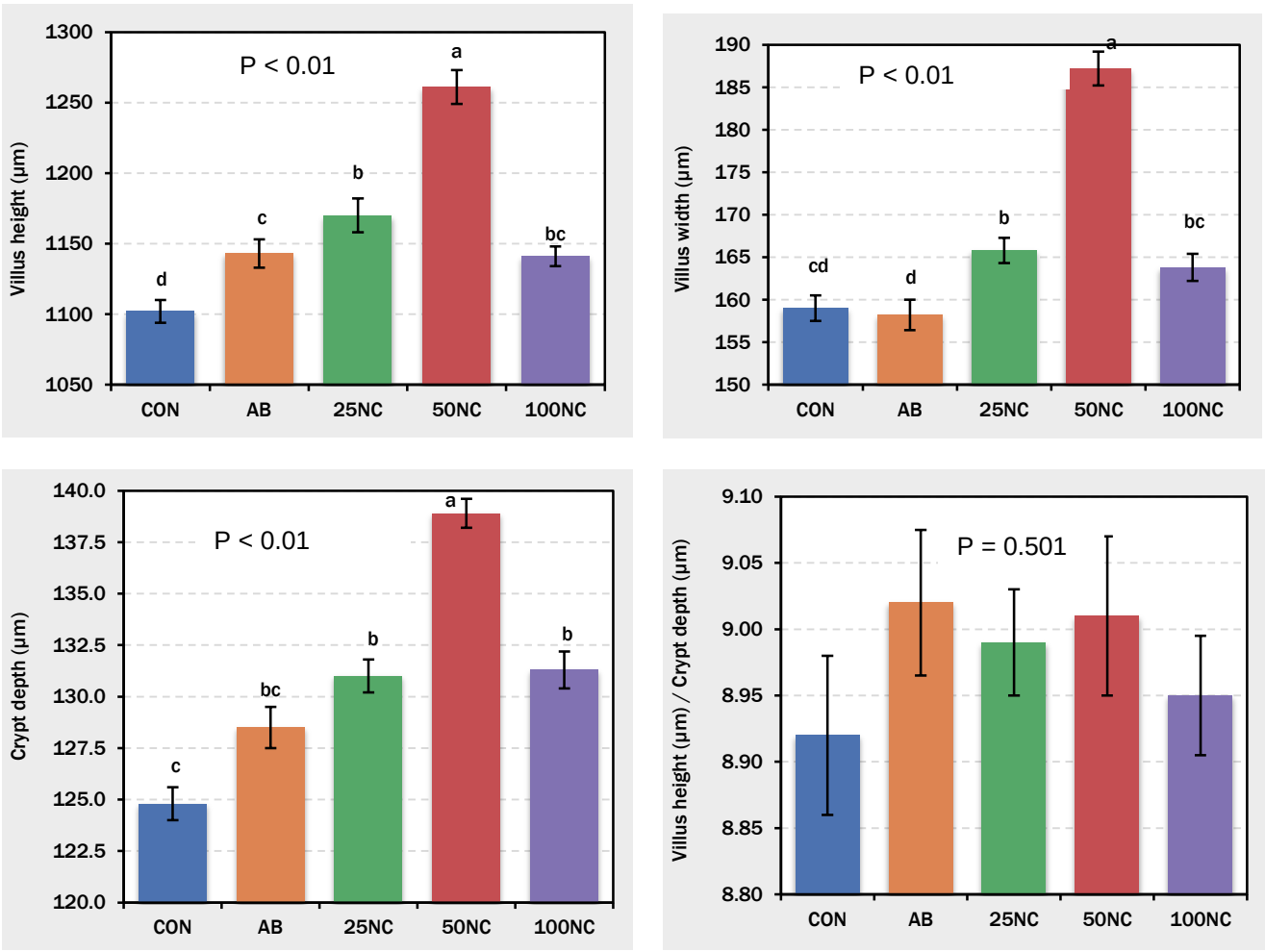


Figure 4 - Ileum histology parameters of broiler chickens supplemented with NC in drinking water. Note: Treatment designations as in Figure 2; values in the bars with different superscripts differ significantly ($P < 0.05$).

Intestinal morphology

Stress-associated villus atrophy was observed in CON, whereas NC improved intestinal architecture. The 100NC group showed the greatest villus height (approximately 18% higher than CON; $P < 0.05$), and the VH:CD ratio was significantly higher in NC-supplemented groups (Figure 4). Villus development generally indicates an improve in the absorptive surface area and more efficient nutrient uptake (El Sabry et al., 2013), providing a plausible mechanism for the improved FCR and ADG observed in 50–100NC. Overall, the findings support the view that phytogenic additives can help maintain gut structure under stress by reducing enteric pathogen burden and stabilizing epithelial turnover and function.

Limitations and future directions

This study relied on culture-based bacterial enumeration and morphometric endpoints; therefore, mechanistic interpretation would be strengthened by integrating microbiome profiling (e.g., 16S rRNA sequencing) and intestinal inflammatory/oxidative stress biomarkers. Because the zeta potential was relatively low, future work should quantify physical stability of NC under commercial waterline conditions (time, temperature, minerals, and disinfectants) and verify active-compound retention. Dose optimization should also be evaluated across genotypes and production systems, together with a cost–benefit assessment to support practical adoption as an antibiotic alternative under cold-humid stress.

CONCLUSION

Daily supplementation of NC in drinking water, particularly at 50 µL/L, enhanced final body weight, weight gain, and FCR in broilers under cold-humid stress, while boosting LAB, eliminating *Salmonella* spp., and improving ileum morphology. The findings propose NC as a promising phytogenic additive for antibiotic reduction strategies and gut health enhancement in challenging microclimatic conditions.

DECLARATIONS

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contribution

Phan Vu Hai: development of the concept and design of the study, analysis of the obtained data, writing the original text of the manuscript. Hoang Van Son: statistical processing of data, literature review, editing of the text. Ngo Mau Dung: participation in the experiment, sample collection and laboratory analysis, correction of the manuscript. All authors approved the final version of the article and are responsible for the accuracy of the presented data.

Ethical regulations

All procedures involving animals complied with Article 72 of the Vietnamese Law on Animal Husbandry (No. 32/2018/QH14) and institutional guidelines. All processes of experiments were in accordance with animal welfare rules under monitoring of Hue University ethical board.

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Competing interests

The authors have not declared any competing interests.

Abbreviations: NC: nanoemulsion of chive essential oil; FCR: feed conversion ratio; LAB: lactic acid bacteria; VH: villus height; CD: crypt depth; ADG: average daily gain; RH: relative humidity; TEM: transmission electron microscopy; PDI: polydispersity index; DLS: dynamic light scattering; HE: hematoxylin and eosin; CFU: colony-forming unit; ANOVA: analysis of variance; SPSS: Statistical Package for the Social Sciences; HSD: honestly significant difference; CON: negative control; AB: antibiotic control; ppm: parts per million; SSA: Salmonella Shigella agar; MRS: de Man Rogosa Sharpe agar; ISO: International Organization for Standardization.

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CLINICOPATHOLOGICAL CHARACTERIZATION OF CUTANEOUS PAPILLOMATOSIS IN CATTLE FROM NEW VALLEY GOVERNORATE, EGYPT

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ABSTRACT: Bovine papillomatosis is a contagious proliferative skin disease of cattle caused by bovine papillomaviruses (BPVs). It is characterized by exophytic, papillary, or cauliflower-like lesions affecting the skin or mucous membranes and occurs worldwide, particularly in young animals. Histopathology remains a reliable confirmatory diagnostic tool in field settings lacking molecular techniques. This descriptive observational field study included 50 cattle with lesions clinically consistent with cutaneous papillomatosis. Animals of both sexes, aged 9 months to 4 years, were evaluated for sex, age, lesion size, multiplicity, and anatomical distribution. Representative lesions were surgically excised, fixed in 10% neutral buffered formalin, processed by routine paraffin embedding, sectioned at 4µm, and stained with hematoxylin and eosin. Data are presented as counts and percentages, and the study followed STROBE guidelines. Most affected cattle were female (76.0%) and aged 9–18 months (74.0%). Lesions smaller than 10 cm^3 predominated (78.0%). Multiple lesions were more common than solitary ones, with three animals presenting up to 20 lesions each. A total of 211 lesion sites were recorded, with the highest frequency in the head and neck region (51.7%), followed by the chest, abdomen, and withers (23.2%). Histopathology revealed epidermal hyperplasia, hyperkeratosis, acanthosis, elongated rete pegs, papillary projections with fibrovascular cores, fibroblastic proliferation, and variable inflammatory infiltration, including occasional vascular thrombosis. This study showed that bovine cutaneous papillomatosis in this setting predominantly affects young cattle and shows a marked predilection for the head and neck. Histopathological features align with classical papillomatous and fibropapillomatous lesions, supporting their value as a practical diagnostic tool in field conditions.

Keywords: Cattle, Molecular typing, Mucous membranes, Papillomatosis, Skin.

INTRODUCTION

Bovine cutaneous papillomatosis is a contagious proliferative skin disease of cattle characterized by exophytic or papillary lesions that may appear as pedunculated, sessile, or cauliflower-like warts. The disease is caused by bovine papillomaviruses (BPVs), a group of small (50–60 nm), non-enveloped, icosahedral double-stranded DNA viruses belonging to the family Papillomaviridae. The clinicopathological spectrum extends from localized epithelial papilloma to fibro papillomatous lesions involving both epithelial and stromal components (Jelínek and Tachezy, 2005; Borzacchiello and Roperto, 2008; Ugochukwu et al., 2019). To date, at least 14 BPV genotypes have been identified, classified into four genera: Deltapapillomavirus (BPV-1,2,13), Xipapillomavirus (BPV-3,4,6,9–12), Epsilonpapillomavirus (BPV-5,8), and one unassigned type (BPV-7) (Gharban et al., 2023; Carvajal-Reina et al., 2025). The clinical and histopathological presentation varies with BPV type: Deltapapillomaviruses cause fibropapillomas characterized by concurrent epithelial and dermal stromal proliferation, Xipapillomaviruses produce pure epithelial papilloma, while Epsilonpapillomaviruses generate intermediate forms involving both components (Jelínek and Tachezy, 2005; Borzacchiello and Roperto, 2008; Khattab et al., 2023).

The disease occurs worldwide and affects cattle of all ages; however, young animals below two years are disproportionately affected (Jelínek and Tachezy, 2005; Sameeh, 2010; Araldi et al., 2017; Al-Salihi et al., 2020). Heifers aged one to two years are considered the most susceptible group, and disease incidence typically declines with age as protective cell-mediated immunity develops following primary BPV exposure. Both sexes are affected, but females, particularly lactating dairy animals, tend to show higher prevalence owing to repeated epithelial trauma through milking procedures, teat cup liner contact, and handling, which create portals of viral entry (Campo, 2002; Sameeh, 2010; Beytut, 2017; Ugochukwu et al., 2019; Bianchi et al., 2020). The disease carries significant economic consequences: teat and udder lesions interfere with milking, reduce milk production, and predispose affected animals to mastitis; generalized cutaneous lesions impair body condition and reduce market value; and severe or complicated lesions may cause

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morbidity sufficient to necessitate culling (Fenner et al., 1987; Campo, 2002; Sameeh, 2010; Gharban et al., 2023). Transmission occurs through direct contact with infected animals and indirectly through contaminated milking equipment, fencing, feeding and watering fixtures, needles, and in young stock through skin abrasions at common contact points; arthropod vectors have also been implicated as mechanical transmitters (Studdert et al., 1988; Fields et al., 2007; Sameeh, 2010; Ugochukwu et al., 2019).

In field settings, the diagnosis of bovine cutaneous papillomatosis is mostly based on clinical inspection of characteristic gross lesion morphology. However, histopathological examination of excised or biopsied tissue remains central to confirmatory diagnosis and to the differentiation of papillomatous lesions from other proliferative skin conditions such as dermatophilosis, squamous cell carcinoma, fibrosarcoma, and equine sarcoid-like lesions in cattle (Somvanshi et al., 1986; Jelínek and Tachezy, 2005; Hesham et al., 2024). Characteristic microscopic features of bovine papillomatous lesions include epidermal hyperplasia, hyperkeratosis, acanthosis, elongated rete pegs, and papillary projections supported by fibrovascular stromal cores; fibro papillomatous lesions additionally show pronounced fibroblastic proliferation and stromal expansion (Jelínek and Tachezy, 2005; Fields et al., 2007; Hesham et al., 2024; Elghmry et al., 2025). In resource-limited field settings where molecular BPV typing by PCR is not routinely accessible, histopathological classification of lesions into fibropapilloma versus epithelial papilloma provides a clinically meaningful approximation of the contributing viral type and informs management and treatment decisions (Salib and Farghali, 2011; Panchal et al., 2024; Usman and Gwadabawa, 2024).

The management includes control communal watering points, shared feeding equipment, and concentration of young stock may favor skin abrasion and horizontal BPV transmission. The present study was conducted to describe the clinicodemographic characteristics (sex, age), lesion burden (size, multiplicity), anatomical distribution, and histopathological features of bovine cutaneous papillomatosis in cattle, with the aim of contributing baseline field data applicable to disease recognition, reporting, and management planning in this setting.

MATERIALS AND METHODS

Study design and animals

This study included 50 cattle presenting with skin lesions clinically consistent with bovine cutaneous papillomatosis. Animals were of both sexes and ranged in age from 9 months to 4 years. Cases were referred to by local farmers or identified during routine veterinary inspections. The study was conducted in accordance with STROBE guidelines for observational research (Cuschieri, 2024).

Clinical data collection

A standardized clinical record was completed for each animal, documenting sex, age (grouped as 9-18 months, 18 months-2.5 years, and >2.5 years), and lesion characteristics following the framework described in horses (Knottenbelt, 2009). Lesion size was estimated by three-dimensional external measurement using a flexible tape measure. The maximum length (L), width (W), and height (H) of each lesion were recorded, and lesion volume was calculated using the prolate ellipsoid formula: $V = (\pi/6) \times L \times W \times H$ Then lesions were grouped into four categories: <10 cm^3, 10–30 cm^3, 30–100 cm^3, and 100-300 cm^3. The 100–300 cm^3 category was pre-specified in the study protocol prior to data collection, based on lesion size ranges reported in the prior literature, and is retained in the classification framework to document the observed absence of intermediate-sized lesions between the 30–100 cm^3 and >300 cm^3 categories in this series.". Lesion multiplicity was recorded as the number of discrete lesion sites per animal. Anatomical location was classified into five body regions: head and neck; forelimb; chest, abdomen, and withers; hind limb; and genital and perineal region. The total number of individual lesion sites across all animals was computed to generate an anatomical distribution map independent of animal count.

Histopathological examination

Representative lesions from each affected animal were surgically excised under the effect of local anesthesia. Tissue samples were immediately fixed in 10% neutral buffered formalin, processed by routine paraffin embedding, sectioned at 4 µm thickness using a rotary microtome, and stained with hematoxylin and eosin (H&E) (Bancroft and Gamble, 2008). Microscopic examination was performed by a single veterinary pathologist using a binocular light microscope. Epidermal and stromal changes were systematically evaluated, with particular attention to epidermal hyperplasia, keratinization pattern (orthokeratotic hyperkeratosis versus parakeratotic hyperkeratosis), degree of acanthosis, elongation of rete pegs, papillary projections, fibrovascular core formation, fibroblastic proliferation, vascular changes, and inflammatory cell infiltration. Photomicrographs were acquired at ×4, ×10, and ×40 objective magnifications. The study is descriptive in design. Results are expressed as counts and proportions (percentages). To examine the association between demographic variables (age group, sex) and lesion characteristics (multiplicity category, lesion size category), the Chi-square test or Fisher's exact test (used when any expected cell count was <5) was applied. All tests were two-tailed with a significance threshold of $P < 0.05$. Statistical analyses were performed using IBM SPSS v.26 software.

RESULTS

Clinicodemographic findings

Clinicodemographic and lesion distribution data are summarized in Tables 1-4. The most notable demographic finding was the predominance of females (76.0%) and of the 9–18 month age group (74.0%), indicating a strong age-related pattern of susceptibility. Among lesion characteristics, most lesions were small (<10 cm^3, 78.0%), and multiple lesions per animal were more common than solitary presentation: only 26.0% of animals had a single lesion, while 6.0% (n = 3) presented with 20 lesions each, representing the most severe end of the distribution. The anatomical distribution showed concentration in the cranial and thoracic regions: the head and neck together with the chest, abdomen, and withers accounted for approximately 75% of all lesion sites.

Table 1 - Sex and age distribution of cattle affected with bovine cutaneous papillomatosis (n = 50).

Age group	Animals (n)	Percent (%)	Males (n)	Females (n)	% of group that are male
9–18 months	37	74.0%	8	29	21.6%
18 months–2.5 years	7	14.0%	2	5	28.6%
>2.5 years	6	12.0%	2	4	33.3%
Total	50	100.0%	12	38	24.0%

Table 2 - Distribution of lesion size categories among affected cattle (n = 50).

Lesion Size Category	Cattle (n)	Percent (%)
<10 cm^3	39	78.0%
10–30 cm^3	5	10.0%
30–100 cm^3	3	6.0%
100–300 cm^3	0	0.0%
>300 cm^3	3	6.0%
Total	50	100.0%

No lesions were recorded in the 100–300 cm^3 range. The category is retained for completeness to confirm the absence of intermediate-sized lesions in this series.

Table 3 - Lesion multiplicity per affected animal, stratified by sex (n = 50).

Lesions per animal	Male (n)	Female (n)	Total (n, %)
1	3	10	13 (26.0%)
2	2	0	2 (4.0%)
3	2	8	10 (20.0%)
4	3	6	9 (18.0%)
5	2	8	10 (20.0%)
6	0	3	3 (6.0%)
20	0	3	3 (6.0%)
Total	12	38	50 (100%)

Table 4 - Anatomical distribution of lesion sites across all affected animals (n = 211 lesion sites, 50 animals).

Anatomical Region	Total sites (n)	Percent of 211 (%)	Male sites (n)	Female sites (n)	% Male	% Female
Head and neck	109	51.7%	12	97	11.0%	89.0%
Chest, abdomen, and withers	49	23.2%	14	35	28.6%	71.4%
Hind limb	22	10.4%	5	17	22.7%	77.3%
Genital and perineal region	17	8.1%	2	15	11.8%	88.2%
Forelimb	14	6.6%	2	12	14.3%	85.7%
Total	211	100.0%	35	176	16.6%	83.4%

Histopathological findings

Hematoxylin and eosin-stained sections of excised papillomatous lesions revealed the following microscopic features: epidermal hyperplasia with marked orthokeratotic hyperkeratosis; acanthosis with elongation and broadening of rete pegs; papillary projections supported by central fibrovascular cores; fibroblastic proliferation within the dermis; focal ulceration of the overlying epidermis in some sections; perivascular and diffuse inflammatory cell infiltration comprising lymphocytes, neutrophils, and eosinophils; vascular thrombosis in some sections; and extension of inflammatory infiltrate to the underlying muscle in sections from severely ulcerated or secondarily infected lesions. Representative histopathological findings are illustrated in Figures 1a–e. Koilocytic changes (perinuclear cytoplasmic vacuolation in superficial keratinocytes, the hallmark cytopathic effect of productive BPV infection) were sought but were not a consistent or prominent feature in the present study.

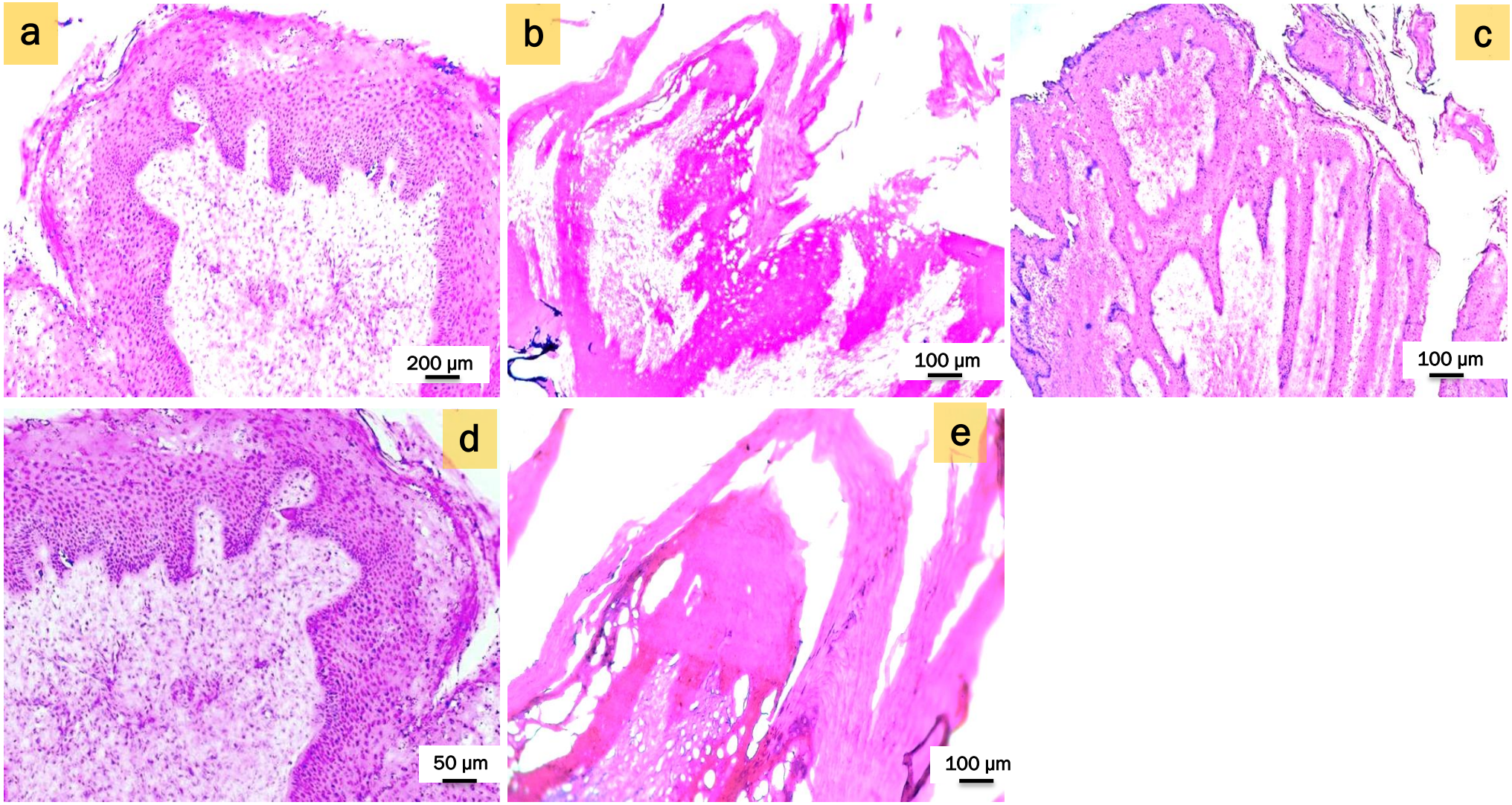


Figure 1 - Histopathological features of bovine cutaneous papillomatosis (HandE stain). (a) Epidermal hyperplasia and orthokeratotic hyperkeratosis. (b) Acanthosis with club-shaped elongation of rete pegs. (c) Papillary projections supported by fibrovascular dermal cores. (d) Mixed inflammatory infiltrate (lymphocytes, neutrophils, and eosinophils) with dermal fibroblastic proliferation. (e) Intraluminal vascular thrombosis and extension of inflammatory infiltrate to the underlying musculature in a severely ulcerated lesion.

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DISCUSSION

The arid environmental conditions of New Valley Governorate, Egypt, are comparable to those of the Saudi Arabian regions where bovine papillomatosis has similarly been reported. The extreme arid conditions of New Valley Governorate like various Saudi areas are characterized by high ambient temperatures often exceeding 40°C during summer months and intense solar ultraviolet (UV) radiation due to the region's low latitude and clear-sky prevalence may exert multiple influences on bovine cutaneous papillomatosis epidemiology. First, chronic UV-B irradiation induces oxidative stress and keratinocyte DNA damage, thereby compromising skin barrier integrity and potentially creating micro abrasions that facilitate BPV entry, particularly in areas of sparse hair coat such as the face, muzzle, and ear pinnae consistent with the head and neck lesion predominance observed here. Second, elevated ambient temperatures accelerate epidermal desquamation and increase skin fold micro-trauma during insect activity peaks, both of which are recognized portals of BPV transmission. Third, BPV virion stability in the environment on fencing, feeding equipment, and pasture soil may be enhanced or reduced by extreme heat and UV exposure; while UV radiation degrades viral DNA, desiccated virions in shaded micro-environments under troughs and shelters may remain infectious for extended periods, sustaining horizontal transmission in concentrated young stock. These environmental factors represent a biologically plausible explanatory layer for the high disease burden in this setting and merit prospective quantification in future studies. The marked predominance of affected animals in the 9-18 month age group (74.0%) is in close agreement with classical descriptions of bovine cutaneous papillomatosis as a disease primarily of young stock. The higher susceptibility of cattle below two years of age has been attributed to relative immunological immaturity: effective BPV-specific cell-mediated immunity, which is the principal mechanism of both wart regression and resistance to reinfection, is not yet fully developed in animals that have not undergone prior antigenic exposure (Jelínek and Tachezy, 2005; Namgyel et al., 2021; Gharban et al., 2023). The observed decline in disease prevalence with age reflects the progressive development of protective adaptive immune responses following primary BPV infection, a pattern that has been documented consistently across geographic and management settings (Sameeh, 2010; Aydin et al., 2020). Comparable field studies in India and Ethiopia have similarly reported that most papillomatosis cases occur in cattle below 18 months. The very low proportion of affected animals older than 2.5 years (12.0%) in the present study is consistent with these observations and suggests that surviving natural exposure in this age group confers at least partial protective immunity.

The female predominance observed in this study (76.0%) warrants specific attention. The higher proportion of affected females is consistent with reports from other field settings (Campo, 2002; Sharma et al., 2020) and is likely to reflect a combination of factors rather than innate sex-linked susceptibility to BPV. In the present study population, females were most of the young stock cohort (29 of 37 animals in the 9-18 month age group), and this demographic imbalance may partially explain the finding. Additionally female cattle are handled more frequently for reproductive examination, vaccination, and milking preparation, creating cutaneous abrasions that serve as viral entry points (Sameeh, 2010). The concentration of lesions in the head and neck and thoracic regions rather than the teat and udder which would be expected to predominate in adult lactating dairy females supports the interpretation that the affected females in this series were predominantly pre-lactating heifers in whom milking-associated teat trauma was not a relevant exposure pathway.

To formally separate biological susceptibility from demographic imbalance, a comparison of sex-specific incidence density (new cases per animal-time at risk, stratified by sex) would be necessary. In the present study, the herd composition data confirm that 78.4% (29/37) of animals in the most affected age cohort (9–18 months) were female, meaning the observed female excess in lesion counts (83.4% of all 211 lesion sites) is substantially and possibly entirely attributable to the sex imbalance in the source population rather than to differential biological susceptibility. This limitation is acknowledged, and future studies should record total herd census data by sex to enable incidence density calculations.

The strong predilection for the head and neck region (51.7% of all lesion sites) in this study is consistent with the known affinity of BPV infection for areas susceptible to contact abrasion in young, group-housed stock. Similar anatomical predominance has been described in comparable field series involving young cattle (Hatama et al., 2018; Babu et al., 2020). In adult dairy herds, teat and udder lesions caused by BPV-1, BPV-5, and BPV-6 often predominate (Campo, 2002; Jelínek and Tachezy, 2005), but in the present study population, which was heavily weighted toward animals aged 9-18 months, teat-associated BPV transmission through milking was not a relevant exposure pathway. Instead, horizontal transmission in this age group is most plausibly facilitated by skin abrasion during social interaction, rubbing against shared fencing, and contact at communal feeding troughs and watering points all of which favor trauma-prone areas of the cranial and thoracic body surface. The chest, abdomen, and withers represented the second most affected region (23.2%), which is consistent with rubbing behavior at horizontal barriers. Lesions of the genital and perineal region (8.1%) were predominantly recorded in females, which may be attributable to BPV-1 or BPV-2 involvement in the perineal and para genital skin in susceptible animals (Borzacchiello and Roperto, 2008; Sameeh, 2010).

A subset of animals (3/50, 6.0%) presented with 20 discrete lesion sites, representing a pattern of extensive disseminated papillomatosis that warrants specific clinical comment. Such severe multifocal disease is generally associated with local or systemic immunosuppression, whether attributable to concurrent endoparasitism, nutritional deficiency, management stress, or primary failure of cell-mediated immune responses against BPV (Sameeh, 2010). In immunocompetent cattle, most papillomatous lesions regress spontaneously within 1–2 years following the development

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of BPV-specific T-lymphocyte-mediated immunity (Nicholls and Stanley, 2000; Elghmry et al., 2025), and persistence or proliferation of lesions should therefore prompt investigation of potential immunosuppressive comorbidities. The BPV genotypes associated with extensive cutaneous dissemination particularly BPV-2 and BPV-3 were not characterized in the present study, as molecular typing was not available. Animals with disseminated papillomatosis present a therapeutic challenge and are at increased risk of secondary bacterial superinfection, body condition loss, and reduced market value (Campo, 2002; Sameeh, 2010; Corteggio et al., 2013).

The histopathological features documented in the present study epidermal hyperplasia, orthokeratotic hyperkeratosis, acanthosis, elongated rete pegs, papillary projections with fibrovascular cores, and dermal fibroblastic proliferation represent the canonical microscopic architecture of bovine papillomatous lesions and are consistent with previously published descriptions (Özsoy et al., 2011; Crossland and Adenomyosis, 2024). The coexistence of fibrovascular stromal cores with fibroblastic dermal proliferation indicates that at least a proportion of the lesions examined were fibropapillomas rather than purely epithelial papilloma, a distinction that is of practical relevance because fibropapillomas are associated with Delta-papillomaviruses (BPV-1, 2, 13) which have broader host tropism and oncogenic potential than the Xipa-pillomaviruses responsible for purely epithelial lesions (Borzacchiello and Roperto, 2008; Yıldırım et al., 2022; Gharban et al., 2023). The vascular thrombosis and deep inflammatory extension to the underlying musculature observed in some sections most likely reflects secondary bacterial superinfection of ulcerated lesions and is consistent with the clinical observation of surface crusting in a proportion of animals. Histopathological examination in this manner provides a reliable field-level confirmatory tool and allows meaningful differentiation of papillomatous lesions from other proliferative skin conditions when molecular typing is unavailable (Somvanshi, 2011; Khattab et al., 2023).

The present study has several limitations that should be acknowledged. First, its cross-sectional descriptive design precludes estimation of incidence rates, assessment of temporal trends, or determination of risk factors for disease acquisition or severity. Second, molecular typing of BPV was not performed; PCR-based genotyping of the BPV strains would substantially strengthen the epidemiological and virological characterization of this disease in the region and is a priority for future investigation. Third, the study was conducted at a single center, which may limit generalizability to other areas. Fourth, systematic data on herd management practices, vaccination history, concurrent disease burden, and nutritional status were not collected, precluding multivariable analysis of potential predictors of lesion multiplicity or distribution. Notwithstanding these limitations, this descriptive series provides the first formal clinicopathological field report of bovine cutaneous papillomatosis and establishes a baseline for future prospective and molecularly informed studies in this region.

CONCLUSIONS

Bovine cutaneous papillomatosis in this observational field series, predominantly affected young cattle (74.0% in the 9–18 month cohort), with a female predominance consistent with demographic and management patterns of the local cattle population. Multiple lesions per animal were more common than solitary presentation, and a subset of severely affected animals exhibited disseminated disease warranting further immunological investigation. Anatomically, lesions showed a strong predilection for the head and neck region, reflecting the likely mode of horizontal transmission through contact abrasion in young, group-managed stock. Histopathological examination confirmed the papillomatous nature of the lesions, documenting the characteristic features of fibro papillomatous and epithelial papillomatous involvement, and provided a reliable basis for confirmatory field-level diagnosis. These findings contribute baseline clinicopathological data for this under-characterized region and provide a foundation for future molecular and epidemiological studies.

DECLARATIONS

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Consent to Publish

The current research project was performed with collaboration of junior researcher joint between the faculty departments to research supposed common surgical diseases and disorders in Egypt and Saudi Arabia. All authors read and approved publication.

Ethics committee approval

This research project was conducted through a collaborative effort between the Department of Clinical Studies, Coll. Vet. Med., King Faisal University, and the Department of Surgery, Fac. Vet. Med., New Valley University. The study involved junior researchers from both institutions and aimed to investigate prevalent surgical diseases and disorders of shared clinical significance in Egypt and Saudi Arabia. All experimental procedures were approved by the IACUC of King Faisal University (Ref. No. 19-Ma-2026). The authors complied with the ARRIVE guidelines and or the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

M. Marzok and M. El-Sherif conceptualization and supervision. A. AL Ghaythan, M. Saber and M. Hassan study design, methodology, and data acquisition. A. AL Ghaythan, R. Elkhidr, and S. Shousha, manuscript drafting and revision. A. AL Ghaythan, R. Elkhidr, and S. Shousha statistical analysis, manuscript review and editing.

Competing interests

All authors have reviewed the manuscript and approved its final version for publication.

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GROWTH PERFORMANCE OF NATIVE CHICKENS FED DIETS CONTAINING FERMENTED PUTAK (*Corypha gebanga*)

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ABSTRACT: Putak, a potential feed ingredient obtained from the inner pith of the gebang palm (*Corypha gebanga*), is traditionally used on Timor Island, Indonesia. However, its low crude protein content and high fibre level restrict its inclusion in poultry diets. Previous studies have indicated that fermentation with 10% palmyra sap for six days enhances the crude protein content of putak. This study evaluated the effects of graded dietary inclusion levels of fermented putak on the growth performance of native chickens during the starter phase. A completely randomized design was applied with four dietary treatments and five replications (7 birds per replicate): 0% (P0), 10% (P1), 20% (P2), and 30% (P3) fermented putak inclusion in the diet. Increasing levels of fermented putak significantly affected feed conversion ratio (FCR; $P < 0.05$) but did not significantly influence feed intake or body weight gain ($P > 0.05$). The FCR of birds fed the control diet did not differ significantly from that of birds fed diets containing 10% fermented putak, whereas poorer FCR values were significantly observed at inclusion levels of 20%. In conclusion, fermented putak can be included at levels of up to 10% in the diets of native chickens during the starter phase without adverse effects on feed efficiency. Future studies should aim to optimize the inclusion level of fermented putak beyond 10% by improving its nutritional quality, particularly through enhanced fermentation techniques or the use of feed additives such as enzymes. Research should also investigate its long-term effects across different growth phases of native chickens, as well as its impact on carcass characteristics and overall health.

Keywords: Feed efficiency, Gebang palm, Growth performance, Native chickens, Palmyra sap.

INTRODUCTION

In rural livestock systems, the utilization of locally available feed resources plays a crucial role in reducing production costs and enhancing sustainability. One such traditional feedstuff in East Nusa Tenggara, Indonesia, is putak. Putak is derived from the pith (central portion) of the trunk of the gebang palm (*Corypha gebanga*) and has been traditionally used as an energy source in livestock diets, particularly for pigs and ruminants (Ginting-Moenthe et al., 2004). The nutritional composition of putak varies among studies but consistently indicates a high carbohydrate content accompanied by low protein levels. For example, per 100 g of putak flour, it provides approximately 351.55 kcal of energy and 85.40 g of carbohydrates, but only 0.36–2.3% crude protein and 0.09 g crude fat (Lalel and Rubak, 2024; Lalel et al., 2024)

Furthermore, its relatively high crude fibre content, which may reach up to 16% (Fuah and Pattie, 2013), constitutes a major limitation to its utilization in monogastric animal diets. Due to these nutritional constraints, the inclusion level of putak in poultry diets is generally restricted. Nevertheless, previous studies have reported that putak can be used as a partial substitute for maize at inclusion levels of up to 20% (Nalle et al., 2017). Fermentation has been proposed as an effective processing strategy to improve the nutritional quality of low-protein, high-fibre feed resources. Previous studies have demonstrated that fermentation can increase crude protein content, reduce crude fibre, and improve feed digestibility (Lateef et al., 2008; Samadi et al., 2015). The effectiveness of fermentation is strongly influenced by the use of additives that serve as both nutrient sources and microbial inoculants. Palmyra sap (*Borassus flabellifer*), a sugar-rich liquid tapped from the male inflorescence of the palmyra palm, represents a potential additive for this purpose (Das and Tamang, 2021).

Palmyra sap contains approximately 77.81 g total sugars per 100 g, mainly sucrose (65.26 g), fructose (6.64 g), and glucose (5.91 g) (Le et al., 2021). Following tapping, the sap undergoes spontaneous fermentation mediated by naturally occurring microorganisms, including *Saccharomyces cerevisiae*, *Leuconostoc mesenteroides*, *Acetobacter*, and *Gluconobacter* species (Sornsenee et al., 2021; Lalel et al., 2024). These microorganisms have been associated with improvements in the nutritional quality of fermented feed materials. In line with this, Koni et al. (2023) reported that the inclusion of 2% urea and 10% palmyra sap during rice bran fermentation increased crude protein content from 8.69% to 10.58% and reduced crude fibre content from 29.43% to 24.59%.

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Recent studies have shown that fermenting putak with 10% palmyra sap for six days significantly increases crude protein content, mineral availability, and lactic acid concentration, while reducing pH and ammonia levels (Koni et al., 2025). These improvements indicate that fermentation may enhance the suitability of putak for poultry diets. Therefore, the objective of this study was to evaluate the effects of graded dietary inclusion levels of putak fermented with palmyra sap on the growth performance of KUB chickens (Kampung Unggul Balitbangtan) during the starter phase.

MATERIALS AND METHODS

Ethical approval

This research was conducted following the poultry rearing regulations outlined in the Regulation of the Minister of Agriculture of the Republic of Indonesia, Number 31/Permentan/OT.140/2/2014. Nutritional requirements for the diets were based on the Indonesian National Standard for Native Chickens (SNI 7783.2:2022) (National Standardization Agency of Indonesia, 2022)

Experimental site and animals

This research was conducted using 140 day-old KUB chickens, obtained from PT. Mekar Jaya Unggas, Indonesia. The experiment lasted for 35 days during the starter phase. The birds were reared in individual pens (80 × 70 × 70 cm) in a semi-open housing system with controlled lighting and ventilation. Each pen was equipped with a 25-watt bulb, feeder, and drinker.

Materials

The main feed ingredient tested was fermented putak (the inner pith of *Corypha gebanga* palm). Other materials used included yellow maize, commercial layer concentrate (K204 Premium), pollard, palm oil, topmix (vitamin-mineral premix), salt, and water. Additives included palmyra sap (*Borassus flabellifer*), and rice husks for bedding.

Fermentation procedure

The fermentation procedure followed the methods described by Koni et al. (2025). Fresh putak was sun-dried for two days, ground into flour, and analyzed for dry matter (DM) content, which was 91.69% DM (8.31% moisture). Fermentation was carried out by mixing putak flour with 10% palmyra sap on a DM basis, and the moisture content of the final mixture was adjusted to 35%. For a 71-kg batch of material (65% DM; 46.15 kg DM), the mixture consisted of 50.33 kg putak, 4615 mL palmyra sap, and 16.05 L of water. The mixture was thoroughly homogenized, compacted into plastic bags, sealed airtight using adhesive tape, and stored away from direct sunlight for six days under anaerobic conditions. After fermentation, the material was air-dried and subsequently used for diet formulation.

Feed formulation

Four diets were formulated with varying inclusion levels of fermented putak (0%, 10%, 20%, and 30%) as shown in Table 1.

Table 1 - Formulation and composition of treatment feed nutrients

Ingredient \ Dietary treatment (%)	PF0	PF10	PF20	PF30
Fermented putak	0.00	10.0	20.0	30.0
Yellow maize	41.0	33.5	26.0	18.5
Layer concentrate (K204)	37.0	37.0	37.0	37.0
Vegetable oil	2.00	2.00	2.00	2.00
Vitamin-mineral premix	0.50	0.50	0.50	0.50
Pollard	19.3	16.8	14.3	11.8
Salt	0.30	0.30	0.30	0.30
Total	100	100	100	100
Nutrient component (calculate value)				
Dry matter (%)	88.2	87.7	87.3	86.8
Crude protein (%)	18.2	17.7	17.1	16.6
Crude fat (%)	6.86	6.20	5.54	4.88
Crude fiber (%)	8.38	8.09	7.80	7.51
Calcium (%)	3.34	3.37	3.41	3.44
Phosphorus (%)	0.31	0.29	0.27	0.24
Metabolizable energy (kcal/kg)	3166.6	3189.4	3212.2	3234.9
Feed cost (IDR/kg)	8000.5	7663	7325.5	6988

PF0: Control feed without fermented putak; PF10: Feed with 10% fermented putak; PF20: Feed with 20% fermented putak; PF30: Feed with 30% fermented putak

Experimental design

The study employed a completely randomized design (CRD) with a single factor consisting of four treatments and five replicates per treatment. Each replicate contained seven chickens, totaling 140 birds. The four treatments were: PF0: Control feed without fermented putak, PF10: Feed with 10% fermented putak, PF20: Feed with 20% fermented putak, PF30: Feed with 30% fermented putak.

Data analysis

Data on feed consumption, body weight gain, and feed conversion ratio were subjected to analysis of variance (ANOVA). If significant differences were detected ($P < 0.05$), means were further compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Feed consumption

The effects of graded inclusion levels of fermented putak on feed intake are presented in Table 2. The results showed that increasing levels of fermented putak (0–30%) did not significantly affect ($P > 0.05$) weekly feed intake of chickens. The similar feed intake observed across treatments indicates that diet palatability remained acceptable at all inclusion levels. This comparable feed intake may be attributed to the relatively similar nutrient composition of the experimental diets, particularly their metabolizable energy contents, which ranged from 3166.64 to 3234.91 kcal/kg (Table 1). These findings are consistent with those of Ferket and Gernat (2006), who reported that dietary energy level plays a major role in regulating feed intake in poultry. Similarly, Gao et al. (2025) stated that dietary energy content is one of the key factors influencing feed consumption in chickens. The present results are also in agreement with Nalle et al. (2017), who observed no significant differences ($P > 0.05$) in feed intake of broiler chickens fed putak meal at inclusion levels ranging from 50 to 200 g/kg. In addition, Ginting-Moenthe et al. (2004) reported no differences in feed intake of pigs fed diets containing up to 20% fermented putak.

The average feed intake of native chickens reared for six weeks in this study was 202.13 g/bird/week. This value was within the range of feed consumption reported for KUB chickens fed diets containing 16 to 20% crude protein, which ranged from 202.75 to 222.50 g/bird/week (Sinurat et al., 2022). However, the feed intake observed in the present study was lower than that reported in native chickens fed fermented banana peel supplemented with palmyra sap, where feed intake ranged from 215.82 to 250.98 g/bird/week (Koni et al., 2026). Differences in feed intake among studies may be influenced by variations in feed composition, nutrient content, palatability, and the physiological response of chickens to the dietary treatments provided

Table 2 - Effect of fermented putak (%) on feed intake, weight gain, FCR and final weight of KUB chicken

Parameters \ Dietary treatment (%)	PF0	PF10	PF20	PF30	SEM	P-value
Initial weight (g)	40.5	40.9	40.4	40.6	0.15	0.519
Feed intake (g/bird/week)	196	202	200	211	3.84	0.554
Weight gain (g/bird/week)	66.7	67.7	59.9	61.6	1.34	0.094
Feed conversion ratio	2.99^{b}	3.12^{b}	3.57^{a}	3.89^{a}	0.09	0.001
Final weight (g)	320	328	299	306	6.54	0.329

PF0: Control feed without fermented putak; PF10: Feed with 10% fermented putak; PF20: Feed with 20% fermented putak; PF30: Feed with 30% fermented putak. ^{a,b,} means in the same row without a common letter are different at $p<0.05$; SEM: Standard error of the mean; p: probability

Body weight gain

Although there were no significant differences ($P > 0.05$) in body weight gain among treatments, numerically higher gains were observed in birds fed 0% and 10% fermented putak (66.70 and 67.71 g/bird/week, respectively) compared to those fed 20% and 30% inclusion levels (59.89 and 61.58 g/bird/week). The average body weight gain in this study was 63.97 g/bird/week., The body weight gain observed in this study was lower than that reported by Saragih et al. (2024), who found that KUB chickens fed commercial diets achieved body weight gains ranging from 88.00 to 101.33 g/bird/week. However, the present result was higher than the findings of Sinurat et al. (2022), who reported average body weight gains of 49.38 to 56.50 g/bird/week in KUB chickens fed diets containing 16 to 20% crude protein. These differences may be related to variations in feed quality, nutrient composition, digestibility, and the efficiency of nutrient utilization by the chickens

Feed conversion ratio

The inclusion of fermented putak in the diet increased the feed conversion ratio (FCR). This was because feed intake did not correspond proportionally to body weight gain. Duncan's test showed that the 10% fermented putak treatment

had a similar FCR to the control (PF0), while the 20% and 30% putak treatments had significantly higher FCR values ($P < 0.05$) compared to the 0-10% treatments. These results differ from Nalle et al. (2017), who reported no significant differences in FCR of broilers fed putak at levels of 0, 5, 10, 15, and 20%, with FCR values of 2.15, 2.18, 2.097, and 2.094, respectively.

Final body weight

The inclusion of fermented putak at different levels had no significant effect ($P > 0.05$) on the final body weight of six-week-old KUB chickens. The results of this study indicate that the use of fermented putak up to a level of 30% can maintain the final body weight of KUB chickens. The absence of significant differences in final body weight observed in this study is presumed to be due to the similar feed intake and weekly body weight gain among treatments during the experimental period. This is likely because the similar feed intake resulted in comparable nutrient absorption.

These findings are supported by Sugiharto et al. (2020), who stated that the amount of feed consumed influences the final body weight of KUB chickens. Prasetyo et al. (2021) also reported that feed quantity, feed quality, nutrient content, and feed intake level play important roles in determining the final body weight of KUB chickens. In the present study, the average final body weight at six weeks of age was 313.17 ± 26.78 g, which was lower than the final body weight reported in KUB chickens fed fermented sago waste diets, ranging from 401.6 to 455.5 g (Erwan et al., 2023). Differences in final body weight may be associated with variations in feed ingredients, nutrient digestibility, dietary energy and protein levels, as well as the growth response of chickens to different feeding treatments.

CONCLUSION

The inclusion of fermented putak up to 30% in the diets of indigenous chickens (KUB) during the starter phase did not significantly affect feed intake or body weight gain. However, higher inclusion levels (20–30%) significantly worsened feed conversion ratio, indicating reduced feed efficiency. The optimal inclusion level was found at 10%, which provided similar growth performance and feed efficiency to the control diet without fermented putak. Therefore, fermented putak can be used as an alternative energy source in poultry feed up to 10% without compromising performance. Further research should focus on increasing the allowable inclusion level of fermented putak beyond 10% by enhancing its nutritional value, particularly through improved fermentation methods or the incorporation of feed additives such as enzymes. Additionally, studies are needed to evaluate its long-term effects across various growth stages of native chickens, including its influence on carcass traits and overall health status.

DECLARATIONS

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Authors' contribution

Theresia Nur Indah Koni contributed to creating the research idea, designing experiments, analyzing data, and writing this article; Tri Anggarini Yuniwati Foenay, and Eni Rohyati contributed data collection and corrected the article; Asri Apriana Widu helped with data analysis, and corrected the article.

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Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Competing interests

The authors declare no competing interests in this research and publication.

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INFLUENCE OF DRY MICROBIAL COMPOSITION BASED ON LACTOBACILLI STRAINS ON THE PHYSIOLOGICAL AND MICROBIOLOGICAL STATUS OF ROMANIAN PHEASANTS

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ABSTRACT: This study presents the results of evaluating the effects of a dry microbial composition based on lactobacilli strains (*Loigolactobacillus coryniformis* and *Lactobacillus johnsonii*), isolated from the intestinal tract of pheasants, on productivity, physiological status, and gut microbiocenosis of Romanian pheasants. The experiment was conducted on 140 birds divided into four groups: one control group and three experimental groups receiving the microbial composition at inclusion levels of 0.5–1.5% of the feed mixture. The trial duration was 91 days. Survival rate, body weight dynamics, feed intake, hematological and biochemical blood parameters were assessed, along with bacteriological analysis of the cecal contents. The results demonstrated that supplementation with the microbial composition at a dose of 1.0% yielded the most pronounced effects, including an increase in survival rate up to 100%, a statistically significant increase in body weight by 7.2%, and a reduction in feed conversion ratio by 4.2% compared to the control group. Hematological and biochemical analyses revealed no signs of toxicity or adverse effects of the microbial additive on the experimental birds. Microbiological analysis of the cecal chyme showed that administration of the microbial composition induced a restructuring of the gut microbiome, characterized by an increase in beneficial microbiota, namely *Lactobacillus* spp. (1.1–2.9-fold) and *Bifidobacterium* spp. (1.4–3.6-fold), accompanied by a reduction in opportunistic microorganisms. The study shows the efficacy of the developed dry microbial composition in Romanian pheasant production, with the optimal effect, at a dosage of 1.0% of the compound feed.

Keywords: Body weight, Dry microbial composition, Feed conversion ratio, Gut microbiota, Lactobacillus.

INTRODUCTION

Modern poultry production, including the breeding of commercial and ornamental bird species such as pheasants, requires the implementation of effective feeding strategies and approaches to maintaining flock health (Pennycott et al., 2012; Gheța et al., 2020). Romanian pheasants are valued both as a resource for hunting farms and for biodiversity enrichment; therefore, improving their productivity and viability remains an important goal (Gheța et al., 2020; Yakovets et al., 2022). One of the most promising approaches is the use of probiotic feed additives based on autochthonous microbial strains, which contribute to the optimization of gastrointestinal microbiota, enhancement of natural resistance, and improved nutrient digestibility (Maquiné et al., 2024; Idowu et al., 2025).

Attentions have been paid to microbial formulations containing lactobacilli strains isolated from the intestinal tract of birds (Betancur-Hurtado et al., 2022; Lysenko et al., 2025). Being adapted to the host species, such strains may exhibit more pronounced probiotic properties compared to allochthonous cultures. Their application can not only modulate gut microbiota but also exert systemic beneficial effects on growth performance, development, and overall physiological status of poultry (Xu et al., 2022, Sirisopapong et al., 2023). A comprehensive evaluation of their effects on productivity indicators and physiological status under controlled rearing conditions is therefore required (Fathima et al., 2022).

Despite extensive research on probiotics in poultry production, the optimal dosages, efficacy, and safety of complex microbial formulations based on autochthonous lactobacilli for pheasants remain insufficiently studied.

The aim of the present study was to evaluate the effects of a dry microbial composition based on a consortium of *Lactobacillus* strains isolated from the intestinal tract of pheasants on survival rate, growth performance, feed conversion ratio, hematological and biochemical blood parameters, as well as modulation of the gut microbiome of Romanian pheasants, in order to determine its optimal dosage and assess its efficacy and safety.

MATERIALS AND METHODS

Experimental studies on the target agricultural poultry were conducted at the Center for Preclinical Research of Veterinary Medicinal Products, while selected laboratory analyses, including microbiological assays, were performed at the Department of Veterinary Medicine of the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy.

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The object of the present study was a dry microbial preparation consisting of a consortium of two lyophilized lactobacilli strains, (*Loigolactobacillus coryniformis* VKM B-3724D and *Lactobacillus johnsonii* VKM B-3725D), combined in a 1:1 ratio. Both strains were originally isolated from the intestinal tract of pheasants and were uniformly dispersed throughout a prebiotic matrix composed of plant-derived polysaccharides. The biological model consisted of domesticated Romanian pheasants (Figure 1).

Ethical consideration

All experimental procedures involving vertebrate animals were approved by the Bioethics Commission of the Russian State Agrarian University – Moscow Timiryazev Agricultural Academy (Order No. 549 dated September 30, 2021; Protocol No. 65 dated November 14, 2025). All animal experiment was carried out in accordance with Directive 2010/63/EU (2010).



Figure 1 - Rearing of pheasants under laboratory conditions.

The experimental design is presented in Table 1. The duration of the experiment was 91 days. The experimental procedures in poultry were conducted in accordance with the guidelines developed by the All-Russian Research Institute of Poultry Farming (Egorov et al., 2013). The clinical status of birds in control and experimental groups was assessed daily by visual inspection, including evaluation of behavior, feather condition, feed intake, and drinking activity.

Body weight gain was monitored weekly by individual weighing using laboratory scales. Total weight gain over the experimental period was calculated as the difference between the initial body weight of day-old pheasants and their final body weight at 91 days of age. In cases of mortality, necropsy was performed to determine the cause of death, and survival rate was calculated for the entire experimental period. Feed intake was recorded daily, and feed conversion ratio (FCR) was calculated at the end of the experiment as the amount of compound feed consumed per 1.0 kg of body weight gain.

On day 91, morphological and biochemical blood analyses were performed. Biochemical parameters of blood serum were determined using an automated biochemical analyzer (Zybio EXC200) with veterinary reagent kits (DiaVetTest). The analysis of selected representatives of the intestinal microbiome in the cecal contents of pheasants was conducted at the end of the rearing period in accordance with established methodological guidelines (Ministry of Agriculture of the Russian Federation, 2004).

Table 1 - Experimental design for evaluating the effects of a dry microbial composition on Romanian pheasants

Group	Number of birds	Feeding and watering conditions
Control	35	Compound feed (CF) + drinking water
Experimental 1	35	CF + microbial composition at 0.5% of feed weight + drinking water
Experimental 2	35	CF + microbial composition at 1.0% of feed weight + drinking water
Experimental 3	35	CF + microbial composition at 1.5% of feed weight + drinking water

Statistical analysis

The obtained data were processed using standard methods of mathematical statistics applied in biology and medicine, employing Microsoft Office Excel 2019 software. Statistical significance was assessed using Student's *t*-test, and differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The results on survival rate, body weight, weight gain, feed intake, and feed conversion ratio (FCR) in pheasants are presented in Table 2 and Figure 2.

At the end of the experiment, the survival rate was 85.7% in the control group, 94.3% in experimental group 1, 100.0% in experimental group 2, and 97.1% in experimental group 3. Compared to the control, survival rate increased by 8.6%, 14.3%, and 11.4%, respectively. At the beginning of the experiment (day 1), the body weight of chicks was comparable across all groups. From day 7 onward, pheasants in experimental groups 2 and 3 showed significantly higher body weight compared to the control (by 4.4% and 5.7%, respectively; $P \leq 0.05$). Starting from day 14, all experimental groups demonstrated a statistically significant increase in body weight relative to the control. This trend persisted throughout the entire experimental period.

By day 21, body weight exceeded the control by 8.7%, 13.8%, and 20.1% in experimental groups 1, 2, and 3, respectively ($P \leq 0.001$). Similar patterns were observed at subsequent time points (days 28–84), with consistently higher values in all experimental groups. By the end of the experiment (day 91), the body weight in the control group reached 972.50 g, whereas it was significantly higher in experimental group 1 (998.17 g; +2.6%), experimental group 2 (1042.08 g; +7.2%), and experimental group 3 (1026.10 g; +5.5%) ($P \leq 0.001$). The average body weight gain over the experimental period was 950.21 g in the control group, compared to 975.32 g, 1019.56 g, and 1004.61 g in experimental groups 1, 2, and 3, respectively. This corresponds to an increase of 2.64%, 7.29%, and 5.72% relative to the control.

The average feed intake per bird was 7431 g in the control group and 7549 g, 7645 g, and 7812 g in experimental groups 1, 2, and 3, respectively, exceeding the control values by 1.59%, 2.88%, and 5.13%. Feed conversion ratio (FCR) was 7.82 kg/kg in the control group and decreased to 7.74, 7.49, and 7.77 kg/kg in experimental groups 1, 2, and 3, respectively, corresponding to improvements of 1.02%, 4.22%, and 0.64%.

The results of hematological and biochemical blood analyses (Table 3) indicated no adverse effects of the microbial composition on the physiological status of pheasants. Morphological blood parameters remained within ranges comparable to the control group, with no statistically significant deviations. The erythrocyte count in the control group was 2.49×10^{12}/L, while in the experimental groups it increased by 4.0–8.0%, reaching a maximum of 2.69×10^{12}/L in experimental group 3. Hemoglobin concentration ranged from 92.07 g/L in the control group to 94.61 g/L in experimental group 3 (+2.8%). Leukocyte levels increased slightly from 17.69×10^9/L in the control group to 18.12×10^9/L in experimental group 3 (+0.9–2.4%). Hematocrit values varied between 33.81% and 35.24%, with no statistically significant differences between groups. These findings are in agreement with Wang et al. (2023) on Lingnan yellow chicken which increases specific immunoglobulin level after supplementation of *Lactobacillus plantarum*. Also, present findings are in agreement with Akter et al. (2023) reports for catfish which increases in blood cell, white blood cell and lymphocyte counts were observed in *Lactobacillus acidophilus* supplemented group.

The analysis of the leukocyte differential revealed no pathological changes. The proportion of lymphocytes in the control group was 65.16%, while in the experimental groups it ranged from 65.94% to 66.75%, showing a slight increase compared to the control. The monocyte content decreased from 9.74% in the control group to 8.63–8.87% in the experimental groups. The proportion of heterophils remained consistent across all groups (24.58–25.20%). Eosinophils and basophils were not detected in any samples.

The biochemical profile of blood serum also showed no statistically significant differences between groups. Alanine aminotransferase (ALT) activity was 11.76 U/L in the control group and ranged from 11.69 to 13.28 U/L in the experimental groups. Aspartate aminotransferase (AST) activity varied between 177.14 and 182.08 U/L, remaining close to the control value (178.48 U/L). Alkaline phosphatase levels were comparable across all groups (2470.91–2493.10 U/L). Serum cholesterol concentration slightly decreased in the experimental groups (2.99–3.03 mmol/L) compared to the control (3.10 mmol/L). A similar trend was observed for glucose, which decreased from 17.96 mmol/L in the control group to 17.63–17.70 mmol/L in the experimental groups. In contrast, total protein content increased from 29.76 g/L in

the control group to 30.24–31.19 g/L in the experimental groups, with the highest value recorded in experimental group 3. Total bilirubin levels ranged from 6.66 to 7.13 µmol/L, remaining within the control range. In the study of Eglite et al. (2023) serum total cholesterol decreased following supplementation of *lactobacillus farciminis* and *lactobacillus rhamnosus.* Present finding on pheasants is in agreement with Eglite et al. (2023) report.

Mineral metabolism parameters were also comparable across groups, with no statistically significant differences. Calcium levels ranged from 2.34 to 2.48 mmol/L, while phosphorus concentrations increased slightly from 1.94 mmol/L in the control group to 2.01–2.05 mmol/L in the experimental groups. Overall, the use of the dry microbial composition did not adversely affect hematological or biochemical blood parameters in pheasants. The observed trends indicate a normal physiological status of the birds. The results of the intestinal microbiota analysis in pheasants are presented in Figure 3.



Figure 2 - Weighing of pheasants using laboratory scales

Table 2 - Survival rate, body weight dynamics, weight gain, and feed utilization in Romanian pheasants (n = 35, M ± m)

Parameter \ Groups	Control	Experimental 1	Experimental 2	Experimental 3
Survival rate, %	85.7	94.3	100.0	97.1
Relative to control, %	–	+8.6	+14.3	+11.4
Body weight dynamics, g (M ± m)				
Day 1	22.29±0.33	22.85±0.33	22.52±0.32	21.49±0.32
Day 7	39.24±0.49	39.98±0.51	40.97±0.48*	41.46±0.50**
Day 14	85.43±0.56	87.74±0.46**	92.41±0.93***	95.67±0.90***
Day 21	133.72±1.16	145.31±1.25***	152.14±1.48***	160.59±1.35***
Day 28	192.08±2.08	209.13±2.24***	217.06±1.80***	224.58±2.20***
Day 35	335.59±2.33	343.49±2.17*	354.09±2.14***	353.42±2.21***
Day 42	432.38±1.68	438.64±1.63*	450.16±1.57***	457.70±1.63***
Day 49	512.47±1.83	525.86±1.88***	537.90±2.54***	546.87±2.64***
Day 56	614.12±2.16	621.58±2.49*	647.76±2.02***	657.11±2.71***
Day 63	700.36±3.47	711.56±2.89*	731.90±3.00***	739.13±2.32***
Day 70	760.13±3.30	771.30±3.21*	786.87±2.42***	793.64±3.31***
Day 77	807.70±3.77	824.12±3.05*	850.19±3.53***	862.61±4.12***
Day 84	905.71±3.13	925.72±3.22***	948.25±3.48***	954.11±3.94***
Day 91	972.50±4.15	998.17±4.79***	1042.08±5.36***	1026.10±6.25***
Body weight gain over the rearing period (Day 0–91)				
Average per bird, g	950.21	975.32	1019.56	1004.61
Relative to control, %	–	+2.64	+7.29	+5.72
Feed intake over the rearing period (Day 0–91)				
Average per bird, g	7431	7549	7645	7812
Relative to control, %	–	+1.59	+2.88	+5.13
FCR, kg/kg	7.82	7.74	7.49	7.77
Relative to control, %	–	−1.02	−4.22	−0.64

Note: Differences compared to the control group are statistically significant at * – P≤0.05, ** – P≤0.01, *** – P≤0.001.

Table 3 - Hematological and biochemical parameters of Romanian pheasants (n = 10, M ± m)

Parameter \ Groups	Control	Experimental 1	Experimental 2	Experimental 3
Hematological parameters				
Erythrocytes, ×10¹²/L	2.49±0.07	2.59±0.08	2.66±0.09	2.69±0.08
Hemoglobin, g/L	92.07±0.92	92.47±1.19	94.40±0.78	94.61±0.88
Leukocytes, ×10⁹/L	17.69±0.21	17.88±0.15	17.85±0.16	18.12±0.15
Hematocrit, %	34.17±0.91	33.81±0.53	35.24±0.57	35.04±0.73
Lymphocytes	65.16±0.69	66.23±0.96	66.75±0.64	65.94±1.08
Monocytes	9.74±0.96	8.63±1.35	8.67±0.85	8.87±1.06
Heterophils	25.20±0.67	25.14±0.79	24.58±0.85	25.19±0.65
Eosinophils	0	0	0	0
Basophils	0	0	0	0
Biochemical parameters				
ALT, U/L	11.76±0.56	13.28±0.53	11.69±0.32	12.40±0.47
AST, U/L	178.48±3.33	181.11±2.43	177.14±3.48	182.08±3.01
Alkaline phosphatase, U/L	2491.57±58.19	2470.91±62.56	2493.10±60.15	2480.60±61.20
Cholesterol, mmol/L	3.10±0.12	3.03±0.13	3.01±0.11	2.99±0.16
Glucose, mmol/L	17.96±0.23	17.66±0.29	17.70±0.18	17.63±0.14
Total protein, g/L	29.76±0.59	30.24±0.58	30.86±0.84	31.19±0.55
Total bilirubin, µmol/L	6.83±0.23	6.66±0.21	6.94±0.20	7.13±0.19
Calcium, mmol/L	2.36±0.07	2.47±0.07	2.34±0.09	2.48±0.06
Phosphorus, mmol/L	1.94±0.05	2.04±0.03	2.05±0.05	2.01±0.03

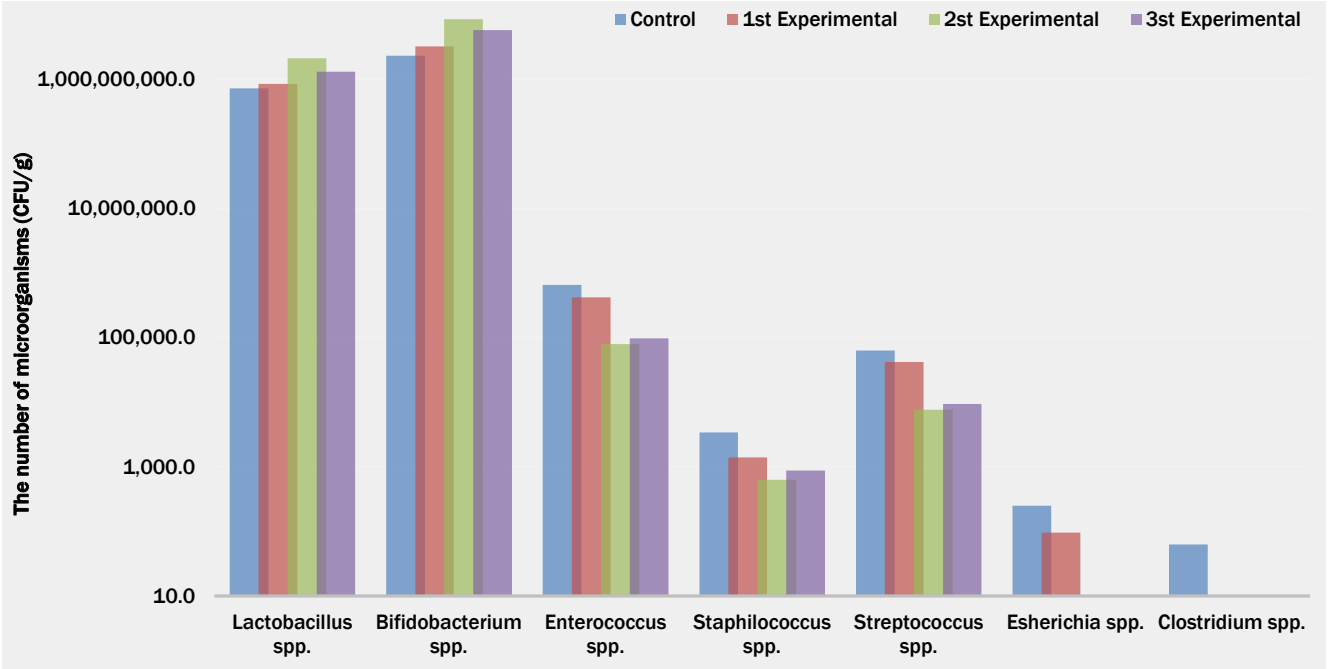


Figure 3 - Microbiological analysis of cecal contents in Romanian pheasants (n = 5, M ± m)

The results of the microbiological analysis of cecal contents demonstrated a pronounced effect of the microbial composition on both the quantitative and qualitative structure of the intestinal microbiome. Similar findings are reported for other bird species such as Guinea Fowls (Galosi et al., 2021) and Shaoxing duck (Sun et al., 2022). In all experimental groups, a significant increase in indigenous microbiota was observed. The abundance of *Lactobacillus* spp. increased by 1.1-fold in experimental group 1 ($P \leq 0.01$), 2.9-fold in experimental group 2 ($P \leq 0.001$), and 1.8-fold in experimental group 3 ($P \leq 0.001$) compared to the control group. Similarly, *Bifidobacterium* spp. increased by 1.4-fold ($P \leq 0.05$), 3.6-fold ($P \leq 0.001$), and 2.4-fold ($P \leq 0.001$) in experimental groups 1, 2, and 3, respectively.

A key outcome of the application of the dry microbial composition was a statistically significant reduction in opportunistic microorganisms. This effect is likely associated not only with the probiotic activity of the lactobacilli strains included in the formulation but also with the increased abundance of beneficial microbiota (*Lactobacillus* spp. and *Bifidobacterium* spp.), which are known to produce biologically active compounds with antagonistic activity against opportunistic and pathogenic microorganisms in the gastrointestinal tract.

Specifically, the abundance of *Enterococcus* spp. decreased by 1.5-fold in experimental group 1 ($P \leq 0.01$), 8.2-fold in experimental group 2 ($P \leq 0.001$), and 6.7-fold in experimental group 3 ($P \leq 0.001$) compared to the control. A significant reduction in *Staphylococcus* spp. was also observed: from $3.4 \pm 0.1 \times 10^3$ CFU/g in the control group to $1.4 \pm 0.2 \times 10^3$ CFU/g, $6.3 \pm 0.3 \times 10^2$ CFU/g, and $8.8 \pm 0.2 \times 10^2$ CFU/g in experimental groups 1, 2, and 3, respectively, corresponding to decreases of 2.4-fold ($p \leq 0.05$), 5.4-fold, and 3.8-fold ($P \leq 0.001$). The level of *Streptococcus* spp. in the cecal chyme of the control group ($6.3 \pm 0.4 \times 10^4$ CFU/g) was significantly higher than in the experimental groups by 1.5-fold ($P \leq 0.05$), 8.2-fold, and 6.7-fold ($P \leq 0.001$), respectively. The findings are in agreement with Perera et al. (2024) for preventing microbial infection in birds (by supplemented special probiotic), and also in agreement with Saeed et al. (2025) for evaluating new Lactiplantibacillus based nutritional strategies. Notably, complete elimination of certain opportunistic and pathogenic microorganisms, including *Escherichia* spp. and *Clostridium* spp., was observed in experimental groups 2 and 3. In the control group, these microorganisms were detected, while in experimental group 1, *Escherichia* spp. were present but at levels 2.6-fold lower than in the control ($P \leq 0.05$). Representatives of *Pseudomonas* spp. were not detected in any of the groups.

CONCLUSION AND RECOMMENDATION

The results of the present study demonstrate that the inclusion of a dry microbial composition in the diet of Romanian pheasants exerts a positive effect on productivity, physiological status, and modulation of the intestinal microbiota. The most pronounced effects were observed in the group receiving the microbial additive at a dosage of 1.0% of compound feed (experimental group 2), where survival rate reached 100%, final body weight increased by 7.2% ($P \leq 0.001$), body weight gain increased by 7.3%, and feed conversion ratio improved by 4.2% compared to the control. Additionally, the abundance of beneficial microbiota, particularly *Lactobacillus* spp. and *Bifidobacterium* spp., increased by 2.9- and 3.6-fold, respectively ($P \leq 0.001$). Hematological and biochemical blood parameters in all experimental groups remained within the physiological range and did not differ significantly from the control values, confirming the safety and good tolerability of the microbial composition. Overall, the developed dry microbial composition is recommended for practical application in Romanian pheasant production at a dosage of 1.0% of compound feed, as it ensures improved production performance with reduced feed costs.

DECLARATIONS

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Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution
A.V. Luneva, Y.A. Lysenko, and E.Y. Marchenko contributed to the research, data analysis, and manuscript writing.
Conceptualization, data curation and funding acquisition: A.V. Luneva.
Formal analysis: Y.A. Lysenko, E.Y. Marchenko.
Methodology: Y.A. Lysenko, E.Y. Marchenko.
Resources, Software and Visualization: E.Y. Marchenko.
Supervision: A.V. Luneva, Y.A. Lysenko.
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Investigation, Project administration, Validation, Writing – review & editing: Y.A. Lysenko.

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Competing interests
The authors declare no competing interests in this research and publication.

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PHYSIOLOGICAL RESPONSES OF SHEEP'S MILK TO FORAGE-BASED DIETS PRODUCED USING TREATED WASTEWATER

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ABSTRACT: The study aimed to investigate the effects of different feeding strategies and irrigation methods of forage on sheep milk yield and composition. Specifically, it evaluated the impact of alfalfa (*Medicago sativa* L.) and guinea grass (*Panicum maximum*)-based diets irrigated with either treated wastewater (TWW), after physical, biological, and chemical methods to remove contaminants or fresh water. 30 lactating ewes were assigned to different feeding groups, and milk yield and composition parameters including fat, solids-not-fat (SNF), lactose, protein, and total solids were analyzed over a 25-day period. Results indicated that while milk yield remained stable across feeding groups, significant differences ($P \leq 0.05$) were observed in milk composition. The highest milk yield (3.5 kg/day) was recorded in ewes supplemented with 100% *M. sativa* irrigated with TWW, while the lowest yield (1.5 kg/day) was observed in the 50% straw irrigated with freshwater treatment. Milk fat percentage and lactose did not significantly differ between feeding treatments, meanwhile SNF, density, total solids, and protein content were significantly affected. Ewes consuming *Panicum maximum* irrigated with TWW had higher SNF and protein levels, suggesting a nutritional advantage of drought-resistant grasses in dairy production. A significant decline ($P \leq 0.05$) in milk fat percentage was observed over time, particularly in ewes consuming 100% *M. sativa* irrigated with TWW. These findings suggest that feeding strategy plays a more decisive role in milk composition than irrigation type, with *P. maximum* improving SNF and protein levels. Irrigated with treated wastewater did not negatively impact milk yield or major composition parameters, indicating its potential as a sustainable irrigation source for forage plants.

Keywords: Feeding strategies, Medicago sativa, Panicum maximum, Sheep, Wastewater.

INTRODUCTION

Milk production and quality are key determinants of both livestock management efficiency and the long-term sustainability of the dairy industry (Priyashantha, 2025). Among the many factors influencing milk yield and composition, feeding strategies play a particularly important role (Mukasafari et al., 2025). The choice of forage type, along with its cultivation conditions, impacts not only the efficiency of milk production but also its nutritional composition (Casper et al., 2025). However, some studies have explored how different forage species, particularly alfalfa, *Medicago sativa* L. and guinea grass, *Panicum maximum*, influence milk composition when irrigated with treated wastewater (TWW) (Guzatti et al., 2018; Neto et al., 2018). Understanding these interactions is crucial for optimizing dairy production in water-scarce regions while promoting sustainable agricultural practices.

The *M. sativa* is widely recognized as a high-quality forage legume, often referred to as the "queen of forages" due to its high protein content, digestibility, and drought resistance. It is extensively used in ruminant feeding systems to improve milk yield and quality. Studies have reported that *M. sativa*-based diets can enhance milk composition, particularly fatty acid profiles, without significantly altering milk yield (Babiker et al., 2017; Guzatti et al., 2018). On the other hand, *P. maximum* is a perennial C4 grass known for its high biomass yield and adaptability to tropical and subtropical climates. Studies have indicated that *P. maximum*-based diets can influence milk yield and composition, though their impact on specific milk parameters remains underexplored (De Souza et al., 2014; Neto et al., 2018). Despite their agronomic advantages, comparative studies on the effects of *M. sativa*- and *P. maximum*-based diets on milk composition are limited, particularly under different irrigation systems considering indigenous sheep such as Awassi sheep (Al-Atiyat et al., 2012 and 2014).

Water scarcity is a major constraint in forage production for small ruminants, leading to an increasing reliance on alternative irrigation sources, such as TWW (Benaafi et al., 2024). The reuse of TWW in agriculture has gained attention as a sustainable solution to freshwater shortages, providing essential nutrients for plant growth while reducing environmental pollution (Ganjegunte et al., 2017; El Moussaoui et al., 2019). Previous studies have confirmed that wastewater irrigation enhances soil fertility and forage biomass production (Mohammad and Mazahreh, 2003; Ganjegunte et al., 2017), but concerns remain regarding potential heavy metal accumulation, microbial

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contamination, and effects on animal health (Sdiri et al., 2023). Despite the impacts of TWW on plant physiology and soil health have been widely studied, its effects on milk production and composition remain largely unexplored. Addressing this gap is essential to determine the feasibility of TWW-irrigated forage as a viable feed source in dairy production.

This study investigates the effects of different feeding strategies and irrigation methods on the milk yield and composition of sheep. Specifically, it evaluates how *M. sativa*- and *P. maximum*-based diets irrigated with TWW or freshwater influence key milk parameters, including milk' yield, fat percentage, lactose, solids-not-fat (SNF), protein content, total solids and density. It is hypothesized that forage type may significantly affect milk composition, while wastewater irrigation may indirectly influence these parameters through its impact on forage nutrient content. By integrating agronomic and animal performance perspectives, this research offers new insights into sustainable feeding practices of small ruminants, particularly for regions facing water scarcity and increasing dependence on wastewater irrigation.

MATERIALS AND METHODS

Experimental site and ethical considerations

This study was carried out in National Agriculture Research Center of Ministry of Agriculture, Palestine from January to November 2021. This study was approved by the Institutional Review Board (IRB) / ethical committee at An-Najah National university, Nablus, Palestine. The approval letter (No. IRB-12/2021) ensures that the procedures for sample collection, sheep feeding trials, and wastewater handling conform to safety and welfare regulations.

Feeding trial

Two experimental fields were established for the cultivation of *M. sativa* and *P. maximum* under TWW and freshwater irrigation. Three random samples were collected to enable a comparative assessment of nutritional composition, including dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude fat, and mineral content (calcium and phosphorus). These parameters provided a comprehensive assessment of the productivity and nutritional value of the forages, enabling a direct comparison between TWW-irrigated and fresh water-irrigated crops. The wastewater was tertiary treated by physical, biological, and chemical methods to remove contaminants and then disinfection was made. A total of 30 lactating ewes (initial body weight: 43.2 ± 0.97 kg, age: 4 years old), each nursing a single lamb of third party, were selected for the feeding trial. To ensure reproductive synchronization, ewes underwent estrous synchronization using intravaginal sponges. The animals were then randomly assigned to five experimental dietary groups (n = 10 ewes per group) as follows: group 1: Control diet (straw as a traditional ration used in local ewe farms), group 2: 50% of traditional roughage replaced with *M. sativa*, group 3: 100% of traditional roughage replaced with *M. sativa*, group 4: 50% of traditional roughage replaced with *P. maximum*, and group 5: 100% of traditional roughage replaced with P. maximum.

The ewes were individually housed in shaded pens (0.75 × 1.5 m), each equipped with plastic feed and water buckets. Diets were formulated according to NRC (NRC, 2007) guidelines to meet the nutritional requirements of lactating ewes. Feed was offered ad libitum at 08:00 AM with a 10% refusal margin, and fresh water was available at all times. Nutrient intake was daily recorded, and lambs relied exclusively on their dams' milk for nourishment during the trial. At the end of the experimental period, lambs were gradually transitioned to concentrate- and roughage-based diets to initiate the weaning process. The feeding trial lasted eight weeks, with the first week serving as an adaptation period and data collection were conducted during the subsequent seven weeks. Feed refusals were weighed, recorded, and sampled daily to determine nutrient intake accurately. The ewe body weight was measured at the beginning of the trial and thereafter bi-weekly. A digestion trial was conducted at the end of the feeding study. Three ewes from each dietary group were randomly selected for the digestion experiment. A one-week adaptation period was followed by a 7-day total fecal collection period to estimate nutrient digestibility. Fecal samples were collected, weighed, and analyzed for DM, CP, fiber fractions (NDF, ADF), and ether extract to determine nutrient utilization efficiency. The treatments were: 1) STRW1: 100% straw irrigated with fresh water; 2) STRW2: 50% straw irrigated with fresh water; 3) FST50: 50% *M. sativa* irrigated with TWW, 50% straw; 4) FT100: 100% *M. sativa* irrigated with TWW; 5) FFW50: 50% *M. sativa* irrigated with fresh water, 50% straw; 6) FFW100: 100% *M. sativa* irrigated with fresh water; 7) PST50: 50% P. maximum irrigated with treated water, 50% straw; 8) FSFW50: 50% *P. maximum* irrigated with fresh water, 50% straw, and 9) PFW100: 100% *P. maximum* irrigated with fresh water.

Milk yield and composition analysis

Milk yield was recorded bi-weekly at 08:00 AM using hand-milking techniques. To standardize the process, lambs were separated from their dams 12 hours before milking. The recorded yield was extrapolated to a 24-hour basis for accurate comparison. From each ewe, a 125 ml milk sample was collected and analyzed for total solids by drying at 55°C in a forced-air oven. Milk fat content was determined using the Gerber method, while CP was calculated via the Kjeldahl procedure using a nitrogen-to-protein conversion factor of N × 6.38.

Chemical analysis of diets and feed refusals

To determine the chemical composition of diets and feed refusals, samples were processed as follows: for the DM the samples were oven-dried at 105°C overnight (AOAC, 2006). The CP was measured using the Kjeldahl method (N × 6.25 conversion factor). The NDF and ADF were analyzed using an ANKOM2000 fiber analyzer. Ether extract (fat content) was determined using the Soxtec procedure, while the metabolizable energy (ME) was estimated using NRC (NRC, 2007) tabular values. The DM and nutrient intakes were calculated by subtracting feed refusals from total feed offered.

Statistical analysis

The statistical analysis was performed using the SAS statistical software package (version 9.2) (SAS, 2012). Data normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. As the data did not follow normal distribution, a non-parametric test was applied. Comparisons of milk yield and composition among feeding strategy groups were conducted using the least significant difference (LSD) test at a probability level of $P \leq 0.05$ (Zar, 1999).

RESULTS

The chemical composition of forage grown under different irrigation methods is presented (Table 1). The main difference between *M. sativa* (alfalfa) and *P. maximum* (Guinea grass) lies in their CP content. The *M. sativa* contains a high protein level (23.6-24.8% of DM), whereas *P. maximum* provides a moderate protein content (11.5-13.9% of DM). Other components, such as crude fiber, NDF, and ADF, are also reported (Table 1).

The milk yield and composition (mean ± SEM) of lactating ewes under different feeding strategies and irrigation methods (Table 2). Milk yield varied significantly among feeding treatments ($P = 0.001$), ranging from 1.5 to 3.5 kg per ewe per day. The highest yield (3.5 kg/day) was recorded in ewes supplemented with FT100, while the lowest yield (1.5 kg/day) was observed in the STRW2 treatment. These findings suggest that partially replacing straw under freshwater irrigation with *M. sativa* under TWW irrigation improved milk yield by approximately 2.5-fold, potentially due to enhanced digestibility or reduced metabolic imbalances. In contrast, no significant differences were observed between ewes fed forages irrigated with fresh water and those irrigated with TWW, indicating that irrigation source alone had no effect on total milk yield. Rather, the composition of the diet appeared to play the dominant role in determining production levels.

Milk composition analysis showed considerable variation in fat percentage, SNF, density, lactose, solids, and protein content across treatments. Fat percentage ranged from 4.10% to 9.09%, with the highest values observed in ewes receiving the FT100 treatment, followed by those supplemented with *P. maximum* irrigated with FSFW50 (8.43%) and PST50 (8.42%). The lowest fat percentage (4.10%) was recorded in ewes supplemented with PFW100, suggesting that legume-based diets may be associated with reduced milk fat content. Nevertheless, differences in fat percentage among feeding treatments were not statistically significant ($P = 0.089$), indicating that factors beyond diet composition, such as metabolic adaptations, may play a role in regulating milk fat content over time (Table 2).

The SNF content differed significantly among feeding treatments ($P = 0.029$), ranging from 8.41 to 9.62. The highest SNF values were observed in ewes supplemented with STRW2, suggesting a potential link between fiber-rich diets and elevated SNF levels, while the lowest values were recorded in ewes fed FSFW50. Milk density also varied significantly ($P = 0.040$), with the highest values in ewes fed FFW50 (30.93) and STRW2 (30.84), and the lowest in those fed FSFW50 (26.26). In contrast, lactose content remained relatively stable across treatments (4.87–5.50%), with no significant differences detected ($P = 0.470$), indicating that dietary interventions had little influence on lactose synthesis. Total solids were significantly affected by diet ($P = 0.030$), ranging from 0.73 to 1.12, with STRW2 showing the highest values and FFW50 the lowest. Similarly, protein percentage varied significantly ($P = 0.036$), with the highest levels in STRW2 (3.17%) and FFW50 (3.16), while FSFW50 yielded the lowest protein content (2.41%). These findings highlight the role of forage type and diet composition in shaping milk quality traits, particularly SNF, density, total solids, and protein content (Table 2).

The results show that four parameters, i.e. milk yield, fat, total solids, and protein content were significantly affected ($P < 0.05$) by both feeding strategy and irrigation method (Figure 1). The LS-means test indicated that ewes fed alfalfa irrigated with treated water (FT100) produced a significantly higher milk yield. A similar trend was observed for milk fat, with the highest fat content also recorded under the FT100 strategy. For total solids, the STRW2 strategy resulted in significantly higher values. Regarding protein content, the highest levels were reported for STRW2, FFW50, and FT100 feeding strategies (Figure 1).

Table 1 - Chemical composition of offered fodder of different irrigation methods.

Feed type	Sample replicate	Fiber (%)	Moisture	Ash (%)	Protein (%)	ADF (%)	NDF (%)
M. sativa fresh water irrigated	1	14.1	82.0	10.06	24.8	19.7	24.6
M. sativa fresh water irrigated	2	14.5	82.1	10.0	24.2	20.2	25.3
M. sativa fresh water irrigated	3	15.2	83.0	10.1	23.9	21.2	26.5
M. sativa TWW irrigated	1	19.1	81.2	10.3	23.6	26.7	33.4
M. sativa TWW irrigated	2	18.2	81.0	10.1	23.96	25.4	31.8
M. sativa TWW irrigated	3	18.6	81.1	10.12	24.6	26.02	32.5
P. maximum fresh water irrigated	1	16.0	82.0	15.8	11.5	22.4	28.0
P. maximum fresh water irrigated	2	16.1	82.1	16.9	11.8	22.5	28.17
P. maximum fresh water irrigated	3	17.0	81.1	16.0	12.09	23.6	29.7
P. maximum TWW irrigated	1	13.0	84.0	14.3	12.8	18.0	22.7
P. maximum TWW irrigated	2	13.1	82.0	14.0	13.0	18.3	22.9
P. maximum TWW irrigated	3	13.2	83.1	14.5	13.9	18.4	23.1

TWW: treated wastewater; ADF: acid detergent fiber; NDF: neutral detergent fiber,

Table 2 - Milk yield and composition (mean ± SEM) of lactating ewes fed under different feeding strategies and irrigation methods.

Treatment	Yield	Fat content (%)	SNF (%)	Total solids (%)	Lactose (%)	Protein (%)
FFW100	2.00±0.19^{b}	6.71±0.78^{bc}	8.87±0.38	28.20±1.70	5.10±0.21	2.56±0.15^{c}
FFW50	2.00±0.19^{b}	7.39±0.78^{b}	9.36±0.38	30.93±1.70	5.27±0.21	3.16±0.15^{a}
FSFW50	3.00±0.19^{a}	8.43±0.78^{a}	8.41±0.38	26.26±1.70	4.87±0.21	2.41±0.15^{d}
FST50	3.00±0.19^{a}	6.15±0.78^{c}	8.67±0.38	28.58±1.70	4.89±0.21	2.93±0.15^{b}
FT100	3.50±0.19^{a}	9.09±0.78^{a}	8.95±0.38	28.26±1.70	5.16±0.21	3.09±0.15^{a}
PFW100	2.60±0.13^{ab}	4.10±0.55^{d}	8.90±0.27	29.83±1.21	4.98±0.15	2.92±0.11^{b}
PST50	2.00±0.19^{b}	8.42±0.78^{a}	8.94±0.38	28.20±1.70	5.16±0.21	2.90±0.15^{b}
STRW1	2.00±0.19^{b}	5.98±0.78^{c}	9.25±0.38	28.99±1.70	5.35±0.21	2.98±0.15^{b}
STRW2	1.50±0.19^{c}	5.77±0.78^{c}	9.62±0.38	30.84±1.70	5.50±0.21	3.17±0.15^{a}
P-Value	*: 0.001	*: 0.0007	NS: 0.520	NS: 0.63	NS: 0.408	*: 0.0199

FFW100: 100% *M. sativa* irrigated with fresh water; FFW50: 50% *M. sativa* irrigated with fresh water, 50% straw; FSFW50: 50% *P. maximum* irrigated with fresh water, 50% straw; FST50: 50% *M. sativa* irrigated with TWW, 50% straw; FT100: 100% *M. sativa* irrigated with TWW; PFW100: 100% *P. maximum* irrigated with fresh water; PST50: 50% *P. maximum* irrigated with treated water, 50% straw; STRW1: 100% straw irrigated with fresh water; STRW2: 50% straw irrigated with fresh water; SNF solids-not-fat.***:** Indicates significant differences among the different treatments with the same column (LSD, $p \leq 0.05$). **NS:** Indicates no significant differences among the different treatments with the same column (LSD, $p > 0.05$).

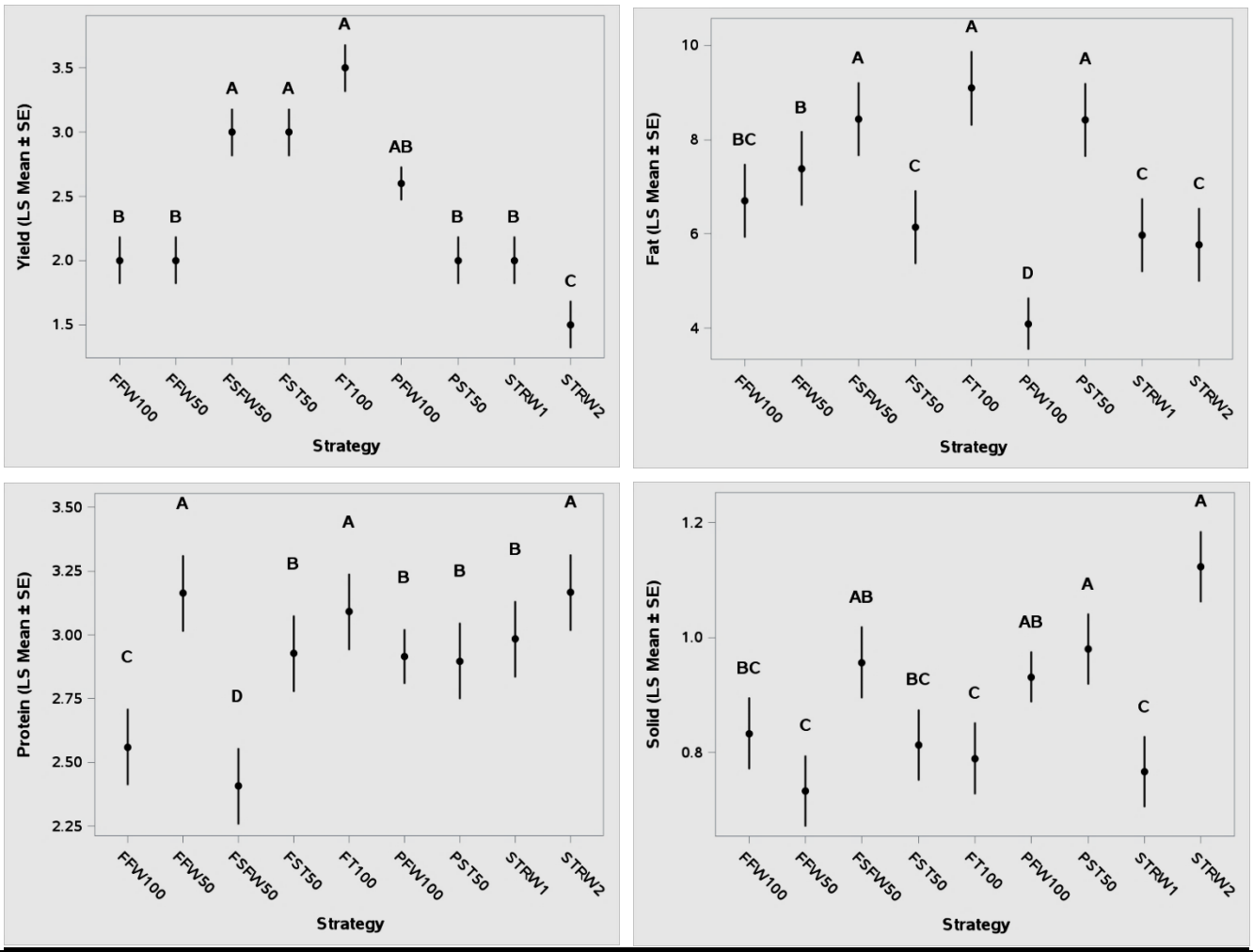


Figure 1 - Long-term effects of different feeding strategies on sheep milk yield and composition. FFW100: 100% *M. sativa* irrigated with fresh water; FFW50: 50% *M. sativa* irrigated with fresh water, 50% straw; FSFW50: 50% *P. maximum* irrigated with fresh water, 50% straw; FST50: 50% *M. sativa* irrigated with TWW, 50% straw; FT100: 100% *M. sativa* irrigated with TWW; PFW100: 100% *P. maximum* irrigated with fresh water; PST50: 50% *P. maximum* irrigated with treated water, 50% straw; STRW1: 100% straw irrigated with fresh water; STRW2: 50% straw irrigated with fresh water

DISCUSSION

The findings of this study provide essential insights into the effects of different feeding strategies and irrigation methods on milk yield and composition in sheep. Over the 25-day experimental period, specific feeding combinations resulted in notable variations in milk composition, while overall milk yield remained largely stable. These results indicate that both diet composition and irrigation type can influence milk quality, particularly regarding fat content and total solids percentage.

It is important to first consider the general chemical composition of both forage species. Alfalfa (*M. sativa*) is notably richer in protein, bioactive compounds (such as isoflavones, phenolics, and saponins), vitamins, and minerals compared to Guinea grass (*P. maximum*) (Horvat et al., 2022). The protein content of *M. sativa* typically ranges between 18-25% of DM and is accompanied by higher concentrations of essential amino acids, particularly lysine. Moreover, its protein is more degradable in the rumen, although tannins and saponins present in the plant can influence its utilization (Liu et al., 2024). In contrast, *P. maximum* contains higher levels of carbohydrates and fiber, but lower protein concentrations (8-15% of DM), and it provides far fewer bioactive phytochemicals (Johnson et al., 1967). The NDF and ADF contents typically range from 35-45% and 25-35% for *M. sativa*, and 55-70% and 35-45% for *P. maximum*, respectively (Johnson et al., 1967; Horvat et al., 2022). Furthermore, the ME and TDN values are relatively similar between the two species, ranging from ~9-11 MJ/kg DM and ~55-65%, respectively (Kumara et al., 2022; Liu et al., 2024). Overall, these reported findings on the chemical composition of both species are consistent with the results obtained in our study.

The effects of *M. sativa*- and *P. maximum*-based diets on milk yield and composition have been widely studied in the literature for sheep and goat (Al-Araimi et al. 2017; Babiker et al. 2017 Guzatti et al. 2018). In this regard, Guzatti et al. (2018) stated that diets containing *M. sativa* and red clover silage did not significantly affect milk yield but altered the fatty acid profile of milk. Similarly, Babiker et al. (2017) found that the addition of Moringa leaves to *M. sativa*-based diets increased milk yield without and significant effect on the composition of milk. Consistent with these findings, our study demonstrates that *M. sativa*-based diets do not significantly impact milk yield but induce specific changes in milk components. Previous research on *P. maximum*-based feeding strategies also supports our results. De Souza et al. (2014) found that *P. maximum*-based diets increased milk yield but had no major effects on milk composition. Our findings align with this, indicating significant differences in milk fat content but minimal changes in milk yield when *P. maximum* was included in the diet. These results confirm that forage composition can influence milk quality, whereas its effect on milk production remains limited.

A key aspect of this study was the evaluation of TWW as an irrigation source for forage plants. Prior research has demonstrated that TWW can enhance soil nutrient levels and improve forage growth (Ganjegunte et al., 2017; El Moussaoui et al., 2019). However, Sdiri et al. (2023) suggested that TWW irrigation may lead to metal accumulation in the soil, potentially altering plant nutritional composition. Our findings indicate that sheep consuming TWW-irrigated forage exhibited variations in milk fat content, but no significant differences were observed in protein, lactose, or total solids percentages compared to sheep fed with fresh water-irrigated forage. These results are consistent with previous studies suggesting that TWW enhances forage nutritional content; however, further research is necessary to evaluate its potential long-term impact on milk quality.

This study represents one of the few investigations that simultaneously assess the effects of *M. sativa*- and *P. maximum*-based diets alongside TWW irrigation. While previous studies have focused on the effects of TWW on soil properties and plant growth (Bedbabis et al., 2014; Soufan et al., 2019), fewer studies have explored its influence on animal nutrition and milk production. By examining the combined effects of feeding strategies and irrigation methods, this study fills an important gap in literature. Moreover, Bevilacqua et al. (2014) and Ceci et al. (2023) have examined the effects of TWW-irrigated forage on animal health and milk safety. Our results indicate that the impact of TWW-irrigated forage on milk composition is limited, with no significant negative effects detected. However, long-term monitoring is necessary to assess the potential risks of metal accumulation and microbial contamination in milk.

Despite these valuable contributions, this study has some limitations. The small sample size (three animals per group), the specific geographic conditions, and the relatively short duration (25 days) may restrict the generalizability of the findings. Furthermore, the long-term effects of TWW irrigation on micronutrient profiles in forage were not fully assessed. Future research should address these limitations by focusing on the following points: 1) conducting studies with larger sample sizes to improve statistical power; 2) evaluating feeding strategies across diverse geographic regions to assess environmental impacts; 3) performing detailed chemical and microbiological analyses of milk composition to ensure food safety; 4) implementing long-term follow-up studies to monitor the potential effects of TWW irrigation on livestock health; and 5) investigating genetic and environmental factors that may influence milk yield and composition.

CONCLUSION

This study examined the effects of different feeding strategies and irrigation methods on sheep milk yield and composition. The results indicate that *M. sativa*- and *P. maximum*-based diets can influence milk composition, particularly

fat content and total solids, while overall milk yield remained largely unaffected. Regarding irrigation, no adverse effects on milk yield were observed in sheep consuming TWW-irrigated forage, though minor variations in milk composition were noted, suggesting that TWW may be a viable irrigation source for forage production. As one of the few studies assessing both feeding strategies and TWW irrigation, this work provides valuable insights into the interplay between animal nutrition, milk quality, and sustainable sheep production. Nevertheless, future research with larger sample sizes and longer durations is needed to fully evaluate the nutritional and safety implications of these practices. Overall, optimizing feeding strategies and selecting appropriate forage types and irrigation methods are critical for enhancing milk yield and composition while supporting sustainable small ruminant production and mitigating potential risks associated with TWW irrigation. However, further research is needed to assess long-term impacts on animal health and milk safety, particularly in regions where water scarcity necessitates alternative irrigation sources.

DECLARATIONS

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Data availability

The data are available upon request from corresponding author

Authors' contribution

All authors have read and approved the final version of the manuscript and agree to its submission to the journal.

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Ethical considerations

This study was ethically approved by the Institutional Review Board (IRB) and ethical committee at An-Najah National university, Nablus, Palestine (Approval No. IRB-12/2021).

Declaration of generative AI in scientific writing

The authors declare that no generative artificial intelligence (AI) tools were used in the writing and editing of this manuscript except for grammar correction, which was conducted under the authors' supervision. The authors are responsible for all intellectual contributions, data analysis, and interpretations this manuscript presents.

Competing interests

The authors declare no conflict of interest.

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IMPACT OF *Bacillus subtilis* AND *Bacillus licheniformis* SUPPLEMENTATION ON REPRODUCTIVE AND PRODUCTIVE QUALITIES OF SOWS

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ABSTRACT: In modern livestock management, biotechnological products, including feed additives, are widely utilized to enhance productivity. One such agent is probiotics, which have been employed for a long time in both human food and livestock. Their effects are based on various biological mechanism–including the reduction of intestinal acidity, inhibition of pathogenic microorganisms, and stimulation of the immune response. Unlike antibiotics, which are prohibited in animal feed, probiotic supplements are regarded as safe alternatives for maintaining animal health and prophylaxis disease. In particular, in the diets of sows, probiotics contribute to the improvement of overall physiological condition and stimulate the development of beneficial intestinal microflora. Consequently, there has been increasing interest in studying effective probiotic bacteria and developing advanced biotechnological preparations. Among promising candidates are probiotics based on *Bacillus subtilis* and *Bacillus licheniformis*, which have been evaluated in various animal species for their potential beneficial effects on host physiology. The use of such probiotic feed additives has demonstrated favorable effects in sows across different ages and physiological stages. The administration of this probiotic for both young and adult animals during periods of preparation for fertilization, as well as before and after farrowing, positively affected their reproductive function. Improvements were observed in indicators such as sexual reflex expression, fertilization rates, litter size, and the number of live births. Additionally, significant enhancements in growth, weight gain, and the survival of piglets up to two months of age were recorded. The optimal doses of probiotic use were determined to be 1.0% of the daily ration for young sows and 1.5% for adults. Therefore, the results of this study substantiate the inclusion of a probiotic supplement combining these two bacteria in the diets of sows to enhance their productivity and the quality of their offspring.

Keywords: Biotechnological products, Gastrointestinal microflora, Immune system, Probiotics, Sows, Reproductive capacity.

INTRODUCTION

Managing the reproductive function of sows to increase the production of meat in accordance with consumers demand is influenced by numerous factors. The reproductive cycle affects the overall condition of sows, the manifestation of estrus after farrowing and subsequent meat productivity. Therefore, intestinal nutrient absorption and immunity are of great importance and require an optimal balance of the intestinal microflora (Gardiner et al., 2020; Szabó et al., 2023). Such balance can be achieved through the administration of probiotics, defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (Kritas et al., 2015).

Probiotics exert their beneficial effects through various mechanisms, including the reduction of intestinal pH, competitive exclusion of pathogenic microorganisms, and modulation of the host immune response (Hu et al., 2021) Moreover, unlike antibiotics, which are banned in animal feed production in several countries, probiotic additives offeran alternative means of improving productivity and preventing disease in sows by promoting overall health and increasing beneficial microbial populations (Pereira et al., 2022). The use of probiotics, antimicrobial metabolites of beneficial microorganisms, or other biotechnological products represents a sustainable approach to enhance animal health and modulate intestinal function. This, in turn, may reduce reliance on antimicrobial agents and support globally to limit the spread of antimicrobial resistance (O'Connor et al., 2020).

According to Mazur-Kuśnirek et al. (2023), the weaning process can beparticularly stressful for piglets because it involves separation from the sow and a dietary transition from milk to plant-based feed. Such stress factors may adversely affect the immune system and disrupt the intestinal microbiota balance, leading to gastrointestinal disorders, infections and diarrhea. Zhu et al. (2022) reported that Lactobacillus species improve growth rates and reduce diarrhea incidence in piglets by enhancing nutrient digestibility and intestinal barrier function. Among available probiotics, Lactobacillus species are considered among the most promising alternatives and are regarded as safe substitutes for antibiotic use in pig husbandry. Hayakawa et al. (2016) also indicated that the most significant impact of probiotics on the intestinal microbiota and host animals occurs when they are included in the diet during stressful periods, such as

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weaning or early lactation. Likewise, Saladrigas-García et al. (2022) demonstrated that supplementing sow feed with probiotics particularly *Bacillus amyloliquefaciens* may benefit fertility. Daily feeding of *B. amyloliquefaciens was* associated with an increased number of weaned piglets. Strong correlations between the microbiota composition of sows and their piglets increased maternal imprinting, with effects persisting beyond weaning. Liu et al. (2025) reported that the intestinal microbiota might influence the return to estrus in sows after weaning through its effects on sex hormones, suggesting its potential for identifying sows with superior reproductive performance. Additionally, Chang et al. (2024) recommended supplementing sows with yeast-derived postbiotics from late pregnancy through lactation to help regulate the intestinal microbiota of both sows and piglets, thereby improving overall health and resilience during the weaning period.

In addition, the prohibition of antibiotics and zinc oxide, which is an extremely effective antimicrobial agent that plays a key role in alleviating intestinal pathologies and dysbiosis associated with piglet weaning, has stimulated the search for new alternative dietary interventions that support health and development. To date, most studies have focused on post-weaning diets. However, maternal dietary intervention offers a promising alternative. Supplement to sows during pregnancy and lactation with microbiological additives can promote intestinal development and improve offspring health before weaning, thereby enhancing productivity and resilience immediately after weaning and later in life (Kiernan et al. 2023).

A large group of probiotic bacteria is based on the *Bacillus* genus, among which *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus pumilus*, *Bacillus licheniformis* and *Bacillus clausii* are frequently studied. These species are of growing scientific interest and hold strong potential or industrial application. Moreover, probiotic spore-forming agents such as *B. subtilis* and *B. licheniformis* are ideal model microorganisms capable of surviving stabilization methods used in producing powdered products, such as lyophilization or drying, including cell dehydration. Modern trends in probiotic delivery involve various microencapsulation techniques, which can considerably increase cell viability during freezing or drying processes (Łubkowska et al., 2023).

According to Li et al. (2025), supplementation with *B. subtilis* and *B. licheniformis* significantly reduced the number of low–birth-weight piglets and the incidence of diarrhea and increased the frequency of successful weaning. These outcomes were accompanied by higher nutrient and immunoglobulin levels in colostrum. In addition, probiotic supplement modulated the microbiota composition of both the sows and their piglets, promoting vertical transmission of beneficial bacteria such as Verrucomicrobiota and Akkermansia. Moreover, the probiotic supplement improved the expression of glucose and amino acid transporters in the placenta and enhanced the activity of the mTOR signaling pathway.

The search for new biotechnological products and probiotic formulations remains relevant in Ukraine. A new domestically developed probiotic supplement used in the present study contains *B. subtilis* and *B. licheniformis* along with the enzymes protease and lipase. Its effectiveness in animals of different species has been under investigation Sidashova et al. (2022), used this probiotic in in bee feed and found that it increased the survival of bees during wintering. Pogranichniy et al. (2023) reported that feeding this probiotic to black-and-brown dairy cows improved not only milk yield, fat content, and general health, but also enhanced stress resistance and serum biochemical parameters. According to Kananykhina and Turpurova (2024) proteases help animals absorb nutrients more efficiently from feed. The authors suggest that this enzyme-probiotic complex exerts a multifaceted effect on the digestive tract, particularly in the rumen. Tsvihun et al. (2025) further revealed that the use of this probiotic in feeding dairy *Lacaune* lambs led to a 16.1% increase in average daily weight gain, a 15.8% rise in total protein content, and a 35.4% increase in globulin levels, indicating enhanced immune resistance.

According to the review of literatures, no available studies have yet been conducted on the use of probiotic supplements in sows during farrowing and lactation. This gap has generated interest in investigating the effects of a therapeutic and prophylactic probiotic supplement containing *Bacillus subtilis* and *Bacillus licheniformis* on the reproductive performance of sows of different physiological stages.

MATERIALS AND METHODS

Materials

At the private agricultural enterprise "Techmet-Yug", 30 young pigs (8 months old, 120-125 kg) and 30 adult sows (2.5-3 years old, 190-200 kg) were selected for the study. Animals were divided into three groups for each category (young pigs and adult sows), with 10 animals per group, based on the principle of analogues. The study was conducted in two stages, as depicted in Figure 1.

Components of probiotic supplement

One kilogram of the preparation contained the following active ingredients: *B. subtilis* and *B. licheniformis* bacteria, with a minimum concentration of 6×10^{12} CFU/kg (6×10^{9} CFU per g); xylanase, 300,000 units/kg; protease, 5,000 units/kg; and amylase, 1,000 units/kg.

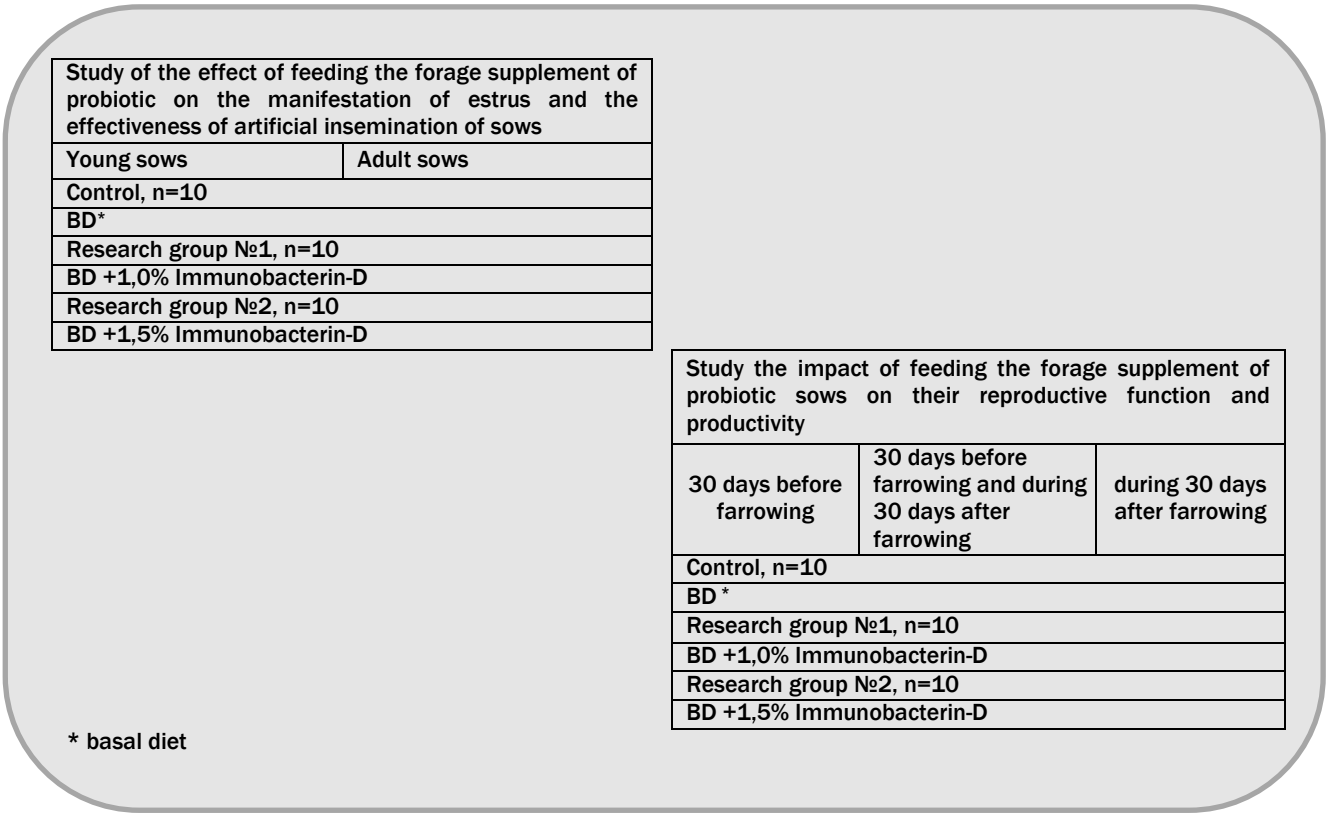


Figure 1 - Scheme of studies conducted.

Methods

The study utilized a balanced analogue group design with adult sows (selected 1 month prior to their expected farrowing). Three groups of 10 sows each were formed. The first group (control) received a traditional diet that met established feeding standards and was balanced for all nutrients. Sows in the experimental groups (second and third) received the probiotic supplement mixed with their feed for 30 days before farrowing and for 30 days postpartum, at inclusion rates of 1.0% and 1.5%, respectively.

Following farrowing, sows were selected based on the balanced analogue group principle, and three groups of 10 sows each were formed. All experimental sows were maintained under identical conditions, but their feeding regimens differed. Sows in the control group (first) received a diet balanced for all nutrients. Sows in the experimental groups (second and third) were supplemented with the probiotic feed additive at rates of 1.0% and 1.5% (w/w), respectively, for one month postpartum. The probiotic mixture was prepared as follows: For a 1.0% inclusion rate, 10 grams of probiotic were weighed and thoroughly mixed with 100 grams of concentrate. This mixture was then added to 1 kilogram of concentrate and blended well. Subsequently, an additional 9 kilograms of concentrate were added and mixed thoroughly. Finally, the total concentrate mass was brought to 100 kg, ensuring all components were well incorporated. A similar procedure was followed for the 1.5% inclusion rate. The control group did not receive any probiotic supplementation.

The growth intensity of the experimental piglets was monitored through individual weighing at birth, at 1 month of age, and at 60 days of age. Average daily weight gain was calculated using the conventional method. To assess growth and development, morphometric measurements were taken on the day of weighing, including height at the withers, chest girth (behind the shoulder blades), chest width (behind the shoulder blades), chest depth, and body length. Body structure indices were calculated from these measurements (Putra and Ilham, 2019).

The following reproductive parameters were evaluated: percentage of sows exhibiting estrus; fertilization rate (calculated as the number of confirmed pregnancies divided by the number of inseminations); number of live-born piglets per sow; number of stillborn piglets; litter mortality up to 30 days postpartum; and farrowing interval (days) (Khalak et al., 2022). Metritis, mastitis, and agalactia (MMA) syndrome in sows were diagnosed through clinical examination post-farrowing. This included monitoring body temperature, assessing feed intake, observing vaginal discharge (purulent, mucous, or with an unpleasant odor), and performing udder palpation 12–24 hours after farrowing (Büttner et al., 2024).

Statistical analysis

All statistical analyses were performed according to the methodologies described in the manuals of Klein and Moeschberger (2003) and Machin et al. (2006) using the Analysis of Molecular Variance JAMOVI software v. 2.6.19 (Navarro and Foxcroft, 2025).

RESULTS AND DISCUSSION

One of the approaches that enhances the nutritional adequacy of farm animals is the use of biotechnological preparations or feed additives with a broad biological spectrum of action. Among these additives are bacterial-based probiotics that serve both therapeutic and nutritional purposes. Such probiotics can be effectively used as complete feed additives that, through the activity of *Bacillus* spp., inhibit the growth of pathogenic and toxin-producing bacteria in feed, create favorable conditions for digestion, improve feed utilization efficiency, reduce the effects of toxins on the animal's body, and stimulate metabolic activity and overall vitality. Pogranichniy et al. (2023). The use of probiotic feed additives in livestock diets is therefore considered a relevant and practically significant area of research. In this study, the effect of feeding probiotic supplements on the manifestation of estrus and the efficiency of insemination in young and adult sows was investigated.

The inclusion of probiotic feed additive (1.0% and 1.5% of the main diet) in feeding young sows resulted in an increased manifestation of estrus by 10.0% and 20.0%, respectively, compared with the control group. However, the effect of probiotic supplementation on the duration of estrus in young sows was not statistically significant, as the duration in animals of the control group and experimental group No. 2 was identical (53 hours), and in sows of experimental group No. 3 it was 54 hours (Table 1).

Table 1 - The impact of probiotic feed additive on the manifestation of estrus and efficiency.

Indicator / Research group		№1 (control)	№2	№3
Number of animals, heads		10	10	10
Came in estrus for 21 days	Heads	6	7	8
	%	60.0	70.0	80.0
The duration of estrus, hrs		53	53	54
The number of sows that were inseminated, heads		6	7	8
The number of sows that were farrowing	Heads	3	5	5
	%	50.0	71.43	62.5
Duration of farrow		2 hrs 20 min	2 hrs 14 min	2 hrs 21 min
Time between the birth of piglets, min		15	14	15
Obtained pigs, heads	Total	28	48	47
	For 1 farrow	9.3 ± 5.49	9.6 ± 4.25	9.4 ± 4.26
Manifestation, kg		1.2 ± 2.06	1.2 ± 1.57	1.2 ± 1.59

The results also indicated that feeding young pigs with the probiotic contributed to an increase in sow fertilization rates by 21.43% and 12.5%, with respective increases of 3.23% and 1.08% in comparison with the control group. In contrast, the feeding of probiotic supplements did not significantly influence fertilization rates among adult sows. Additionally, the duration of farrowing among sows in the experimental groups was unaffected by probiotic supplementation, with piglets being born at intervals of approximately 14–15 minutes. Overall, these results suggest that dietary inclusion of the probiotic was associated with heightened estrus manifestation, improved fertilization rates, and increased litter size in young sows. The best insemination results were achieved when young sows received the probiotic supplement at a concentration of 1.0% in addition to their main diet. Similar trials were conducted with adult sows.

When adult sows were given probiotic supplementation (1.0% and 1.5% of the main diet) after weaning, the manifestation of sexual reflexes increased by 10.0% in both experimental groups compared with the control group (Table 2). The effect of probiotic addition on the duration of estrus was not statistically significant, as sows in the control and experimental group No. 2 exhibited estrus for 61 hours, while sows in group No. 3 showed estrus for 62 hours. Feeding adult sows the probiotic supplement at 1.0% and 1.5% concentrations during their preparation for fertilization increased the fertilization rate by 1.39% and improved litter size (multiple births) by 9.33% and 10.59%, respectively. This led to an increase in total piglets born from 10 sows by 25.0% and 26.3%, respectively. Differences among experimental groups in farrowing duration were not significant, indicating that this parameter was independent of probiotic supplementation. Piglets were born at intervals of 15–16 minutes in all groups. Feeding sows during lactation is one of the most influential factors affecting their entire production cycle and farm productivity. Adequate nutrition during lactation influences milk production, piglet viability, and body weight at weaning. It also impacts fertility and litter size in subsequent reproductive cycles, body reserves, and the overall longevity of sows. Milk production increases gradually after farrowing, reaching its peak approximately two weeks into lactation. It is generally accepted that feeding during lactation should follow a rising feed curve after farrowing, reaching its maximum in the second week of lactation.

Balanced nutrition is crucial for providing adequate growth for both the sow and her offspring until weaning. Insufficient growth in sows can lead to lower live weight post-lactation and prolonged estrus periods. Conversely, excessive obesity is undesirable, as obese sows exhibit poor feed intake during lactation, produce less milk, lose significant weight, and are often culled prematurely (Povod et al., 2024). Common issues in swine production include an increasing number of stillbirths, prolonged farrowing durations, endometritis, mastitis, agalactia, hypogalactia, high piglet mortality, reduced weaning weights, and delayed or absent estrus post-weaning (Kramarenko et al., 2024).

The supplementary introduction of a probiotic to the sows diet one month prior to expected farrowing, at rates of 1.0% and 1.5%, increased the number of live-born piglets by 2.9% and 1.9%, respectively, compared to the control group. This improvement is attributed to a reduction in the number of stillbirths in the experimental groups (No. 2 and 3) by 1.86% and 0.95%, respectively (Table 3).

Table 2 - The Impact of probiotic feed additive on estrus and efficiency of insemination of adult sows

Indicator / Research group		№1 (control)	№2	№3
Number of animals, heads		10	10	10
Came in estrus for 21 days	Heads	8	9	9
	%	80.00	90.00	90.00
The duration of estrus, hrs		61	61	62
The number of sows that were inseminated, heads		8	9	9
The number of sows that were farrowing	Heads	7	8	8
	%	87.50	88.89	88.89
Duration of farrow		2 hrs 46 min	2 hrs 51 min	2 hrs 53 min
Time between the birth of piglets, min		16	15	15
Obtained pigs, heads	Total	72	90	91
	For 1 farrow	10.29 ± 1.481	11.25 ± 1.174	11.38 ± 1.237
Manifestation, kg		1.2 ± 0.20	1.3 ± 0.19	1.3 ± 0.19

Table 3 - The probiotic feed additives at 30 days before sow productivity

Indicator / Research group	№1 (control)	№2	№3
Number of animals, heads	10	10	10
Live weight of sows 30 days before farrowing, kg	196.0 ± 2.1	197.5 ± 0.8	198.3 ± 2.5
Live weight of sows 30 days after farrowing, kg	181.6 ± 3.82	182.1 ± 3.45	182.8 ± 1.05
Average daily feed consumption with sowing sows, kg	3.17	3.17	3.17
Feed units	3.4	3.4	3.4
Crude protein, g	366	366	366
Lysine, g	14.5	14.5	14.5
Obtained pigs, heads			
Total	109	110	110
Living	104	107	106
Dead	5	3	4
Alive on 1 farrow	10.4	10.7	10.6
% of the stillbirth	4.59	2.73	3.64
Nest weight on day 21, kg	43.7 ± 1.77	49.2 ± 1.54*	50.1 ± 1.62*
The average live weight of a pig at birth, kg	1.26 ± 0.011	1.35 ± 0.017***	1.36 ± 0.016***

* P≤0.05; ** P≤0.01; ***P≤0.001

Nest weight on day 21 in experimental groups №2 and № 3 also increased by 12.59% and 14.65% compared to the control group. In addition, feeding the probiotic supplement 30 days before planned farrowing contributed to an increase in piglet birth weight by 7.14% and 7.94% in groups' No. 2 and No. 3, respectively, compared to the control group. The average daily feed consumption of sows across all groups did not differ, remaining at 3.17 kg or 3.4 feed units. All sows received 366 g of crude protein and 14.5 g of lysine. The total number of piglets born across all groups was similar, ranging from 109 to 110. Notably, the control group had the lowest number of live-born piglets (104), while the groups receiving the probiotic had higher numbers (107 and 106, respectively). The incidence of stillborn piglets in the control group was 5, representing the highest stillbirth percentage (4.59%). In experimental groups No. 2 and No. 3, where the probiotic supplement was administered, the number of stillborns was lower (3 and 4, respectively), with their proportion decreasing to 2.73% and 3.64%. The average number of live piglets per farrowing in the control group was 10.4, while in the experimental groups this figure was higher - 10.7 and 10.6. These results collectively indicate a positive effect of the probiotic supplement on sow reproductive performance, evidenced by an increased number of live-born piglets and a reduced rate of stillbirths.

The growth and survival of piglets up to 2 months of age were assessed in relation to the administration of the probiotic feed additive to sows 30 days prior to farrowing (Table 4). The supplementary inclusion of varying amounts of probiotic in the sows' diet a month before farrowing also influenced piglet live weight at birth and subsequent growth, as well as their survival up to 2 months of age.

Table 4 - The Influence of probiotic feed additives 30 days before farrowing on growth and survival of piglets

Indicator / Research group		№1 (control)	№2	№3
Number of animals, heads		104	107	106
The average live weight of piglets, kg	At birth	1.26 ± 0.011	1.35 ± 0.017***	1.36 ± 0.016***
	In 2 months	16.7 ± 0.21	17.5 ± 0.25*	17.6 ± 0.24*
Mortality of piglets up to 2 months	heads	9	9	8
	%	11.65	7.55	8.45
Weaned piglets per sow, heads		9.1 ± 0.54	9.8 ± 0.35	9.8 ± 0.42
Average daily increase in piglets from birth to 2 months, kg		0.26 ± 0.501	0.27 ± 0.500	0.27 ± 0.500
Gross increase in 2 months, kg		15.44 ± 0.59	16.15 ± 0.77	16.24 ± 0.28

* P≤0.05; ** P≤0.01; ***P≤0.001

Specifically, when the probiotic additive was used in the sow rations 30 days before farrowing at concentration of 1.0% and 1.5%, the live weight of piglets at birth increased by 7.14% and 7.94% in experimental groups' No. 2 and 3 respectively. By 2 months of age, the live weight in these groups had increased by 4.79%. The average daily weight gain of piglets from birth to 2 months of age in the second and third research groups increased by 4.67% and 5.45%, respectively, compared to the first (control) group. The gross increase in piglet live weight over the 2-month period in experimental groups' No. 2 and 3 was also higher, by 4.6% and 5.18%, compared to the control. The number of weaned piglets per sow increased by 7.69% in the groups receiving the feed additive compared to the control. Furthermore, in the experimental groups (No. 2 and 3), piglet mortality up to 2 months of age decreased by 4.64% and 3.67%, respectively, compared to the control group.The study also examined the effect of administering probiotic feed additive to sows 30 days before farrowing and continuing for 30 days after farrowing on their reproductive function and productivity (Table 5). Sows that received the probiotic feed additive during this extended period showed an increase in the number of live-born piglets at birth. Additionally, the live weight of piglets at birth in groups' No. 2 and 3 increased by 5.51% and 6.30%, respectively, compared to the control group. It is plausible that the increased birth weight and number of piglets in the experimental groups are due to the probiotic feed additive promoting enhanced development and survival of piglets during the final month of gestation. Nest weight on day 21 in experimental groups' No. 2 and 3 increased by 8.31% and 13.03% compared to the control. The average daily feed consumption of farrowing sows across all groups remained consistent at approximately 3.2 kg or 3.3 feed units. This intake provided a nutritional profile of 367 g of crude protein and 14.6 g of lysine.

Table 6 presents the growth and survival of piglets up to two months of age, dependent on the introduction of the probiotic feed additive to the sows' rations 30 days before farrowing and continuing for 30 days after farrowing. The inclusion of varying amounts of the probiotic feed additive in the sows' diet during this period affects piglet mass at birth and their subsequent growth, as well as piglet mortality up to two months of age.

When probiotic supplementswere used in the feeding of sows, starting 30 days before expected farrowing and continuing for 30 days after, the live weight of piglets at birth in experimental groups No. 2 and 3 (receiving 1.0% and 1.5% of the feed supplement, respectively) increased by 5.51% and 6.30% compared to the control group. This positive effect on live weight was also observed at 2 months of age. From birth to 2 months, the average daily gain in live weight of piglets in experimental groups' No. 2 and 3 increased by 10.63% and 11.42%, respectively, compared to the control. Similarly, in these groups, the mortality of piglets up to 2 months of age decreased by 2.27% and 2.35%, respectively, compared to the data obtained in the control group.

The prevention of diseases in sows after farrowing is a critical aspect, as this period is vital for the health of both sows and piglets. Preventing diseases in sows after farrowing significantly influences the quantity and quality of mammary and gland secretion, which leads to a reduction in sickness among young piglets during the postnatal period and an increase in their viability. In this regard, it is noteworthy that the addition of the probiotic feed additive to the diet of sows has a positive effect on preventing their incidence of disease in the early postpartum period. Clinical examinations of the sows revealed disease incidence across all experimental groups; however, the control group exhibited a higher incidence rate of 20.0%, which was 10.0% greater than that observed in experimental groups' No. 2 and 3, where the incidence was 10.0% (Table 7).

Table 5 - The probiotic feed additives 30 days before farrowing and continued for 30 days after farrowing on a reproductive function and sows performance

Indicator \ Research group	№1 (control)	№2	№3
Number of animals, heads	10	10	10
Live weight of sows 30 days before farrowing, kg	196.2 ± 2.10	197.1 ± 2.82	198.2 ± 2.51
Live weight of sows 30 days after farrowing, kg	181.7 ± 3.82	181.8 ± 3.45	182.6 ± 1.05
Average daily feed consumption with sowing sows, kg	3.2	3.2	3.2
Feed units	3.3	3.3	3.3
Crude protein, g	367	367	367
Lysine, g	14.6	14.6	14.6
Obtained pigs, heads			
Total	111	111	112
Living	103	107	108
Dead	8	4	4
Alive on 1 farrow	10.3	10.7	10.8
% of the stillbirth	7.21	3.60	3.54
Nest weight on day 21, kg	44.5 ± 1.02	48.2 ± 1.34*	50.3 ± 1.62**
The average live weight of a pig at birth, kg	1.27 ± 0.43	1.34 ± 0.78	1.35 ± 0.81

* P≤0.05; ** P≤0.01; ***P≤0.001

Table 6 - The Impact of probiotic feed additive 30 days before farrowing and during 30 days after farrowing on the growth and survival of piglets

Indicator \ Research group		№1 (control)	№2	№3
Number of animals, heads		103	107	108
The average live weight of piglets, kg	At birth	1.27 ± 1.103	1.34 ± 1.112	1.35 ± 1.110
	In 2 months	16.5 ± 3.66	18.2 ± 3.73	18.3 ± 3.72
Mortality of piglets up to 2 months	heads	11	9	9
	%	10.68	8.41	8.33
Weaned piglets per sow, heads		9.2 ± 2.85	9.8 ± 2.87	9.9 ± 2.87
Average daily increase in piglets from birth to 2 months, kg		0.25 ± 0.492	0.28 ± 0.511	0.28 ± 0.508
Gross increase in 2 months, kg		15.2 ± 3.54	16.7 ± 3.60	17.0 ± 3.62

* P≤0.05; ** P≤0.01; ***P≤0.001

Table 7 - The effect of feed additives probiotic on the incidence of sows in the early postpartum period

Indicator \ Research group	№1 (control)	№2	№3
Number of sows, heads	10	10	10
Number of sick sows			
Heads	2	1	1
%	20.0	10.0	10.0
Sick with metritis-mastitis-agalactia			
Heads	1	-	1
%	50.0	-	100.0
Sick with postpartum endometritis			
Heads	1	1	-
%	50.0	100.0	-

* P≤0.05; ** P≤0.01; ***P≤0.001

Table 8 - The impact of feeding feed additive the probiotic sows during 30 days after farrowing on growth and survival of piglets

Indicator \ Research group		№1 (control)	№2	№3
Number of piglets, heads		104	108	107
The average live weight of piglets, kg	At birth	1.23 ± 1.081	1.26 ± 1.073	1.26 ± 1.078
	In 2 months	16.4 ± 3.63	17.2 ± 3.63	17.3 ± 3.66
Mortality of piglets up to 2 months	heads	12	9	7
	%	11.54	8.33	6.54
Weaned piglets per sow, heads		9.2 ± 2.83	9.9 ± 2.87	10.0 ± 2.90
Average daily increase in piglets from birth to 2 months, kg		0.25 ± 0.490	0.27 ± 0.499	0.27 ± 0.502
Gross increase in 2 months, kg		15.2 ± 1.52	16.0 ± 2.31	16.0 ± 2.10

* P≤0.05; ** P≤0.01; ***P≤0.001

Among the sows in the control group, one animal (50.0%) was detected with metritis-mastitis-agalactia and postpartum endometritis. In experimental groups' No. 2 and 3, one sow each was detected with endometritis (100.0%) and metritis-mastitis-agalactia (100.0%), respectively. The effectiveness of administering the probiotic supplement to sows within 30 days from the moment of farrowing on their performance indicators was studied (Table 8). The supplementary feeding of the probiotic feed additive for one month, starting from the moment of farrowing, at inclusion rates of 1.0% and 1.5%, positively affected the growth and survival of the resulting litter.

Pigs of experimental groups No. 2 and 3 exceeded the control group in live weight at birth by 2.44%. At 2 months of age, these pigs exceeded their counterparts from the control group in this indicator by 4.88% and 5.49, respectively. The average daily gain of piglets in research groups No. 2 and 3 from birth to 2 months increased by 5.14% and 5.53% compared to the control. The gross increase in the live weight of piglets at 2 months in research groups' No. 2 and 3 increased compared to the control by 5.08% and 5.74%, respectively. The number of weaned piglets per sow, when fed the probiotic feed additive in experimental groups No. 2 and 3, increased by 7.61% and 8.70%, respectively, compared to the control. Additionally, piglets under two months of age in the second and third groups exhibited lower mortality rates (by 3.21% and 5.0%, respectively) compared to the control group.

While assessing the intensity of piglet growth by changes in live weight is important, it does not provide a complete picture of their overall development. According to numerous studies (Van Steenbergen, 1989; Alimzhanova et al., 2018; Polupan et al., 2024), evaluating animals based on their exterior is a method to determine their advantages and disadvantages, health status, physiological strength, breed characteristics, and productivity direction. Furthermore, for greater clarity on body physique features within a single breed, describing the exterior profile is useful. In this regard, at 60 days of age, basic body structure measurements were taken from piglets of all groups to determine the features of their linear growth. Piglets from experimental groups No. 2 and 3, whose mothers received different doses of the probiotic feed additive, showed an increase in all measured indicators compared to their peers in the control group. Notably, a significant difference in height at the withers was observed in experimental group No. 2 (*P≤0.05) and No. 3 (P≤0.01), and in chest girth in groups No. 2 and 3 (P≤0.001; Table 9).

Table 9 - The impact of feeding the fodder supplement of the probiotic sows for 30 days after farrowing on the arms of the body of piglets at the age of 2 months, cm

Indicator \ Research group	№1 (control)	№2	№3
Height in the withers	45.1 ± 0.79	47.3 ± 0.47*	48.1 ± 0.58**
Girth of the breast behind the shoulder blades	67.1 ± 0.29	69.8 ± 0.59***	70.4 ± 0.67***
Breast width behind the shoulder blades	14.6 ± 0.36	14.7 ± 0.27	15.3 ± 0.27
Breast depth	24.2 ± 0.34	24.0 ± 0.39	24.9 ± 0.38
The length of the trunk	67.9 ± 1.56	70.5 ± 1.54	71.5 ± 1.37

* P≤0.05; ** P≤0.01; ***P≤0.001

In general, all results corresponded to the phenotypic evaluation of the breed and its production orientation - the meat type. Regarding height at the withers, chest girth behind the shoulder blades, chest width, and body length of piglets in experimental group No. 2 (from sows fed 1.0% probiotic feed additive for 30 days post-farrowing) exceeded those in the control group by 4.89%, 4.02%, 0.68%, and 3.83%, respectively.

Measurements of piglets in experimental group No. 3 (from sows fed 1.5% probiotic for 30 days post-farrowing) were also higher than the control group: height at the withers (6.65%), chest circumference behind the shoulder blades (4.92%), chest width behind the shoulder blades (4.79%), chest depth (2.89%), and body length (5.30%). However, results regarding chest depth were inconsistent. In experimental group No. 2, piglets were slightly behind their peers in the control group by 0.83%, showing a volume deficiency of only 0.2 cm. In contrast, animals in experimental group No. 3 showed a slight positive tendency, performing 2.89% better than the control. Additionally, a slight difference was noted in the piglets of the experimental groups compared with the control in chest width behind the shoulder blades: 0.68% for group No. 2 and 4.79% for group No. 3. These data, which describe the body characteristics of the piglets, are illustrated in the constructed exterior profile (Figure 2). The external forms of piglets in groups' No. 2 and 3 were visibly more developed than those of their peers in the control group. Analyzing these results, we conclude that the development of the piglets followed standard ontogenic patterns. It is characteristic of the meat (bacon) pig type that chest girth is smaller than trunk length, the trunk is elongated, the chest depth is reduced, and the anterior torso is lighter than the massive hindquarters.

The most objective judgments regarding body structure can be made by evaluating body indices (Table 10). The long-legged index reflects the relative development of the limbs and is used to characterize the body type and assess the developmental degree of animals within the same breed. In this study, pigs in experimental groups' No. 2 and 3 did not differ from each other (0.82%), but they exceeded peers in the control group by 3.95% and 3.1%, respectively. The relative length of the animal (compared to height at the withers) characterizes the stretch index.

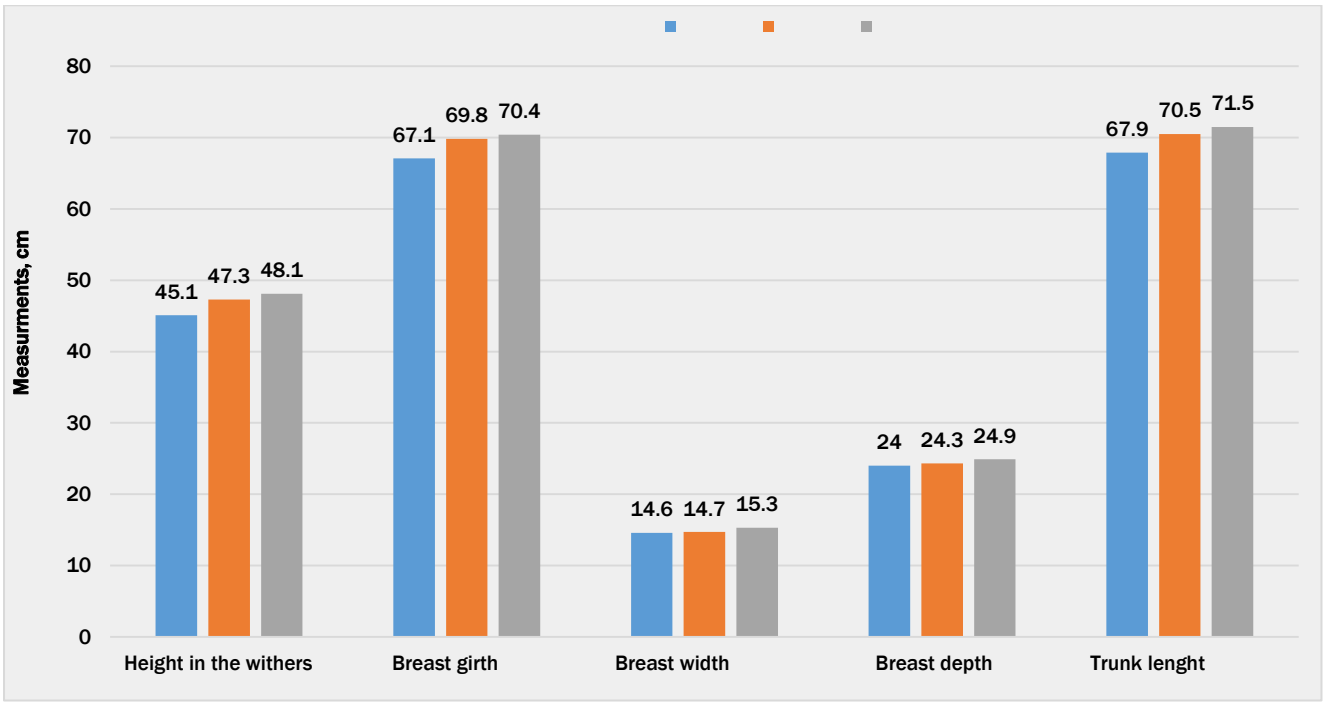


Figure 2 - Exterior piglets profile at the age of 2 months.

Table 10 - Pigerous body indices of piglets at the age of 2 months

Indicator / Research group	№1 (control)	№2	№3
Long-legged	46.78 ± 0.27	48.63 ± 0.38	48.23 ± 0.44
Stretch	150.55 ± 1.18	149.05 ± 0.98	148.65 ± 1.13
Thoracic	60.83 ± 0.95	60.49 ± 0.74	61.45 ± 0.93
Body compactness index	98.82 ± 1.21	99.01 ± 1.14	98.46 ± 1.57
Massiveness	148.78 ± 1.11	147.57 ± 0.88	146.36 ± 1.41

* P≤0.05; ** P≤0.01; ***P≤0.001

For the control group. This index was 150.55%, while in groups' No. 2 and 3 it was 149.05% and 148.65%, respectively. Animals in experimental groups' No. 2 and No. 3 showed a significant decrease in the stretch index compared with the control by 1.0% and 1.26%, respectively. The thoracic index is of greatest interest for adult animals as it serves to determine the chest ratio. Research results recorded an increase in this index in experimental group No. 3 compared with the control by 1.02%, whereas in group No. 2 this indicator was 0.66% lower than the control. The body compactness index evaluates the relative development of body mass expressed as the ratio of chest girth to trunk length. This index was 98.82% in the control group, 99.01% in group No. 2, and 98.46% in group No. 3. In general, piglets in the experimental groups' No. 2 and No. 3 exceeded the control by 0.19% and 0.36%, respectively. Finally, the massiveness index, defined as the ratio of chest girth to height at the withers, indicates the relative development of the torso. Analysis of this index suggests that piglets from sows fed 1.0% and 1.5% probiotic additives post-farrowing showed a decrease in massiveness compared with the control group by 0.81% and 1.63%, respectively.

DISCUSSION

According to scientists. probiotics are biotechnological products. or "live" active ingredients. used as food additives that consist of living microorganisms. They exert beneficial effects on host physiological functions by improving intestinal microbial equilibrium (Fuller, 1989; Kritas. et al., 2006; Anee. et al., 2021)

Gu et al., (2019) investigated the effects of isomaltooligosaccharide (IMO) and Bacillus on sow productivity. serum metabolites. and placental antioxidant capacity. Their findings indicated that IMO and *Bacillus* supplementation improved protein metabolism. Furthermore, the increase in dietary components led to a higher concentration of growth hormone in the serum of the umbilical cord vein, resulting in an increased birth weight of piglets. These microorganisms also play a crucial role in regulating sow health by selectively promoting the growth and metabolic functions of one or more types of bacteria in the colon. (Roberfroid, 1999).

The use of a multi-strain probiotic in pregnant sows, as demonstrated by Pereira et al. (2024) proved to be a beneficial feeding strategy to improve their welfare. Sows fed probiotic supplements exhibited better human-animal interactions and experienced reduced stress levels. Probiotic supplementation lowered cortisol levels and increased serotonin levels in sows. Furthermore, pigs born from sows fed probiotic additives tended to show reduced indicators of negative microflora during bacteriological testing.

The positive effect of a combined probiotic feed additive is also supported by Tang et al. (2024) in their research on preventing stress in pigs. Their study found that probiotics had a significant anti-stress effect. In both weaned piglets and pregnant sows receiving a combination probiotic consisting of *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Bifidobacterium longum*, changes in fecal microbiota composition were observed, influencing the piglet group.

In addition. feeding probiotic additives positively impacts sperm quality. Helli et al. (2022) reported that oral administration of probiotics can help combat oxidative damage of sperm. Probiotic additives contribute to a significant increase in semen volume, quantity, concentration and motility, as well as an increase in the overall antioxidative capacity of plasma, while significantly decreasing plasma malondialdehyde concentrations and inflammatory markers.

Research by Ngo et al. (2023) found that probiotics containing *B. amyloliquefaciens* TOA5001 improved intestinal morphology. intestinal microflora. oxidative activity (biological antioxidant potential) and seperm quality in male broiler-bidders. The findings of Sanchez-Rodriguez et al. (2024) in male mice indicated a higher percentage of normal sperm with fewer anomalies in mice that received probiotics. This suggests that, on one hand, probiotics reach the intestine, and through their metabolism microorganisms produce proteins, amino acids, and short-chain fatty acids such as propionate, acetate, and butyrate, which maintain intestinal integrity and reduce intestinal inflammation.

The use of probiotic mixture "Sviteco-PWC" in poultry significantly reduced the amount of *Eimeria aservulina* in the intestine of broilers. It also modulated intestinal microbiota composition and reduced colonization by pathogenic bacteria, thereby enhancing intestinal immunity. This led to an increase in intestinal immunity in broilers and improved production indicators (Luhovyi et al., 2025).

The impact of probiotics extends beyond the intestine and reproductive capacity. They can reach other organs and systems, leading to their use in treatment of various diseases, including heart disease and cardiovascular system disorder (Oniszczuk et al., 2021). Probiotic substances are involved in regulating the host's lipid metabolism and are utilized in managing hyperlipidemia (Jia et al., 2021), diabetes, and are particularly recommended for preventing associated joint diseases linked to insulin deficiency (Wang et al., 2022). Furthermore, probiotic intervention can mitigate hypertension, regulate intestinal flora to increase beneficial bacteria and modulate metabolites of intestinal microbial flora, such as trimethyl trimethylamine oxide, short-chain fatty acids, and polyphenols, which contribute to reducing blood pressure (Yuan et al., 2023).

CONCLUSION

The results of the study indicate the high efficiency of using probiotic feed additives in feeding sows of all ages and physiological conditions. Supplementing the diet of both young and adult sows during preparation for fertilization, before and after farrowing, had a positive effect on their reproductive performance, including increased estrus, fertilization rates, litter size, and the number of live piglets at birth. Improvements were also observed in growth rate, live weight, and piglet survival up to two months of age. Under the conditions of this study, inclusion levels of 1.0% for young sows and 1.5% for adult sows were associated with the most pronounced productive responses. Statistically significant improvements in the exterior performance of piglets were demonstrated indicating a broadly positive impact of the probiotic additive on the development of young animals. The finings substantiate the feasibility of adding probiotic supplements into technological schemes for sow feeding to enhancetheir productivity and improve product quality.

DECLARATIONS

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Authors' contribution

The first four authors should be considered as first authors, S. Kramarenko planned, designed, and spell-checked and supervised this study. I. Liuta collected data and samples, performed laboratory studies, analyzed and visualized the data. O. Karateieva described the results, formulated the initial version of the manuscript and critically edited the final version of the manuscript. A. Kramarenko also managed the data and reviewed the manuscript. All authors read and approved the submitted version of the manuscript.

Ethical approval:

All animal handling procedures were conducted in accordance with European legislation (European Union Council Directive No. 98/58/EC, 1998; Nalon and Stevenson, 2019). The study protocol, evaluating the effects of the probiotic supplement on the reproductive performance of sows and the growth of piglets, was approved by the local Bioethics Committee of Mykolaiv National Agrarian University, Ukraine, adhering to Good Clinical Practice (GCP) guidelines for the protection of humane treatment of experimental animals. The entire experimental process was carried out in accordance with animal welfare regulations under the supervision of the ethics team of the Mykolaiv National Agrarian University No. 27 dated 05.06.2025.

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Competing interests

The authors did not declare any competing interests.

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GENETIC AND BIOCHEMICAL CHARACTERISTICS OF SIRE BULLS (*Bos taurus)* IN REPRODUCTIVE BIOTECHNOLOGIES

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RESEARCH ARTICLE

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ABSTRACT: This study aims to investigate the relationship between polymorphic serum protein systems – transferrin *(Tf)*, amylase (*Am*), and ceruloplasmin, (*Cp*)- and sperm production parameters, as well as fertilization rates, in Red Steppe and Angeln cattle. Electrophoresis was employed to determine protein phenotypes and calculate levels of heterozygosity. The relevance of this study is justified by the necessity of establishing immunogenetic markers to enhance selection efficiency. Analysis revealed breed-specific immunogenetic markers. Regarding the Transferrin (*Tf*) locus in Red Steppe bulls, the highest sperm production indicators were observed in homozygous individuals (Tf^{AA}, Tf^{DD}), whereas in the Angeln breed, superior sperm volume was noted in heterozygous individuals (Tf^{AD}). Cows inseminated with sperm from heterozygous pairs generally exhibited significantly higher fertility rates than those inseminated with sperm from homozygous pairs. The Amylase (*Am*) system confirmed the negative influence of the Am^{CC} phenotype, which is associated with the lowest level of sperm production and fertility. In the Red Steppe breed, homozygous Am^{BB} sire bulls possessed the highest reproductive qualities. The study of the Ceruloplasmin (*Cp*) system revealed that in the Red Steppe breed, heterozygotes (Cp^{BC}) demonstrated the highest fertility, despite the superior sperm productivity of homozygotes (Cp^{AA}). Conversely, in the Angeln breed, Cp^{BB} homozygotes prevailed in both indicators. These results suggest that the optimal level of heterozygosity may depend on breed-specific genetic backgrounds. Red steppe bulls that were homozygous or possessed a 25% heterozygosity level exhibited the highest sperm production and fertilization ability, whereas the optimal level of Angeln bulls was 50%. It is recommended that homozygosity levels of *Tf*, *Am*, and *Cp* be incorporated into selection indices when purchasing sire bulls to improve the reproductive qualities of the herd.

Keywords: Amylase, *Bos taurus*, Ceruloplasmin, Heterozygosity, Semen quality, Sire bull, Transferrin.

INTRODUCTION

In the current globalized and climate changing world, maintaining the genetic diversity of farm animal populations requires a systematic approach. This involves not only obtaining objective information about existing populations, but also creating detailed databases for analyzing their structure and studying genetic processes. The modern strategy for protecting livestock biodiversity in Ukraine necessitates scientific justification that account for internal economic reforms, European integration, and real changes in livestock species composition (Polupan et al., 2017).

The rational preservation and reproduction of domestic breeds that carry unique, economically valuable traits are of increasing importance (Davidescu et al., 2023; Engdawork et al., 2024). Red steppe cattle, for example, were developed under the conditions of a dairy sector with one-sided specialization (Suyunova et al., 2024) and are distinguished by exceptional endurance and adaptability to extreme heat (Bodnaruk et al., 2020).

In improvement and refinement of cattle breeds and types, significant attention is given to genetic and population analysis. Employing various genetic methods, such as studying blood groups and polymorphic systems of proteins and enzymes, allows for a more objective analysis of the current state and outlines prospects for genetic and selection processes at different stages of breed development and functioning (Chumel, 2003; Shcherbatyj et al., 2016; Zhmur et al., 2025).

One of the most effective tools for addressing these issues is the implementation of modern genetics achievements, particular the use of polymorphic protein systems as markers of breeding value (Bodnaruk et al., 2022). The study of systems such as transferrin (*Tf*), amylase (*Am*) and ceruloplasmin (*Cp*) allows not only for the assessment of population genetic structure (Guha et al., 2012, Hrytsiienko et al., 2019, Sanni et al., 2023), but also for the identification of relationships between these markers and the reproductive function and sperm productivity of sire bulls.

The investigation of sperm production indicators and factors affecting the quality of sire bulls of farm animals remains a pertinent issue (Piddubna and Zakharchuk, 2020). Recently, increased attention has been directed towards the immunology and immunogenetics of male reproductive qualities, particularly the distribution of phenotypes and the frequency of polymorphic protein systems (e.g., transferrin, amylase, and ceruloplasmin). These systems determine the

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level of heterozygosity and establish relationships with sperm production and the fertility of sire bulls of the Red Steppe and Angler breeds.

Studies by Fedorovych et al. (2008) have demonstrated a relationship between genotype specificity and the fertilization of cows, as well as the volume of ejaculates in breeding bulls. Hrytsiienko et al. (2019) concluded that allelic variants of the studied polymorphic genetic and biochemical systems can be used to evaluate the genetic composition of breeds and intra-breed groups. This is crucial for organizing breeding and selection activities in animal husbandry and enables the practical application of genetic-mathematical methods to predict the milk productivity of cows.

Recent studies by Stadnytska and Kaplinsky (2021) indicated that certain genotypes at serum protein loci (e.g., *AA* homozygotes for transferrin) exhibit better fertility rates and shorter service periods compared to other variants. Therefore, combining strategic biodiversity management with the application of genetic markers is necessary to increase the competitiveness of domestic livestock and preserve its gene pool (Kryvoruchko et al., 2024).

An analysis of the genetic structure of the Black-and-White dairy breed by Bodnaruk et al. (2022) revealed specific features: the transferrin locus demonstrates the highest heterozygosity (0.729) and was in a balanced state according to the Hardy–Weinberg law (*P* – 0.104). The dominant allele *Tf A* had a frequency of 0.438 owing to heterozygotes AD_1 and AD_2. The allele *Tf* D_1 had a low frequency (0.208), while *Tf* D_2 was quite high (0.333), which is typical for meat breeds.

The *Tf E* allele is rare, with a frequency of 0.021 in heterozygotes. The amylase locus (*Am-l*) is in an unbalanced state due to a deficiency of heterozygotes and an excess of homozygotes (P – 0.013), with equal frequencies of the *Am-l B* and *Am-l C* alleles (0.500 each). The heterozygosity for this locus is 31.8%. The ceruloplasmin (CP) locus is also unbalanced (*P* – 0.041) and shows an excess of *AB* heterozygotes. The frequency of the *Cp A* allele is 0.489, while that of the *Cp B* allele is 0.511.

The relationship between transferrin types and reproductive abilities has also been studied in other farm animals (Weitkamp et al., 1982; Demir and Mert, 2015; Jawad et al., 2025). Research on the genetic polymorphism of local goat breeds in Turkey revealed significant variability of biochemical markers, indicating three hemoglobin phenotypes and six transferrin phenotypes (*AA*, *AB*, *AC*, *BB*, *BC*, *CC*). Notably, interbreed differentiation in ceruloplasmin levels was observed: in the Honamli breed, the level was 1.793 ± 0.109 mg/dL, which is twice that of the Kilis breed (0.822 ± 0.055 mg/dL). These findings indicate significant differences in metabolic process intensity and antioxidant protection (Demir et al., 2015).

To study potential relationships between transferrin type and litter size (multiparity) across five breed groups (Durok, selected Durok, Yorkshire, selected Yorkshire, and selected Durok × Yorkshire crosses), transferrin (*Tf*) typing was performed on 343 boars and 1094 gilts, which were sires of 1208 litters (Huang and Rasmusen, 1982). Boars with the BB genotype produced a higher number of live-born piglets (8.67) than those with the *AB* genotype (7.76). Crossbreeding *BB* males with *AB* females resulted in the highest number of live-born (8.71) and weaned (8.39) piglets in the selected Duroc group. Crossbreeding *BB* males with *AB* females in crossbreeds resulted in higher multiparity rates (10.85 born; 8.87 live-born; and 8.19 weaned) compared to breeding *AB* males with *BB* females (9.84; 7.65; and 7.13, respectively).

However, no statistically significant effects of paternal or maternal transferrin genotype, or their interaction, was found on the number of piglets born, born alive, or weaned per litter in any of the five breed classes. This suggests that transferrin typing may have limited value in increasing multiparity in pigs. In Ukraine, the use of molecular genetic markers is integral to preserving the animal gene pool by identifying valuable genotypes and creating the conditions necessary for their targeted use in biodiversity conservation programs for cattle populations (Kopylova et al., 2013).

This study aims to establish a connection between polymorphic serum systems and the reproductive function of bulls to develop molecular genetic criteria for selecting highly productive animals.

MATERIALS AND METHODS

The research was conducted at JSC "Oblplempidpryemstvo" and the breeding enterprises of Mykolaiv Oblast, focusing on semen and blood serum of *Bos Taurus* sire bulls. A total of 233 breeding bulls, comprising Red Steppe (*n*=165) and Angeln (*n*=68) breeds, were examined. Data were also sourced from laboratory records pertaining to the breeding bulls' utilization, sperm production evaluation, and breeding cards (1 MOL). Experimental groups were formed considering breed, age, and heterozygosity level for marker genes, adhering to all zootechnical requirements. Feeding, housing, and the management of breeding bulls complied with generally accepted zootechnical standards (Ibatullin et al., 2014).

Semen was collected from the bulls using an artificial vagina, following the procedures outlined in the artificial insemination instructions (Koreyba, 2016). The ejaculate volume, sperm concentration, and activity were evaluated using methods described by Koreyba (2016), after which samples were collected for further analysis.. Testing for polymorphic protein systems (*Tf*, *Am*, and *Cp*) in sire bulls was performed at the immunogenetics laboratory of the M.F. Ivanov Institute of Animal Husbandry of Steppe Regions, "Askania-Nova" NAAS. This was conducted according to the current procedures described by Harris and Hopkinson (1976) and Gahne et al. (1977). The level of heterozygosity was calculated as the percentage of heterozygous loci among the examined *Tf*, *Am*, and *Cp* loci relative to the total number of loci analyzed. For

instance, if one heterozygous locus was identified out of four analyzed, the heterozygosity level was considered 25%; if two were found, it was 50%, and so on.

RESULTS AND DISCUSSION

An analysis of the distribution of phenotypes and transferrin gene frequencies in breeding bulls (Table 1) revealed the presence of Red Steppe breeds with possible phenotypes *AA, AD, AE, DD* and *DE*. The *EE* phenotype was absent; while bulls of the Angeln breed exhibited *AE* and *EE* phenotypes. Male offspring of both breeds showed the highest concentrations of the *D* gene allele (0.519 and 0.516), whereas the concentrations of *A* and *E* were lower (0.446 and 0.476 and 0.035 and 0.008, respectively). Red Steppe bulls displayed a higher level of heterozygosity at the *Tf* locus (50.9%) compared to Angeln bulls (39.7%). Similar genotypes were observed in studies by Chumel (2003), Shcherbatyj et al. (2016), Hrytsiienko et al. (2019), Stadnytska and Kaplinsky (2021), although the frequencies differed.

The analysis of sperm production in relation to transferrin types in Red Steppe bulls (Table 2) showed that males with the *DD* type produced the fewest ejaculates per year compared to other groups. This difference was significant - 10.6 ejaculates fewer (t_d = 2.236; $P < 0.05$), than in bulls with the *AA* type.

The largest total volume of semen per year was obtained from bulls with the Tf^{AA} type (718.4 ± 26.4 ml), and the smallest from bulls of the *AE* or *DE* type (506.0 ± 60.5 and 570.8 ± 53.8 ml). These differences were significant (t_d = 3.218 and 2.502). Bulls with Tf^{AD} phenotype also significantly outperformed their Tf^{AE} and Tf^{DE} counterparts (t_d = 2.784 and 1.966). The largest average ejaculate volume was recorded in bulls with the *Tf AA* and *DD* type (3.88 ± 0.13 and 3.78 ± 0.12 ml), while the lowest was in bulls with the *AE* type (2.73±0.28 ml). The differences were statistically significant (t_d = 3.720 and 3.322; $P<0.001$). Sperm concentration was highest in bulls with the *Tf AE* type (1.100 ± 0.032 billion/ml) significantly exceeding those of bulls with the *AA* and *AD* types (t_d = 2.209 and 2.654; $P<0.05$ and $P<0.01$). Regarding total sperm count, bulls with the Tf^{DD} and Tf^{AA} types (4.049 ± 0.158 and 3.912 ± 0.182 billion, respectively) had higher counts than bulls with the Tf^{AE} type (3.010 ± 0.383 billion; t_d = 2.509 and 2.127; $P<0.02$ and $P<0.05$). Bulls with the *AA* and *AD* types also exhibited the highest sperm motility (8.39 ± 0.08 and 8.33 ± 0.04, respectively), significantly exceeding that of bulls with the *DE* type (8.13 ± 0.07; t_d = 2.452 and 2.439; $P<0.02$).

The fertilization rate of cows from the first insemination was highest when sperm from bulls with the *AA* and *DD* types was used (64.3 ± 1.04% and 63.7 ± 1.01%, respectively), and lowest when semen from bulls with the Tf^{AE} type was used (61.0 ± 3.8%). These patterns were consistent with the observed correlations between transferrin types and sperm quality (Table 3). Notably, Red Steppe bulls with the *D1D1* transferrin type produced the greatest number of ejaculates (195.7 ± 0.9 per year) and the largest total volume of semen (828.3 ± 49.2 ml). They also had the highest ejaculate volume (4.23 ± 0.25 ml), total sperm count (4.24 ± 0.57 billion), and sperm activity (8.43 ± 0.09). In contrast, bulls with the *D2E* transferrin type produced the fewest ejaculates (175.8 ± 5.9) and the least semen volume (496.0 ± 38.8 ml), with the lowest ejaculate volume (2.81 ± 0.15 ml), sperm concentration (0.98 ± 0.04 billion/ml), total sperm count (2.74 ± 0.18 billion), and sperm activity (8.08 ± 0.07). Interestingly, cows inseminated with semen from the *D1D1* bulls had lower fertilization rates, whereas those inseminated with semen from *D2E* bulls exhibited the highest rates.

This observation may be influenced by additional factors, such as female's reproductive status and insemination management practices, which were not directly controlled in this study. No significant differences were foun in the number of ejaculates obtained from Angeln bulls with different transferrin types (Table 4). However, bulls with the *AD* type demonstrated a significant advantage over those with the *AA* type in terms of total annual sperm volume (799.2 ± 89.6 ml and 690.2 ± 32.7 ml, respectively; td = 2.472, $P < 0.02$). Consequently, the average ejaculate volume was significantly higher in bulls with the *AD* type (4.35 ± 0.09 ml) compared to the *AA* type (3.76 ± 0.16 ml; td = 3.207). Sperm concentration was highest in bulls *with* the *AA* type (1.073 ± 0.038 billion/ml), although this difference was not statistically significant. The total number of sperm per ejaculate was highest in bulls with the *AD* type (4.442 ± 0.152 billion), with slightly lower counts in bulls of the *DD* and *AA* types. Sperm activity was highest in bulls with the *AA* type (8.44 ± 0.06), but no significant difference was observed compared to other transferrin types. The fertilization rate of females following the first insemination was highest when using sperm from bulls with the *TfDD* type (66.7 ± 1.4%), a result that differed significantly (td = 2.827) from that of bulls with the *TfAD* phenotype (61.3 ± 1.3%) and also significantly (td = 1.902) from bulls with the *TfAA* type (62.8 ± 1.5%). Therefore, based on the results in Table 4, establishing a consistent and close relationship between quantitative and qualitative sperm production indicators and transferrin types in Angeln bulls is not always possible across all parameters.

A comparison of gene frequencies at the amylase locus (Table 1) indicates that both breeds are characterized by a higher frequency of the *B* allele. For Red Steppe bulls, this frequency is 0.630, and for Angeln bulls, it is 0.792, respectively. The level of heterozygosity at the amylase locus for Red Steppe bulls is 33.3%, and for Angeln bulls, it is 31.7%, respectively. These findings may differ from those reported in studies by Bodnaruk et al. (2020), Bodnaruk et al. (2022), Zhmur et al. (2025).

Table 1 - Phenotype distribution and frequency of in sire bulls

Tf alleles

Breed	n	Distribution of phenotypes										Allele frequency, $\overline{X} \pm S_{\overline{X}}$		
		AA		*AD*		*AE*		*DD*		*DE*				
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*A*	*D*	*E*
Red Steppe	157	34	21.7	69	43.9	3	1.9	43	27.4	8	5.1	0.446±0.0280	0.519±0.0301	0.035±0.0104
Angeln's	63	18	28.6	24	38.1	-	-	20	31.7	1	1.6	0.476±0.0443	0.516±0.0445	0.008±0.0079

Am alleles

Breed	n	Distribution of phenotypes						Allele frequency, $\overline{X} \pm S_{\overline{X}}$	
		BB		*BC*		*CC*			
		n	%	*n*	%	*n*	%	*B*	*C*
Red Steppe	144	68	47.2	48	33.3	28	19.4	0.630±0.0283	0.361±0.0283
Angeln's	60	38	63.3	19	31.7	3	5.0	0.792±0.0371	0.208±0.0371

Cp alleles

Breed	n	Distribution of phenotypes						Allele frequency, $\overline{X} \pm S_{\overline{X}}$	
		AA		*AB*		*BB*			
		n	%	*n*	%	*n*	%	*A*	*B*
Red Steppe	147	59	40.1	64	43.5	24	16.3	0.619±0.0283	0.381±0.0283
Angeln's	66	38	57.6	23	34.8	5	7.6	0.750±0.0119	0.250±0.0119

Table 2 - Quantitative and qualitative indicators of sperm production of sire bulls of the Red Steppe breed different genetic predisposition according to Tf types

Transferrin type	Number of bulls, criterion of reliability	Number of ejaculates	Semen received from bull per year, ml	Average volume ejaculate, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, scores	Fertility of cows after 1st insemination, %
AA	34	184.9±3.4	718.4±26.4	3.88±0.13	1.005±0.028	3.912±0.182	8.39±0.08	64.3±1.04
AD	69	183.5±5.8	681.0±17.1	3.69±0.07	1.005±0.016	3.713±0.091	8.33±0.04	62.9±1.0
	td	0.208	1.189	1.284	-	0.978	0.674	0.972
AE	3	185.3±7.2	506.0±60.5	2.73±0.28	1.100±0.032	3.010±0.383	8.30±0.15	61.0±3.8
	t d	0.050	3.218**	3.720***	2.209*	2.127*	0.529	0.837
	$t d_1$	0.196	2.784**	3.322***	2.654*	1.784	0.194	0.483
	td_2	1.388	2.563*	3.443***	0.956	2.509*	-	0.687
DD	43	174.3±3.3	672.6±23.7	3.78±0.12	1.057±0.021	4.049±0.158[a]	8.30±0.054	63.7±1.01
	t d	2.236*	1.290	0.560	1.485	0.568	0.957	0.414
	td_1	1.379	0.288	0.647	1.970	1.846	0.469	0.563
DE	8	179.0±4.4	570.8±53.8	3.37±0.30	1.021±0.033	3.485±0.386	8.13±0.07	63.3±1.8
	t d	1.061	2.502	1.560	0.372	1.001	2.452*	0.714
	$t d_1$	0.618	1.966	1.038	1.416	0.574	2.409*	0.015
	$t d_2$	0.855	1.731	1.269	0.795	1.352	1.977	0.679

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$), (**- $P < 0.01$), (*** - $P < 0.001$) based on *t*-Student criteria (t_d).

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Table 3 - Quantitative and qualitative indicators of sperm production of sire bulls of the Red Steppe breed different genetic predisposition according to *Tf* types

Transferrin type	Number of bulls, heads	Number of ejaculates	Semen received from bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, scores	Fertility of cows after 1st insemination, %
A A	34	184.9±3.4	718.4±26.4	3.88±0.13	1.005±0.028	3.912±0.182	8.39±0.08	64.3±1.04
A D₁	21	173.2±5.8	619.9±24.4	3.77±0.22	1.035±0.027	3.884±0.242	8.32±0.07	61.3±2.0
A D₂	48	186.2±2.3	707.8±21.2	3.78±0.09	0.992±0.020	3.760±0.120	8.33±0.06	63.6±1.2
A E	3	185.3±7.2	506.0±60.5	2.73±0.28	1.100±0.032	3.010±0.383	8.30±0.15	61.0±3.8
D₁D₁	3	195.7±0.9	828.3±49.2	4.23±0.25	0.990±0.082	4.240±0.570	8.43±0.09	59.3±0.9
D₂D₂	34	169.5±3.9	628.3±28.1	3.70±0.14	1.074±0.024	3.978±0.188	8.28±0.06	63.2±1.2
D₁D₂	6	184.0±7.1	732.2±52.9	3.98±0.24	1.002±0.047	4.027±0.386	8.33±0.07	68.5±2.5
D₁E	3	184.3±6.8	695.3±97.7	4.31±0.24	1.097±0.015	4.72±0.240	8.20±0.15	59.3±2.6
D₂E	5	175.8±5.9	496.0±38.8	2.81±0.15	0.980±0.040	2.74±0.180	8.08±0.07	64.0±2.0

Table 4 - Quantitative and qualitative indicators of sperm production of sire bulls of the Angeln breed of different genetic predisposition by *Tf* types

Transferrin type	Number of bulls, heads	Number of ejaculates	Semen received from bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, scores	Fertility of cows after 1st insemination, %
A A	18	184.6±5.4	690.2±32.7	3.76±0.16	1.073±0.038	4.017±0.207	8.44±0.06	62.8±1.5
A D	24	183.1±4.3	799.2±29.6	4.35±0.09	1.022±0.025	4.442±0.152	8.42±0.08	61.3±1.3
	t d	0.217	2.472*	3.207**	1.133	1.655	0.200	0.758
D D	20	181.2±5.7	761.3±35.6	4.23±0.21	1.011±0.024	4.278±0.250	8.42±0.09	66.7±1.4[a]
	t d	0.433	1.471	1.780	1.381	0.803	0.185	1.902
	t d₁	0.268	0.819	0.526	0.257	0.560	-	2.827**
A A	18	184.6±5.4	690.2±32.7	3.76±0.16	1.073±0.038	4.017±0.207	8.44±0.06	62.8±1.5
A D₁	8	174.9±12.5	788.3±67.4	4.58±0.35	0.969±0.035	4.438±0.398	8.44±0.08	64.5±1.9
A D₂	16	183.5±5.4	804.6±30.7	4.38±0.09	1.048±0.032	4.596±0.190	8.40±0.10	59.7±1.5
A E	-	-	-	-	-	-	-	-
D₁D₁	1	194	848	4.37	1.07	4.68	8.3	59.3±0.9
D₂D₂	12	184.3±7.8	715.3±40.8	3.88±0.12	1.018±0.028	3.943±0.169	8.41±0.12	66.8±1.7
D₁D₂	7	184.0±7.1	732.2±52.9	3.98±0.24	1.002±0.047	4.027±0.386	8.33±0.07	68.5±2.5
D₁E	1	198	870	4.39	1.12	4.92	7.9	

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$), (**- $P < 0.01$) based on *t*-Student criteria (t_d).

A study of the sperm production of Red Steppe bulls in relation to amylase types (Table 5) determined that bulls with the Am^{CC} phenotype produced the least ejaculate volume (180.6 ± 2.8 ml) and sperm count (666.4 ± 23.7 ml) annually. In contrast, homozygous *BB* sires produced the largest ejaculate volume (3.79 ± 0.08 ml), which significantly exceeded that of males with the Am^{BC} (3.55 ± 0.09 ml; t_d = 2.000*; P < 0.05)* and Am^{CC} (3.54 ± 0.11 ml, t_d = 1.838) types. It was also observed that homozygous *BB* bulls for the amylase locus secreted the largest number of sperm per ejaculate (3.968 ± 0.116 billion) with a higher concentration (1.025 ± 0.018 billion/ml) and sperm activity (8.35 ± 0.04). Fertilization rates of females after the first insemination with their sperm were also the highest (62.8 ± 0.94). Sperm production indicators of homozygous Am^{CC} bulls were, as noted, worse across all parameters. They exhibited the lowest sperm concentration (0.989 ± 0.25 billion/ml, t_d =1.61) and sperm count per ejaculate (3.482 ± 0.124 billion, t_d = 2.862), as well as the lowest sperm activity (8.28 ± 0.06, t_d = 0.970). Consequently, the lowest fertilization rate was obtained when inseminating females with their sperm (60.4 ± 1.6, t_d = 1.293). Bulls with the Am^{BC} phenotype occupied an intermediate position for most of the studied indicators.

In the Angeln breed, it was not possible to establish a consistent and close relationship between different amylase types and sperm production quality (Table 5). Although bulls with the Am^{CC} phenotype produced the fewest ejaculates (179.3 ± 17.7), and the least amount of sperm (669.7 ± 125.9 ml) per year, they also had the smallest ejaculate volume (3.68 ± 0.41 ml), the lowest total sperm count in the ejaculate (3.807 ± 0.571 billion), sperm activity (8.37±0.03) and the lowest fertilization rate of females from the first insemination (59.0 ± 5.3%). Homozygous *BB* and heterozygous *BC* males at the amylase locus outperformed their *CC* counterparts in both quantitative and qualitative sperm production parameters. However, this advantage was not consistent: either *BB* bulls prevailed in one case or *BC* bulls in another. These minor differences among these fertile individuals explain the variability in the outcomes.

The genetic polymorphism of ceruloplasmin, characterized by two codominant alleles, *A* and *B,* was determined (Table 1). The data show that the frequency of allele *A* was higher in both breeds (0.619 and 0.750) compared to allele *B* (0.381 and 0.250, respectively). The level of heterozygosity at the ceruloplasmin locus was 43.5% for Red Steppe bulls and 34.8% for Angeln bulls. These frequencies and heterozygosity levels are consistent with previous studies by Hrytsienko et al. (2019), Bodnaruk et al. (2022), and Zhmur et al. (2025).

Analysis of the relationship between bulls' sperm production parameters and ceruloplasmin types (Table 6) revealed that the highest number of ejaculates (185.1 ± 2.1) and sperm (696.9 ± 17.9 ml) per year were obtained from bulls with the *AA* genotype. These sire bulls also produced the largest ejaculate volumes (3.76 ± 0.08 ml) the highest sperm concentration (1.040 ± 0.19 billion/ml) the greatest total sperm count in the ejaculate (3.930 ± 0.128 billion), and the highest sperm activity (8.37 ± 0.05). In contrast, all these indicators were lowest in homozygous Cp^{BB} bulls, with only 661.1 ± 18.9 ml of sperm obtained (t_d = 1.144), an ejaculate volume of 3.61 ± 0.10 (t_d = 1.172), sperm concentration of 0.985 ± 0.034 billion/ml (t_d = 1.413), total sperm count of 3.582 ± 0.168 billion, and sperm activity of 8.27 ± 0.07. The fertility rate of females following the first insemination was also lowest in this group, at 60.5% ± 1.4% compared to 62.6 ± 0.9% in the *AA* group (t_d = 1.265).

Similar patterns were observed in the Angeln breed (Table 6): the quantitative and qualitative sperm production indicators were closely associated with the ceruloplasmin types. The highest number of ejaculates (191.0 ± 4.1) and sperm volume (830.2 ± 58.8 ml) per year was obtained from homozygous Cp^{BB} bulls. These bulls also exhibited the highest sperm concentration (1.106 ± 0.056 billion/ml), total sperm count (4.762 ± 0.239 billion), sperm activity (8.40 ± 0.07), and the highest fertilization success rate after the first insemination (65.8 ± 2.1%). Lower quantitative and qualitative sperm production indicators were observed in heterozygous Cp^{AB} bulls, from which 183.5 ± 5.4 ejaculates with a total volume of 742.1 ± 33.6 ml (td = 1.302) were obtained per year. Each ejaculate had an average volume of 4.05 ± 0.14 ml (td = 1.064), with a sperm concentration of 1.013 ± 0.022 billion/ml (td = 1.407), and a total sperm count of 4.075 ± 0.141 billion (td = 2.475). These parameters were minimal, and the fertilization rate after the first insemination was lower than that of the Cp^{BB} group, at 64.1 ± 1.3%. The lowest fertilization rate was observed in the homozygous Cp^{AA} bulls, at 62.5 ± 1.2%.

The potential importance of different locus combinations in the studied systems for determining sperm production indicators in breeding bulls necessitates further analysis. Genetic markers were therefore used to assess the complexity of heterozygosity at these loci and to analyze the relationship with sperm production in breeding bulls (Tables 7 and 8). The entire population of Red Steppe breeding bulls was divided into five groups based on their degree of heterozygosity, while the Angeln breed was divided into four groups. The study's findings indicated that, in the Red Steppe breed, there was a slight decrease in sperm production indicators as the level of heterozygosity for marker genes increased. The largest number of ejaculates was obtained from bulls with a 25% level of heterozygosity (181.5 ± 2.8), while the smallest number was obtained from bulls with 100% heterozygosity (166.0 ± 11.4, td = 1.320). In terms of total sperm volume obtained (674.3 ± 39.7 ml), homozygous bulls (with 0% heterozygosity) surpassed all other groups. Subsequently, a linear decrease in volume was observed, with a significant difference compared to bulls with 100% heterozygosity (486.0 ± 83.5 ml, t_d = 2.036). Similar results were found for ejaculate volume, which was largest in homozygous bulls (3.83 ± 0.156 ml) and significantly exceeded that of bulls with 25% heterozygosity (3.74 ± 0.08 ml). It also exceeded the volumes from bulls with 75% heterozygosity (3.41 ± 0.13 ml) and 100% heterozygosity (2.85 ± 0.35 ml). The linear relationship for sperm concentration was not consistently maintained. It decreased from homozygosity to a 50% heterozygosity level (from 1.052 ± 0.37 to 1.013 ± 0.016 billion/ml), increased at 75% heterozygosity (to 1.068 ± 0.24 billion/ml), and then decreased again at 100% heterozygosity.

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Table 5 - Quantitative and qualitative indicators of sperm production of sire bulls of different genetic predisposition by types Am

Amylase type		Number of bulls, heads	Ejaculates received	Semen obtained from one bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	General number of sperm in ejaculate, billion	Sperm activity, points	Fertility of cows after 1st insemination, %
Red Steppe breed	*BB*	68	184.1±2.1	692.3±17.9	3.79±0.08	1.025±0.018	3.968±0.116	8.35±0.04	62.8±0.94
	BC	48	185.9±1.8	668.4±18.5	3.55±0.09	1.036±0.021	3.708±0.124	8.32±0.05	62.1±1.07
		td	0.650	0.930	2.0*	0.397	1.529	0.469	0.492
	CC	28	180.6±2.8	666.4±23.7	3.54±0.11	0.989±0.025	3.482±0.124	8.28±0.06	60.4±1.6
		td	1.000	0.872	1.838	1.161	2.862**	0.970	1.293
		td_1	1.592	0.066	0.070	1.424	1.291	0.500	0.895
Angeln breed	*BB*	38	188.5±2.9	775.2±24.2	4.11±0.10	1.037±0.023	4.249±0.135	8.49±0.09	62.98±0.96
	BC	19	185.6±4.8	801.2±36.5	4.30±0.15	1.022±0.019	4.384±0.162	8.42±0.08	62.9±1.45
		td	0.516	0.605	0.333	0.503	0.640	0.583	-
	CC	3	179.3±17.7	669.7±125.9	3.68±0.41	1.030±0.065	3.807±0.571	8.37±0.03	59.0±5.3
		td	0.512	0.823	1.018	0.102	0.753	1.263	0.724
		td_1	0.344	0.317	1.419	0.118	0.971	0.588	0.710

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$), (**- $P < 0.01$) based on *t*-Student criteria (t_d).

Table 6 - Quantitative and qualitative indicators of sperm production of sire bulls of different genetic predisposition by Cp types

Ceruloplasmin type		Number of bulls, heads	Number of ejaculates	Semen received from bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, points	Fertility of cows after 1st insemination, %
Red Steppe breed	*AA*	59	185.1±2.1	690.9±17.9	3.76±0.08	1.040±0.019	3.930±0.128	8.37±0.05	62.6±0.9
	AB	64	181.7±1.9	666.1±18.1	3.66±0.09	1.010±0.023	3.748±0.115	8.28±0.04	63.34±1.0
		td	1.201	0.939	0.833	1.007	1.057	1.406	0.548
	BB	24	182.9±3.7	661.1±18.9	3.61±0.10	0.985±0.034	3.582±0.168	8.27±0.07	60.5±1.4
		td	0.517	1.144	1.172	1.413	1.648	1.163	1.265
		td_1	0.288	0.184	0.370	0.610	0.814	0.123	1.651
Angeln breed	*AA*	38	186.5±2.8	780.7±27.4	4.17±0.11	1.035±0.019	4.317±0.145	8.39±0.06	62.5±1.2
	AB	23	183.5±5.4	742.1±33.6	4.05±0.14	1.013±0.022	4.075±0.141	8.40±0.06	64.1±1.3
		td	0.492	0.890	0.674	0.759	1.198	0.117	0.791
	BB	5	191.0±4.1	830.2±58.8	4.33±0.24	1.106±0.056	4.762±0.239	8.40±0.07	65.8±2.1
		td	0.907	0.763	0.606	1.203	1.592	0.109	1.369
		td_1	1.106	1.302	1.064	1.407	2.475*	-	0.688

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$) based on *t*-Student criteria (t_d).

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Table 7 - Characteristics of sperm production of sire bulls of the Red Steppe breed at different levels of heterozygosity for marker genes

Heterozygosity, %	Number of bulls, heads	Ejaculates received	Semen received from bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, points	Fertility of cows from the 1st insemination, %
0	22	173.7±5.1	674.3±39.7	3.83±0.156[a]	1.052±0.037	3.995±0.237	8.38±0.06	64.4±1.56
25	57	181.5±2.8	671.3±20.7	3.74±0.08[b]	1.026±0.018	3.890±0.108	8.39±0.05	63.8±0.95
	td	1.345	0.067	0.514	0.634	0.404	0.128	0.327
50	51	180.3±2.2	667.8±17.6	3.62±0.08[c]	1.013±0.016	3.674±0.105	8.31±0.05	62.1±0.91
	td	1.179	0.149	1.200	0.975	1.239	0.897	1.271
	td_1	0.337	0.129	1.062	0.542	1.912	1.127	1.288
75	22	178.9±4.8	646.2±26.2	3.41±0.13	1.068±0.024	3.634±0.159	8.25±0.06	60.6±1.25
	td	0.743	0.630	2.068*	0.364	1.267	1.529	1.900
	td_1	0.467	0.751	2.156*	1.400	0.813	1.795	2.038
	td_2	0.265	0.684	1.373	1.910	0.211	0.769	0.968
100	8	166.0±11.4	486.0±83.5	2.85±0.35[a]	1.035±0.064	2.904±0.331	8.31±0.12	56.3±2.99
	td	0.616	2.036	2.559*	0.230	2.973**	0.522	2.403*
	td_1	1.320	2.154*	2.479*	0.136	2.833**	0.615	2.389*
	td_2	1.233	2.131*	2.145*	0.333	2.219*	-	1.924
	td_3	1.040	1.831	1.514	0.485	1.989	0.448	1.327

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$), (**- $P < 0.01$) based on *t*-Student criteria (t_d).

Table 8 - Characteristics of sperm production of sire bulls of the Angeln breed at different levels of heterozygosity for marker genes

Heterozygosity, %	Number of bulls, heads	Ejaculates received	Semen received from bull per year, ml	Average ejaculate volume, ml	Sperm concentration, billion/ml	Total number of sperm in ejaculate, billion	Sperm activity, scores	Fertility of cows from the 1st insemination, %
0	13	187.5±2.8	779.8±51.9	4.14±0.25	1.062±0.028	4.388±0.273	8.36±0.08	60.6±1.78
25	31	184.9±3.5	758.4±22.6	4.09±0.11	1.050±0.020	4.200±0.142	8.37±0.07	63.9±1.04
	td	0.580	0.378	0.185	0.353	0.610	0.094	1.602
50	14	175.9±7.9	782.0±44.3	4.43±0.12	0.994±0.020	4.394±0.127	8.43±0.05	64.6±1.19
	td	1.384	0.023	1.036	2.000	0.020	0.638	1.869
	td_1	1.042	0.474	2.086*	2.800**	1.016	0.698	0.443
75	4	189.5±6.2	786.8±88.1	4.13±0.37	0.980±0.075	3.985±0.430	8.35±0.03	62.8±6.2
	td	0.294	0.680	0.022	1.025	0.729	0.118	0.341
	td_1	0.646	0.301	0.103	0.897	0.475	0.263	0.175
	td_2	1.360	0.049	0.771	0.180	0.912	1.379	0.285

Note: Values are presented as Mean \pm SE; Asterisks (*) indicate significant differences ($P < 0.05$), (**- $P < 0.01$) based on *t*-Student criteria (t_d).

Thus, the highest sperm concentration was observed in bulls with a 75% heterozygosity level, and a relatively high concentration was found in homozygous bulls. However, no significant differences were established between the groups for this indicator. A similar pattern was observed for other indicators. In terms of the total number of sperm per ejaculate (3.995 ± 0.237 and 3.890 ± 0.108 billion), bulls with 0% and 25% heterozygosity bulls exceeded other groups, with a significant difference compared to bulls with 100% heterozygosity level (2.904 ± 0.331 billion, t_d = 2.973 and 2.833). The highest sperm activity (8.38±0.06 and 8.39±0.05) was also found in bulls with 0% and 25% heterozygosity, while the lowest (8.25 ± 0.06, t_d = 1.529 and 1.795) was observed in bulls with 75% heterozygosity. With an increase in the level of heterozygosity, there was also a decrease in the fertilizing ability of bull sperm. The highest fertilization rate of females at the first insemination (64.4 ± 1.56 and 63.8 ± 0.95%) was observed in the group using sperm from homozygous bulls and bulls with a 25% heterozygosity. The lowest rates were recorded in the 4th and 5th groups (75% and 100% heterozygosity, respectively), with rates of 60.6 ± 1.25% (td = 1.900 and 2.038) and 56.3 ± 2.99% (td = 2.403 and 2.389). Studies by Gopinathan et al. (2018) and Piddubna and Zakharchuk (2020) have established the dependence of sperm productivity in breeding bulls on genotypic factors.

When examining the sperm production indicators of Angeln bulls (Table 8), a similar pattern related to heterozygosity levels was not found. The largest ejaculate volume (4.43 ± 0.12 ml) was observed in bulls with a 50% level of heterozygosity, and the smallest (4.09 ± 0.11 ml, t_d = 2.086) was in bulls with a 25% level. Regarding sperm concentration, a linear decrease was observed as the level of heterozygosity increased. The highest concentration (1.062 ± 0.028 billion/ml) was characteristic of homozygotes, while the lowest concentrations (0.994±0.02 billion/ml and 0.980 ± 0.075 billion/ml) were found in bulls with 50% and 75% heterozygosity. In terms of the total number of sperm in the ejaculate (4.394 ± 0.127 and 4.388 ± 0.273 billion/ml), bulls with 50% heterozygosity and homozygotes showed an advantage. Sperm activity was higher in bulls with 50% heterozygosity (8.43 ± 0.05), and they also exhibited higher fertilization ability (64.6 ± 1.19%). The lowest fertilization rate of females from the first insemination was obtained in homozygous bulls (60.6 ± 1.78%, t_d = 1.869).

CONCLUSIONS

1. A significant difference was observed in the volume of semen obtained per year between bulls with the transferrin types *AA*, *AB*, *AE* and *DE*. The ejaculate volume and the total number of sperm count were highest in homozygous bulls (*AA*, *DD*) of the Red Steppe breed, while in the Angeln breed, the highest values were found in heterozygous bulls (*AD*). The highest sperm activity was characteristic of bulls with the *AA* transferrin type. The fertilization rate of females from the first insemination was the highest in Red Steppe bulls with the *AA* and *AD* transferrin types, and in Angeln bulls with the *DD* type.
2. Homozygous *BB* at the *Am* locus in the Red Steppe breed, and heterozygous *BC* bulls in the Angeln breed exhibited the highest sperm production and fertilization ability.
3. Homozygous bulls of the Red Steppe breed with the *Cp AA* type showed the highest ejaculate volume, sperm concentration, total sperm count in the ejaculate, and sperm activity. The fertilization ability from the first insemination of cows and heifers was highest in heterozygous (*BC*) bulls. Homozygous (*BB*) bulls in the Angeln breed demonstrated the highest sperm production and fertilization ability.
4. Homozygous bulls and those with a 25% level of heterozygosity for marker genes in the Red Steppe breed exhibited the highest indicators of sperm production and fertilization ability. An increase in the level of heterozygosity in these bulls was associated with a decrease in the noted indicators of reproductive function. In the Angeln breed, bulls with a 50% level of heterozygosity demonstrated the highest indicators of sperm production and fertilization ability.

Suggestions

1. These findings suggest that consideration of homozygosity levels at the *Tf*, Am, and Cp loci may be useful when selecting sire bulls, although validation in independent populations is warranted.
2. To perform typing of genetic and biochemical features of sperm production of bulls of Bos taurus breeds currently widespread in Ukraine.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contributions

V. Melnyk focused on the conceptual and methodological part of the study, as well as on the primary data processing, development of the main scientific concept of the work and definition of key tasks. Direct participation in the collection of material. M. Gill was critical for the analysis, interpretation of results and their scientific justification, conducting

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comprehensive statistical analysis, including calculating the reliability of differences and correlations. Interpreting the obtained numerical data and comparing them with literary sources. Formulation of final conclusions based on the data obtained and development of practical recommendations (proposals for breeding enterprises), structuring and critical review of the scientific text. M. Tymofiiv provided the collection, systematization, and critical analysis of existing scientific publications used in the literature review. Carrying out general scientific and stylistic editing of the text, ensuring its compliance with publication requirements, and being responsible for the final version of the article.

Consent to publish

All authors agreed to the publication of this manuscript.

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Ethical regulations

Animal maintenance and data collection were carried out in accordance with the Law of Ukraine "On Veterinary Medicine and Animal Welfare" of 04.02.2021 No. 1206-IX. Authors complied with the ARRIVE guidelines and or the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee.

Competing interests

The authors declare that they have no competing interests.

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ENHANCING NUTRIENT DIGESTIBILITY AND MICROBIAL FERMENTATION IN RICE STRAW-BASED DIETS USING CASSAVA, COPRA, AND PALM KERNEL MEALS

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ABSTRACT: This study assessed the effects of cassava-urea and protein meal supplementation at different proportions on the *in vitro* fermentation characteristics and digestibility of rice straw-based diets. Five diets were prepared with 20% rice straw as the base, and cassava-urea to protein meal ratios of 80:0 (T1), 70:10 (T2), 60:20 (T3), 50:30 (T4), and 40:40 (T5). *In vitro* digestibility and fermentation traits were analyzed using a two-stage method. The results showed that higher levels of protein meal improved crude protein digestibility from 67.5 to 81.1%. However, this increases reduced dry matter and organic matter digestibility, dropping from 82.8 to 75.0% and from 83.4 to 74.4%, respectively ($P < 0.05$). The pH remained within the optimal range of 7.29 to 7.41, with no significant change in total volatile fatty acids (VFA) production, which ranged from 40.9 to 44.8 mM. The ammonia nitrogen (NH_3-N) levels rose significantly from 7.86 to 11.4 mg/100 mL. This led to a decrease in the VFA:NH_3-N ratio, which changed from 5.30 to 4.05 ($P < 0.05$). This suggests an energy-to-nitrogen imbalance at higher protein meal levels. Microbial protein synthesis improved from 1.17 to 1.35 mg/mL ($P < 0.05$), likely due to increased nitrogen availability despite the imbalance. Including a moderate amount of protein meals, specifically 60% cassava-urea and 20% protein meal, provided a better balance in digestibility and fermentation efficiency. This highlights the importance of optimizing the energy-to-protein ratio in ruminant feed formulations.

Keywords: Cassava meal, *In vitro* digestibility, Microbial protein synthesis, Rice straw, Rumen fermentation.

INTRODUCTION

The rising demand for animal protein has created a greater need for sustainable and cost-effective feed resources, especially for ruminants. Rice straw, a common agricultural by-product, is often used as a basic feed ingredient because it is abundant and inexpensive. However, rice straw has its drawbacks. It has low crude protein content, which ranges from 4.0 to 4.7%, high lignocellulose levels, and low digestibility (Hasan et al., 2006, Kumar et al., 2021). These factors limit its use as the only feed source. To address these issues, it is crucial to supplement rice straw-based diets with energy- and protein-rich feed components. This will improve their nutritional value and fermentation efficiency.

Cassava-urea, a composite feed made from cassava mixed with urea, is a high-energy supplement used in ruminant nutrition. It provides easily fermentable carbohydrates and non-protein nitrogen, promoting microbial growth in the rumen (Wanapat and Khampa, 2007). Additionally, protein sources like copra and palm kernel meal act as bypass proteins and supply fiber, complementing the energy from cassava-urea. Finding the right balance between energy and protein is essential for improving fermentation efficiency, nutrient digestibility, and overall animal performance (Ortega et al., 2022), highlighting the importance of precise formulation in ruminant diets.

Despite these advantages, the ideal levels of cassava-urea and protein meals for maximizing digestibility and fermentation in rice straw-based diets are still uncertain. Excessive protein addition may cause nitrogen wastage, while insufficient energy can impair microbial activity and feed utilization. This research investigates how different amounts of cassava-urea and protein meal affect *in vitro* fermentation and digestibility of rice straw-based diets. By identifying the optimal energy-protein ratio, the study offers valuable insights to enhance feed formulation for ruminant nutrition.

MATERIALS AND METHODS

Treatments and experimental design

Five dietary treatments utilized rice straw as the main fiber source, constituting 20% of the total diet. The other 80% consisted of a mixture of cassava-urea and protein meal. This ratio was chosen to enhance the rapid availability of carbohydrates and protein in the ration. The cassava-urea was prepared by combining 99% cassava flour with 1% urea, offering fermentable carbohydrates and non-protein nitrogen. The protein meal included 75% copra meal and 25% palm kernel meal, providing crude protein and structural fiber. The ratios of cassava-urea to protein meal were 80:0 (T1), 70:10

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(T2), 60:20 (T3), 50:30 (T4), and 40:40 (T5). This mixture was then mixed with 20% rice straw to create the dietary treatments. All treatments were assessed using a one-way completely randomized design. The composition of the dietary treatments is shown in Table 1, and the chemical composition is presented in Table 2.

Table 1 - Formulation of rice straw-based diets supplemented with cassava-urea and protein meal in varying proportions

Feedstuffs	T1	T2	T3	T4	T5
Rice straw (%)	20	20	20	20	20
Cassava-urea (%) [1]	80	70	60	50	40
Protein meal (%) [2]	0	10	20	30	40

[1] Cassava-urea = 99% cassava + 1% urea. [2] Protein meal = 75% copra meal + 25% palm kernel meal

Table 2 - Chemical composition of rice straw-based diets supplemented with cassava-urea and protein meal at varying proportions

Nutrients	T1	T2	T3	T4	T5
Dry matter (%)	94.3	93.5	92.5	92.8	92.3
Organic matter (%DM)	93.4	93.0	92.8	92.1	91.8
Crude protein (%DM)	5.16	7.38	8.82	8.97	10.7
Ether extract (%DM)	2.00	2.38	2.40	2.90	2.90
Crude fiber (%DM)	9.19	10.0	12.0	13.0	13.6
Nitrogen-free extract (%DM) [1]	77.1	73.2	69.6	68.3	64.7

T1 = 20% rice straw + 80% cassava-urea, T2 = 20% rice straw + 70% cassava-urea + 10% protein meal, T3 = 20% rice straw + 60% cassava-urea + 20% protein meal, T4 = 20% rice straw + 50% cassava-urea + 30% protein meal, T5 = 20% rice straw + 40% cassava-urea + 40% protein meal. [1] Nitrogen-free extract (NFE) = dry matter - (crude protein + ether extract + crude fiber + ash).

Chemical composition analysis

The analyses of chemical composition followed the methods described by the Association of Official Agricultural Chemists (AOAC, 2005). Samples from each treatment group were dried at 55°C and ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA), both before and after *in vitro* incubation. The analyses included measurements of dry matter (DM) (method 934.01), ash (method 942.05), crude protein (CP) (method 984.13), ether extract (EE) (method 920.39), and crude fiber (CF) (method 978.10). The nitrogen-free extract (NFE) was also calculated using the following formula:

NFE = dry matter – (crude protein + ether extract + crude fiber + ash) (1)

***In vitro* digestibility and fermentation characteristics analysis**

Two Ongole crossbred cows served as donors of ruminal fluid for *in vitro* incubations. The cows received a diet of Napier grass (*Pennisetum purpureum*) and commercial concentrate in an 80:20 ratio. Rumen fluid was collected before morning feeding, filtered with four layers of cheesecloth, and mixed with artificial saliva following the method described by Tilley and Terry (1963).

The *in vitro* digestibility was assessed using Tilley and Terry's two-stage method, focusing only on rumen degradation; thus, only the first stage was conducted. Each dietary treatment was tested in seven replications. At the end of the incubation period, fermentation characteristics were measured, which included pH, total volatile fatty acids (VFA), individual VFAs (acetic, propionic, and butyric acids), ammonia nitrogen (NH_3-N), and microbial protein synthesis. Ruminal VFAs were separated and quantified using gas chromatography (GC 2010 Plus, Shimadzu Corporation, Kyoto, Japan) following the methods described by Filípek and Dvořák (2009). Ammonia nitrogen concentration was determined using the spectrophotometric method of Chaney and Marbach (1962). Microbial protein synthesis was analyzed using a method described by Plummer (1987).

Statistical analysis

All data were analyzed using the SAS software (SAS Institute Inc., Cary, NC). The statistical model applied was:

$Yij = \mu + Ti + Ej$ (2)

Yij represents the individual response variable measured, μ is the overall mean, Ti denotes the fixed effect of treatment (i = 1 to 5), and Ej is the error term. Mean comparisons were performed using Duncan's multiple range test when the model found significant treatment effects ($P \leq 0.05$). Significant differences were noted at $P \leq 0.05$.

RESULTS AND DISCUSSION

In vitro digestibility

The *in vitro* digestibility results in Table 3 show significant differences in dry matter digestibility (DMD), organic matter digestibility (OMD), and crude protein digestibility (CPD) among the five dietary treatments. The DMD decreased from 82.8% in T1 to 75.0% in T5. This change shows that increasing protein meal inclusion lowers dry matter digestibility. The high DMD in T1 comes from its high cassava-urea content (80%), which provides easily fermentable carbohydrates, as shown in the nitrogen-free extract (NFE) in Table 2. Cassava has a high NFE level and ferments easily in the rumen, giving a quick energy source for rumen microbes (Rosmalia et al., 2023). However, the higher fiber content in diets with more protein meals (T4 and T5; Table 2) reduces DMD by lowering the availability of digestible energy.

The OMD data also showed a decline, dropping from 83.4% in T1 to 74.4% in T5. This highlights the trade-off between energy availability and fiber inclusion as the levels of protein meal increase. These changes align with the lower NFE values observed in Table 2, decreasing from 77.1% DM in T1 to 64.7% DM in T5. Conversely, CPD showed notable improvement with increased inclusion of protein meal, rising from 67.5% in T1 to 81.1% in T5. This trend is related to the higher crude protein content in diets with more protein meals, with values increasing from 5.16% DM in T1 to 10.7% DM in T5, as detailed in Table 2. The findings suggest that higher cassava-urea levels (T1 and T2) enhance energy digestibility but may restrict protein availability. Studies have indicated that cassava tubers, rich in carbohydrates, can substantially boost the digestibility of organic matter and gross energy in ruminants (Thang et al., 2010, Putridinanti et al., 2019, Santos et al., 2019). In contrast, diets high in protein meals (T4 and T5) improve CPD but lower overall energy digestibility due to increased fiber content. Earlier research indicates that higher dietary protein, particularly rumen undegradable protein, can improve protein digestibility in ruminants. For instance, increasing rumen undegradable protein (RUP) in diets led to higher milk yield and fat content in lactating cows, even though nutrient digestibility declined with increased RUP levels (Akhtar et al., 2017, Araújo et al., 2020).

These *in vitro* digestibility results demonstrate a nutritional trade-off from the treatments. While increasing protein meals enhance crude protein availability and utilization, it simultaneously decreases carbohydrate availability and overall fermentability, as shown by DMD and OMD measurements. This emphasizes the need to balance fermentable energy, such as NFE from cassava, with nitrogen sources like true protein from meals. Maintaining such a balance is crucial for enhancing microbial activity and nutrient digestibility in ruminants. While T5 might provide better protein digestibility, intermediate formulations like T3 could offer a better balance of energy and protein use for practical feeding systems in tropical ruminant production. Diet T3, which contains 60% cassava-urea and 20% protein meal, shows improved results in DMD, OMD, and CPD. This suggests it could be a good option for balancing energy and protein in diets using rice straw. These results highlight the importance of modifying cassava-urea and protein meal levels to enhance nutrient utilization in ruminant diets.

Table 3 - *In vitro* digestibility of rice straw-based diets supplemented with cassava-urea and protein meal at varying proportions

Digestibility (%)	T1	T2	T3	T4	T5
Dry matter	$82.8^{c} \pm 0.34$	$79.6^{b} \pm 0.79$	$78.7^{b} \pm 1.39$	$75.3^{a} \pm 0.63$	$75.0^{a} \pm 1.45$
Organic matter	$83.4^{c} \pm 0.78$	$80.3^{b} \pm 0.64$	$79.1^{b} \pm 1.25$	$75.5^{a} \pm 0.78$	$74.4^{a} \pm 0.64$
Crude protein	$67.5^{a} \pm 0.52$	$73.8^{b} \pm 0.80$	$80.3^{c} \pm 1.02$	$74.8^{b} \pm 0.52$	$81.1^{c} \pm 0.82$

$^{a,b,c:}$ Means within a column with different superscripts differ significantly ($P<0.05$). T1 = 20% rice straw + 80% cassava-urea, T2 = 20% rice straw + 70% cassava-urea + 10% protein meal, T3 = 20% rice straw + 60% cassava-urea + 20% protein meal, T4 = 20% rice straw + 50% cassava-urea + 30% protein meal, T5 = 20% rice straw + 40% cassava-urea + 40% protein meal.

In vitro fermentation characteristics

Table 4 presents the *in vitro* fermentation characteristics of rice straw-based diets with varying levels of cassava-urea and protein meal, as outlined in Table 1. The parameters assessed – such as pH, total and individual volatile fatty acids (VFAs), ammonia nitrogen (NH_3-N), the VFA:NH_3-N ratio, and microbial protein – offer valuable insights into rumen fermentation and nutrient utilization.

The pH levels ranged from 7.29 to 7.41 (Table 4), remaining within the optimal range for rumen microbial activity. This indicates effective buffering capacity despite dietary changes. Total volatile fatty acid (VFA) concentrations varied from 40.9 mM (T1) to 44.8 mM (T5) with no significant differences between treatments, suggesting consistent fermentation activity across all diets. Similarly, individual VFAs (acetic, propionic, and butyric acids) showed no significant variation, implying that alterations in cassava-urea and protein meal ratios did not adversely affect these fermentation end-products. The acetate-to-propionate (A:P) ratio ranged from 2.63 (T3) to 3.13 (T2), with no significant differences. Nevertheless, the numerically lower A:P ratio observed in T3 may indicate a tendency toward more energy-efficient fermentation. A lower A:P ratio is often associated with improved energy utilization due to higher propionate production,

reduced methane formation, and enhanced fermentation stability, which collectively promote efficient microbial syntrophy and reduce competition among methanogens (Meng et al., 2013, Wagner et al., 2014, Zakaria et al., 2022). As a result, it creates a more effective and stable fermentation environment.

Table 4 - *In vitro* fermentation characteristics of rice straw-based diets supplemented with cassava-urea and protein meal at varying proportions

Item	T1	T2	T3	T4	T5
pH	7.34 ± 0.18	7.35 ± 0.14	7.29 ± 0.19	7.41 ± 0.21	7.40 ± 0.16
Total VFA (mM)	40.9 ± 0.78	42.6 ± 4.00	42.9 ± 2.27	43.5 ± 0.30	44.8 ± 0.11
Acetate (mM)	24.4 ± 0.34	26.3 ± 3.00	25.2 ± 1.16	26.3 ± 1.01	27.2 ± 1.30
Propionate (mM)	8.75 ± 0.99	8.40 ± 0.90	9.61 ± 0.96	9.12 ± 0.53	9.64 ± 1.06
Butyrate (mM)	7.84 ± 0.13	7.82 ± 0.14	8.04 ± 0.15	8.00 ± 0.18	8.04 ± 0.13
A:P	2.80 ± 0.36	3.13 ± 0.03	2.63 ± 0.14	2.88 ± 0.26	2.85 ± 0.45
NH_3-N (mg/100mL)	7.86^{a} ± 0.23	8.07^{a} ± 0.70	9.33^{a} ± 1.25	9.54^{a} ± 0.11	11.4^{b} ± 1.00
VFA:NH_3-N	5.30^{b} ± 0.06	5.27^{b} ± 0.24	5.22^{b} ± 0.06	4.72^{b} ± 0.30	4.05^{a} ± 0.53
Microbial protein (mg/mL)	1.17^{a} ± 0.07	1.17^{a} ± 0.09	1.23^{ab} ± 0.11	1.30^{ab} ± 0.11	1.35^{b} ± 0.05

^{a,b}: Means within a column with different superscripts differ significantly (P<0.05). T1 = 20% rice straw + 80% cassava-urea, T2 = 20% rice straw + 70% cassava-urea + 10% protein meal, T3 = 20% rice straw + 60% cassava-urea + 20% protein meal, T4 = 20% rice straw + 50% cassava-urea + 30% protein meal, T5 = 20% rice straw + 40% cassava-urea + 40% protein meal.

Ammonia nitrogen concentrations increased significantly with higher protein meal inclusion, moving from 7.86 mg/100 mL in T1 to 11.4 mg/100 mL in T5. This change shows more protein breakdown as crude protein content rose (Table 2). However, the decreasing VFA:NH_3-N ratio, which dropped from 5.30 in T1 to 4.05 in T5, suggests an imbalance between energy and nitrogen at higher protein meal levels. This imbalance could limit microbial protein production. Microbial protein synthesis in the rumen works best when the degradation rates of dietary carbohydrates and proteins are synchronized (Shete et al., 2012, Brassard et al., 2015). This synchronization gives rumen microbes a steady supply of energy and nitrogen, which is essential for their growth and protein production.

Microbial protein concentrations increased from T1 (1.17 mg/mL) to T5 (1.35 mg/mL), with significant improvements observed in T4 and T5 ($P < 0.05$). These results reflect the combined effects of increased crude protein digestibility and NH_3-N availability (Table 3), which together support enhanced microbial growth. Although energy (NFE) levels declined with higher protein inclusion (Table 2), the slight increase in microbial protein indicates that true protein sources such as copra and palm kernel meal are more effective in supporting microbial synthesis than cassava-derived non-protein nitrogen (cassava-urea) alone. Previous researchers reported that true protein sources like copra and palm kernel meals support microbial synthesis more effectively than cassava-derived NPN alone. They provide higher protein content and better digestibility, leading to more efficient nitrogen utilization and microbial protein synthesis (Stein et al., 2015, Rebelo et al., 2019).

Overall, the fermentation characteristics shown in Table 4 confirm that changes in protein and carbohydrate sources, as outlined in Table 1, greatly affect ruminal fermentation patterns. Protein supplementation improves nitrogen use and microbial growth. However, too much protein compared to fermentable energy can lower fermentation efficiency, which is indicated by lower VFA:NH_3-N ratios. Thus, achieving the right balance of fermentable carbohydrates and degradable protein, as in T3, is essential for efficient rumen fermentation and to maximize microbial protein yield.

CONCLUSION

This study shows that rice straw diets with higher cassava-urea levels (T1 and T2) enhance fermentation efficiency by improving energy digestibility. Conversely, diets richer in protein meals (T4 and T5) increase crude protein digestibility but decrease overall efficiency. Diet T3, with 60% cassava-urea and 20% protein meal, strikes a good balance between digestibility, fermentation, and nutrient utilization. These findings highlight the importance of combining degradable protein with fermentable energy to improve rumen performance. This strategy also enhances the nutritional value of low-quality tropical forages like rice straw.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethical Regulations

The authors confirm that the journal's ethical policies, as noted on the journal's author guidelines page, have been adhered to. Ethical approval was not required, as this was an *in vitro* study in which no animals were used in the main experiment.

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Authors' contribution

All authors worked together on the manuscript and contributed equally to its development. Each of them played an active role at different stages of the research, including generating ideas, collecting data, analyzing information, and writing. Their contributions were shared evenly, ensuring a balanced effort in shaping the final paper.

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Competing interests

The authors declare that there is no competing interest regarding the publication of this article.

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GROWTH PERFORMANCE, MINERAL COMPOSITION AND HEAVY METAL ACCUMULATION IN JAMBO GRASS (*Sorghum bicolor* × *S. sudanense*) IRRIGATED WITH INDUSTRIAL EFFLUENT

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ABSTRACT: The use of industrial wastewater for forage production is increasing in peri-urban livestock systems, yet its implications for fodder safety remain uncertain. This study evaluated the effects of graded industrial effluent irrigation on growth characteristics, nutrient composition, and heavy metal accumulation in Jambo grass (*Sorghum bicolor* × *Sorghum sudanense*). A pot experiment was conducted using five irrigation treatments comprising fresh water (control) and 25%, 50%, 75%, and 100% industrial effluent. Morphological traits, biomass yield, proximate composition, and concentrations of Pb, Cd, Cr, Ni, Cu, Zn, and Fe were determined in soil and plant tissues. Moderate effluent application (25–50%) improved plant height, leaf production, and total biomass yield compared with the control. However, progressive increases in effluent concentration resulted in elevated accumulation of Pb and Cd, particularly in roots and leaves. At higher effluent levels (≥75%), Pb and Cd concentrations in edible plant parts exceeded recommended limits for livestock feed, despite proximate composition remaining largely unaffected. Crude protein and fiber fractions showed no significant variation among treatments. The results indicate that diluted industrial effluent may support Jambo grass productivity, but unrestricted use poses potential risks to livestock health due to heavy metal contamination. Careful dilution and routine monitoring are therefore essential if wastewater-irrigated fodder is to be used in animal feeding systems.

Keywords: Forage quality, Jambo grass, Heavy metals, Livestock feed safety, Wastewater irrigation.

INTRODUCTION

Industrial effluent discharge has become a persistent challenge in many livestock-producing regions, particularly where agricultural land is located near industrial zones (Qadir et al., 2010; Aftab et al., 2023; Islam et al., 2023). In such areas, wastewater is frequently used for irrigation due to limited freshwater availability and its perceived nutrient value (Pedrero et al., 2010; Ungureanu et al., 2020). While industrial effluents may supply essential minerals that support plant growth, they often contain potentially toxic elements that can accumulate in soil and fodder crops (Nagajyoti et al., 2010; Alloway, 2013; Penzy et al., 2023; Islam et al., 2023).

Forage crops grown under contaminated irrigation are of particular concern because they serve as the primary feed source for ruminant animals (Khan et al., 2008; Othman et al., 2021). Heavy metals such as lead and cadmium are not biologically degradable and may persist in plant tissues, ultimately entering animal products (McLaughlin et al., 1999; Islam et al., 2023). Chronic exposure, even at low concentrations, can impair animal performance and pose risks to food safety (NRC, 2005; Atta et al., 2022). Despite these concerns, limited information is available on the response of commonly cultivated fodder grasses to industrial effluent irrigation under controlled conditions.

Jambo grass (*Sorghum bicolor* × *Sorghum sudanense*) is widely used in tropical livestock systems due to its high biomass yield, adaptability, and suitability for cut-and-carry feeding (FAO, 2011). Its rapid growth and extensive root system suggest a degree of tolerance to suboptimal soil and water conditions (Skerman and Riveros, 1990). However, information on its mineral uptake behavior and heavy metal accumulation under effluent irrigation remains scarce, although previous findings on morphological responses of fodder crops under industrial effluent stress have been documented (Islam et al., 2023).

Therefore, the present study was undertaken to evaluate the effects of industrial effluent irrigation on growth performance, nutrient composition, and heavy metal accumulation in Jambo grass, with specific emphasis on its suitability as a livestock feed resource.

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MATERIALS AND METHODS

Experimental location and climatic conditions

The experiment was conducted at the Livestock and Poultry Farm of Gazipur Agricultural University (GAU), Gazipur, Bangladesh. The experimental period (February to June, 2024) represented the transition from late winter to early monsoon, marked by increasing temperature, relative humidity, and occasional rainfall, which are conducive to enhanced growth of irrigated fodder crops (BBS, 2023). All experimental procedures were conducted in accordance with the methodology described by Islam et al. (2023), ensuring consistency with established protocols.

Soil collection and pot preparation

Surface soil (0-15 cm) was collected from the university research field, air-dried, crushed, and sieved to remove debris. Plastic pots (36 × 36 × 32.5 cm) were filled with 18 kg of prepared soil. During pot preparation, well-decomposed cow dung was incorporated at 400 g per pot to improve soil organic matter and nutrient availability. Basal fertilization was applied using urea, triple superphosphate, and muriate of potash at 10, 15, and 12 g per pot, respectively, two weeks before sowing. The fertilizer rates were selected based on recommended nutrient requirements for sorghum-sudangrass hybrids and adjusted proportionally to pot soil weight to ensure balanced nutrient supply under controlled conditions (FAO, 2011; Havlin et al., 2014; FRG, 2018).

Planting material and experimental design

Jambo grass (*Sorghum bicolor* × *S. sudanense*) seeds were obtained from the Department of Animal Nutrition, Bangladesh Agricultural University. Three seeds were sown per pot in a triangular arrangement. The experiment followed a completely randomized design with five irrigation treatments and eight replications.

Irrigation treatments

Industrial effluent was collected from a nearby garment factory discharge point within the university area. During the irrigation period, effluent samples were consistently collected from the same location of a local drainage channel situated near the Livestock and Poultry Farm, GAU, and were applied directly for irrigation. The collected effluent was thoroughly mixed before application to ensure homogeneity. The effluent was used as irrigation water at five different concentrations (0%, 25%, 50%, 75%, and 100%) by mixing with fresh water according to the treatment design. Water samples (industrial effluent and fresh water) were collected weekly, preserved in clean plastic containers, and stored at 4°C for subsequent laboratory analysis. Five irrigation treatments were established using different proportions of fresh water and industrial effluent: **T_1:** 100% fresh water; **T_2:** 75% fresh water + 25% effluent; **T_3:** 50% fresh water + 50% effluent; **T_4:** 25% fresh water + 75% effluent; **T_5:** 100% effluent. All pots received equal volumes of irrigation water at 3-day intervals, depending on moisture conditions.

Crop management and harvesting

Routine intercultural operations, including weeding and fencing, were conducted throughout the experimental period. No pesticides were applied. Jambo grass was harvested 75 days after sowing. Fresh biomass from each pot was weighed immediately after harvest.

Measurement of growth parameters

Plant height, number of tillers, number of leaves, leaf length, leaf yield, and stem yield were recorded following harvest. The leaf-to-stem ratio was calculated from dry matter yield.

Sample preparation and laboratory analysis

Irrigation water analysis

Irrigation water samples were collected and stored in plastic bottles that had been pre-washed with 10 mol/L HCl, rinsed thoroughly with deionized water, and air-dried. The collected samples were filtered through a 0.45 µm membrane filter using a 100 mL syringe and subsequently stored in clean plastic bottles. To prevent precipitation and preserve heavy metals and trace elements, the samples were acidified by adding 10 mol/L HCl to each 50 mL of water sample. The effluent samples were analyzed following standard analytical methods as described by Thomas (1982), Hossain et al. (2015).

Soil and plant sample analysis

Soil and plant samples were collected from each pot. The soil samples were air-dried, cleaned, homogenized, and ground using a grinding machine, then passed through a 20-mesh sieve. Similarly, the plant samples were oven-dried at 65°C for approximately 48 hours until a constant weight was achieved, after which they were ground and sieved through a 20-mesh sieve. For digestion, 0.5 g of both soil and plant samples were treated with a di-acid mixture of HNO_3-$HClO_4$ (2.5:1) following the method of Norvell et al. (2000) using a block digester (Model: VELP). After adding the acid mixture,

samples were left overnight and subsequently heated at 120°C for about 4 hours until the evolution of white fumes. The digested samples were then filtered through Whatman No. 1 filter paper, and the volume was adjusted to 50 mL with deionized water. The prepared extracts were used for the determination of heavy metals (Pb, Cd, Cr, Ni, Cu, Zn, and Fe). The concentrations of these metals were analyzed using an atomic absorption spectrophotometer equipped with a Buck Scientific 210 VGP continuous flow hydride generation system (HG-AAS). The proximate composition of plant samples was analyzed following the method of AOAC (2019) and expressed on a dry matter basis.

Statistical analysis

Data were analyzed using analysis of variance appropriate for a completely randomized design. Treatment means were compared using Duncan's multiple range test, and differences were considered significant at $P < 0.05$.

RESULTS

Heavy metal and mineral composition of fresh water and industrial effluent before irrigation

Industrial effluent exhibited significantly higher ($P < 0.01$) concentrations of Pb, Cd, Cr, Ni, Cu, Fe, Mg, Na, K, and Ca compared to fresh water used for irrigation (Table 1). In contrast, no significant differences ($P > 0.05$) were observed between fresh water and industrial effluent with respect to Zn and Mn concentrations.

Soil heavy metal and mineral status after harvest

Post-harvest soil analysis revealed significant effects of effluent irrigation on Pb ($P < 0.01$), Cd ($P < 0.01$), Ni ($P < 0.01$), Fe ($P < 0.05$), Mg ($P < 0.01$), K ($P < 0.05$), and Ca ($P < 0.01$) concentrations (Table 2). Lead and cadmium levels increased with effluent application, particularly at higher concentrations. Nickel showed maximum accumulation under the 50% effluent treatment; moreover, iron and magnesium showed higher accumulation under the 100% effluent treatment (Table 2). Chromium, copper, zinc, manganese, and sodium did not differ significantly ($P > 0.05$) among treatments. However, after harvest, there was minimal accumulation of potassium and calcium in the soil.

Growth and biomass yield of Jambo grass

Plant height differed significantly ($P < 0.01$) among treatments, with the tallest plants observed under 25% effluent irrigation. Total biomass yield was highest ($P < 0.01$) at 50% effluent and lowest under 100% effluent irrigation. The number of tillers showed no significant ($P > 0.05$) variation, while leaf number and leaf length were significantly higher under moderate effluent treatments (Table 3). Leaf yield increased ($P < 0.01$) with effluent dilution up to 50%, whereas stem yield declined progressively at higher effluent (100%) concentrations. The leaf-to-stem ratio increased ($P < 0.01$) with increasing effluent proportion.

Heavy metal accumulation in plant tissues

The concentrations of heavy metals and macro-minerals in different parts of Jambo grass were influenced to varying degrees by industrial effluent irrigation (Table 4). Lead (Pb) concentration in whole grass remained unaffected ($P > 0.05$), although significant differences were observed in plant parts, with higher accumulation in leaves under T_4 ($P < 0.01$), stems under T_5 ($P < 0.05$), and roots under T_3 ($P < 0.01$). Cadmium (Cd) concentration in whole grass did not vary significantly ($P > 0.05$), whereas leaf Cd content differed markedly ($P < 0.01$), showing the highest value in T_1 and the lowest in T_5; root Cd accumulation was also significant ($P < 0.01$), with greater values in T_1 and T_4. Chromium (Cr) remained statistically unchanged across all treatments and plant parts ($P > 0.05$). Nickel (Ni) concentration varied significantly in whole grass and all plant components ($P < 0.01$), with maximum accumulation in whole grass and roots under T_3, in leaves under T_5, and in stems under T_2 and T_3. Copper (Cu) concentration showed no significant variation in whole grass and stems ($P > 0.05$), but differed in leaves ($P < 0.05$) and roots ($P < 0.01$), with relatively higher values under moderate effluent treatments. Zinc (Zn) concentration decreased significantly in whole grass with increasing effluent levels ($P < 0.01$), with the highest value in T_1, while significant differences were also observed in leaves ($P < 0.01$), stems ($P < 0.05$), and roots ($P < 0.01$), with root Zn peaking under T_3. Iron (Fe) concentration showed a slight but significant reduction in whole grass at higher effluent levels ($P < 0.05$), whereas significant variation was observed in leaves ($P < 0.01$), stems ($P < 0.01$), and roots ($P < 0.01$), with maximum accumulation generally under intermediate to higher effluent treatments. Among macro-minerals, magnesium (Mg) concentration in whole grass did not differ significantly ($P > 0.05$), although significant variation was observed in leaves ($P < 0.05$), stems ($P < 0.01$), and roots ($P < 0.01$), with stem Mg increasing under effluent application and root Mg declining with increasing effluent levels. Manganese (Mn) concentration decreased significantly in whole grass ($P < 0.05$), while significant differences were observed in leaves ($P < 0.01$), stems ($P < 0.01$), and roots ($P < 0.01$), with maximum accumulation generally recorded under T_3. Sodium (Na) concentration was significantly affected in whole grass and all plant parts ($P < 0.01$), with higher values in control and higher effluent treatments, depending on plant component. Potassium (K) concentration showed highly significant variation ($P < 0.01$) in all plant parts, with whole grass and root K increasing under higher effluent levels, while leaf and stem responses varied among treatments. Calcium (Ca) concentration differed significantly in whole grass ($P < 0.05$), leaves ($P < 0.01$), stems ($P < 0.01$), and roots ($P < 0.01$), with increased accumulation generally observed under moderate effluent treatments. Overall, industrial effluent irrigation significantly influenced the distribution of both heavy metals and essential minerals in Jambo grass, with distinct partitioning patterns among leaves, stems, and roots.

Table 1 - Heavy metal and mineral composition of irrigation water used in the experiment (ppm)

Component (ppm) \ Irrigation water	Fresh water	Waste water	P-value*
Pb	0.031±0.03^{b}	3.508±0.68^{a}	0.000
Cd	0.023±0.01^{b}	1.639±0.29^{a}	0.000
Cr	0.002±0.00^{b}	3.082±0.72^{a}	0.000
Ni	0.038±0.02^{b}	5.193±1.05^{a}	0.000
Cu	0.759±0.28^{b}	3.596±0.93^{a}	0.000
Zn	0.228±0.1	0.300±0.07	0.136
Fe	0.564±0.21^{b}	1.396±0.35^{a}	0.000
Mg	2.958±0.97^{b}	5.574±1.01^{a}	0.000
Mn	5.672±0.95	5.887±1.03	0.692
Na	7.025±0.68^{b}	8.566±0.51^{a}	0.000
K	1.509±0.42^{b}	4.017±0.26^{a}	0.000
Ca	2.653±0.21^{b}	4.645±0.83^{a}	0.000

*P-values indicate differences between fresh water and industrial effluent.

Table 2 - Heavy metal and mineral composition of soil after harvest as influenced by industrial effluent irrigation (ppm)

Component (ppm) \ Treatment*	T_1	T_2	T_3	T_4	T_5	P-value
Pb	3.27±0.10^{b}	3.39±0.11^{b}	3.99±0.12^{a}	3.86±0.16^{a}	3.26±0.14^{b}	0.0001
Cd	0.35±0.10^{b}	0.46±0.11^{ab}	0.34±0.10^{b}	0.64±0.10^{a}	0.66±0.08^{a}	0.005
Cr	0.05±0.01	0.05±0.01	0.05±0.01	0.06±0.01	0.04±0.01	0.646
Ni	3.19±0.17^{c}	3.17±0.25^{c}	4.20±0.15^{a}	3.88±0.20^{ab}	3.47±0.14b^{c}	0.0001
Cu	0.36±0.03	0.47±0.04	0.43±0.06	0.43±0.09	0.43±0.09	0.467
Zn	2.19±0.19	2.10±0.12	2.05±0.12	1.96±0.07	2.20±0.09	0.168
Fe	28.25±1.04^{ab}	27.24±1.03^{b}	28.75±1.11^{ab}	29.20±0.77^{ab}	30.44±0.39^{a}	0.017
Mg	377.48±2.29^{c}	338.68±2.96^{d}	374.47±3.86^{c}	417.19±4.30^{b}	471.82±2.41^{a}	0.000
Mn	0.28±0.03	0.28±0.06	0.35±0.03	0.30±0.08	0.31±0.05	0.464
Na	203.62±4.56	201.93±3.08	196.31±1.60	200.39±1.89	200.38±3.06	0.119
K	125.65±1.63^{a}	118.58±4.16^{b}	118.48±3.13^{b}	118.48±3.09^{b}	123.79±2.97^{ab}	0.041
Ca	265.06±3.33^{a}	225.42±1.69^{c}	204.46±4.29^{e}	214.46±4.05^{d}	242.61±3.77^{b}	0.000

*T_1: 100% fresh water; T_2: 75% fresh water + 25% effluent; T_3: 50% fresh water + 50% effluent; T_4: 25% fresh water + 75% effluent; T_5: 100% industrial effluent. Means within a row bearing different superscripts differ significantly ($p < 0.05$)

Table 3 - Effect of industrial effluent irrigation on morphological traits and biomass yield of Jambo grass at 75 days

Parameters \ Treatment*	T_1	T_2	T_3	T_4	T_5	P-value
Plant height (cm)	102.58±3.35^{b}	114.33±2.42^{a}	106.92±4.36^{b}	106.00±4.20^{b}	103.67±4.37^{b}	0.000
No of Tillers/ Plant	4.33±0.56	3.89±0.81	3.89±0.50	3.83±0.41	3.61±0.65	0.355
Number of leaves/ Plant	17.44±1.33^{b}	21.11±2.56^{a}	18.17±2.34^{ab}	18.67±2.07^{ab}	17.00±2.13^{b}	0.023
Longest Leaf length (cm)	64.00±2.52^{ab}	66.94±2.54^{a}	66.06±2.40 ^{a}	65.72±2.65^{ab}	61.89±2.10^{b}	0.012
Leaf wide (cm)	6.08±0.40	6.98±0.39	6.78±0.86	6.96±0.66	6.22±0.86	0.073
Biomass Yield /plant (gm)	151.44±7.76^{b}	165.44±6.83^{b}	196.00±12.31^{a}	155.56±8.57^{b}	98.56±8.86^{c}	0.000
Leaf Yield/ Plant (gm)	40.24±2.62^{d}	70.67±4.74^{b}	92.33±5.99^{a}	52.44±4.94^{c}	56.00±4.61^{c}	0.00
Stem Yield/ Plant (gm)	294.50±4.66^{a}	238.33±3.27^{c}	254.67±4.03^{b}	215.67±4.03^{d}	131.67±4.80^{e}	0.00
Leaves to stem ratio	0.53±0.03^{d}	0.78±0.07^{c}	0.91±0.04^{b}	0.73±0.09^{c}	1.28±0.14^{a}	0.000

*T_1: 100% fresh water; T_2: 75% fresh water + 25% effluent; T_3: 50% fresh water + 50% effluent; T_4: 25% fresh water + 75% effluent; T_5: 100% industrial effluent. Values are mean ± SD. Different superscripts within a row indicate significant differences ($p < 0.05$).

Proximate composition of Jambo grass

Moisture content showed a non-significant (P = 0.06) increasing trend with higher effluent levels. Crude protein, crude fiber, crude fat, ash, nitrogen-free extract, neutral detergent fiber, and acid detergent fiber did not differ significantly among treatments (P > 0.00). All proximate values remained within typical ranges for tropical forage grasses (Table 5).

Table 4 - Heavy metal and mineral concentration in different parts of Jambo grass under varying irrigation treatments (ppm).

Component (ppm)	Different parts	*Treatment					P-value
		T_1	T_2	T_3	T_4	T_5	
Pb	Whole grass	4.20±0.53	3.74±0.24	4.00±0.51	3.82±0.24	3.89±0.12	0.61
	Leaf	3.54±0.08^{c}	3.87±0.08^{b}	3.87±0.11^{b}	4.08±0.08^{a}	3.75±0.05^{b}	0.00
	Stem	4.26±0.28^{ab}	4.09±0.12^{ab}	4.18±0.09^{ab}	3.83±0.23^{b}	4.49±0.12^{a}	0.017
	Root	3.59±0.02^{d}	4.15±0.12^{c}	4.66±0.04^{a}	4.19±0.05^{c}	4.49± 0.06^{b}	0.000
Cd	Whole grass	0.48±0.20	0.48±0.27	0.36±0.24	0.36±0.11	0.33±0.06	0.78
	Leaf	0.75±0.04^{a}	0.37±0.05^{bc}	0.37±0.06^{bc}	0.47±0.06^{b}	0.29±0.03^{c}	0.00
	Stem	0.72±0.08	0.66±0.05	0.67±0.13	0.78±0.22	0.84±0.08	0.417
	Root	1.04±0.02^{a}	0.73±0.17^{b}	0.73±0.14^{b}	1.10±0.06^{a}	0.61±0.04^{b}	0.001
Cr	Whole grass	6.56±0.60	0.02±0.01	0.02±0.01	0.02±0.00	0.02±0.01	0.85
	Leaf	0.02±0.00	0.03±0.01	0.03±0.01	0.02±0.00	0.02±0.01	0.56
	Stem	0.04±0.00	0.05±0.01	0.05±0.02	0.03±0.03	0.03±0.02	0.487
	Root	0.02±0.01	0.03±0.01	0.04±0.00	0.04±0.02	0.02±0.01	0.304
Ni	Whole grass	4.40±0.62^{d}	4.89±0.45^{cd}	6.57±0.29^{a}	5.33±0.41^{bc}	5.71±0.25^{b}	0.00
	Leaf	6.24±0.22^{b}	5.92±0.31^{bc}	4.64±0.19^{d}	5.56±0.17^{c}	6.93±0.12^{a}	0.00
	Stem	5.99±0.20^{b}	6.94±0.10^{a}	6.89±0.20^{a}	6.33±0.15^{b}	5.10±0.21^{c}	0.000
	Root	4.90±0.22^{c}	4.33±0.08^{d}	7.71±0.10^{a}	6.29±0.19^{b}	4.99±0.13^{c}	0.000
Cu	Whole grass	0.80±0. 23	0.88±0.05	0.73±0.06	0.89±0.19	0.88±0.07	0.60
	Leaf	0.86±0.08^{a}	0.75±0.05^{ab}	0.83±0.03^{a}	0.84±0.07^{a}	0.64±0.05^{b}	0.01
	Stem	0.90±0.03	0.94±0.05	0.86±0.07	0.92±0.10	0.92±0.14	0.803
	Root	0.87±0.11^{ab}	0.71±0.02^{c}	0.92±0.02^{a}	0.81±0.02^{b}	0.89±0.02^{ab}	0.005
Zn	Whole grass	24.29±0.5^{a}	21.39±0.7^{b}	22.23±0.36^{b}	22.32±0.5^{b}	19.96±0.4^{c}	0.00
	Leaf	24.40±0.37^{a}	23.08±0.1^{b}	20.31±0.36^{d}	22.18±0.1^{c}	23.66±0.14^{b}	0.00
	Stem	23.43±0.16^{ab}	21.76±0.8^{b}	22.81±1.44^{ab}	24.98±0.92^{a}	23.79±0.37^{ab}	0.012
	Root	19.11±0.84^{c}	22.65±2.28^{b}	26.04±0.09^{a}	21.85±0.21^{b}	23.64±1.07^{ab}	0.000
Fe	Whole grass	320.00±1.68^{a}	319.97±2.13^{a}	316.77±1.94^{a}	316.52±4.71^{a}	310.84±1.95^{b}	0.01
	Leaf	308.25±3.21^{c}	329.17±0.78^{a}	316.93±1.88^{b}	310.05±1.14^{c}	303.73±1.13^{d}	0.00
	Stem	300.53±2.60^{c}	318.70±4.23^{b}	307.76±4.38^{c}	331.42±1.59^{a}	331.03±3.16^{a}	0.000
	Root	319.21±3.07^{ab}	314.82±2.79^{bc}	311.29±3.74$_{c}$	325.51±3.20$_{a}$	309.98±2.11$_{c}$	0.001
Mg	Whole grass	172.00±1.17	174.99±1.97	176.03±1.78	176.11±2.74	176.84±2.83	0.13
	Leaf	177.01±2.99^{ab}	173.64±0.43^{bc}	174.86±0.78^{abc}	171.61±0.36^{c}	177.81±0.74^{a}	0.00
	Stem	176.88±2.86^{c}	201.11±1.04^{a}	191.28±2.34^{b}	189.18±3.06^{b}	194.22±2.15^{b}	0.000
	Root	203.36±0.42^{a}	195.63±3.47^{b}	183.02±1.17^{c}	175.66±2.90^{d}	180.12±2.56^{cd}	0.000
Mn	Whole grass	9.90±0.62^{a}	8.87±0.19^{b}	9.03±0.30^{b}	8.68±0.28^{b}	8.55±0.12^{b}	0.01
	Leaf	9.42±0.15^{a}	8.97±0.12^{b}	9.44±0.09^{a}	8.50±0.15^{c}	9.47±0.13^{a}	0.00
	Stem	5.24±0.27^{b}	4.39±0.56^{b}	6.22±0.29^{a}	4.49±0.54^{b}	4.89±0.37^{b}	0.002
	Root	4.86±0.13^{b}	4.90±0.13^{b}	6.26±0.06$_{a}$	4.98±0.19$_{b}$	4.17±0.16$_{c}$	0.000
Na	Whole grass	468.24±3.92^{a}	442.03±3.35^{c}	451.64±2.49^{b}	432.95±2.16^{d}	441.37±3.27^{c}	0.00
	Leaf	477.13±2.79^{a}	439.23±1.15^{c}	434.89±1.94^{c}	435.24±1.96^{c}	465.41±1.60^{b}	0.00
	Stem	472.93±3.13^{b}	434.80±3.42^{d}	449.12±4.30^{c}	497.48±2.98^{a}	468.18±3.94^{b}	0.000
	Root	472.75±3.94^{a}	430.73±1.82^{c}	422.51±2.47^{d}	468.60±3.47^{a}	452.37±2.22^{b}	0.000
K	Whole grass	844.85±3.78^{bc}	841.61±3.64^{c}	849.23±2.12^{b}	827.32±2.38^{d}	930.78±2.31^{a}	0.00
	Leaf	952.54±1.88^{a}	784.25±1.10^{d}	817.94±3.26^{c}	910.68±1.26^{b}	816.96±2.35^{c}	0.00
	Stem	810.37±3.04^{e}	984.01±3.70^{a}	937.02±4.29^{b}	878.32±4.41^{c}	858.79±3.03^{d}	0.000
	Root	932.17±3.18^{d}	985.97±3.89^{c}	998.14±4.53^{b}	796.39±4.09^{e}	1048.02±3.65^{a}	0.000
Ca	Whole grass	144.49±4.14^{b}	144.90±3.61^{b}	146.35±2.10^{b}	153.41±3.06^{a}	145.15±3.09^{b}	0.04
	Leaf	141.84±1.53^{c}	153.77±0.81^{b}	162.16±0.85^{a}	142.96±1.18^{c}	142.91±1.32^{c}	0.00
	Stem	149.06±2.19^{b}	157.14±1.91^{ab}	150.56±4.18^{b}	149.25±3.63^{b}	161.33±3.80^{a}	0.003
	Root	152.92±2.47^{d}	164.02±3.05^{b}	172.37±3.12^{a}	147.86±0.43^{d}	158.87±0.53^{c}	0.000

*T_1: 100% fresh water; T_2: 75% fresh water + 25% effluent; T_3: 50% fresh water + 50% effluent; T_4: 25% fresh water + 75% effluent; T_5: 100% industrial effluent. Whole grass values represent composite samples. Means with different superscripts differ significantly (p < 0.05).

Table 5 - Proximate and fiber composition of Jambo grass under different irrigation treatments (% DM).

Parameters (%) \ Treatment*	T_1	T_2	T_3	T_4	T_5	P-value
Moisture	18.71±1.18	18.68±0.32	19.55±0.79	21.14±1.11	19.57±1.09	0.06
Crude protein	7.48±0.32	6.22±0.24	6.56±0.60	6.70±0.57	7.01±0.80	0.13
Crude fiber	30.86±0.75	32.04±1.51	31.36±1.51	30.43±1.62	31.70±1.57	0.67
Crude fat	1.01±0.06	1.09±0.03	0.93±0.16	0.96±0.06	0.96±0.13	0.43
Ash	8.95±0.86	8.40±0.90	8.53±0.47	8.67±0.70	8.67±0.36	0.89
NFE	32.98±1.36	33.58±2.01	33.06±0.34	32.09±0.97	32.08±0.79	0.53
NDF	63.28±2.20	62.42±1.53	65.71±0.99	64.98±2.64	63.80±2.05	0.32
ADF	44.09±1.08	45.77±2.15	44.80±2.15	43.47±2.31	45.28±2.25	0.67

*T_1: 100% fresh water; T_2: 75% fresh water + 25% effluent; T_3: 50% fresh water + 50% effluent; T_4: 25% fresh water + 75% effluent; T_5: 100% industrial effluent. DM, dry matter; NFE, nitrogen-free extract; NDF, neutral detergent fiber; ADF, acid detergent fiber.

DISCUSSION

Moderate industrial effluent irrigation enhanced the growth and biomass production of Jambo grass, while higher concentrations induced physiological stress. Thus, industrial effluent irrigation exerted a dual effect on the growth and nutrient dynamics of Jambo grass. The observed enhancement in plant height, leaf development, and biomass under moderate effluent (25-50%) application can be attributed to the increased availability of essential nutrients such as potassium, calcium, and magnesium, which commonly enrich soil fertility and contribute to improved plant metabolism and growth (Marschner, 2012; Yerli et al., 2025).

The decline in biomass and stem yield at higher effluent levels indicates a shift from nutrient benefit to toxicity, likely associated with excessive accumulation of heavy metals such as Pb, Cd, and Ni, which are known to disrupt root function, physiological processes, enzyme activity, and nutrient uptake processes (Shanker et al., 2005; Ali et al., 2013; Nowwar et al., 2023; Islam et al., 2023). Nickel and zinc showed maximum accumulation under moderate effluent levels (50%), suggesting that metal availability and plant uptake efficiency are enhanced at intermediate contamination levels. Yadav et al. (2002), who noted that moderate metal concentrations could stimulate uptake mechanisms before reaching toxicity thresholds, reported similar observations. In contrast, the decline in Cd and Zn concentrations at higher effluent levels (100%) may be attributed to physiological stress and reduced root permeability, which limits metal absorption under toxic conditions (Kabata-Pendias, 2000; Nagajyoti et al., 2010). The predominance of heavy metal accumulation in roots observed in the present study reflects an inherent protective tolerance mechanism whereby plants restrict the translocation of metals to aerial tissues, a phenomenon widely documented in forage species exposed to metal stress (Sharma et al., 2006; Yusuf et al., 2011). However, this physiological barrier may become progressively less effective with prolonged exposure or elevated effluent concentrations, leading to increased metal movement into shoots at higher treatment levels, as reported in recent findings (Kanwal et al., 2024).

From a feed safety perspective, the elevated concentrations of Pb and Cd detected in leaves under higher effluent treatments (100%) are of particular concern, as wastewater-irrigated crops have been reported to accumulate toxic metals beyond safe limits, thereby posing risks to livestock and human health through food chain transfer (Sharma et al., 2015; Singh et al., 2024). The recommended maximum levels in complete livestock diets are generally 8 mg kg^{-1} for Pb and 0.4 mg kg^{-1} for Cd (0.2 mg kg^{-1} for horses), thresholds established to minimize toxicity and residue transfer into edible animal products (CFIA, 2024). Moreover, from a livestock nutrition perspective, exceeding these thresholds may impair feed intake, growth, reproduction, renal and hepatic function in animals, while chronic consumption can lead to toxicity, reduced productivity, and the transfer of contaminants into animal-derived products such as milk, meat, and offal (Food and Agriculture Organization of the United Nations and World Health Organization, 2001; Faye and Sinyavskiy, 2008), thereby elevating human dietary exposure.

In contrast, essential micronutrients such as Zn, Cu, Fe, and Mn remained within acceptable limits, indicating that wastewater irrigation may enhance certain aspects of mineral nutrition without compromising overall forage quality, as also reported in similar studies on wastewater-irrigated fodder crops (Yadav et al., 2009; Bhatnagar et al., 2020); however, long-term application may still lead to bioaccumulation and associated health risks, particularly for elements such as Zn when present in excess (Akhtar et al., 2025). According to FAO (2001) guidelines, elevated levels of heavy metals in forage crops pose risks to animal productivity and food safety. The relatively stable proximate composition across treatments further supports the notion that basic nutritional attributes are less sensitive to effluent-induced changes than mineral composition.

Overall, these findings confirm a threshold response, where moderate effluent use can be beneficial for forage production, but higher concentrations result in heavy metal accumulation and potential toxicity. Nevertheless, the imbalance between beneficial nutrients and toxic metals under higher effluent levels underscores a critical threshold beyond which the risks outweigh the agronomic advantages. Therefore, the findings highlight the importance of controlled dilution and continuous monitoring of industrial effluent when used for forage production to ensure both productivity and feed safety within sustainable livestock systems.

CONCLUSION

Industrial effluent irrigation influenced both growth performance and mineral accumulation in Jambo grass. Moderate effluent dilution improved biomass production without markedly altering proximate composition. However, elevated concentrations of lead and cadmium in plant tissues at higher effluent levels raise concerns regarding fodder safety. The findings indicate that while diluted industrial effluent may be used cautiously for forage production, unrestricted application is not advisable. Regular monitoring of heavy metal concentrations is essential before wastewater-irrigated fodder is incorporated into livestock feeding systems.

DECLARATIONS

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Ethical approval
This study did not involve the use of live animals or human participants.

Data availability
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Authors' contributions
Shilpi Islam conceived, designed, and implemented the experiment. Shilpi Islam and Susmita Sarker Bristi participated in data collection and laboratory analyses. Md. Rashidul and Abu Sadek Md. Selim performed the statistical analysis and interpretation of the results. Shilpi Islam prepared the initial draft of the manuscript. All authors contributed to manuscript revision, read, and approved the final version.

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All authors have reviewed and approved the final manuscript and consent to its publication.

Competing Interests
The authors declare that they have no competing interests.

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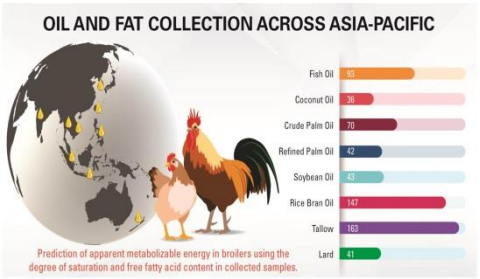
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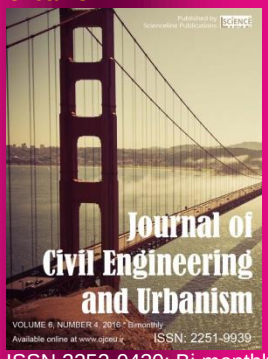
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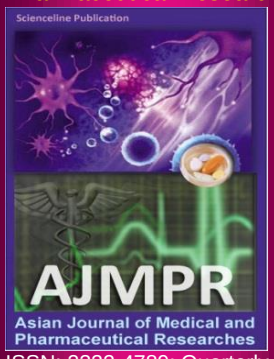
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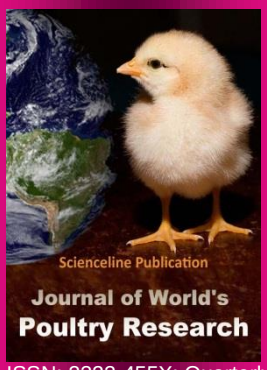
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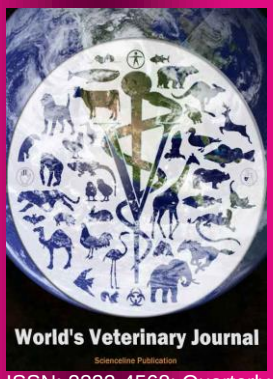
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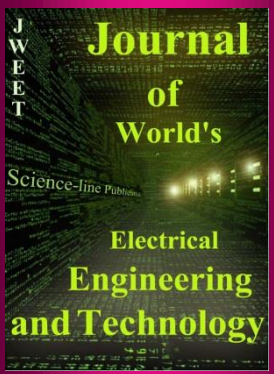
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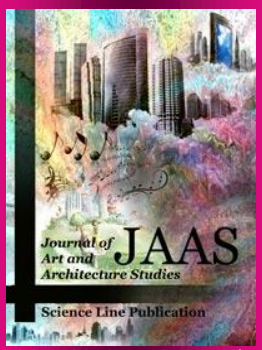
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