

BOOKLET





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Research Paper

Impact of the housing system and applied precision technologies on productive and reproductive indicators in dairy herds

Mitev E, Popova Y, and Laleva S.

Online J. Anim. Feed Res., 16(5): 261-268, 2026; pii: S222877012600031-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.31>

Abstract

The objective of this study is to examine the associations of the number of applied precision technologies, housing systems, and 3.6%-fat-standardized milk yield groups with the farm-level average duration of the service period and the average semen doses per conception. For the purpose, a survey was held in 2025 by direct standardized interview among 180 owners of cattle farms in Bulgaria and the gathered information was analyzed by one-way analysis of variance (ANOVA). The results showed that high average daily milk yield over 30 kg was associated with a longer service period of 126 days, while for farms with low productivity (up to 20 kg) it was the shortest 102.17 days. Farms with tie-stall housing maintained the shortest service period (104.69 days) but showed lower conception efficiency, requiring a higher mean of 3.73 semen doses per conception. On the other hand, in free-stall housing, the service period was the longest (129.79 days), but the lowest mean of 3.26 doses per conception was recorded. The implementation of three or more precision technologies optimized reproductive management, demonstrating a lower mean of 3.36 doses per conception, whereas farms using only one or two technologies utilized a higher number of 3.71 and 3.75 doses, respectively. In conclusion, high farm-level productivity does not inevitably compromise fertility, provided that intensive milk production is balanced with precise technological monitoring and strategic management decisions.

Keywords: Precision livestock farming, dairy farms, service period, number of doses for insemination, housing system.



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Research Paper

Assessment of quality of poultry feed manufactured in registered and unregistered feed mills in Bangladesh

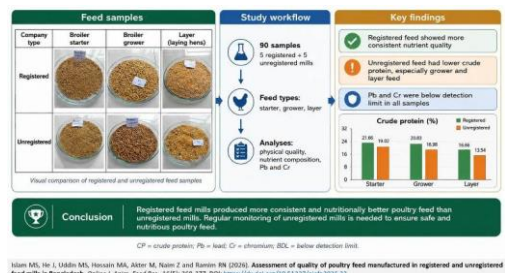
Islam MS, He J, Uddin MS, Hossain MA, Akter M, Naim Z and Ramim RN.

Online J. Anim. Feed Res., 16(5): 269-277, 2026; pii: S222877012600032-16

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Abstract

The study evaluated poultry feed quality and health-hazardous elements from registered and unregistered feed mills in Savar, Kaliakoir, and Sreepur upazilas of Dhaka Division, Bangladesh. A total of 90 feed samples, including broiler starter, grower, and layer feeds, were randomly collected from five registered and five unregistered feed mills, considering three samples in each category. The feed samples were evaluated for physical attributes, including color, odor, particle size, and the presence of foreign materials, and were subsequently subjected to proximate analysis for the determination of moisture, crude protein (CP), ether extract (EE), and ash. The concentrations of two heavy metals, lead (Pb) and chromium (Cr), were determined in the feed samples. Both registered and unregistered feed samples showed acceptable physical quality, characterized by normal color and smell, particle size and absence of visible mold contamination or foreign materials. Proximate analysis showed the significant variation in terms of moisture, CP, EE, and ash within and between feed groups, indicating comparatively lower quality of feed obtained from unregistered group. In broiler feed (starter and grower), mean moisture, CP, EE, and ash contents ranged from 10.45–11.35%, 16.98–21.66%, 5.24–8.20%, and 6.13–7.20%, respectively, across registered and unregistered categories. In layer feed, the corresponding values in registered and unregistered categories were 11.05% and 10.81% moisture, 16.86% and 13.54% CP, 5.45% and 5.78% EE, and 11.77% and 8.16% ash, respectively. The concentrations of Cr and Pb were found to be below the detection level for all types of feed in both categories. The



present study found that feed from unregistered mills, particularly in crude protein, was below standard, whereas registered-mill feed largely met nutritional standards. Based on the measured physicochemical parameters and Pb and Cr levels, no significant public health risk was evident.
Keywords: Chicken feed, Health hazard, Nutritional quality, Physical quality, Proximate analysis.

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Research Paper

Digestibility and feeding value of improved tropical grasses and *Leucaena leucocephala* cultivars for goat nutrition in Vietnam

Liem TN, Dung NM, Duc LM, Hai DT, and An LV.

Online J. Anim. Feed Res., 16(5): 278-288, 2026; pii: S222877012600033-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.33>

Abstract

This study evaluated the nutritive value and digestibility of five tropical grass cultivars, including Ruzi (*Brachiaria ruziziensis*), Mulato II (*Brachiaria ruziziensis* × *B. decumbens* × *B. brizantha*), and three *Panicum maximum* cultivars (Purple, Mombasa and Mun River), together with five *Leucaena leucocephala* cultivars (Tarramba, Peru, Cunningham, KU15 and KU56). *In vitro* digestibility was determined using ANKOM DAISYII incubator system, and an *in vivo* feeding trial evaluated the effects of increasing dietary inclusion of Tarramba leaves (0, 10, 20 and 30% of dietary dry matter) in Mombasa-based diets on nutrient intake and apparent digestibility in goats. Grass cultivars differed significantly ($P < 0.05$) in dry matter and fibre digestibility. No significant differences were detected among grass cultivars for *in vitro* dry matter, neutral detergent fibre or acid detergent fibre digestibility, although numerical differences were observed. Similarly, digestibility did not differ significantly among the five *Leucaena* cultivars, whereas *Leucaena* cultivars had substantially higher crude protein concentrations than the grasses. Increasing Tarramba inclusion resulted in a linear increase in crude protein intake and apparent crude protein digestibility while apparent dry matter and organic matter digestibility remained unaffected. Dry matter and organic matter intake showed quadratic responses to increasing Tarramba inclusion, whereas ADF digestibility decreased linearly and NDF digestibility showed a numerical decline. Under the conditions of the short-term digestibility trial, inclusion of Tarramba leaves up to 30% of dietary dry matter did not adversely affect apparent dry matter and organic matter digestibility. These findings indicate that improved tropical grasses and *Leucaena* can provide complementary forage resources for improving the nutritional quality of grass-based diets for goats.
Keywords: Apparent digestibility, Crude protein, Goat nutrition, Tropical forages, *Leucaena leucocephala*.

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Research Paper

Valorization of young prickly pear (*Opuntia ficus-indica*) cladodes as a sustainable livestock feed supplement: physicochemical, nutritional value and antioxidant activity

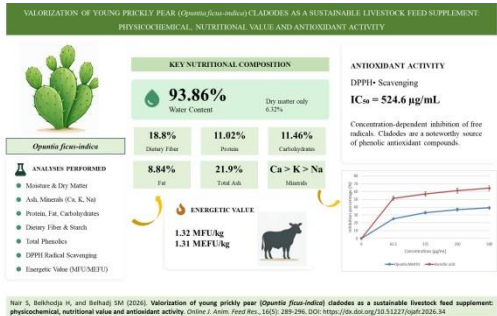
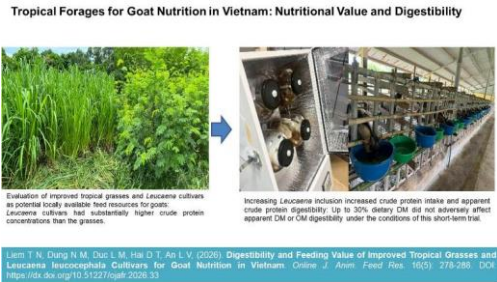
Nair S, Belkhodja H, and Belhadj SM.

Online J. Anim. Feed Res., 16(5): 289-296, 2026; pii: S222877012600034-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.34>

Abstract

The prickly pear cactus (*Opuntia ficus-indica*) is prevalent in arid and semi-arid environments, known for its drought tolerance and ability to thrive in poor soils. The cladodes, or pads, are particularly relevant as a nutritional supplement for animals due to their high nutrient content and water-retention capacity. This study aims to analyze the nutritional qualities of young *Opuntia ficus-indica* cladodes growing in the Chorfa region of Mascara Province, Algeria for prospective integration into feedstuff. The physicochemical study was based on the determination of moisture, dry matter, sugar, protein, fat, ash, minerals, starch, and fiber content. The phytochemical analysis was performed by total phenolic content determination. It was followed by antioxidant potential evaluation using DPPH free radical scavenging assay and energetic value assessment. The physicochemical study revealed that the cladodes had a high-water content ($93.86 \pm 0.15\%$) and very low dry matter ($6.32 \pm 0.2\%$). The total ash content was $21.9 \pm 0.03\%$, with calcium as the most abundant mineral, followed by potassium and sodium. The cladode had a good amount of dietary fiber ($18.8 \pm 1.1\%$) and presented values of $11.46 \pm 0.2\%$, $11.02 \pm 1.1\%$, and $8.84 \pm 0.3\%$ for carbohydrate, protein, and fat content, respectively. Based on the phytochemical characterization, cladodes were a noteworthy source of



antioxidant compounds. The estimated energy values are 1.32 MFU/kg and 1.31 MEFU/kg. The methanolic extract was effective in inhibiting the free radical DPPH• in a concentration-dependent manner with an IC50 value of 524.6 µg/mL. *Opuntia ficus-indica* cladodes were found to be a good source of sugars, proteins, and dietary fiber, making them potentially valuable ruminant feed.

Keywords: Cladodes, Fiber content, *Opuntia ficus-indica*, Physicochemical analysis, Livestock nutrition.

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Research Paper

The physical, nutritional, phytochemical, and microbiological properties of water mimosa (*Neptunia oleracea* Lour) silage using different moisture contents for poultry feed

Yosi F, Muhakka M, Gofar N, Hasanah N, Nababan A, and Lestari I.

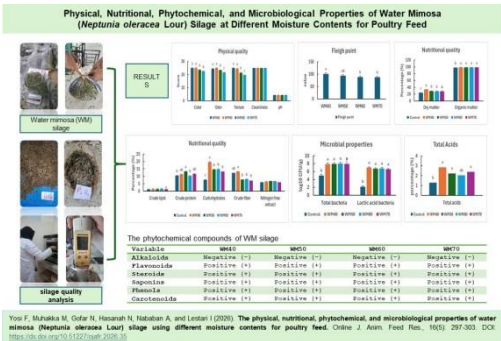
Online J. Anim. Feed Res., 16(5): 297-303, 2026; pii: S222877012600035-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.35>

Abstract

The potential of water mimosa (WM; *Neptunia oleracea* Lour) swamp legumes, as a source of green protein, phytochemical compounds, and probiotics in the form of silage juice for waterfowl such as ducks is quite large, but to date it has not been further investigated. This study aimed to evaluate the physical, nutritional, phytochemical, and microbiological characteristics of WM silage fermented at different moisture levels. This study used a completely randomized design consisting of 5 treatments and 3 replicates. The treatments consisted of control group (fresh WM legumes), WM40, WM50, WM60, and WM70: WM silage with moisture levels of 40%, 50%, 60%, and 70%, respectively. The results of the analysis of variance showed that differences in silage moisture content had a significant effect on color, aroma, and texture scores ($P < 0.05$). Qualitative tests showed that WM silage fermented at moisture levels of 40-70% contained several phytochemical/antibacterial compounds, including flavonoids, steroids, saponins, phenols, and carotenoids. Differences in WM silage moisture content significantly affected dry matter, organic matter, crude protein, crude fiber, carbohydrates, and Fleigh point ($P < 0.05$). Total acid, total bacteria count, and lactic acid bacteria count in WM silage fermented at different moisture contents increased compared to the control group ($P < 0.05$) but showed similar results between moisture levels of 40 and 70%. In conclusion, the making of WM silage with a moisture content of up to 50% provided optimal yields in terms of the physical, microbiological, nutritional, and phytochemical properties of WM silage.

Keywords: Microbiological characteristics, Moisture content, *Neptunia oleracea* Lour, Nutritional content, Phytochemical compounds, Silage.



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Research Paper

Nile tilapia (*Oreochromis niloticus*) seed production and growth performance of fry and grower broodstocks in Northwest Ethiopia

Aynalem Y, Yalew A, and Tilahun G.

Online J. Anim. Feed Res., 16(5): 304-313, 2026; pii: S222877012600036-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.36>

Abstract

Small-scale Nile Tilapia (*Oreochromis niloticus*) fry production is essential for improving seed availability and supporting sustainable aquaculture. This study evaluated the biological performance and economic feasibility of Nile tilapia fry production in earthen ponds using a locally formulated diet under small-scale farming conditions in Ethiopia. Thirty-three broodstock fish (11 males and 22 females) collected from the Geray Reservoir region were stocked in a 100 m² earthen pond at a male-to-female ratio of 1:2 and fed a locally formulated diet containing 35% crude protein at 3% of body weight per day. The diet consisted of fish meal (38.1%), soybean meal (38.1%), and wheat bran (24.0%), offered twice daily. Male brood stocks showed significantly greater growth performance than females ($P < 0.001$). Each spawning cycle produced approximately 3,300 fry, and broodstocks spawned an average of eight times annually, yielding about 1,200 fry per female and an estimated annual production of 26,400 fingerlings. Fry reared in the best-performing on-farm pond (researcher) achieved significantly greater final weight, final length, and daily growth rate than those reared at the other sites (farmers) ($P < 0.001$), while survival remained consistently high (98.75–100%) with no significant differences among sites. In water



Aynalem Y, Yalew A, and Tilahun G (2026). Nile tilapia (*Oreochromis niloticus*) seed production and growth performance of fry and grower broodstocks in Northwest Ethiopia. Online J. Anim. Feed Res., 16(4): 304-313. DOI: <https://dx.doi.org/10.51227/ojafr.2026.36>

quality, no significant differences among sites for temperature, dissolved oxygen, pH, total dissolved solids and ammonia ($P > 0.05$), although electrical conductivity differed significantly ($P < 0.001$). Total feed consumption was 251.16 kg, corresponding to a total feed cost of US\$105.94. The selling price was US\$0.079 per fingerling. The production system generated positive economic returns. These findings demonstrate that earthen-pond-based Nile tilapia fry production using locally formulated feeds is a technically feasible and economically viable approach for increasing the availability of quality fingerlings. The technology has strong potential for dissemination through Farmers Training Centers to support sustainable aquaculture development, fisheries enhancement, and improved rural livelihoods in Ethiopia. Keywords: Economic feasibility, Ethiopia, Formulated feed, Fry production, Growth performance, *Oreochromis niloticus*

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Review

Husbandry and Nutritional Management of Hobby and Backyard Poultry Flocks: Genotype, Natural Feed Resources, Health, and Performance

Mevliyaoğulları E, Yenilmez F, and Yıldırım A.

Online J. Anim. Feed Res., 16(5): 314-321, 2026; pii: S222877012600037-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.37>

Abstract

Hobby and backyard poultry keeping encompasses systems ranging from a few urban laying hens kept for companionship to low-input rural flocks that generate income and provide animal-source protein. This review examines how flock purpose, genotype, life stage, feed resources, housing, and health considerations inform husbandry and feeding decisions in these diverse systems. Egg production, companionship, and the use of household scraps predominate in urban hobby flocks, whereas income, household nutrition, and low-cost production are central to rural systems. Indigenous breeds are important for adaptation to low-input conditions and genetic resource conservation, although small, dispersed breeding populations are vulnerable to inbreeding and genetic loss. The amount and nutritional value of scavenged feed and separately supplied household scraps vary with season, region, genotype, and flock size; these resources do not guarantee a balanced diet. Balanced feeding is particularly important during chick rearing, while adequate energy, amino acids, minerals, and clean water are required during lay. Evidence on feeding behavior, gut microbiota, stress, biosecurity, and soil lead exposure indicates that nutritional management extends beyond ration composition. Geographic and methodological differences among studies limit generalization. For practical flock management, the review emphasizes defining flock purpose, selecting a suitable genotype, assessing local feed resources, providing life-stage-specific supplementation, ensuring secure housing and feed access, and managing health and environmental risks. Future studies should address genotype-specific nutrient requirements, seasonal feed resources, and the effects of feeding and management interventions. Existing evidence does not establish a universal feed formula for all hobby and backyard poultry. Keywords: Animal welfare, Backyard poultry keeping, Hobby poultry, Indigenous breeds, Scavenging feed resource, Supplementary feeding.



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Research Paper

Dynamics of dairy cow milk productivity and frequency of visits to the milking robot under a voluntary milking system

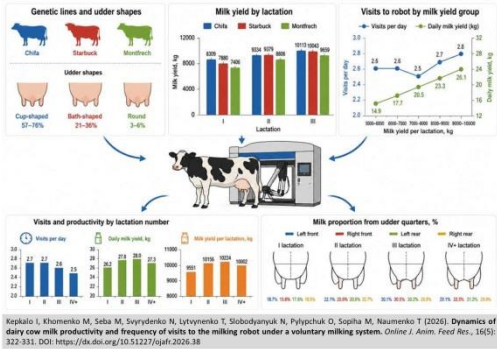
Kepkalo I, Khomenko M, Seba M, Svyrydenko N, Lytvynenko T, Slobodyanyuk N, Pylypchuk O, Sopiha M, Naumenko T.

Online J. Anim. Feed Res., 16(5): 322-331, 2026; pii: S222877012600038-16

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Abstract

The study analyzed the frequency of voluntary visits to an automated milking system (AMS) by Holstein cows, along with milk yield indicators, functional characteristics of the udder, and the duration of intervals between milkings. The research was conducted at a farm in the Kyiv region using a loose-housing system and robotic milking. Trends in average daily milk yield and the frequency of visits to the milking robot were analyzed using data from the VMS-2012 automated control system, while milk yield was recorded using "Fullflow" electronic meters. Milk flow rate was determined based on the amount of milk obtained from individual udder quarters and the duration of milking. To evaluate the relationship between productivity and milking frequency, cows were categorized into five groups according to lactation milk yield (5000–6000; 6000–7000; 7000–8000; 8000–9000; and 9000–10000 kg) and according to lactation number (I,



II, III, IV or higher). Animals belonging to the Chifa, Starbuck, and Montfrecht lines were evaluated separately, taking into account udder conformation, milk flow rate, and the proportion of milk obtained from individual udder quarters. It was found that cows with a lactation yield of 9000–10000 kg visited the milking robot more frequently—2.8 times per day—compared with animals yielding 6000–8000 kg, which visited it 2.6 times per day. As lactation number increased, the frequency of visits decreased, reaching 2.5 visits per day in cows in their fourth and subsequent lactations. The highest 305-day milk yield was recorded in cows in their third lactation, which visited the robot 2.6 times per day. First-lactation cows, despite having the highest visit frequency of 2.7 times per day, produced 9551 kg of milk during the 305-day lactation. Milk flow rates from individual udder quarters were similar, with slightly higher values observed in the rear quarters.

Keywords: Average daily milk yield, Cows, Lactation, Mammary gland, Milking robots, Udder.

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Research Paper

Physiological response and heat shock expression in the Nigerian indigenous goat breeds raised under thermal stress in Southwest Nigeria

Fatoki DO, Ayoola MO, and Berena GA.

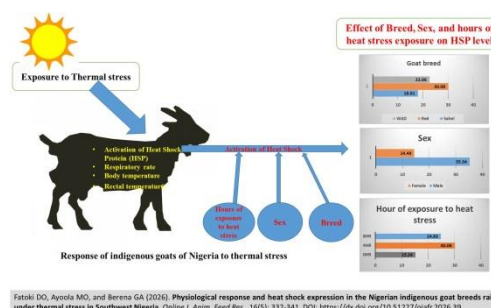
Online J. Anim. Feed Res., 16(5): 332-341, 2026; pii: S222877012600039-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.39>

Abstract

Environmental stress remains a major threat to optimum productivity of livestock in tropical climates. Generally, goats often develop adaptive mechanisms to thrive in their native environment. However, little is known about the adaptation of the Nigerian indigenous goat breeds to different environmental conditions. The influence of varying periods of heat exposure on physiological response and HSP70 gene expression in the Nigerian indigenous goat breeds was therefore examined in this study. A total of 45 goats comprising West African dwarf (WAD), Red Sokoto (RS), and Sahel (SH) breeds were assigned to three treatments; 0, 4, and 8 hours of daily exposure to environmental temperature. Each treatment had two males and 3 females per breed of goats. Environmental variables were monitored while Temperature-Humidity-Index (THI) was calculated. Also, body temperature (BST), rectal temperature (RT), respiratory rate (RR), pulse rate (PR) were monitored while serum HSP70 level was determined. Average temperature (29.86 °C), relative humidity (73.57%) and THI (79.34) were recorded. Also, there was no significant effect of breed on all the physiological parameters, hours of exposure to heat had significant influence on BST, RT, RR and PR. Also, physiological indices monitored significantly ($P < 0.05$) increased with days of exposure while effects of sex on physiological responses of the various breeds exposed to environmental stress was not significant. For HSP70 expression, Red Sokoto goats had the highest level (30.08 ± 0.75 ng/mL), compared to WAD goats (22.66 ± 0.53 ng/mL) and Sahel goats (18.01 ± 0.55 ng/mL) while male goats (35.34 ± 0.50) had higher HSP level compared to female goats (14.49 ± 0.44). In conclusion, duration of exposure to heat influenced physiological responses and serum HSP expression in the Nigerian indigenous goats.

Keywords: Adaptation, Heat stress, HSP70 expression, Indigenous goat breeds, Physiological response.



Fatoki DO, Ayoola MO, and Berena GA (2026). Physiological response and heat shock expression in the Nigerian indigenous goat breeds raised under thermal stress in Southwest Nigeria. *Online J. Anim. Feed Res.*, 16(5): 332-341. DOI: <https://dx.doi.org/10.51227/ojafr.2026.39>

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Research Paper

Polymorphism of POU1F1 genes and its relationship with growth traits of Bach Thao goat breed

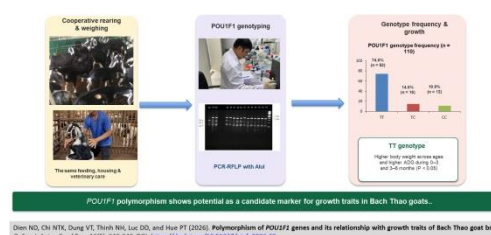
Dien ND, Chi NTK, Dung VT, Thinh NH, Luc DD, and Hue PT.

Online J. Anim. Feed Res., 16(5): 342-348, 2026; pii: S222877012600040-16

DOI: <https://dx.doi.org/10.51227/ojafr.2026.40>

Abstract

This study aimed to evaluate the polymorphism of the POU1F1 gene and its association with body weight and weight gain in Bach Thao goats. A total of 110 animals (64 males and 46 females) were used, and ear tissue samples were collected for DNA extraction and PCR-RFLP analysis to determine POU1F1 genotypes. Allele and genotype frequencies were subsequently calculated. Three genotypes (TT, TC, and CC) were identified, with the T allele and TT genotype showing the highest frequencies. Body weight at birth, and at 3, 6, and 9 months of age, as well as average daily gain (ADG) during the corresponding growth period were recorded. Goats with the TT genotype had significant higher body weight at birth, and at 3, 6, and 9 months of age, together with higher ADG during the corresponding period, than those with the CC genotypes ($P < 0.05$). These findings indicate that POU1F1 polymorphism



Dien ND, Chi NTK, Dung VT, Thinh NH, Luc DD, and Hue PT (2026). Polymorphism of POU1F1 genes and its relationship with growth traits of Bach Thao goat breed. *Online J. Anim. Feed Res.*, 16(5): 342-348. DOI: <https://dx.doi.org/10.51227/ojafr.2026.40>

is associated with growth performance in Bach Thao goats and may have potential as a molecular marker for marker-assisted selection to improve growth traits in this breed.

Keywords: POU1F1, Bach Thao goat, Weight, Gene polymorphism, PCR-RFLP.

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Research Paper

Potential risk factors associated with large intestinal impaction in working donkeys at Egypt

Al-Antaif W, Marzok M, Hamad Y, Almubarak A and El-khodery S.

Online J. Anim. Feed Res., 16(5): 349-356, 2026; pii: S222877012600041-16

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Abstract

Large intestinal impaction is a principal cause of abdominal pain in donkeys, associated with high morbidity and mortality. The objective of this study was to investigate potential risk factors associated with large intestinal impaction in working donkeys in northern Egypt. A case-control study was conducted on 75 donkeys (4–12 years, weighing 150–290 kg) between September 2020 and August 2024. Clinical, parasitological, and biochemical examinations were performed. Logistic regression was applied to identify independent predictors of impaction. Fifty-three donkeys were diagnosed with large intestinal impaction, confirmed by clinical signs and elevated serum triglycerides (224.6 ± 36.0 vs. 196.0 ± 23.0 mg/dl, $P < 0.05$). Significant risk factors included older age (>10 years; OR = 16.8, 95% CI: 8.17–33.64, $P = 0.001$), female sex ($P < 0.05$), poor body condition ($P < 0.01$), heavy workload ($P < 0.05$), dental disease ($P < 0.05$), orthopedic problems ($P < 0.05$), and feeding rice husk (OR = 39.5, 95% CI: 1.1–14.13, $P = 0.044$). Seasonal variation also increased risk (OR = 5.8, 95% CI: 1.2–27.45, $P = 0.026$). Clinically, constipation (OR = 277.8, $P < 0.0001$), anorexia (OR = 92.16, $P < 0.0001$), and absent gut sounds (OR = 61.36, $P < 0.0001$) were the strongest diagnostic indicators. Poor nutritional management, particularly rice husk feeding, advanced age, and seasonal variations are key predictors of large intestinal impaction in working donkeys in northern Egypt. Clinical vigilance, especially in geriatric animals, is essential to reduce morbidity and improve welfare.

Keywords: Colic; Epidemiology; Equine; Forages; Risk factors

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IMPACT OF THE HOUSING SYSTEM AND APPLIED PRECISION TECHNOLOGIES ON PRODUCTIVE AND REPRODUCTIVE INDICATORS IN DAIRY HERDS

Emil MITEV , Yovka POPOVA , and Stayka LALEVA 

Institute of Agriculture – Stara Zagora, 6000, Academy of Agriculture, Sofia, Bulgaria

Email: emilmittev@gmail.com

Supporting Information



ABSTRACT: The objective of this study is to examine the associations of the number of applied precision technologies, housing systems, and 3.6%-fat-standardized milk yield groups with the farm-level average duration of the service period and the average semen doses per conception. For the purpose, a survey was held in 2025 by direct standardized interview among 180 owners of cattle farms in Bulgaria and the gathered information was analyzed by one-way analysis of variance (ANOVA). The results showed that high average daily milk yield over 30 kg was associated with a longer service period of 126 days, while for farms with low productivity (up to 20 kg) it was the shortest 102.17 days. Farms with tie-stall housing maintained the shortest service period (104.69 days) but showed lower conception efficiency, requiring a higher mean of 3.73 semen doses per conception. On the other hand, in free-stall housing, the service period was the longest (129.79 days), but the lowest mean of 3.26 doses per conception was recorded. The implementation of three or more precision technologies optimized reproductive management, demonstrating a lower mean of 3.36 doses per conception, whereas farms using only one or two technologies utilized a higher number of 3.71 and 3.75 doses, respectively. In conclusion, high farm-level productivity does not inevitably compromise fertility, provided that intensive milk production is balanced with precise technological monitoring and strategic management decisions.

Keywords: Precision livestock farming, dairy farms, service period, number of doses for insemination, housing system.

INTRODUCTION

In recent years, dairy cattle farming has undergone large-scale transformation aimed at increasing milk productivity from cows. An example of this is the United States, where the Holstein breed reaches levels of 12,934 kg of milk produced per cow (Bórawski et al., 2021; Ruban et al., 2023). However, the absolute leader in intensive production worldwide remains Israel with an average annual milk yield of 13,112.80 kg (Bayramoğlu and Esen, 2025). In the European Union, the average productivity is about 7,208 kg, while Denmark reports levels of up to 9,504 kg (International Scientific Days 2020, 2020). Despite the drive for modernization, Bulgaria's indicators remain significantly lower than the European average. With a yield of around 3,640 kg of milk per cow, our country achieves less than 50% of the EU average (Kubala and Stanuch, 2023). While the national average is low, studies in various intensive farms in Bulgaria show a radically different picture. Studies of structural convergence in the EU have found that in countries like Bulgaria the sector is divided into two clearly defined poles. The first one encompasses a large proportion of small farms with a yield of around 3,000 kg, and the second one includes modernizing farms, whose productivity has stabilized around 7,500 kg and continues to grow (Jong et al., 2019).

A study by Karamfilov and Kanev (2025) on Holstein cattle in Southern Bulgaria for the period 2019–2023 reported an average lactation milk yield of 10,840 kg, with some farms exceeding 12,150 kg per animal. This is almost three times the national average.

This focus on milk yield, according to Ruban et al. (2023), presents modern cattle breeding with a critical paradox: approaching the genetic ceiling of production can be associated with high metabolic demands, which may affect their health and compromises their reproductive capacity. Britt et al. (2021) emphasize that in the highest-producing herds, the management of physiological stress is a key factor in preventing reproductive problems. With just 15.4% of the world's dairy cows producing nearly 45% of the global milk supply, the enormous pressure on these animals requires constant monitoring of their welfare. This is further emphasized by recent regional findings demonstrating the necessity of routine screening even in traditional production frameworks, where 60% of 196 analyzed milk samples exceeded a threshold of 500,000 somatic cells/mL (Mevliyaogullari & Göncü, 2025a).

Overcoming these economic and biological challenges, is only possible through the full digitalization of farms. Precision livestock farming offers automated monitoring systems that replace subjective visual observation. State-of-the-

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art developments in the field of computer vision, using YOLOv5 algorithms, allow the identification of behavioral signs of estrus with an accuracy of 97.6%, even under difficult lighting conditions in the barn (Clayton et al., 2025). According to Patel et al. (2026), the integration of these digital technologies is the only way to achieve “smart” and sustainable dairy production that balances high milk yield with reproductive health.

The aim of the present study is to analyze the differences in the farm-level average duration of the service period and the average number of semen doses per conception among different precision technology classes, housing systems, and fat-standardized milk yield (FSMY) groups.

MATERIALS AND METHODS

Design and Data Collection

For the purpose of the study, a survey was conducted among 180 owners of cattle farms from all over the country with different farm capacities in the period from 01 Jan 2025 to 31 Dec 2025. The survey included a total of 80 questions related to general characteristics of the farms, productive and reproductive indicators, technologies and technological equipment, animal nutrition and health, etc. The empirical information was collected through direct standardized face-to-face interviews. All respondents were asked the same questions in the same sequence. Strict instructions were used, which allowed the collection of comparable data across the surveyed farms. The data were gathered through a non-probability convenience sampling approach utilizing the PAPI (Pen and Paper Interview) format, which ensured objectivity and a high degree of control over the interviewing process. The individual information gathered in this way was entered in the form of electronic records and analyzed using selected statistical and mathematical methods. For some groups of indicators, the data were taken from the farm documents (productive and reproductive indicators, number of cattle by category, etc.).

Grouping and Factor Classes

Since the cows are of different breeds both between farms and within individual herds, milk fat content exhibits natural fluctuations depending on both the breed and the lactation stage. As demonstrated by Mevliyaogullari and Gönçü (2025a), milk fat traits change significantly during weeks 2–32 of lactation even under identical dietary rations. Therefore, to reduce the effect of the breed and the lactation stage on the milk productivity parameters, the farm-level mean daily milk yield was converted to a 3.6%-fat-standardized milk yield (FSMY) using the formula:

$$\text{FSMY (kg)} = (\text{daily milk yield (kg)} \times \text{fat content (\%)}) / 3.6$$

For a better approximation, the average daily FSMY was categorized into three factor classes, as follows: 1 – up to 20 kg, 2 – from 20 to 30 kg, and 3 – over 30 kg. A total of 19 advanced precision electronic components and technical solutions were evaluated in the survey, and the owners indicated which options were implemented on their farms. For statistical analysis, each component was documented as a binary variable (1 = present, 0 = absent) to represent the total number of distinct advanced systems utilized. These items comprised automated milking helpers (including milk meters, automatic cup detachers, conductivity and temperature indicators), computerized tracking software (including herd logs, electronic tags, pedometers, neck collars, rumen boluses), and precision feeding automation (including concentrate stations, smart wagon mixer scales, automated calf feeders). Due to the distribution of responses, the farms were classified into four consistent categories based on their advanced technology counts: Class 0 (none are used), Class 1 (one device), Class 2 (two devices), and Class 3 (three or more devices).

Statistical analysis

Data management and graphical illustrations were performed using MS Excel. Descriptive statistics and analysis of variance (ANOVA) were conducted using the relevant modules of STATISTICA by StatSoft (Copyright 1984–2011, StatSoft, Inc.). To evaluate the impact of individual factors, the following one-way ANOVA model was applied:

$$Y_{ij} = \mu + A_i + e_{ij}$$

where Y_{ij} is the dependent variable (service period and mean number of semen doses per conception, analyzed at farm level); μ is the overall mean of the trait; A_i is the fixed effect of the studied factor class (housing system, applied precision technologies, or FSMY group); e_{ij} is the random effect of factors not included in the model.

For each trait, the least squares means were estimated for each class of the fixed factors from the corresponding analysis of variance model. The variations among these classes were evaluated based on the sums of squares, calculated as the deviations from the overall mean value of the trait obtained from the model.

RESULTS AND DISCUSSION

Table 1 presents the descriptive statistical metrics for the studied productive and reproductive parameters. The column “Number” reflects the number of farms that provided specific data on the respective indicator within the framework of the survey.

The average service period was 115.37 ± 1.46 days, varying from 73 to 152 days with a standard deviation of 18.76 days. The average number of semen doses per conception was 3.50 ± 0.06 , ranging from 1 to 5 with a standard deviation of 0.72. The FSMY was 28.23 ± 0.56 kg on average, with a standard deviation of 7.51 kg and a variation from 13.73 kg to 49.10 kg. The results obtained about the service period are in line with the research of [Gukezhev et al. \(2025\)](#), according to which in modern high-productivity herds this indicator naturally stabilizes in the range between 115 and 120 days. The author adds that a significant percentage of farms report an average of three to four doses of semen. [Mikolaychik et al. \(2021\)](#) specified that the extension of the period to insemination is a common consequence of the high intensity of milk production and is associated with a higher number of necessary inseminations.

The applied one-factor analysis of variance (Table 2) revealed a significant association between the housing system and the service period ($P < 0.001$) and the semen doses per conception ($P < 0.01$).

Table 1 - Average values and variation of the studied indicators and factors

Parameters	n	$\bar{x} \pm Se$	SD	min - max
Farm-average service period (days)	164	115.37 ± 1.46	18.76	73 - 152
Farm-average semen doses per conception	161	3.50 ± 0.06	0.72	1 - 5
Farm-average daily FSMY (kg)	180	28.23 ± 0.56	7.51	13.73 - 49.10

n = number of reporting farms; FSMY= 3.6%-fat-standardized milk yield; $\bar{x} \pm Se$ = mean $\pm$ standard error; SD = standard deviation

Table 2 - Analysis of variance for the studied operational factors and reproductive parameters

Variation source	Degrees of freedom	Service period			Semen doses per conception		
	df	MS	F	P	MS	F	P
Housing system	2	6861	25.32***		2.48	5.06**	
Precision technologies	3	3165.1	10.58***		1.58	3.29*	
FSMY	2	8437	33.56***		0.66	1.29	

FSMY = 3.6%-fat-standardized milk yield; df = degrees of freedom; MS = mean square; F = F-statistic; P = P-value; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

A significant effect on the service period ($P < 0.001$) and the semen doses per conception ($P < 0.05$) was also found depending on the number of applied technical devices and sensors. Regarding the factor 3.6%-fat-standardized milk yield (FSMY), significant differences were reported only for the service period ($P < 0.001$), and the effect on the semen doses per conception was not statistically significant. In support of present results, [Bielfeldt et al. \(2004\)](#) found that the housing system (free versus tie) was a statistically significant factor ($P < 0.05$) for reproductive performance, including the success of the first insemination. In addition, [Huba et al. \(2023\)](#) noted that the factors “pasture rearing” and “herd” had a statistical effect ($P < 0.01$) on the trait “number of lactations”. Using ANOVA and F-test, [Palombi et al. \(2013\)](#) found that the introduction of calving monitoring systems had a statistically significant association with the number of inseminations to conception. On the other hand, [Kiyici et al. \(2020\)](#) found in their model that the level of milk production did not differ statistically ($P > 0.05$) in relation to reproductive results (number of inseminations).

The average values for the service period depending on the housing system applied on the farm were presented in Figure 1. The longest average service period was recorded on farms with free-stall housing systems 129.79 days, followed by those with free group housing 115.33 days. The shortest average service period was observed on farms with tie-stall housing 104.69 days. The results obtained are in line with the research of [Naviya et al. \(2025\)](#), who found that in free-range systems, significant variations in this indicator are often observed. In free-stall housing in summer conditions, an average service period of about 121.2 days was recorded, with the detection of estrus and heat stress at the herd level remaining critical factors. [Litvinenko et al. \(2020\)](#) added that in highly productive dairy herds under similar housing systems, the service period often reaches levels of about 130 days, which is frequently associated with a prolonged intercalving period and a decrease in the coefficient of reproductive capacity. [Sivkin and Arutyunyan \(2026\)](#) found that, unlike free systems, tie-stall housing allows for more intensive individual monitoring, which under certain conditions is associated with a shorter service period by 12.0–14.2 days compared to free-range systems.

LS-mean values for the service period depending on the number of precision technologies applied are shown in Figure 2. Farms with the largest number of applied technical devices and sensors (3 and more) had the longest average service period 121.14 days, and the shortest period was recorded on farms without any technologies (Class 0) 97.11 days. This effect is also due to the large difference in the 3.6%-fat-standardized milk yield among these groups. The lowest daily milk yield is on farms that do not use any devices and sensors in their work 23.95 kg.

Morozov (2024), in line with our results, found that in digitalized farms using health control systems, the service period often varies within 128–135 days, which significantly exceeds standard technological norms. Edwards et al. (2026) added that the implementation of additional sensors does not always result in automatic shortening of reproductive intervals, and in some cases there is even an increase in the period to insemination by up to 20 days without a significant improvement in fertility levels. Kleen and Guatteo (2023) noted that the effectiveness of precision livestock farming depends to a large extent on management decisions based on the submitted data, and in less technologically advanced farms, direct visual control of animals still ensures high insemination efficiency. Through correlation analysis ($r = 0.15$) Gorelik et al. (2021) also confirmed that high daily milk yield is consistently associated with an extension of the service period.

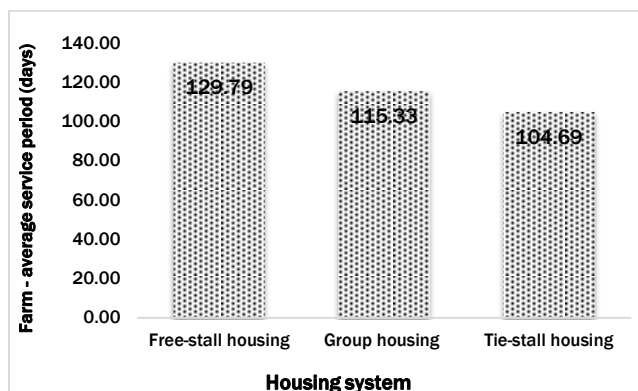


Figure 1 - LS-mean values for the service period depending on the housing systems used on the farm ($F = 25.32$; $P < 0.001$).

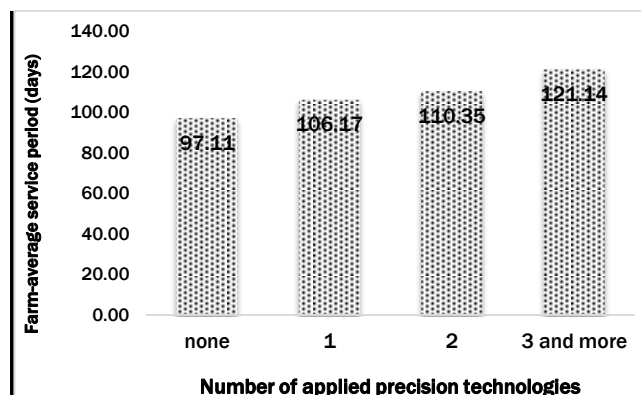


Figure 2 - Farm-average service period (days) depending on the number of applied precision technologies (none, 1, 2, or 3 and more devices; $F = 10.58$; $P < 0.001$).

Regarding the relationship between the service period and the average daily milk yield at the farm level, Figure 3 presents the LS-mean values of the service period depending on the 3.6%-fat-standardized milk yield obtained from the analysis of variance model (Table 2). In farms with the lowest average FSMY up to 20 kg, the service period was the shortest 102.17 days, while in farms with the highest daily milk yield, over 30 kg, the average service period was the longest 126.0 days. This significant relationship supports the assumptions made so far that farms with small capacity, tie-stall housing, and no application of any technical devices and sensors, having low daily milk yield, exhibit a correspondingly shorter service period, while longer service periods characterize large, high-yielding technologically intensive operations.

The difference we found of nearly 24 days in the reproductive interval between groups with different productivity was confirmed by the studies of Amma et al. (2024) who found that the group with the highest milk production had an average of 41.5 days longer service period compared to the group with the lowest productivity. Titova (2021) added that the association of the level of milk yield on the variation of the service period was 5.4%, with each increase in milk yield by about 15% being associated with a longer period to conception by 7.2 days.

That relationship, according to Lepesheva et al. (2023), is explained by the high metabolic demands in high-producing herds. They pointed out that the focus on milk production often led to a “subfertility syndrome”, with the statistical correlation between milk yield and fertility indicators varying widely from $r = -0.2$ to -0.7 . This is consistent with the findings of Mevliyaoğulları and Göncü (2025b), who evaluated early-lactation blood parameters in high- and low-yielding Holstein cows and reported no statistically significant differences between the yield groups, which may suggest that under comparable feeding and housing conditions blood metabolic indicators alone may not reliably reflect milk yield variations at the herd level. Although on the highly productive farms in our study the service period reached 126 days, Grinchuk and Nesterova (2021) noted that in modern conditions, values of 120 to 130 days are considered a physiologically justified adaptation that allows herds to realize their genetic potential for milk production without permanently endangering productive longevity. LS-mean values for the semen doses per conception depending on the applied housing system on the farm are presented in Figure 4.

Although the shortest service period was recorded in farms with tie-stall housing, these herds were reported to have the highest semen doses per conception 3.73, while farms with free-stall housing reported the lowest number of doses used 3.26. These results were supported by the research of Bielfeldt et al. (2004), who found that the percentage of conception from the first attempt in barns with tie-stall housing was significantly lower (about 44%) compared to those with free-stall housing (52%), which was associated with higher semen doses per conception. Livchikov and Shishkina (2025) added that the free-stall system achieved significantly better indicators in terms of insemination frequency compared to tie-stall housing, and the differences were statistically significant. Sudarev and Ivanov (2022) reported that the insemination rate in tie-stall systems was higher by 0.26 to 0.42 units compared to free-stall farms, and Admina et al. (2024) added that the differences in fertility between the two farming systems were highly reliable at significance levels

of $P < 0.001$. LS-mean values for the semen doses per conception depending on the degree of implementation of precision technologies are presented in Figure 5. The lowest number was reported in farms applying three or more technical devices and sensors (3.36 doses), while the highest values were observed in farms using one or two technologies 3.71 and 3.75 doses, respectively.

Krpálková et al. (2020) supported our results by stating that precision technologies not only improved the detection of estrus, but also by determining the moment of insemination resulted in significant improvement in the conception rate and, accordingly, to a reduction in the semen doses per conception. Kaewbang et al. (2024) added that the implementation of biosensors was associated with a statistically significant ($P < 0.01$) increase in the conception rate, and in some farms the probability of success increased nearly fivefold compared to traditional methods. Cavallini et al. (2025) specified that the combination of rumination indicators and 3D accelerometers for activity allowed for a more accurate differentiation of physiological states at the herd level. Lovarelli et al. (2020) found that the larger amount of data collected by multiple devices allowed farmers to avoid errors in estrus detection and reduce the number of required semen doses, which is critical for the economic sustainability of large farms. LS-mean values for the semen doses per conception depending on FSMY are presented in Figure 6.

In farms with the lowest FSMY, below 20 kg per day, a higher number of semen doses per conception were used 3.65 compared to those with daily milk yield above 30 kg, 3.41 doses. The results obtained correspond to the studies of Bayramoğlu and Esen (2025), who found that the average semen doses per conception was lower in large farms compared to small ones. The authors explained this with the better organization and technological management in higher-productive farms, which managed to compensate for the physiological stress from the high milk yield. In contrast to our results, Çağatay et al. (2025) found a highly reliable relationship between milk productivity and reproductive indicators ($P < 0.001$). In their study, the average semen doses per conception was 2.29, indicating that the level of milk yield had a direct association with the number of doses used. The relationship between these factors was further supported by the study of Erzurum and Kayar (2024), who indicated that the number of inseminations was influenced not only by genetic factors, but also by a number of environmental factors. Their data showed that effective herd management allowed maintaining high milk productivity without necessarily leading to an increased number of required semen doses.

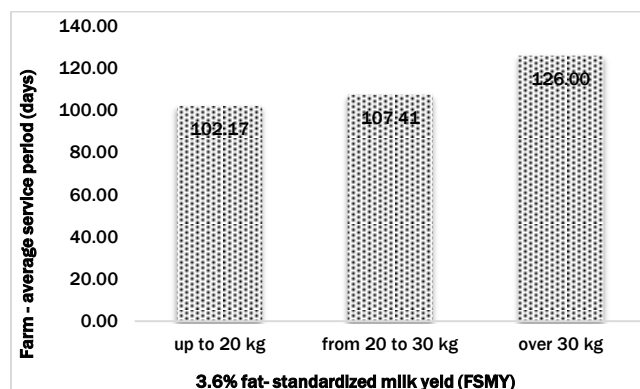


Figure 3 - LS-mean values for the service period depending on FSMY ($F = 33.56$; $P < 0.001$).

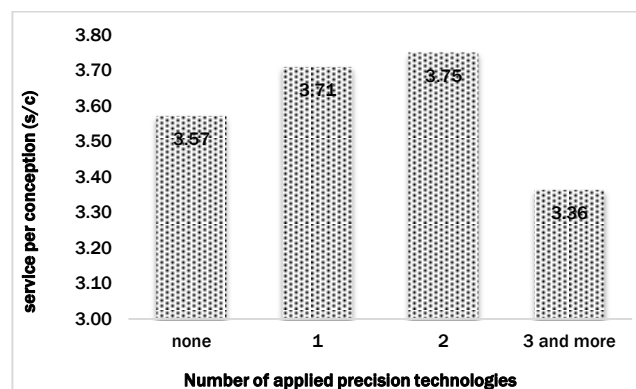


Figure 5 - Semen doses per conception (expressed as services per conception, S/C) depending on the number of applied precision technologies (none, 1, 2, or 3 and more devices; $F = 3.29$; $P < 0.05$).

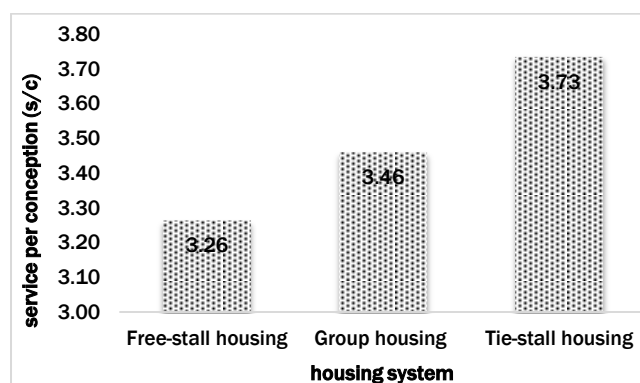


Figure 4 - LS-mean values for semen doses per conception depending on the housing system ($F = 5.06$; $P < 0.01$).

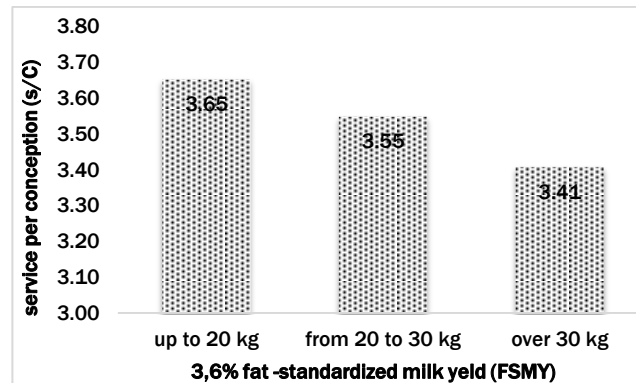


Figure 6 - Semen doses per conception (expressed as service per conception, S/C) depending on the 3.6%-fat-standardized milk yield (FSMY) groups ($F = 0.66$; $P = ns$).

CONCLUSION AND RECOMMENDATION

In conclusion, farm-level average daily FSMY, housing systems, and precision technology implementation significantly influence dairy herd reproductive parameters. A high average daily FSMY (over 30 kg) is associated with a longer service period mean of 126 days, whereas low-productivity farms (up to 20 kg) exhibit the shortest mean of 102.17 days. Regarding infrastructure, tie-stall housing maintains the shortest service period (104.69 days) but requires a higher mean of 3.73 semen doses per conception. Conversely, free-stall housing results in the longest service period (129.79 days) but achieves the lowest mean of 3.26 semen doses per conception. Furthermore, technology adoption optimizes reproductive management; implementing three or more precision technologies reduces semen demand to a mean of 3.36 doses, compared to 3.71 and 3.75 doses when using only one or two technologies, respectively. These results confirm that high farm productivity does not necessarily compromise fertility parameters when combined with precise technological monitoring and strategic management decisions. A limitation of this study is the application of a single-factor framework necessitated by the high multicollinearity among the examined operational factors. Future multivariable research could further clarify the combined associations of housing, technology, and milk yield.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Emil Mitev, Institute of Agriculture, 6000 Stara Zagora, Bulgaria. E-mail: emillmitev@gmail.com; ORCID: <https://orcid.org/0009-0006-1472-639X>

Data availability

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

Authors' contribution

Emil Mitev: statistical processing of data, literature review, writing the original text of the manuscript. Yovka Popova: development of the concept and design of the study, analysis and interpretation of the obtained data, and critical revision of the manuscript. Stayka Laleva: supervision of the experiment, resources, final correction and editing of the text. All authors approved the final version of the article and are responsible for the accuracy of the presented data.

Ethical regulations

Formal ethical approval was not required for this study according to the institutional regulations of the Agricultural Institute – Stara Zagora, as the research strictly involved a non-invasive, voluntary survey of farm owners and collected no sensitive biological or personal data. Participation in the surveys and interviews was entirely voluntary, and informed consent was obtained from all respondents prior to data collection. Absolute confidentiality and anonymity of the participants' personal data were guaranteed throughout the study.

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Competing interests

The authors declare that they have no competing interests.

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ASSESSMENT OF QUALITY OF POULTRY FEED MANUFACTURED IN REGISTERED AND UNREGISTERED FEED MILLS IN BANGLADESH

Md.Saiful ISLAM¹ , Jiangfeng HE² , Md.Shihab UDDIN¹ , Md.Arafat HOSSAIN³ ,
Mofassara AKTER⁴ , Zannatul NAIM¹  and Rahat Nahiyan RAMIM³ 

¹Department of Animal Production and Management, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

²Inner Mongolia Academy of Agriculture and Animal Husbandry Sciences, Hohhot, 010031, China

³Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

⁴Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

[✉]Email: saiful.apma@sau.edu.bd

[➤]Supporting Information



ABSTRACT: The study evaluated poultry feed quality and health-hazardous elements from registered and unregistered feed mills in Savar, Kaliakoir, and Sreepur upazilas of Dhaka Division, Bangladesh. A total of 90 feed samples, including broiler starter, grower, and layer feeds, were randomly collected from five registered and five unregistered feed mills, considering three samples in each category. The feed samples were evaluated for physical attributes, including color, odor, particle size, and the presence of foreign materials, and were subsequently subjected to proximate analysis for the determination of moisture, crude protein (CP), ether extract (EE), and ash. The concentrations of two heavy metals, lead (Pb) and chromium (Cr), were determined in the feed samples. Both registered and unregistered feed samples showed acceptable physical quality, characterized by normal color and smell, particle size and absence of visible mold contamination or foreign materials. Proximate analysis showed the significant variation in terms of moisture, CP, EE, and ash within and between feed groups, indicating comparatively lower quality of feed obtained from unregistered group. In broiler feed (starter and grower), mean moisture, CP, EE, and ash contents ranged from 10.45–11.35%, 16.98–21.66%, 5.24–8.20%, and 6.13–7.20%, respectively, across registered and unregistered categories. In layer feed, the corresponding values in registered and unregistered categories were 11.05% and 10.81% moisture, 16.86% and 13.54% CP, 5.45% and 5.78% EE, and 11.77% and 8.16% ash, respectively. The concentrations of Cr and Pb were found to be below the detection level for all types of feed in both categories. The present study found that feed from unregistered mills, particularly in crude protein, was below standard, whereas registered-mill feed largely met nutritional standards. Based on the measured physicochemical parameters and Pb and Cr levels, no significant public health risk was evident.

Keywords: Chicken feed, Health hazard, Nutritional quality, Physical quality, Proximate analysis.

INTRODUCTION

The production of chicken feed is booming in Bangladesh due to the growing demand for poultry goods, particularly chicken eggs and meat (Hamid et al., 2016). According to the Feed Industry Association Bangladesh (FIAB), poultry uses the most feed because commercial poultry farming depends entirely on industrially produced feed. In Bangladesh, the annual feed demand is approximately 8.0 million metric tons, with commercial poultry farms being the largest consumers (Mamun et al., 2024). There are about 507 registered feed mills which are regulated and maintained by the government authorities (DLS Annual Report 2023-2024). A large number of unregistered feed mills are expanding in addition to registered mills in order to meet the growing demand for poultry feed (Nabihatul Afrooz, 2022). Since many of these feed producers do not hold an official license from the Department of Livestock Services (DLS), there is little control to guarantee that they adhere to quality standards. In the absence of oversight, they frequently disregard advised protocols. Nevertheless, unregistered feeds are commonly used, primarily due to their lower cost compared to feeds from registered feed mills (Nakkazi et al., 2015). They typically use lower quality raw materials to keep expenses down and draw in more customers, which boosts their profit margins (Mengesha and Mammo, 2012; Sigut et al., 2025).

The quality of chicken meat and eggs mostly depends on the quality of feed (Gautron et al., 2022; Maina et al., 2025). To evaluate the quality of poultry feed, several factors need to be considered. The quality of the ingredients used in poultry feed is crucial to its nutritional value (Demissie and Negash, 2020; Hossain et al., 2022). Poultry feed should be free from harmful contaminants, such as toxins, bacteria, and heavy metals (Alam et al., 2021; Islam et al., 2024; Mamun et al., 2024). The texture of the feed should be appropriate for the age and size of the birds (Tadesse et al., 2017). Proper packaging of poultry feed is essential to protect it from moisture, pests, and other contaminants (You et al., 2024). Low quality feed can also enhance the high mortality of chicken (Sultana et al., 2017). Numerous reports have emerged regarding adulterated, poor-quality feed produced by unregistered feed mills, resulting in serious health issues in birds, significant drops in production, and posing a potential risk to public health (Islam et al., 2024; Mamun et al., 2024). In most cases, farmers typically prioritize treating disease or low productivity with antibiotics over enhancing feed quality.

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Additionally, a significant barrier to the sustainable growth of the industry is the insufficient quantity and quality of chicken feed (Sigut et al., 2025).

Multiple studies (Shamim et al., 2024; Islam et al., 2024; Islam et al., 2024; Ochieng et al., 2021) have reported alterations in feed quality and the presence of potential pollutants in poultry feeds in developing countries. However, comparative data on the physical quality, proximate composition, and heavy metal contamination of feeds produced by registered and unregistered feed mills in Bangladesh remain limited, particularly in the Dhaka division. Therefore, the present study was designed to evaluate and compare the physical characteristics, proximate composition, and selected heavy metals (lead and chromium) in poultry feeds collected from registered and unregistered feed mills in the Dhaka division of Bangladesh. The importance of feed quality in sustainable poultry production is emphasized in this study, which also calls on farmers, consumers, and legislators to collaborate in order to preserve uniform feed standards.

MATERIALS AND METHODS

Location

The study was conducted under the Laboratory of Department of Animal Production & Management, Sher-e-Bangla Agricultural University, Dhaka 1207

Collection of feed samples

Broiler starter, broiler grower, and layer feed were collected randomly from 5 registered feed mills of three selected areas, namely at Savar, Kaliakoir, and Sreepur Upazila of Dhaka Division. The same categories of feed samples were also collected from the 5 unregistered feed mills in the same areas as well. A total of 90 feed samples of poultry feeds were collected from the farms, considering three samples in each feed mill, and kept in a zip lock poly bag.

Evaluation of physical properties

Feed samples were evaluated for physical characteristics including color, odor (with no rancid or abnormal smell), the presence of visible foreign materials based on visual inspection and feed form (mash, crumble, and pellet). Feed particle size was evaluated based on the protocol as described previously (Wilcox et al., 1970).

Determination of chemical properties

The feed samples were subjected to proximate analysis by standardized method according to (AOAC, 1990) for the determination of moisture, crude protein (CP), ether extract (EE) & ash. Selected individual feed sample was mixed thoroughly, dried, ground by grinding machine at the size of 0.5mm. Triplicate samples of each commercial feed types were used to determine the following chemical compositions.

The moisture content determination was carried out by keeping feed samples in a thermostat oven at 105°C for 24 hours. This was done by the difference between the net weight and the weight after drying to a constant weight. The final weight determined after cooling in a desiccator for about 35 minutes AOAC (1990). Crude protein was measured using the Kjeldahl method, a typical laboratory procedure for finding total nitrogen content in feed samples. About 1g of each sample was subjected to acid digestion using condensed sulfuric acid (H₂SO₄) and a mixed catalyst. The digested solution was then distilled into a 2% boric acid solution and consequently titrated with 0.1N hydrochloric acid (HCl). The nitrogen content obtained was transformed to crude protein by applying a conversion factor of 6.25 (N × 6.25) according to AOAC (1990). For measuring ether extract, 3g sample was wrapped in filter paper and placed in an extraction thimble with cotton wool on top. It was then set up for Soxhlet extraction using 300 cm³ of 60–80 °C petroleum ether in a pre-weighed flask. The extraction ran for 3 hours with a constant water flow through the condenser. Afterward, the sample was air-dried and oven-dried at 80 °C until a constant weight was achieved. The crude fat content was calculated as a percentage of extractable fat.

During ash determination of feed according to AOAC (1990) method, a 2g sample is placed in a crucible and incinerated in a muffle furnace at 550°C. After approximately 6 hours, the weight stabilizes, indicating complete ashing. The final residue, representing the mineral content, is weighed after cooling in a desiccator. The weights were measured and the weights of the ashes obtained by difference.

Determination of heavy metals

Heavy metal analysis was performed following a wet-acid digestion procedure adapted from standard methods for trace metal determination. Briefly, 2 g of homogenized poultry feed sample was transferred into a digestion flask and treated with 10 mL concentrated nitric acid (65% HNO₃) and 4 mL hydrogen peroxide (30% H₂O₂). The mixture was heated gradually on a hot plate until complete digestion was achieved, as indicated by the formation of a clear solution. After cooling, the digest was filtered through Whatman No. 14 filter paper and diluted to a known volume with distilled water. The resulting solutions were analyzed for heavy metals using an Analytik Jena novAA 400P Atomic Absorption Spectrophotometer (AAS) (United States Environmental Protection Agency. 1996. *Method 3050B: Acid Digestion of Sediments, Sludges, and Soils*. Washington, DC, USA.)

Statistical analysis

All collected data were checked for completeness and entered into Microsoft Excel for data organization and tabulation. Statistical analyses were performed using Statistix 10 software (Analytical Software, Tallahassee, FL, USA). Analysis of variance (ANOVA) was used to evaluate differences among the measured parameters. Results are presented as mean ± standard deviation (SD). Statistical significance was declared at P < 0.05.

RESULTS AND DISCUSSION

Physical quality characteristics of feed samples

Feed form has reported to control feed intake in poultry, with pelleted diets generally improving feed consumption compared with mash diets; however, the magnitude of this effect may vary depending on diet composition, particle size, bird age, and management conditions (Shabani et al., 2015). Both the feed particle size and nutrient density play an important role on feed intake and bird performance (Svihus et al., 2024). The results demonstrated in Table 1 that the color of the feed exhibited slight variations among the samples. The feeds were observed in shades of yellow, dark yellow, dark brown, and light brown. All samples maintained a normal flavor profile, with no foreign particles or molds detected. No significant variation has been observed in particle size between registered and unregistered feed mills (Figure 1). The form of feed presentation and particle size significantly influence broiler performance within the poultry sector (Lv et al., 2015). Particle size has been shown to influence feed intake and the development of the gastrointestinal tract (Naeem et al., 2024). Particle size distribution of the poultry feed samples was determined by sieve analysis and expressed as the percentage of feed particles retained in each sieve-size fraction. The sieve fractions were classified as >4 mm, 3–4 mm, 2–3 mm, 1–2 mm, and 0.5–1 mm. The results showed that particle size distribution varied among starter, grower, and layer feeds. In broiler starter feed, most particles were retained in the 2–3 mm and 1–2 mm fractions. In broiler grower feed, the highest proportion of particles was retained in the 2–3 mm fraction, representing 89.12% in registered feed and 87.86% in unregistered feed. Layer feed showed a wider particle size distribution, with particles distributed across all sieve-size fractions. Overall, the results indicate that grower feed had a more uniform particle size distribution, while layer feed showed greater variation in particle size. Providing broilers with a pelleted diet enhances both weight gain and feed intake (Svihus et al., 2024; Stojkov et al., 2025). In the feed industry, feed formulations, conditioning, and grinding are regarded as critical factors affecting pellet quality. Enhancements in pellet quality can be achieved by refining feed production processes, particularly in grinding and conditioning (Lilly et al., 2011; Rueda et al., 2022; Svihus et al., 2024). It is widely accepted that grinding raw materials to a finer particle size may lead to improved pellet quality (Shabani et al., 2015). A comparative physical analysis of the feeds revealed color variations among different feeds from registered and unregistered companies. Additionally, some unregistered feeds contained foreign particles. While most feed companies produced feeds with a similar aroma, certain unregistered feeds were noted to be hard.

Table 1 - Comparative analysis of physical properties between registered and unregistered feed mill.

Parameter	Feed company	Broiler starter	Broiler Grower	Layer (Layer feed)
Feed form	Registered Unregistered	Crumble Crumble	Pellet Pellet	Mash Mash
Color	Registered Unregistered	Dark brown / Light brown Brown / Dark brown	Light brown / Dark yellow Dark yellow	Yellow/Dark yellow Yellow
Smell	Registered Unregistered	Normal Normal	Normal Normal	Normal Normal
Foreign particles	Registered Unregistered	Absent Absent	Absent Absent	Absent Absent/Present

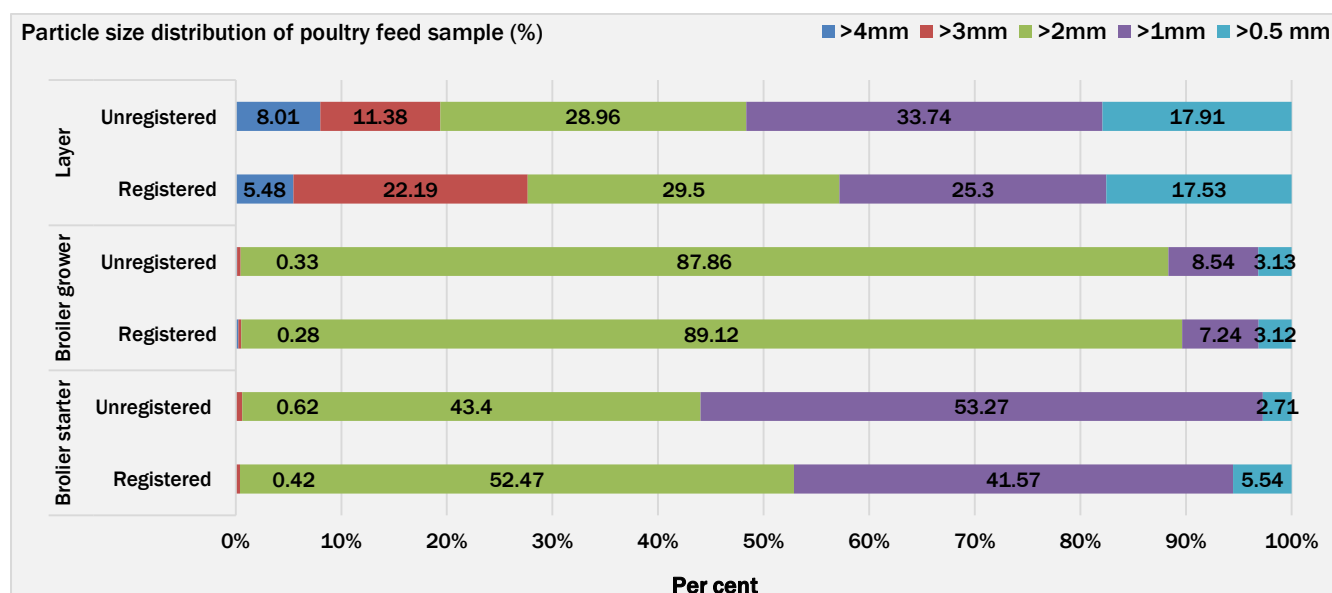


Figure 1 - Particle size distribution of poultry feed samples

Chemical properties of feed

Moisture percentage

Moisture or water contents of feeds are often overlooked. Present study indicates the moisture content ranging 9.09% to 12.11% in different types of chicken feed (Figure 2). The results indicate minor differences in the moisture of chicken feed from various sources. Moisture per cent in chicken feed ranges between 8-12% indicating the good quality feed with longer shelf life, which means that those feeds may store well and resist fungal attack. Moisture content of feed may sometimes be lower or higher than the manufacturers declared. The type of ingredients or the timing of ingredient collection determines the moisture content of feed. Moreover, high moisture percentage may support the growth of fungi in the feed when stored for longer period (Vieira, 2003). Corns are the major ingredients poultry feeds in Bangladesh, which possess more than 20% at the time of harvesting (Saiful et al., 2015).

Crude protein percentage

Typically, starter rations contain higher protein levels compared to grower and finisher rations for both broilers and layers. The crude protein content ranged from 17.71% to 22.03% in broiler starter rations, 15.62% to 21.50% in broiler grower rations, and 11.97% to 17.64% in layer rations were investigated this study (Figure 3). However, according to standard commercial industry standards, the commonly recommended crude protein levels of 20–22% for broiler starter, 18–20% for broiler grower, and 16–18% for layer rations. Based on this comparison, some starter and grower feed samples were below the recommended crude protein range, while some grower samples exceeded the recommended range. Layer rations also showed lower crude protein values in some samples, indicating variation in protein adequacy among the analyzed feed samples. In this study, significantly lower CP value was found in all types of unregistered feed compared to registered feed. This might be because protein feed ingredients for ration formulation are expensive, and some feed producers might employ less expensive protein sources to increase their profits. It is evident that crude protein constitutes a significant and costly element of animal feed (Alhotan et al., 2014). When low-quality protein contains insufficient and unbalanced amino acids, adding more dietary protein to the ration may not have an impact on growth, feed efficiency, or carcass characteristics; instead, it may result in increased mortality (Delezie et al., 2007).

Ether extract percentage

Figure 4 illustrates clear differences in Ether extract percentage among poultry feeds from registered and unregistered feed mills. Registered feeds, particularly for broiler growers, generally contain higher and more consistent fat levels, which are indispensable for supplying energy to poultry (Swamy and Upendra, 2013). However, feeds from unregistered sources are more inconsistent and frequently have lower fat values, particularly in the diets of broiler growers. Significant variations in the nutritional quality of broiler starter and layer feeds were observed.

Ash percentage

Mineral elements are abundant in the bodies of animals, and in a chicken's body, which is around 4%. Minerals are critical nutrients required in specific amounts in the poultry diets needed for muscle contraction, egg shell formation, stronger bones, blood clotting, and enzyme activation (Orban et al., 1993). This study reported the various ranges of ash content in different feed sample (Figure 5). However, it was reported that low ash content in feed causes diseases and poor eggshell formation in birds (Delezie et al., 2015).

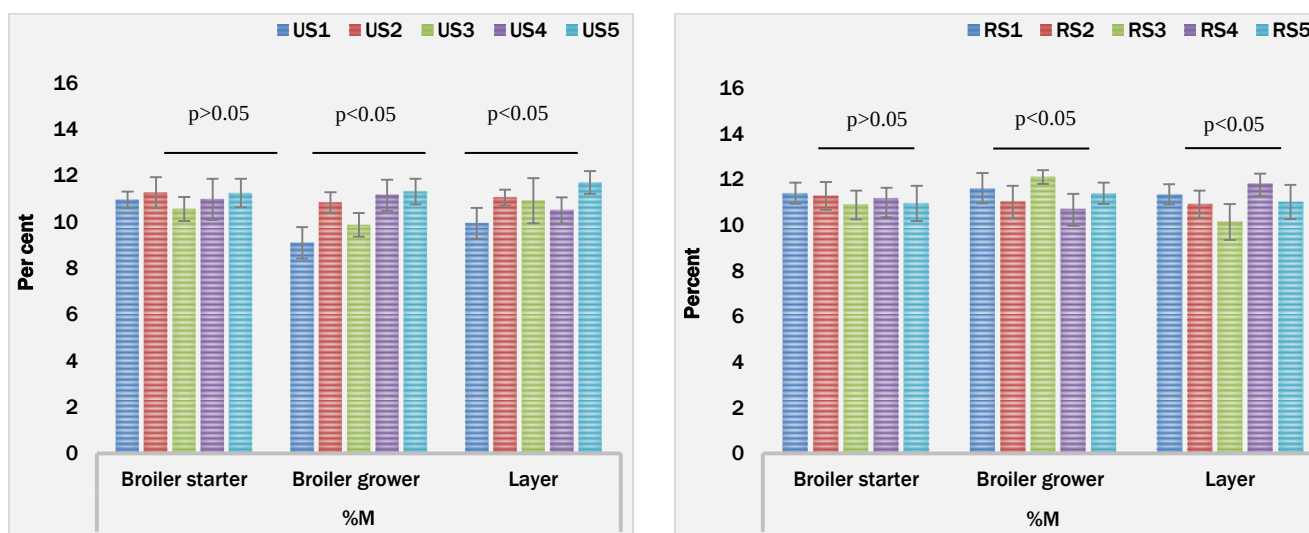


Figure 2 - Moisture percentages of registered (left) and unregistered (right) feed mill samples. RS= Registered feed mill samples; US=Unregistered feed mill samples, M= Moisture.

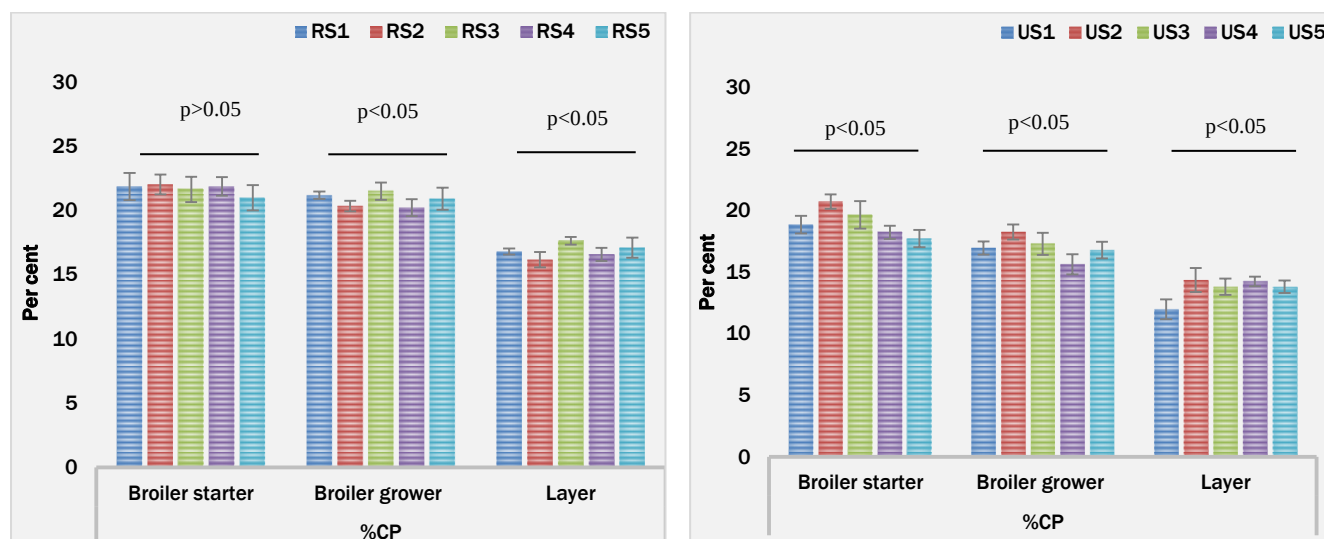


Figure 3 - Crude protein percentage of registered (left) and unregistered (right) feed mill samples. RS= Registered feed mill samples; US=Unregistered feed mill samples, CP= Crude protein.

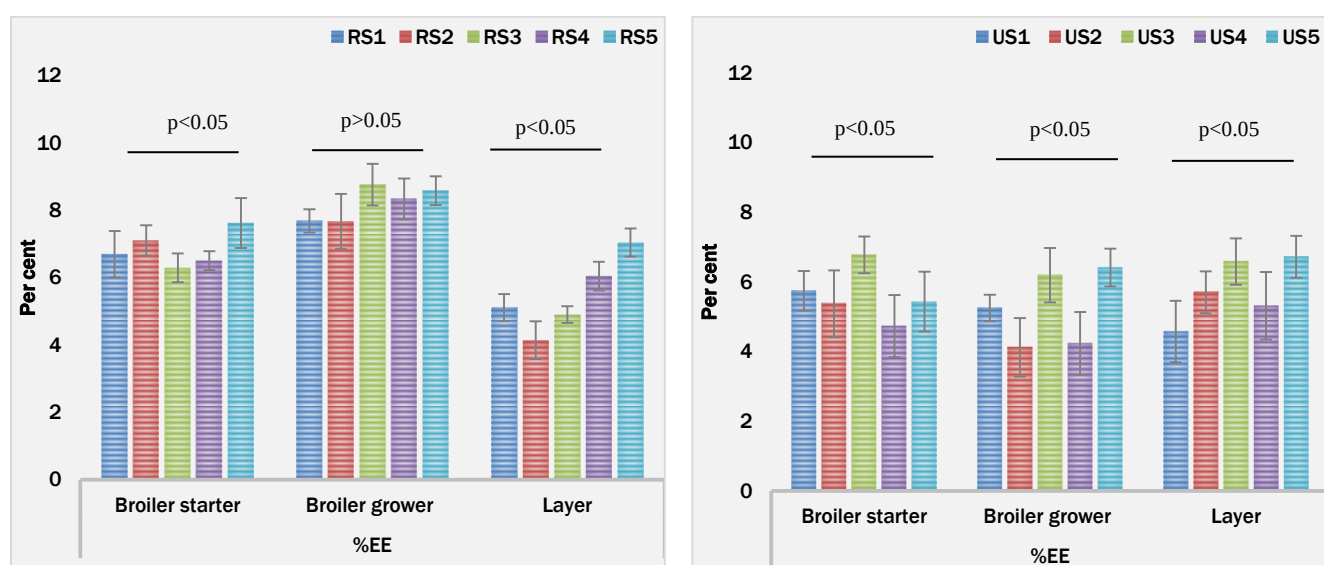


Figure 4 - Ether extract percentage of registered (left) and unregistered (right) feed mill samples. RS= Registered feed mill samples; US=Unregistered feed mill samples, EE=Ether extract.

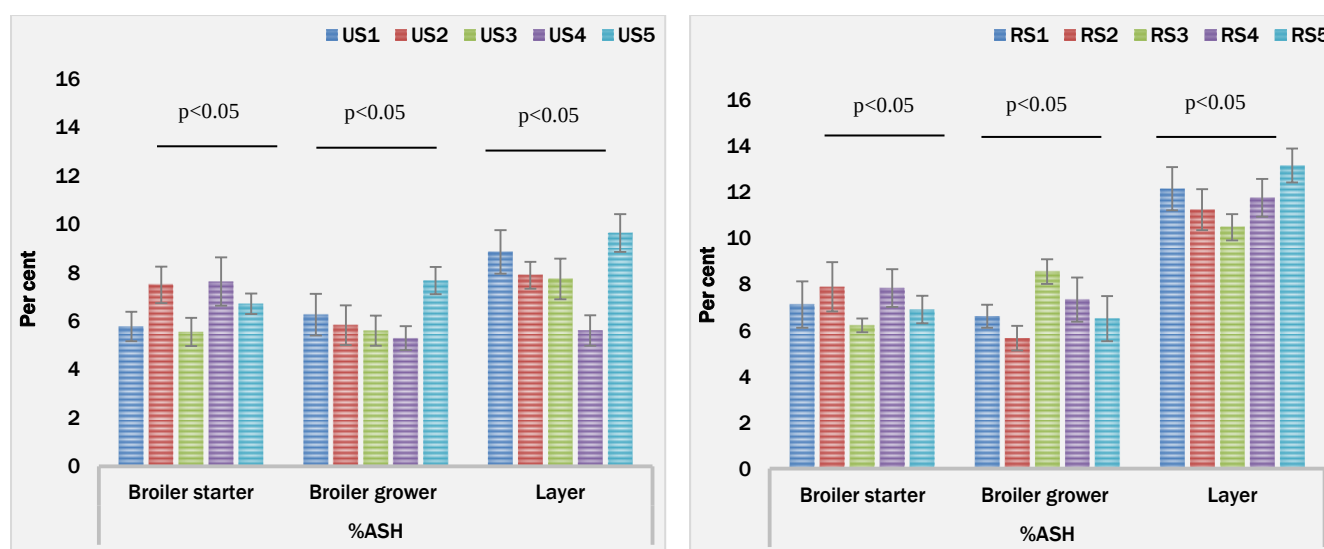


Figure 5 - Ash percentage of registered (left) and unregistered (right) feed mill samples. RS= Registered feed mill samples; US=Unregistered feed mill samples.

Comparative analysis between registered and unregistered feed mill samples

Figure 6 compares the quality of poultry feed across starter, grower, and layer feeds from registered and unregistered feed mills, focusing on important components like moisture, fat, protein, and minerals. All things considered, feed from registered mills appears to be more consistent and balanced overall, particularly in terms of protein and minerals, which are essential for healthy growth, especially in the early stages of chicken (Dutta et al., 2020). Feed from unregistered feed mills, on the other hand, frequently lacks these vital nutrients and varies significantly (Hasan et al., 2022). While the two groups moisture levels are generally comparable, the unregistered group exhibits greater variability. Additionally, some of the unregistered samples had a slightly higher fat content. These results imply that feed quality is not substantially maintained by unregistered feed mills (Dutta et al., 2020).

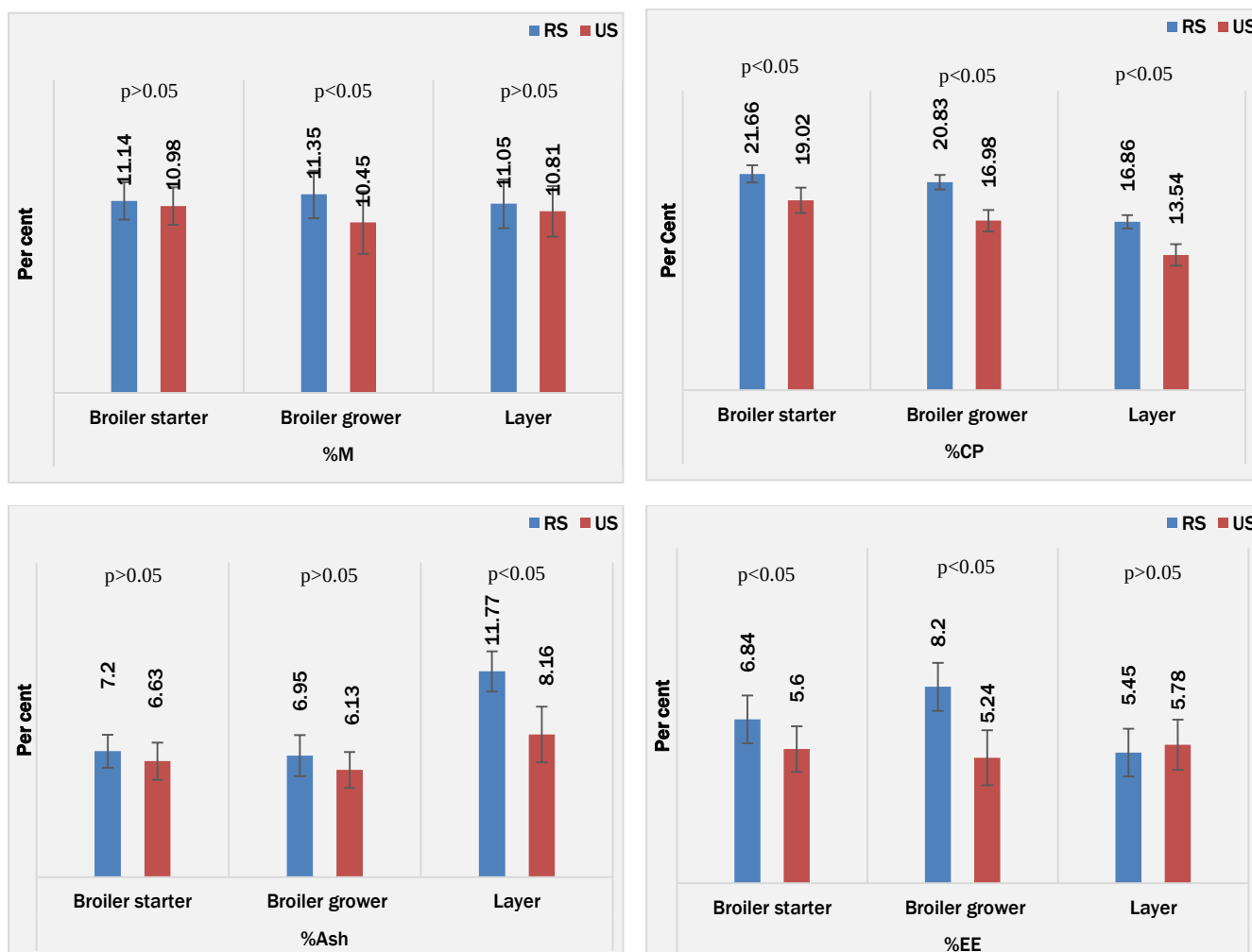


Figure 6 - Comparative nutrient composition between registered and unregistered feed mill samples. RS= Registered feed mill samples; US=Unregistered feed mill samples, M= Moisture, CP=Crude protein, EE=Ether extract.

Heavy metals in feed

Poisonous metals lead and chromium in broiler feed might exhibit toxicity and carcinogenic effects at low concentrations on public health by consumption of poultry products day by day. According to the United States Environmental Protection Agency, metals such as Chromium (Cr), Arsenic (As), Cadmium (Cd), and Lead (Pb) are regarded as some of the most environmentally hazardous elements (Hossain et al., 2022). The concentrations of both lead and chromium were found to be below the detection limit (BDL) in this study (Table 2), indicating the feed is generally considered not hazardous. Therefore, periodic analyses of these toxic metals in poultry feed are necessary for ensuring feed quality and consumer safety as well. However, many works have been carried out regarding the analysis of these metals in different types of poultry feed and found below the detection level (Islam et al., 2024).

Table 2 - Comparative heavy metal composition between registered and unregistered feed

Elements	Feed company	Broiler starter	Broiler Grower	Layer (Layer feed)
Cr	Registered	BDL	BDL	BDL
	Unregistered	BDL	BDL	BDL
Pb	Registered	BDL	BDL	BDL
	Unregistered	BDL	BDL	BDL

BDL=Below detection limit. Values that were below 0.021 ppm and 0.074 ppm were identified as BDL for Cr & Pb respectively.

CONCLUSION

The research included testing broiler feeds by measuring their physical structure, chemical content, concentration of heavy metals and their quality against food safety standards. The study showed that feed from registered feed mills in the Dhaka Division was of a much higher quality than that from unregistered feed mills. Unregistered feed millers are using low-quality ingredients to increase profits, as they are not inspected by the government authority. Because of these factors, the government and involved stakeholders ought to take action to ensure the availability of nutritious and safe animal feed, which contributes to the production of safe protein for human consumption. Further research should include more feed mills and samples from diverse regions to validate quality variability and monitor nutritional consistency, adulteration, and other contaminants, particularly in unregistered feed mills.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Md. Saiful Islam; E-mail: saiful.apma@sau.edu.bd; ORCID: <https://orcid.org/0000-0001-9293-0069>

Data availability

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

Authors' contribution

The first four authors contributed equally and were considered as first author. M. S. Islam planned, designed the research, managed the fund, prepared original draft and reviewed the manuscript. J. He designed the research and reviewed the manuscript. M. S. Uddin collected data, samples and conducted laboratory analysis. M. A. Hossain prepared manuscript, analyzed and visualized data. Z. Naim reviewed the manuscript. M. Akter reviewed and approved the final version of the manuscript. R.N. Ramim reviewed the manuscript. All authors read and approved the submitted version of the manuscript.

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Competing Interests

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

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DIGESTIBILITY AND FEEDING VALUE OF IMPROVED TROPICAL GRASSES AND *Leucaena leucocephala* CULTIVARS FOR GOAT NUTRITION IN VIETNAM

Tran Ngoc LIEM , Ngo Mau DUNG , Le Minh DUC , Duong Thanh HAI , and Le Van AN 

University of Agriculture and Forestry, Hue University, Hue, Vietnam

Email: levanan@hueuni.edu.vn

Supporting Information



ABSTRACT: This study evaluated the nutritive value and digestibility of five tropical grass cultivars, including Ruzi (*Brachiaria ruziziensis*), Mulato II (*Brachiaria ruziziensis* × *B. decumbens* × *B. brizantha*), and three *Panicum maximum* cultivars (Purple, Mombasa and Mun River), together with five *Leucaena leucocephala* cultivars (Tarramba, Peru, Cunningham, KU15 and KU56). *In vitro* digestibility was determined using ANKOM DAISYII incubator system, and an *in vivo* feeding trial evaluated the effects of increasing dietary inclusion of Tarramba leaves (0, 10, 20 and 30% of dietary dry matter) in Mombasa-based diets on nutrient intake and apparent digestibility in goats. Grass cultivars differed significantly ($P < 0.05$) in dry matter and fibre digestibility. No significant differences were detected among grass cultivars for *in vitro* dry matter, neutral detergent fibre or acid detergent fibre digestibility, although numerical differences were observed. Similarly, digestibility did not differ significantly among the five *Leucaena* cultivars, whereas *Leucaena* cultivars had substantially higher crude protein concentrations than the grasses. Increasing Tarramba inclusion resulted in a linear increase in crude protein intake and apparent crude protein digestibility while apparent dry matter and organic matter digestibility remained unaffected. Dry matter and organic matter intake showed quadratic responses to increasing Tarramba inclusion, whereas ADF digestibility decreased linearly and NDF digestibility showed a numerical decline. Under the conditions of the short-term digestibility trial, inclusion of Tarramba leaves up to 30% of dietary dry matter did not adversely affect apparent dry matter and organic matter digestibility. These findings indicate that improved tropical grasses and *Leucaena* can provide complementary forage resources for improving the nutritional quality of grass-based diets for goats.

Keywords: Apparent digestibility, Crude protein, Goat nutrition, Tropical forages, *Leucaena leucocephala*.

INTRODUCTION

Ruminant production plays a vital role in food security, rural livelihoods and the sustainable utilisation of natural resources throughout the tropics. However, productivity is frequently constrained by an inadequate supply of high-quality forage, particularly during periods of seasonal feed shortage (Cooke et al., 2025; Mugoti et al., 2025). This challenge is especially evident in smallholder farming systems, where animal performance depends largely on locally available forage resources rather than purchased concentrates (Devendra and Thomas, 2002).

In Vietnam, increasing demand for meat and milk has driven the expansion of the ruminant sector over the past decade (MARD, 2020; FAO, 2021). Although the country has favourable climatic conditions for forage production, ruminant feeding systems continue to rely predominantly on native grasses, cultivated forages and crop residues, whose availability and nutritional quality vary markedly with season. In central Vietnam, seasonal drought and flooding can constrain forage availability and quality, contributing to fluctuations in the supply crude protein and digestible nutrients. These seasonal constraints can consequently limit feed intake, nutrient utilisation, and growth performance in goats and other ruminants (Dung et al., 2013; Nguyen et al., 2023).

Improving forage quality through the adoption of productive tropical grasses and protein-rich forage legumes has therefore become a key strategy for enhancing the productivity and sustainability of ruminant production systems (Da Silva et al., 2015; De Angelis et al., 2021). Improved cultivars of *Brachiaria* and *Panicum* are recognised for their high forage yield, persistence and adaptation to tropical environments (Da Silva et al., 2015). Among tropical forage legumes, *Leucaena leucocephala* is widely used as a protein-rich forage because of its high crude protein concentration, favourable digestibility, drought tolerance and ability to improve the nutritional balance of grass-based diets (Garcia et al., 1996; De Angelis et al., 2021). Numerous studies have reported that supplementation of grass-based diets with *Leucaena* can improve rumen nitrogen supply, nutrient digestibility, voluntary feed intake and animal performance.

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However, these responses vary among cultivars and dietary inclusion levels, and excessive inclusion may reduce feed utilisation because of anti-nutritional compounds, particularly mimosine (Phaikaew et al., 2012; Casanova-Lugo et al., 2014). Therefore, both cultivar selection and dietary inclusion level are critical for maximising the nutritional benefits of *Leucaena* while minimising potential adverse effects.

Several improved *Leucaena* cultivars have recently been introduced into Vietnam to enhance forage production under tropical conditions. Among these, the cultivar Tarramba exhibited superior adaptation, forage yield and nutritional characteristics compared with other introduced cultivars under local growing conditions (Liem et al., 2026). Previous studies also demonstrated that supplementation with Tarramba leaves improved growth performance, feed efficiency and carcass characteristics of Vietnamese goats (Liem et al., 2025), indicating its considerable potential as a protein supplement for grass-based feeding systems. However, information on the effects of Tarramba inclusion on nutrient intake and apparent nutrient digestibility in grass-based diets remains limited. Importantly, the selection of Tarramba for further feeding evaluation was based primarily on its agronomic performance and practical suitability under local conditions, rather than on superior *in vitro* digestibility relative to other *Leucaena* cultivars.

Despite recent advances in forage evaluation in Vietnam, comprehensive evaluations of the nutritive value and digestibility of improved tropical grass and *Leucaena* cultivars under local environmental conditions remain scarce. Furthermore, previous studies have generally focused on either forage quality or animal performance, whereas few have integrated laboratory evaluation of forage digestibility with *in vivo* assessment of nutrient intake and apparent digestibility. Linking these complementary approaches is essential for identifying forage cultivars with superior feeding value and developing evidence-based recommendations for incorporating protein-rich forage legumes into grass-based diets for small ruminants.

Therefore, this study integrated *in vitro* and *in vivo* approaches to evaluate the nutritive value and digestibility of improved tropical forages for goats in central Vietnam. Specifically, the objectives were to (i) compare the chemical composition and *in vitro* digestibility of five tropical grass cultivars and five *Leucaena leucocephala* cultivars, and (ii) determine the effects of increasing dietary inclusion of Tarramba *Leucaena leucocephala* leaves (0%, 10%, 20% and 30% of dietary dry matter) in a Mombasa grass-based diet on nutrient intake and apparent digestibility in goats. This study provides evidence to support forage cultivar selection and the development of nutritionally balanced and sustainable feeding strategies for goat production in Vietnam.

MATERIALS AND METHODS

Experiment 1: *In vitro* evaluation of tropical grasses and *Leucaena* cultivars

Five tropical grass cultivars, namely Ruzi (*Brachiaria ruziziensis*), Mulato II (*Brachiaria ruziziensis* × *B. decumbens* × *B. brizantha*), Purple (*Panicum maximum* cv. Purple), Mombasa (*Panicum maximum* cv. Mombasa) and Mun River (*Panicum maximum* cv. Mun River), were cultivated at the experimental farm of the University of Agriculture and Forestry, Hue University, Vietnam. The grasses were harvested at a regrowth age of 45 days in May 2024. Five *Leucaena leucocephala* cultivars (Tarramba, Peru, Cunningham, KU15 and KU56) were grown at the same site and harvested in March 2024 after a four-month regrowth period. Only the edible fractions (leaves and young stems) were collected for analysis.

For chemical composition analysis, three independent forage samples were collected from each grass cultivar and four independent forage samples were collected from each *Leucaena* cultivar at harvest. Thus, the chemical composition dataset comprised 15 grass samples and 20 *Leucaena* samples. Samples were collected from different locations within the harvested forage material and were treated as independent samples for statistical analysis.

For the *in vitro* digestibility assay, two independent forage samples were selected from each cultivar, giving a total of 20 forage samples across the five grass and five *Leucaena* cultivars. Each independent forage sample was assigned to one ANKOM F57 filter bag and constituted one experimental observation. The two independent samples from each cultivar were allocated to two digestion jars, with one sample from each cultivar placed in each jar. Thus, each jar contained 10 forage samples, representing one sample from each of the 10 cultivars, and 2 blank bags. All 20 sample-containing bags and 4 blank bags were incubated simultaneously in the two digestion jars during one incubation run in the ANKOM DAISYII system. The two digestion jars were considered incubation vessels within the same run and were not treated as independent biological replicates. All forage samples were oven-dried at 60 °C for 72 h, ground to pass through a 1-mm screen using a laboratory mill, sealed in airtight containers and stored at −20 °C until analysis.

Chemical analysis

Samples of all forage cultivars were analysed for dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) at the Laboratory of the University of Agriculture and Forestry, Hue University, Vietnam. The analytical procedures used for chemical composition are described in the following section.

***In vitro* digestibility**

In vitro digestibility was determined using the ANKOM DAISYII Incubator (ANKOM Technology Corporation, Fairport, NY, USA) at the Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Thailand. Approximately 0.50 g of each dried forage sample was weighed into a pre-weighed ANKOM F57 filter bag that had been pre-rinsed with acetone and dried. Each filter bag contained one of the two independent forage samples collected for each cultivar; no additional filter bags were used as technical replicates of the same forage sample. Empty bags were included as blanks to correct for changes in bag weight during incubation. The 20 sample-containing bags and 4 blank bags were distributed between the two digestion jars, such that each jar contained 10 sample bags and 2 blank bags, representing one independent sample from each cultivar.

The artificial buffer consisted of two solutions. Buffer A contained KH_2PO_4 (10.0 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 g/L), NaCl (0.5 g/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.1 g/L) and urea (0.5 g/L), whereas Buffer B contained Na_2CO_3 (15.0 g/L) and $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (1.0 g/L). Both buffers were prepared with distilled water according to the ANKOM protocol. Fresh rumen fluid was collected before the morning feeding from two rumen-fistulated beef steers weighing approximately 320–330 kg. The steers were fed rice straw *ad libitum* as roughage and 2 kg/day of a concentrate diet containing 12% crude protein. The diet was offered twice daily, and fresh water was available freely. Rumen fluid collected from the two steers was mixed and filtered through four layers of cheesecloth. The pooled rumen fluid was maintained under oxygen-free conditions and continuously flushed with CO_2 until preparation of the rumen inoculum according to the ANKOM DAISYII protocol. The pooled rumen fluid from the two donor steers was used as a common inoculum for all samples; therefore, donor animals were not considered independent experimental replicates.

For each digestion jar, 1330 mL of Buffer A and 266 mL of Buffer B were added to each digestion jar and equilibrated at 39 °C for approximately 30 min before adding 400 mL of rumen fluid. The jars were then incubated in the DAISYII incubator at 39 °C for 48 h. Both digestion jars were incubated simultaneously during the same incubation run. At the end of incubation, the filter bags were thoroughly rinsed with cold water to terminate microbial fermentation and remove residual digesta before being dried to constant weight. *In vitro* dry matter digestibility (IVDMD) was calculated after correcting for blank bag weight changes. Residues remaining after incubation were subsequently analysed for NDF and ADF using the ANKOM fibre analyser to determine *in vitro* neutral detergent fibre digestibility (IVNDFD) and *in vitro* acid detergent fibre digestibility (IVADFD), respectively. For statistical analysis, the two independent forage samples per cultivar were considered the experimental observations. Filter bags and digestion jars were not treated as additional experimental replicates.

In vitro digestibility variables were calculated as follows: $\text{IVDMD (\%)} = [1 - ((W_3 - W_1 \times C_1)/W_2)] \times 100$

where W_1 is the initial weight of the empty filter bag (g), W_2 is the sample weight (g), W_3 is the final weight of the filter bag after incubation and drying (g), and C_1 is the blank bag correction factor.

$\text{IVNDFD (\%)} = [1 - (\text{NDF residue after incubation} / \text{initial NDF content})] \times 100$

$\text{IVADFD (\%)} = [1 - (\text{ADF residue after incubation} / \text{initial ADF content})] \times 100$

Experiment 2: Effects of Increasing Dietary Inclusion of Tarramba *Leucaena leucocephala* on Feed Intake and Apparent Nutrient Digestibility in Goats

Animals and experimental design

Sixteen male Co goats, a local Vietnamese breed, with an initial body weight of 12.4 ± 1.3 kg (mean $\pm$ SD), were used in this experiment. Prior to the experiment, all goats were vaccinated against pasteurellosis, haemorrhagic septicaemia and foot-and-mouth disease and were treated with anthelmintics to ensure uniform health status. The goats were randomly allocated to four dietary treatments in a completely randomized design, with four goats per treatment. Each goat was housed individually in a separate pen equipped with an individual feeder and continuous access to clean drinking water throughout the experimental period.

Experimental diets and feeding management

Guinea grass (*Panicum maximum* cv. Mombasa) and *Leucaena leucocephala* cv. Tarramba used in the feeding trial were cultivated at the Institute for Development Studies, University of Agriculture and Forestry, Hue University, Vietnam. Tarramba was selected for Experiment 2 primarily because of its favourable agronomic adaptation and forage yield under the environmental conditions of central Vietnam, as previously reported by Liem et al. (2026), rather than because of superior *in vitro* digestibility among the *Leucaena* cultivars evaluated in Experiment 1. Guinea grass was harvested at a 45-day regrowth period, whereas Tarramba was harvested after a four-month regrowth period. Only the edible portions were collected daily, chopped and thoroughly mixed before being offered fresh to the animals.

Four experimental diets were formulated by replacing Guinea grass with increasing proportions of Tarramba leaves (0%, 10%, 20% and 30% of dietary DM). This resulted in Guinea grass: *Leucaena* ratios of 100:0, 90:10, 80:20 and 70:30 on a DM basis, respectively. The chemical composition of the feed ingredients and the experimental diets is presented in Tables 1 and 2, respectively.

Goats were fed twice daily at 09:00 and 15:00 h. During the 20-day adaptation period, the daily feed allowance was initially estimated at approximately 4% of body weight on a DM basis and was adjusted according to each goat's observed feed intake. During the subsequent 6-day measurement period, the daily amount offered to each goat was set at 90% of its individual average daily DM intake during the adaptation period to minimise feed refusals and facilitate accurate measurement of nutrient intake. Any feed refusals were collected before the morning feeding, weighed, and recorded daily. The quantities of fresh Guinea grass and *Leucaena* offered were adjusted according to their measured DM concentrations to maintain the intended dietary inclusion levels on a DM basis. Fresh drinking water was available ad libitum throughout the experiment.

Table 1 - Chemical composition of forage ingredients used in the feeding trial

Ingredients	DM (%)	Chemical composition (g/kg DM)					
		CP	EE	NDF	ADF	ADL	Ash
Guinea grass (<i>Panicum maximum</i> cv. Mombasa)	24.3	82	21	722	413	79	74
<i>Leucaena</i> leaves (<i>Leucaena leucocephala</i> cv. Tarramba)	33.2	254	72	330	192	87	70

Note: Values are expressed on a dry matter basis unless otherwise indicated. DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin.

Table 2 - Ingredient composition and calculated chemical composition of the experimental diets

Variables	<i>Leucaena</i> inclusion level (% of dietary DM)			
	0	10	20	30
Ingredient composition (kg/100 kg DM)				
Guinea grass	100	90	80	70
<i>Leucaena</i> leaves	0	10	20	30
Total	100	100	100	100
Calculated chemical composition (g/kg DM)				
OM	926	926	927	927
CP	82	99	116	134
EE	21	26	31	36
NDF	722	683	644	604
ADF	413	391	369	347
ADL	79	80	81	81
ME (MJ/kg DM)	9.10	9.32	9.54	9.76

Note: Values were calculated from the analysed chemical composition of the individual forage ingredients. DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; ME, metabolisable energy.

Digestibility trial and sample collection

The digestibility trial lasted 26 days, including a 20-day adaptation period followed by a 6-day measurement period. Goats were adapted to the experimental diets and housing conditions during the first 20 days. Feed intake was measured from days 21 to 26, while quantitative faecal collection was performed from days 22 to 26. During the measurement period, the amounts of feed offered and refused were recorded daily for each goat. Samples of offered feeds and refusals were collected daily, weighed and oven-dried at 65°C for 24 h to determine DM intake. Faeces were collected through the mesh-floor faecal collection system of individual metabolism cages and recovered at 08:00, 14:00 and 18:00 h each day. The total faecal output from each goat was weighed and recorded daily, thoroughly mixed, and a representative subsample corresponding to 20% of the daily faecal output was collected and stored at -20°C until chemical analysis.

Chemical analysis

Forage samples, feed ingredients, experimental diets and faecal samples were analysed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE) and ash according to AOAC (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to Van Soest et al. (1991). All chemical analyses were conducted at the Laboratory at the University of Agriculture and Forestry, Hue University, Vietnam. For Experiment 2, apparent nutrient digestibility coefficients were calculated as follows:

$$\text{Apparent digestibility (\%)} = [(\text{nutrient intake} - \text{faecal nutrient output}) / \text{nutrient intake}] \times 100$$

Statistical analysis

Statistical analyses were performed using Minitab Statistical Software (Version 16; Minitab LLC, State College, PA, USA). Data from Experiments 1 and 2 were analysed separately using one-way analysis of variance (ANOVA).

For Experiment 1, the five tropical grass cultivars and five *Leucaena leucocephala* cultivars were analysed as two separate forage groups, with cultivar fitted as the fixed effect within each group. For chemical composition, three forage samples per grass cultivar ($n = 3$) and four forage samples per *Leucaena* cultivar ($n = 4$) were used as the experimental observations. For *in vitro* digestibility, two independently collected forage samples per cultivar ($n = 2$) were considered the experimental observations. Filter bags and digestion jars were not treated as additional experimental replicates.

For Experiment 2, dietary treatment was fitted as the fixed effect, and individual goats were considered the experimental units ($n = 4$ per treatment). The assumptions of ANOVA were assessed using the model residuals, including normality and homogeneity of variance, before interpretation of treatment effects. Initial body weight was considered as a potential covariate; however, because goats were randomly allocated to treatments and initial body weight did not differ significantly among treatments ($P = 0.447$), the primary analysis was conducted without covariate adjustment.

When a significant cultivar or treatment effect was detected ($P < 0.05$), means were separated using Tukey's honestly significant difference test. For Experiment 2, orthogonal polynomial contrasts were additionally used to assess linear and quadratic responses to increasing dietary *Leucaena* inclusion (0%, 10%, 20%, and 30% of dietary DM). Statistical significance was declared at $P < 0.05$, while P-values for linear and quadratic responses were reported separately. Data are presented as means $\pm$ standard deviation (SD). For Experiment 1, the standard error of the mean (SEM) values were calculated separately for the grass and *Leucaena* groups and are reported in Table 3 and 4. The SEM is also reported for Experiment 2.

RESULTS AND DISCUSSION

Chemical composition of tropical grass and *Leucaena* cultivars

The chemical composition of the five tropical grass cultivars and five *Leucaena leucocephala* cultivars is presented in Table 3. Among the tropical grasses, OM, CP, EE and ADL concentrations did not differ significantly ($P > 0.05$), whereas NDF and ADF varied markedly among cultivars ($P < 0.05$). Ruzi and Mulato II contained lower NDF (63.3 and 66.3% DM, respectively) and ADF (31.7 and 32.9% DM, respectively) concentrations than the three *Panicum maximum* cultivars (Purple, Mombasa and Mun River), which contained approximately 72% NDF and 41% ADF. These lower fibre concentrations are consistent with a greater potential for fibre degradation and may contribute to higher forage digestibility compared with the *Panicum* cultivars. In contrast, no significant differences were detected among the five *Leucaena* cultivars for any measured chemical component ($P > 0.05$). This suggests that the measured chemical characteristics were relatively consistent among the introduced *Leucaena* cultivars under the conditions of the present study. Although CP concentration ranged numerically from 23.3 to 27.9% DM, all cultivars consistently exhibited high protein concentrations together with substantially lower NDF and ADF concentrations than the grasses. This indicates that the introduced *Leucaena* cultivars showed relatively consistent nutritive characteristics under the environmental conditions of central Vietnam and have considerable potential as protein supplements in forage-based diets.

Table 3 - Chemical composition of five tropical grass cultivars and five *Leucaena leucocephala* cultivars (% DM)

Component	Ruzi	Mulato II	Purple	Mombasa	Mun River	SEM	P
OM	93.35 $\pm$ 0.87	92.78 $\pm$ 0.82	92.62 $\pm$ 0.90	92.60 $\pm$ 1.14	93.13 $\pm$ 1.19	0.57	0.852
CP	7.43 $\pm$ 0.61	8.22 $\pm$ 0.81	8.08 $\pm$ 0.30	8.15 $\pm$ 0.19	8.11 $\pm$ 0.24	0.28	0.350
EE	2.03 $\pm$ 0.06	2.26 $\pm$ 0.12	2.10 $\pm$ 0.20	2.09 $\pm$ 0.32	1.89 $\pm$ 0.38	0.14	0.523
NDF	63.30 $\pm$ 2.36 ^b	66.30 $\pm$ 0.60 ^b	72.70 $\pm$ 0.89 ^a	72.10 $\pm$ 0.76 ^a	72.50 $\pm$ 0.51 ^a	0.71	0.001
ADF	31.70 $\pm$ 1.64 ^b	32.90 $\pm$ 0.63 ^b	41.10 $\pm$ 0.82 ^a	41.29 $\pm$ 0.60 ^a	41.20 $\pm$ 1.06 ^a	0.59	0.001
ADL	7.11 $\pm$ 0.39	8.00 $\pm$ 0.54	7.59 $\pm$ 0.47	7.93 $\pm$ 0.37	7.27 $\pm$ 1.17	0.38	0.430
Component	Tarramba	Peru	Cunningham	KU15	KU56	SEM	P
OM	93.00 $\pm$ 0.58	93.10 $\pm$ 0.36	92.90 $\pm$ 0.68	93.00 $\pm$ 0.25	93.30 $\pm$ 0.61	0.26	0.778
CP	25.40 $\pm$ 3.35	26.90 $\pm$ 4.22	26.40 $\pm$ 3.44	23.30 $\pm$ 4.65	27.90 $\pm$ 1.38	1.79	0.454
EE	7.17 $\pm$ 0.81	6.33 $\pm$ 1.59	6.30 $\pm$ 1.33	6.35 $\pm$ 1.19	6.43 $\pm$ 1.08	0.61	0.836
NDF	33.02 $\pm$ 3.10	33.89 $\pm$ 1.40	33.61 $\pm$ 1.89	35.22 $\pm$ 1.63	36.50 $\pm$ 1.23	1.07	0.198
ADF	19.20 $\pm$ 1.55	19.87 $\pm$ 2.03	18.88 $\pm$ 1.17	20.08 $\pm$ 2.36	19.80 $\pm$ 2.44	0.98	0.898
ADL	8.72 $\pm$ 0.61	9.16 $\pm$ 0.67	9.36 $\pm$ 0.75	9.49 $\pm$ 0.57	9.53 $\pm$ 0.59	0.32	0.410

Note: Values are means $\pm$ SD ($n=3$ for tropical grass cultivars and $n=4$ for *Leucaena leucocephala* cultivars). SD, standard deviation; SEM, standard error of the mean. Means within a row followed by different superscript letters differ significantly according to Tukey's multiple comparison test ($P < 0.05$).

The contrasting chemical composition of grasses and *Leucaena* reflects their complementary roles in tropical ruminant feeding systems. Tropical grasses primarily provide structural carbohydrates required for normal rumen function, whereas *Leucaena* provides a concentrated source of crude protein that can supply nitrogen for microbial protein synthesis and ruminal fermentation. Similar compositional characteristics have been reported previously, with *Leucaena* consistently containing two- to three-fold greater CP concentrations and considerably lower fibre concentrations than tropical grasses (Takawale et al., 2016; Álvarez Ríos et al., 2018; Okoruwa, 2020).

Differences in fibre concentration among the grass cultivars are also consistent with their botanical characteristics. The lower NDF and ADF concentrations observed in Ruzi and Mulato II are consistent with their superior forage quality. Reduced cell wall content generally facilitates microbial degradation in the rumen and is closely associated with improved forage digestibility (Bacorro et al., 2018; Rojas García et al., 2020). These compositional differences therefore provide a biological explanation for the digestibility responses observed in the subsequent *in vitro* evaluation.

In addition to forage nutritive value, biomass productivity is another important criterion for cultivar selection and practical application in smallholder production systems. Under the same environmental conditions, our previous field evaluation showed that the grass cultivars produced approximately 15–24 t DM ha⁻¹ year⁻¹, whereas edible *Leucaena* biomass ranged from 4.0 to 5.3 t DM ha⁻¹ year⁻¹ (Liem et al., 2026). Despite producing less biomass than the tropical grasses, the markedly higher crude protein concentration of *Leucaena* enhances its value as a complementary forage rather than a replacement for grasses. Integrating productive grasses with protein-rich forage legumes has been widely recognised as an effective strategy for improving forage quality and reducing reliance on commercial protein supplements in tropical ruminant production systems (Casanova-Lugo et al., 2014; Cowley and Roschinsky, 2019).

Overall, Ruzi and Mulato II were characterised by lower fibre concentrations than the *Panicum* cultivars, indicating greater potential digestibility, whereas all *Leucaena* cultivars consistently provided high crude protein concentrations suitable for protein supplementation. Together, these compositional characteristics establish the nutritional basis for interpreting the *in vitro* digestibility results presented in the following section.

***In vitro* digestibility of tropical grasses and *Leucaena* cultivars**

The *in vitro* digestibility of the five tropical grass cultivars and five *Leucaena leucocephala* cultivars is presented in Table 4. Among the tropical grasses, cultivar effects were not statistically significant for IVDMD ($P = 0.063$), IVNDFD ($P = 0.052$) or IVADFD ($P = 0.091$), although numerical differences were observed among cultivars. Similarly, none of the digestibility indices differed significantly among the five *Leucaena* cultivars (IVDMD, $P = 0.490$; IVNDFD, $P = 0.141$; IVADFD, $P = 0.231$). Significant differences among grass cultivars were observed for IVDMD, IVNDFD and IVADFD ($P < 0.05$), whereas none of these digestibility indices differed significantly among the *Leucaena* cultivars ($P > 0.05$). The numerical variation in digestibility among the grasses was broadly consistent with differences in fibre composition (Table 3), suggesting that variation in cell wall characteristics may contribute to differences in forage digestibility.

Among the tropical grasses, Ruzi exhibited the numerically highest IVDMD, IVNDFD, whereas Purple had the highest numerical IVADFD. For IVDMD, Ruzi (60.79%) showed the highest value and was significantly higher than Mulato II, Mombasa and Mun River, while Purple had an intermediate value. For IVNDFD, Ruzi (52.39%) was significantly higher than Mombasa (39.06%) and Mun River (42.30%),

Although these differences were not statistically significant, the relatively higher digestibility values observed for Ruzi and Mulato II were consistent with their lower NDF and ADF concentrations. Lower concentrations of structural fibre generally increase the accessibility of plant tissues to rumen microorganisms and are associated with improved forage digestibility (Jung and Allen, 1995). Similar relationships between fibre concentration and digestibility have been reported for tropical *Brachiaria* cultivars (Maranatha et al., 2019; Lemus et al., 2021), supporting the potential feeding value of Ruzi and Mulato II as forage resources for ruminant production.

The five *Leucaena* cultivars showed numerical variation in *in vitro* digestibility, but none of the differences was statistically significant. Peru exhibited the highest IVDMD (56.73%), whereas KU15 had the lowest value (47.33%). Peru also showed the highest numerical IVNDFD (39.16%) and IVADFD (31.87%), while Tarramba had the lowest numerical values for IVNDFD (23.16%) and IVADFD (13.69%). However, the overall effects of cultivar were not significant for IVDMD ($P = 0.490$), IVNDFD ($P = 0.141$) or IVADFD ($P = 0.231$). These numerical differences should therefore not be interpreted as evidence of superior digestibility of any particular *Leucaena* cultivar under the conditions of the present study.

The numerical variation in *Leucaena* digestibility may reflect differences in plant maturity, tissue characteristics or secondary metabolites. One possible contributing factor was the relatively long regrowth interval (four months) used before harvest. As forage legumes mature, increasing cell wall lignification and changes in tissue structure generally reduce microbial accessibility and fibre degradation. Previous studies have likewise reported greater digestibility in younger *Leucaena* harvested at shorter regrowth intervals (Rengsirikul et al., 2011). In addition, secondary metabolites such as mimosine and condensed tannins may modify ruminal fermentation and nutrient utilisation by influencing microbial activity and protein degradation (Barros-Rodríguez et al., 2014; Yanuartono et al., 2019; Lakshmi et al., 2020). However, these compounds were not determined in the present study, and their contribution to the observed differences among cultivars therefore remains speculative.

The feeding value of *Leucaena*, however, should not be assessed solely on the basis of fibre digestibility. All cultivars contained approximately threefold higher crude protein concentrations than the tropical grasses (Table 3), indicating that their principal nutritional contribution is the supply of crude protein and nitrogen for ruminal microbial protein synthesis rather than structural carbohydrates. Supplementation with *Leucaena* can therefore improve rumen nitrogen availability, support microbial protein synthesis and potentially enhance the utilisation of low-protein tropical grasses. This complementary nutritional role has been widely demonstrated in grass-based feeding systems (Takawale et al., 2016; Álvarez Ríos et al., 2018; Okoruwa, 2020).

Although Peru exhibited numerically higher IVDMD, IVNDFD and IVADFD than Tarramba, these differences were not statistically significant. Digestibility alone was therefore not the determining criterion for cultivar selection in the subsequent feeding trial. Under the same environmental conditions in central Vietnam, our previous field evaluation demonstrated that Tarramba combined favourable nutritive value with good adaptation, high forage yield and persistence compared with other introduced cultivars (Liem et al., 2026). These agronomic characteristics are essential for sustainable forage production systems, where reliable biomass supply and field performance are as important as nutritive value. Consequently, Tarramba was selected as the cultivar for evaluating the effects of increasing *Leucaena* inclusion in practical goat feeding systems.

Overall, the present findings demonstrate the complementary nutritional functions of tropical grasses and *Leucaena* in forage-based diets. Ruzi and Mulato II exhibited relatively high fibre digestibility among the grass cultivars, with significant cultivar effects detected for IVDMD, IVNDFD and IVADFD, whereas all *Leucaena* cultivars showed no significant differences in any of the three digestibility indices. Nevertheless, all *Leucaena* cultivars consistently provided high crude protein concentrations despite the variation in their numerical fibre digestibility values. Together, these characteristics provide the nutritional basis the effects of increasing dietary inclusion of Tarramba on nutrient intake and apparent nutrient digestibility in goats under practical feeding conditions.

Table 4 - *In vitro* dry matter and fibre digestibility of five tropical grass cultivars and five *Leucaena leucocephala* cultivars after 48 h incubation using the DAISYII system

Cultivar	IVDMD (%)	IVNDFD (%)	IVADFD (%)
Grasses			
Ruzi	60.79	52.39	51.19
Mulato II	57.97	50.00	50.87
Purple	54.52	48.34	51.53
Mombasa	50.20	39.06	40.27
Mun River	50.99	42.30	44.06
SEM	0.81	1.37	1.45
P-value	0.063	0.052	0.091
<i>Leucaena</i> cultivars			
Tarramba	51.77	23.16	13.69
Peru	56.73	39.16	31.87
Cunningham	51.22	41.67	35.57
KU15	47.33	35.97	19.27
KU56	55.03	35.55	27.98
SEM	1.93	2.55	3.39
P-value	0.490	0.141	0.231

Values are means based on two independent forage samples per cultivar (n=2). IVDMD, *in vitro* dry matter digestibility. IVNDFD, *in vitro* neutral detergent fibre digestibility. IVADFD, *in vitro* acid detergent fibre digestibility. SEM, standard error of the mean.

Effects of increasing *Leucaena leucocephala* leaf inclusion on feed intake and apparent nutrient digestibility in goats

The effects of increasing dietary inclusion of *Leucaena leucocephala* cv. Tarramba leaves on feed intake and apparent nutrient digestibility are presented in Table 5. Increasing dietary inclusion of *Leucaena leucocephala* cv. Tarramba affected several nutrient intake variables, with the most pronounced response observed for crude protein intake, whereas apparent dry matter (DM) and organic matter (OM) digestibility were not affected by dietary treatment. The polynomial contrasts further identified linear or quadratic responses for several nutrient intake and digestibility variables. Dry matter and OM intake differed among dietary treatments (ANOVA; $P < 0.05$), and showed significant quadratic responses to increasing *Leucaena* inclusion (both $P < 0.05$), whereas the linear responses were not significant.

Table 5 - Feed intake and apparent nutrient digestibility of goats fed Mombasa grass-based diets containing increasing levels of *Leucaena leucocephala* leaves

Variables	Leucaena Inclusion levels (% of dietary DM)				SEM	ANOVA P	Linear P	Quadratic P
	0%	10%	20%	30%				
Initial body weight (kg)	12.35 ± 0.50	12.58 ± 0.75	12.65 ± 0.50	12.20 ± 0.43	0.26	0.447		
Feed intake (g/head/day)								
DM	316.7 ± 37.8 ^b	377.6 ± 29.5 ^a	375.2 ± 17.6 ^a	358.9 ± 13.4 ^{ab}	13.2	0.024	0.057	0.013
OM	296.0 ± 35.3 ^b	352.6 ± 27.6 ^a	350.0 ± 16.4 ^a	334.5 ± 12.5 ^{ab}	12.3	0.025	0.063	0.013
CP	30.5 ± 3.67 ^c	42.5 ± 3.4 ^b	48.5 ± 2.3 ^{ab}	52.3 ± 1.9 ^a	1.45	<0.001	<0.001	0.016
NDF	217.9 ± 25.9 ^{ab}	240.4 ± 18.7 ^a	219.1 ± 10.3 ^{ab}	191.0 ± 7.2 ^b	8.59	0.013	0.021	0.012
ADF	122.1 ± 14.5 ^{ab}	135.9 ± 10.5 ^a	125.0 ± 5.9 ^{ab}	110.6 ± 4.0 ^b	4.84	0.023	0.061	0.012
Apparent digestibility (%)								
DM	70.5 ± 6.5	72.8 ± 3.7	72.1 ± 2.9	72.7 ± 2.2	2.09	0.857	0.546	0.692
OM	71.2 ± 6.5	73.4 ± 3.7	72.5 ± 2.8	73.0 ± 2.3	2.07	0.884	0.622	0.692
CP	72.4 ± 6.3 ^b	75.8 ± 3.6 ^{ab}	78.7 ± 2.1 ^{ab}	80.7 ± 1.4 ^a	1.93	0.049	0.007	0.725
NDF	72.8 ± 6.9	72.9 ± 4.4	69.8 ± 3.4	67.5 ± 2.2	2.29	0.336	0.091	0.638
ADF	65.4 ± 7.7	64.7 ± 4.4	59.4 ± 3.8	56.4 ± 4.0	2.62	0.090	0.017	0.658

Note: Values are presented as means ± SD (n=4 per treatment). Different superscript letters within the same row indicate significant differences according to Tukey's multiple comparison test (P < 0.05). Linear and quadratic P-values were obtained using orthogonal polynomial contrasts for the quantitative inclusion levels (0, 10, 20, and 30% of dietary DM). DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; SEM, standard error of the mean.

Goats receiving diets containing 10% and 20% *Leucaena* consumed more DM and OM than those fed the grass-only diet, whereas intake at the 30% inclusion level was intermediate and did not differ significantly from the other treatments. The quadratic pattern indicates that intake increased at moderate inclusion levels and then tended to decline at the highest inclusion level, rather than increasing progressively with *Leucaena* inclusion. This response may reflect changes in the nutritional balance and physical characteristics of the diets following partial replacement of Mombasa grass with *Leucaena*. Comparable increases in feed intake following *Leucaena* supplementation have been reported in tropical small ruminants (Aregheore, 2002; Rusdy et al., 2020). The absence of a significant reduction in DM or OM intake at the 30% inclusion level indicates that feed intake was not adversely affected at the highest inclusion level tested under the conditions of the present study.

CP intake increased markedly with increasing *Leucaena* inclusion, from 30.5 g/head/day in the control diet to 52.3 g/head/day at 30% inclusion, and showed significant linear ($P < 0.001$) and quadratic ($P = 0.016$) responses. This response was consistent with the substantially higher CP concentration of *Leucaena* leaves than Mombasa grass observed in Experiment 1. NDF intake was affected by dietary treatment (ANOVA; $P = 0.013$) and showed both significant linear ($P = 0.021$) and quadratic ($P = 0.012$) responses. ADF intake also differed among treatments (ANOVA; $P = 0.023$) and showed a significant quadratic response ($P = 0.012$). For both NDF and ADF, intake was highest at 10% inclusion and declined at 30% inclusion, consistent with the lower fibre concentration of *Leucaena* relative to Mombasa grass.

Apparent DM and OM digestibility remained relatively high across all treatments (approximately 70–73%) and were not affected by dietary treatment ($P > 0.05$). Neither the linear nor quadratic contrasts were significant. These findings indicate that replacing up to 30% of Mombasa grass with Tarramba leaves did not alter overall DM or OM digestibility.

In contrast, apparent CP digestibility increased significantly from 72.4% in the control diet to 80.7% at 30% *Leucaena* inclusion and was affected by dietary treatment (ANOVA; $P = 0.049$). The significant linear contrast ($P = 0.007$), together with the non-significant quadratic contrast ($P = 0.725$), indicated a linear response in apparent CP digestibility across the inclusion levels. This response is consistent with the greater protein contribution of *Leucaena* leaves and may reflect improved apparent digestion of dietary CP as the proportion of *Leucaena* increased. Similar improvements in protein utilisation have been reported following the incorporation of forage legumes into low-protein tropical grass diets for goats and sheep (Garcia et al., 1996; Takawale et al., 2016; Álvarez Ríos et al., 2018; Okoruwa, 2020).

Although apparent NDF and ADF digestibility showed a numerical decline as *Leucaena* inclusion increased, the treatment ANOVA was not significant ($P > 0.05$). However, the orthogonal polynomial contrasts revealed a significant linear decline for ADF digestibility ($P = 0.017$), whereas NDF digestibility only showed a tendency toward a linear decrease ($P = 0.091$), with no significant quadratic responses ($P = 0.638$ and 0.658 , respectively). Thus, the decline in ADF digestibility was detected as a significant linear response across the inclusion gradient, despite the non-significant overall treatment effect in the ANOVA. NDF digestibility showed a numerical decline, with a non-significant tendency toward a linear response ($P = 0.091$). This pattern may be related to differences in the fibre characteristics of the dietary ingredients observed in Experiment 1. Nevertheless, the absence of a significant effect on DM and OM digestibility indicates that the observed reduction in fibre digestibility did not translate into a measurable reduction in overall organic nutrient digestion.

Overall, increasing Tarramba inclusion modified dietary nutrient intake and several digestibility responses. CP intake and apparent CP digestibility increased linearly with increasing *Leucaena* inclusion, whereas DM and OM intake showed quadratic responses. NDF and ADF intake also changed with increasing *Leucaena* inclusion, accompanied by linear declines in their apparent digestibility. Importantly, DM and OM digestibility remained unaffected across the inclusion levels tested. Under the conditions of the present short-term digestibility trial, inclusion of Tarramba leaves up to 30% of dietary DM did not adversely affect DM intake or apparent DM and OM digestibility.

CONCLUSION

This study demonstrated the complementary nutritional roles of improved tropical grasses and *Leucaena leucocephala* for goat feeding under the conditions of central Vietnam. The evaluated *Leucaena* cultivars contained substantially higher crude protein concentrations, highlighting their potential as protein-rich forage resources for grass-based diets. Although numerical differences in *in vitro* digestibility were observed among cultivars, no statistically significant differences were detected within either the grass or *Leucaena* groups.

In the feeding trial, increasing Tarramba *Leucaena* inclusion increased crude protein intake and apparent crude protein digestibility, with a significant linear response in the latter. Dry matter and organic matter intake showed quadratic responses to increasing Tarramba inclusion, whereas apparent dry matter and organic matter digestibility were not affected by dietary treatment. Apparent acid detergent fibre digestibility showed a significant linear decline, while apparent neutral detergent fibre digestibility showed only a numerical decline without a significant linear or quadratic response.

Overall, the findings provide a basis for considering improved forage cultivars and indicate the potential of Tarramba as a protein-rich, locally available forage resource for grass-based diets in smallholder production systems. Under the conditions of this short-term digestibility trial, inclusion of Tarramba leaves at up to 30% of dietary dry matter did not

adversely affect apparent dry matter or organic matter digestibility. However, the present study does not establish an optimum inclusion level or provide evidence on growth performance, nitrogen utilisation, health responses, or long-term production outcomes. Further studies assessing these responses under longer-term and practical feeding conditions are warranted to determine appropriate inclusion levels of Tarramba in goat diets.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Le Van AN; Email: levanan@hueuni.edu.vn; ORCID: <https://orcid.org/0000-0002-0954-6208>

Data availability

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Author's contribution

T.N.Liem: Conceptualization, Investigation, Data curation, Formal analysis, Writing – original draft. N.M.Dung: Conceptualization, Investigation, Data curation, Writing – original draft. L.M.Duc: Conceptualization, Investigation, Data curation. D.T.Hai: Methodology, Formal analysis. L.V.An: Project administration, Conceptualization, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. All authors read and approved the final manuscript.

Ethical considerations

All animal procedures were reviewed and approved by the Animal Ethics Committee of Hue University, Vietnam (Approval No. HUVN0055). All animal procedures complied with the committee's guidelines for the ethical use of animals in research and were reported in accordance with the ARRIVE guidelines.

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Competing interests

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

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VALORIZATION OF YOUNG PRICKLY PEAR (*Opuntia ficus-indica*) CLADODES AS A SUSTAINABLE LIVESTOCK FEED SUPPLEMENT: PHYSICOCHEMICAL, NUTRITIONAL VALUE AND ANTIOXIDANT ACTIVITY

Samira NAIR¹ , Hamza BELKHODJA² , and Sidi Mohamed BELHADJ³ 

¹Laboratory of Physical Chemistry of Macromolecules and Biological Interfaces, University of Mustapha Stambouli, Mascara, 29000, Algeria

²Laboratory of Bioconversion, Microbiology Engineering and Health Safety, University of Mustapha Stambouli, Mascara, 29000, Algeria

³Department of Biology, University of Mustapha Stambouli, Mascara, 29000, Algeria

✉ Email: samira.nair@univ-mascara.dz

➤ Supporting Information



ABSTRACT: The prickly pear cactus (*Opuntia ficus-indica*) is prevalent in arid and semi-arid environments, known for its drought tolerance and ability to thrive in poor soils. The cladodes, or pads, are particularly relevant as a nutritional supplement for animals due to their high nutrient content and water-retention capacity. This study aims to analyze the nutritional qualities of young *Opuntia ficus-indica* cladodes growing in the Chorfa region of Mascara Province, Algeria for prospective integration into feedstuff. The physicochemical study was based on the determination of moisture, dry matter, sugar, protein, fat, ash, minerals, starch, and fiber content. The phytochemical analysis was performed by total phenolic content determination. It was followed by antioxidant potential evaluation using DPPH free radical scavenging assay and energetic value assessment. The physicochemical study revealed that the cladodes had a high-water content ($93.86 \pm 0.15\%$) and very low dry matter ($6.32 \pm 0.2\%$). The total ash content was $21.9 \pm 0.03\%$, with calcium as the most abundant mineral, followed by potassium and sodium. The cladode had a good amount of dietary fiber ($18.8 \pm 1.1\%$) and presented values of $11.46 \pm 0.2\%$, $11.02 \pm 1.1\%$, and $8.84 \pm 0.3\%$ for carbohydrate, protein, and fat content, respectively. Based on the phytochemical characterization, cladodes were a noteworthy source of antioxidant compounds. The estimated energy values are 1.32 MFU/kg and 1.31 MEFU/kg. The methanolic extract was effective in inhibiting the free radical DPPH• in a concentration-dependent manner with an IC₅₀ value of 524.6 µg/mL. *Opuntia ficus-indica* cladodes were found to be a good source of sugars, proteins, and dietary fiber, making them potentially valuable ruminant feed.

Keywords: Cladodes, Fiber content, *Opuntia ficus-indica*, Physicochemical analysis, Livestock nutrition.

INTRODUCTION

In Algeria, a vast territory rich in largely unexplored flora offers a precious reservoir of active ingredients for various sectors. The prickly pear (*Opuntia ficus-indica* (L.)) is a perfect example of this plant wealth, which is underestimated locally, mainly due to a lack of recognition by the expert community. *Opuntia ficus-indica*, a perennial arborescent cactus native to Mexico, is extensively cultivated in arid and semi-arid regions worldwide. The species is characterized by its flattened, photosynthetic stem segments (cladodes) that function as water-storing organs rich in mucilage, and by its fleshy, edible fruits known as prickly pears. Due to its remarkable drought tolerance and nutritional value, *O. ficus-indica* has become an important species for food, forage, and industrial applications (Martins et al., 2023). In Algeria's arid and semi-arid zones, the availability of forage resources is a major constraint on the development of livestock farming. Faced with climatic variability and the degradation of natural pastures, it is essential to identify and develop local resources adapted to difficult ecological conditions. The prickly pear, a xerophilous plant widely distributed in these regions, offers great potential as alternative forage (Kalli et al., 2023).

One creative and sustainable way to address the lack of forage supplies in these regions is to incorporate prickly pear cladodes into cattle feed (Medjekal et al., 2023). Indeed, this plant is nutrient-dense and extremely heat- and drought-resistant. It can be a significant source of water, fiber, minerals, and some vitamins, which makes it a perfect animal feed supplement, especially during periods when conventional fodder is in short supply (Pastorelli et al., 2022). To assess possible application in animal feed, this study investigates the nutritional qualities of *Opuntia ficus-indica* cladodes grown in Mascara, northwest Algeria.

MATERIALS AND METHODS

Plant material preparation

The plant material consisted of young cladodes of the *Opuntia ficus-indica*. Samples measuring 30–40 cm in length and 15–18 cm in width were collected from the Chorfa region, Sig, Mascara province, Algeria (35° 25' 55" North, 0° 14' 43" West) in January 2024. After harvesting, the cladodes were thoroughly rinsed with running tap water to remove any

adhering impurities, and then blotted dry with absorbent paper to remove residual moisture. The cleaned cladodes were then cut into small sections and either used fresh or homogenized and centrifuged to obtain the fresh supernatant. For the preparation of the dried material, the cladodes were sliced and oven-dried at 40 °C until a constant weight was achieved. The dried samples were ground in an electric grinder, and the resulting powder was passed through a 250 µm sieve to ensure a fine, homogeneous particle size (Figure 1).

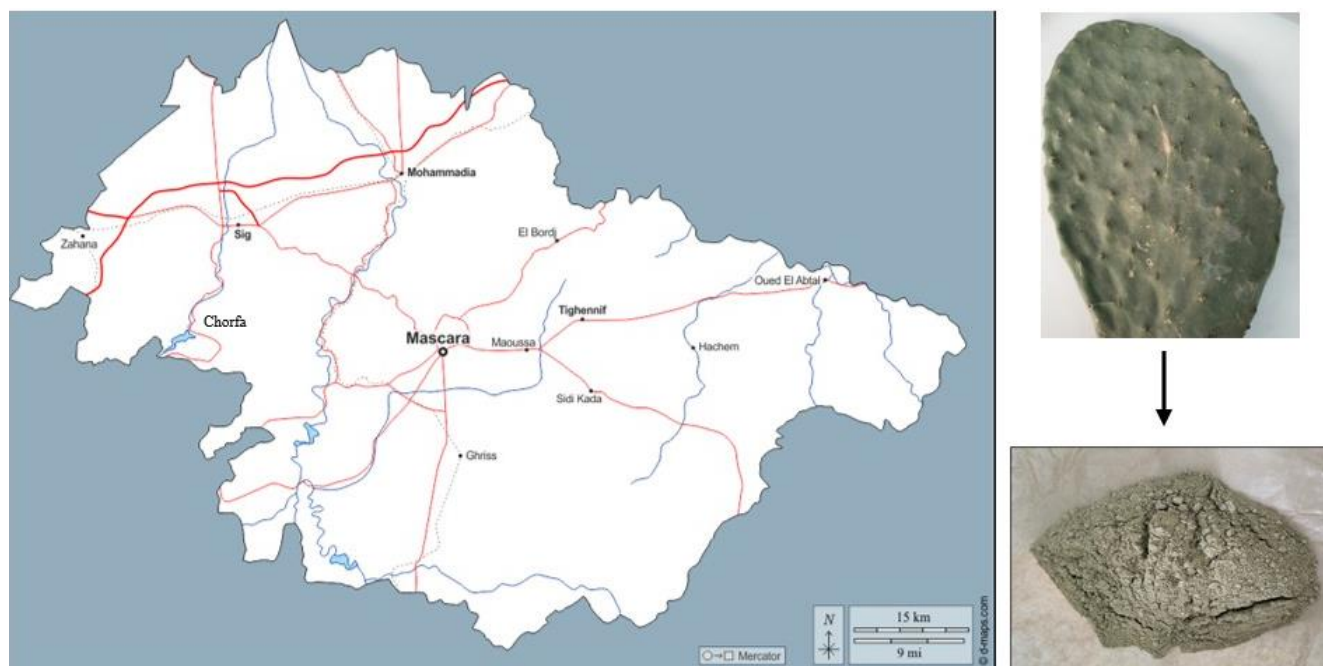


Figure 1 - Location of the study area.

Physicochemical analysis

Several measurements were used to evaluate the physicochemical composition of the cladodes. The following techniques were used to assess moisture, dry matter, ash content, pH, sugar, protein, and lipid content (AOAC, 1995; AOAC, 2002). This was followed by the measurement of fibre, pectin, and chlorophyll content (Yuan et al., 2022). The minerals were quantified by atomic absorption spectrophotometry, and the starch was determined by polarimetry. Every calculation was carried out in triplicate.

Phytochemical screening

Extraction of phenolic substances

The extraction of polyphenols was carried out according to the protocol described by Song et al. (2010), which consists of macerating 10 g of cladode powder in a methanol-water mixture (1.3/8.7: V/V) in a ratio of 1/10 (W/V) for 72 hours, with solvent changes every 24 hours and occasional stirring. The macerates were combined, filtered through filter paper, and the solvent in the filtrate was evaporated under reduced pressure at 40 °C using a rotary evaporator. The resulting dry extract was then weighed and kept in the dark at 4 °C until further analysis.

Determination of total phenolic content

1 ml of the methanolic extract was mixed with 1 ml of Folin-Ciocalteu reagent. After stirring and allowing the mixture to stand for 6 minutes, 8 mL of a 7.5% aqueous Na_2CO_3 solution was added. The mixture was then left to stand for 120 minutes at room temperature and in the dark. Finally, absorbance was measured at 765 nm (Shui and Leong, 2002). A standard curve was prepared with gallic acid, and the total polyphenol content was expressed as mg of gallic acid equivalents (GAE) per gram of extract.

DPPH free radical scavenging assay

Antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (Amzad and Dawood, 2015). 750 µL of the methanolic extract at various concentrations was added to 1.75 mL of DPPH (2 mg DPPH/100 mL methanol). The mixture was incubated in the dark for 30 min. Absorbance was measured at 517 nm against a negative control using a spectrophotometer. Ascorbic acid was used as a positive control in the same concentration range as the extracts. Every calculation was carried out in triplicate. The DPPH free radical scavenging activity was calculated using the formula 1: $\text{DPPH-Inhibition (\%)} = (\text{Abs C} - \text{Abs E}) / \text{Abs C} \times 100$ (1)

Where: Abs C: absorbance of the control (without the sample); Abs E: absorbance of the solution containing the methanolic extract or ascorbic acid.

Estimating energy value

To estimate the energy value of cactus cladodes, we can use Atwater's coefficients, which allow us to determine the energy provided by the following macronutrients: protein, fat, carbohydrates (starch and sugar), and fibre. The Atwater coefficients are as follows: Protein: 4 kcal/g, Fat: 9 kcal/g, Carbohydrates (starch and sugar): 4 kcal/g, Fibre: 2 kcal/g (this coefficient is an approximation because dietary fibre provides little usable energy). The energy provided by each component is calculated based on its percentage. However, we must first convert these percentages to grams for a quantity of 100 grams of dry matter of the cladodes; then, we multiply each value by its respective Atwater coefficient, calculate the energy provided by each component, add all the energy contributions, and calculate the sum to obtain the total energy value. MFU (Milk Feed Units) and MEFU (Meat Feed Units) are units used in animal nutrition to estimate the nutritional value of forages and concentrates for ruminants. They take into account not only the energy value, but also the specific use of this energy for milk (MFU) or meat (MEFU) production (Chibani et al., 2010). To apply the nutritional value prediction equations to a feed, in this case young prickly pear cladodes, we proceeded using the following formulas:

Calculation of the digestibility of organic matter (dOM): $dOM(\%) = -1.14 \times CC + 99.18$

Calculation of the milk feed unit (MFU): $MFU = -0.0018 \times CC + 1.3585$

Calculation of the meat feed unit (MEFU): $MEFU = -0.0021 \times CB + 1.350$

Calculation of metabolizable energy (ME): $ME \text{ (kcal/kg)} = 38.474 \times dOM - 149.4676$

Calculation of the digestible nitrogen (DN) using the total nitrogen content (TN), $DN \text{ (g/kg)} = 8.824 \times TN - 22.43$.

All analyses of energy value were performed in triplicate, and results are expressed as mean $\pm$ standard deviation.

RESULTS AND DISCUSSION

Extraction yield

Drying yield is measured as the percentage of water evaporated relative to the initial weight of the cladodes. The extraction yield of $6.31 \pm 0.2\%$ for *Opuntia ficus-indica* cladodes appears to be higher than those reported by Msaddak (2015) and Ait Maamar and Has (2019), which were 5.20% and 5.7%, respectively. This result is lower than those presented by Khefif and Sedjerari (2023; 7.39%) and by Nair et al. (2024; 6.7%), who worked in the area of Mascara. This percentage may vary, depending on several factors, including the drying method used, environmental conditions, and the variety of the *Opuntia ficus-indica*. In general, the drying process of the cladodes can significantly reduce their water content, making them easier to store and transport while preserving some of their nutritional components. Ayadi et al. (2009) showed that cacti's cladodes are very rich in water; therefore, their drying performance is much lower.

Physicochemical characteristics

Table 1 presents the physicochemical characteristics of *Opuntia ficus-indica* cladodes. The moisture content of the young cladodes of *Opuntia* has been estimated at $93.86 \pm 0.15\%$ of fresh weight, which is very similar to the results reported by Nair et al. (2024; 93.25%). It is superior to that reported by Abdessemed et al. (2014; 91.9%), Khefif and Sedjerari (2023; 91.1%), and to the results reported by Msaddak (2015). The moisture content of the cladodes usually ranges between 88% and 97% (Martins et al., 2023); a large proportion of this can be explained by their role in storing water (Benattia and Arrar, 2018). The results for dry matter content showed a low rate of $6.32 \pm 0.2\%$, indicating that almost the entire weight of cladodes is water (93.86% of fresh weight). From these results, it is observed that the rackets are rich in water. For this reason, this plant is well adapted to hot, dry climates, including desert environments, in arid and semi-arid regions. It is for this reason that the *Opuntia* may be useful in resolving the problem of livestock in these areas. The high-water content in the *Opuntia*'s tissues represents a valuable source of liquid for animals (Lee et al., 2003).

Ash content is a measure of the total mineral content of the cladodes studied. The analyses reveal that the cladodes are mineralized ($21.9 \pm 0.03\%$). These results are superior to those reported by Khefif and Sedjerari (2023) (17.1%) and lower than those reported by Hernández-Urbiola (2011), Toure et al. (2015), Msaddak (2015), and Nair et al. (2024). The variability in these results can be attributed to geographical location, climate, and the physical properties of soils in different matrices (Teklu et al., 2023).

The pH of the young cladodes is 4.46 ± 0.1 , which is slightly lower than Boutakiout (2015) value of 4.7. Khefif and Sedjerari (2023), who worked in the Mascara area, reported that the cladodes in this region have a pH of 5.1. The pH variations are mainly due to differences in climatic conditions and the maturation process (Silva et al., 2023). In addition, acidity increases at night and decreases during the day, varying considerably with time of day (Inglese, 2018). In general, organic acids are mediators of metabolic processes, and the rackets tend to acidify during growth, whereas the fruit acidifies during ripening. Variations in acidity are due to differences in estimation procedures, climatic conditions, and maturation processes (Maiuolo et al., 2024).

Dietary fibres are essential compounds for a balanced diet and contribute to animal digestive health by promoting intestinal transit and regulating digestion. The results also show that the powder of cladodes is a good source of dietary fibre ($18.8 \pm 1.1\%$), a rate in line with 5-20% reported by Inglese et al. (2018). This content is close to that quoted by Moussaoui (2020; 18%) and higher than that reported by Khefif and Sedjerari (2023; 8.5%).

The total content of substances, pectic cladodes of the study area (1.16%), considered as a soluble fibre, is lower than that found by Moussaoui (2020; $1.69 \pm 0.06\%$) and is included in the interval described by Boutakiout (2015) for the Moroccan cladodes (0.1 to 2.25%). The pectin content of the plant under study is higher than that reported by Khefif and Sedjerari (2023; 0.41%). This difference may be due to growth conditions and harvest timing, but it is also related to the assay method.

Table 1 - Physicochemical composition (g/100 g) of young *Opuntia ficus-indica* cladodes.

Parameters	Value
Moisture	93.86 ± 0.15
Total solids	6.32 ± 0.2
Ash	21.9 ± 0.03
Reducing carbohydrate	11.46 ± 0.2
Starch	7.55 ± 0.2
Fats	8.84 ± 0.3
pH	4.46 ± 0.1
Fibers	18.8 ± 1.1
Proteins	11.02 ± 1.1
Chlorophyll a	0.164
Chlorophyll b	0.130
Potassium (K)	2.83
Sodium (Na)	1.29
Calcium (ca)	4.56

Table 2 - Predicted nutritional and energy values of *Opuntia ficus-indica* cladodes.

Nutrient parameter	Value
dOM (%)	77.748 ± 1.5
Crude cellulose (CC)	18.8 ± 1.1
Total Nitrogen	11.02 ± 1.2
MFU	1.32
MEFU	1.31
ME (kcal/kg)	2842.51
DN (g/kg)	948.91

dOM= digestibility of organic matter; MFU= Milk Feed Units; MEFU= Meat Feed Units; ME= metabolizable energy; DN= digestible nitrogen.

Table 2 showed that the content of chlorophyll a (0.164 mg/100 g) is lower than that achieved by Moussaoui (2020; 0.322 ± 0.034 mg/100 g), and by Khefif and Sedjerari (2023; 0.321 mg/100 g). Chlorophyll (b) reached a value of 0.130 mg/100g, which is similar to that obtained by Moussaoui (2020) (0.131 ± 0.069 mg /100 g) and higher compared to those cited by Khefif and Sedjerari (2023; 0.110 mg/100 g). The predominance of chlorophyll a over chlorophyll b in the cladodes of the study area observed in this work has also been reported by Guevara-Figueroa et al. (2010).

The results for the mineral elements of the cladodes in the study area are listed in Table 1. We note the predominance of calcium (4.56%), followed by potassium (2.83%) and sodium (1.29%). Our results are in concordance with those of Moussaoui (2020) who has found the dominance of calcium, with a percentage of ($9.04 \pm 1.21\%$), followed by potassium ($1.10 \pm 0.43\%$) and with those of Khefif and Sedjerari (2023), which showed the predominance of calcium (8.58%), followed by potassium (3.21%) and sodium (2.43%). The exact amount of these mineral elements in the cladodes of *Opuntia ficus-indica* can vary depending on many factors. Still, the cladodes of the young prickly pear are generally regarded as a good source of some of these minerals, especially calcium, potassium and magnesium.

Starch is an important energy source for animals and is widely used in animal feed to provide the calories needed for growth, milk production, and other metabolic functions. The cladodes of the study area had a starch content of $7.55 \pm 0.2\%$ (Table 1). Carbohydrates are a total of 36% to 37% of the total dry weight of cladodes, with an average grade of 85 to 171 mg/ g of dry weight of starch (Ginestra et al., 2009). The starch may contribute to the total energy intake of the

feed, but it must be included in the diet in appropriate amounts and combined with other nutrients to provide a well-balanced diet (Nozière et al., 2026).

According to Table 1, the carbohydrate content in the racks is $11.46 \pm 0.2\%$. This value is lower than those reported by Nair et al. (2024; 23.33%) and by Bouzoubaa et al. (2016), which ranged from 13.5 to 15.87% across different varieties of *Opuntia ficus indica* in Morocco. The carbohydrate content can vary considerably depending on the specific cactus variety, growing conditions, the age of the racks, and other environmental factors (Boutakiout, 2017).

The fat content of the plant studied was $8.84 \pm 0.3\%$ (Table 1). This value is much higher than those reported in the literature by Msaddak (2015; 3.02%), Nair et al. (2024; 0.3%), and Khefif and Sedjerari (2023; 1.3%). However, the thorny racks and the 2-year study by Ayadi (2009) showed higher concentrations in fat, respectively, 3.95% and 4.69%. The highest levels of the cactus in lipids are recorded at the beginning of fruiting. In general, forages have low fat content, and *Opuntia* is no exception. Its content, which is 1.96% for snowshoes aged 1 year, decreases with age; this level also varies across periods (Rodrigues et al., 2023).

Protein is essential for growth, muscle development, reproduction, and general health of the animals (Nozière et al., 2026). The results of the protein are represented in Table 1. We note that the rate of protein cladodes studied, $11.02 \pm 1.1\%$, is higher than that reported by Msaddak (2015; 7.43%) and higher than that reported by Khefif and Sedjerari (2023; 3.15%). This value is in agreement with the results published by El Kharrassi (2015), which range from 4.65% to 14.22% for mature cladodes. A protein level of 11.02% in the racks of cacti can significantly contribute to the overall nutritional intake of animals, especially when combined with other protein sources in their diet.

Phytochemical screening

Total phenolic content

The results showed that the total phenolic content in the cladodes of the fig tree (44.78 mg GAE/g) is much higher than that described by Msaddak (2015; 24.85 mg GAE /g). However, Nair et al. (2024) reported that the total phenolic content of the cladodes of the region of Mascara was 44.17 mg GAE /g.

DPPH free radical scavenging activity

Figure 2 shows that the methanolic extract of *Opuntia ficus-indica* cladodes exhibited a concentration-dependent DPPH• radical-scavenging activity, although it was less potent than ascorbic acid, with IC_{50} values of 524.6 and 295.5 $\mu\text{g/mL}$, respectively. Compared with Nair et al. (2024), who reported a much lower DPPH IC_{50} of 4.816 $\mu\text{g/mL}$ for a methanolic cladode extract from the Mascara region, the present extract showed considerably lower antioxidant potency. Nevertheless, both studies confirm the antioxidant potential of *O. ficus-indica*, while the marked difference in IC_{50} values indicates substantial variability between independent experiments. Other parameters also differed slightly, including ash content (21.9% vs. 22.5%) and total phenolic content (44.78 vs. 44.17 mg GAE/g). The value of the concentration of the inhibitory IC_{50} reported by Messadek (2018) is of the order of 1.45 mg/ml. Previous work reports a positive linear correlation between scavenging activity and total phenol content (Bahloul et al., 2016).

The differences in antioxidant activity may be related to variations in extraction procedures, plant maturity, harvesting and storage conditions, genotype, and analytical methods. Moreover, the similar total phenolic contents but markedly different DPPH responses suggest that total phenolics alone do not determine antioxidant activity; the qualitative composition and interactions of phenolic compounds, flavonoids, betalains, and other antioxidant constituents may also play important roles (Perucini-Avendaño et al., 2021).

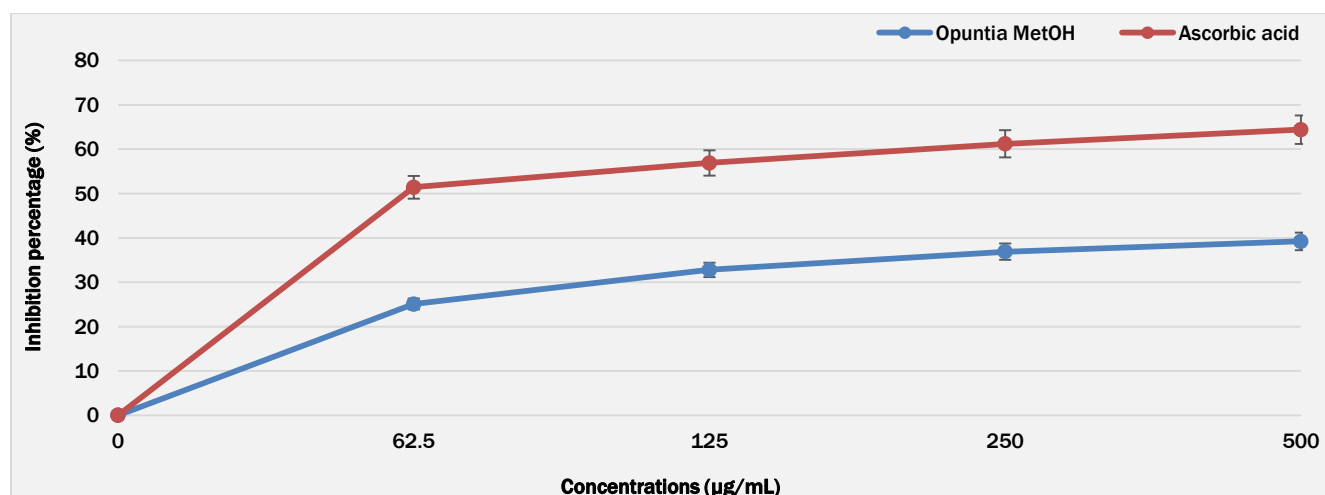


Figure 2 - DPPH free radical scavenging activity (%) of the methanolic extract of *Opuntia ficus-indica* cladodes in comparison with ascorbic acid.

Energy value

The prediction models offer a cost-effective method for estimating the nutritive value of *O. ficus-indica* cladodes. They can play an important role in integrating these food resources into farming systems. The selection of the model of prediction of the nutritive value for the *O. ficus-indica* cladodes was based on extensive work on the chemical composition and digestibility of the organic matter (dOM) of 71 forages, conducted over a period of forty years. This research has enabled the development of mathematical models that are reliable for estimating the energy and nitrogen content of forage, thus facilitating their use in routine processes (Chibani et al., 2010). The models were developed with explanatory variables including crude cellulose (CC), neutral detergent fibre (NDF), acid detergent fibre (ADF), mineral matter (MM), and total nitrogen (TN). The nutritional values were given in Table 2, in which the content of crude cellulose (CC) was 18.8 ± 1.1 g/100 g and the concentration of total nitrogen was 11.02 ± 1.2 g/100 g.

According to Table 2, the prediction models provide a useful estimate of the nutritional value of *O. ficus-indica* cladodes. The digestibility of organic matter is relatively high ($77.74 \pm 1.5\%$). The energy values forage of the *O. ficus indica* was of the order of 1.32 MFU and 1.31 MEFU. These values are higher than the spontaneous early head announced by Bencherchali and Houmani (2017), which are 0.72 in MFU and 0.66 in MEFU, and are superior to the values of the rebus pitted (1.18 in MFU and 1.15 in MEFU). The metabolizable energy (ME) of the plant studied was 2842.51 kcal/kg, and the nitrogen matter digestibility was 948.91 g/kg.

The dOM (%) value of 77.748 ± 1.5 indicated the proportion of the organic (non-mineral) fraction that rumen microbes and the animal can break down and utilize. A value close to 78% is considered good for a succulent forage, showing relatively high digestibility compared to many dry grasses or crop residues common in North Africa. The moderate variability (± 1.5) can arise from factors such as cladode age, variety, soil conditions, or season. Higher dOM generally translates into better energy supply for the animal (Teklu et al., 2023).

Crude cellulose (CC) at 18.8 ± 1.1 represents the cellulose content, a key structural component of plant cell walls (often linked to the acid detergent fibre, ADF, fractions in forage analysis). At approximately 19%, this level provides necessary "bulk" for proper rumen function, supporting microbial activity and preventing digestive disorders. However, excessive fibre can reduce voluntary intake and energy density. Young prickly pear cladodes typically show moderate fibre levels with relatively good digestibility because they contain lower lignin (the indigestible woody component) than mature grasses. Recent studies confirm cellulose values in *Opuntia* cladodes commonly range between 11–20%, aligning well with the table (Pessoa et al., 2022). The total nitrogen content of 11.02 ± 1.2 (most likely in g/kg) is used to estimate crude protein (CP) by multiplying by the factor 6.25. This is a low-to-moderate protein level for forage. Younger cladodes generally contain higher nitrogen/protein than older ones. While sufficient for basic rumen microbial needs (the optimal range is often cited as 6–8%), it is usually too low to fully support high-production animals without supplementation from protein-rich sources such as legume hay, urea, or concentrates. Multiple recent analyses report average CP in *Opuntia* cladodes between 3–10%, with many values clustering around 5–8%, consistent with this table (Rodrigues et al., 2016).

MFU (1.32) and MEFU (1.31) are energy units commonly used in French and North African feeding systems. One MFU approximates the energy provided by 1 kg of standard barley. These values indicate solid energy density for a succulent forage, supporting both lactation and growth/maintenance. The closeness of the two figures suggests balanced utility across production types. The metabolizable energy (ME) of 2842.51 kcal/kg (approximately 11.9 MJ/kg) reflects the energy actually available to the animal after digestive losses (faeces, urine, and methane). This is a moderate-to-good level for cactus forage – comparable to many hays but enhanced by the plant's natural juiciness when fed fresh. It can contribute effectively to maintenance and moderate production when combined with other feeds. Finally, DN at 948.91 g/kg most likely refers to digestible nitrogenous matter or a related measure of the highly utilizable dry matter/nitrogen fraction. The very high value (~95%) reinforces the excellent overall digestibility shown by the dOM figure and indicates that the cladodes are efficiently broken down by rumen microbes (Todaro et al., 2020; Erol et al., 2022).

Overall, the cladodes of prickly pear demonstrate clear strengths in digestibility and energy provision, as well as high moisture content. Their limitations lie mainly in moderate protein and fibre levels. In practice, especially in Algerian livestock systems, they are often fed fresh as a drought-resistant supplement or base feed, combined with higher-protein forages or concentrates to create balanced rations.

CONCLUSION

This study focuses on the valorization of young prickly pear (*O. ficus-indica*) cladodes, often regarded as a by-product, which can be processed into flour rich in bioactive compounds and exhibiting high antioxidant activity. Physicochemical analysis revealed that the cladodes are very rich in water and have a relatively low dry matter. The ash content was high, with calcium being the most abundant mineral, followed by potassium and sodium. Overall, the flour was also found to be a good source of sugars, proteins, and dietary fiber, making it potentially valuable for both ruminant feed. This work contributes to a better understanding of the young prickly pear's characteristics and supports its recovery and sustainable utilization.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Samira Nair; E-mail: samira.nair@univ-mascara.dz; ORCID: <https://orcid.org/0009-0000-6024-3301>

Data availability

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

Authors' contribution

S. Nair: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft.

H. Belkhodja: Formal analysis, Writing - Review & Editing

S. M. Belhadj: Validation, Formal analysis, Writing - Review & Editing

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Competing interests

The authors have not declared any competing interests.

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THE PHYSICAL, NUTRITIONAL, PHYTOCHEMICAL, AND MICROBIOLOGICAL PROPERTIES OF WATER MIMOSA (*Neptunia oleracea* Lour) SILAGE USING DIFFERENT MOISTURE CONTENTS FOR POULTRY FEED

Fitra YOSI¹ , Muhakka MUHAKKA¹ , Nuni GOFAR² , Nurul HASANAH¹ , Alexsandra NABABAN¹ , and Ika LESTARI¹ 

¹Department of Animal Science, Faculty of Agriculture, University of Sriwijaya, Palembang 30662, South Sumatra, Indonesia

²Department of Soil Science, Faculty of Agriculture, University of Sriwijaya, Palembang 30662, South Sumatra, Indonesia

✉Email: fitrayosi@unsri.ac.id

✉Supporting Information



ABSTRACT: The potential of water mimosa (WM; *Neptunia oleracea* Lour) swamp legumes, as a source of green protein, phytochemical compounds, and probiotics in the form of silage juice for waterfowl such as ducks is quite large, but to date it has not been further investigated. This study aimed to evaluate the physical, nutritional, phytochemical, and microbiological characteristics of WM silage fermented at different moisture levels. This study used a completely randomized design consisting of 5 treatments and 3 replicates. The treatments consisted of control group (fresh WM legumes), WM40, WM50, WM60, and WM70: WM silage with moisture levels of 40%, 50%, 60%, and 70%, respectively. The results of the analysis of variance showed that differences in silage moisture content had a significant effect on color, aroma, and texture scores ($P < 0.05$). Qualitative tests showed that WM silage fermented at moisture levels of 40-70% contained several phytochemical/antibacterial compounds, including flavonoids, steroids, saponins, phenols, and carotenoids. Differences in WM silage moisture content significantly affected dry matter, organic matter, crude protein, crude fiber, carbohydrates, and Fleigh point ($P < 0.05$). Total acid, total bacteria count, and lactic acid bacteria count in WM silage fermented at different moisture contents increased compared to the control group ($P < 0.05$) but showed similar results between moisture levels of 40 and 70%. In conclusion, the making of WM silage with a moisture content of up to 50% provided optimal yields in terms of the physical, microbiological, nutritional, and phytochemical properties of WM silage.

Keywords: Microbiological characteristics, Moisture content, *Neptunia oleracea* Lour, Nutritional content, Phytochemical compounds, Silage.

INTRODUCTION

Feed constitutes the most significant factor in supporting the productivity of poultry. Fishmeal and soybean meal are the primary sources of protein and are extensively utilized in poultry feed formulations (Ravindran, 2013). However, the procurement of these ingredients is often challenging due to their necessity for importation, which introduces variability in availability and sustainability. Consequently, there is a compelling need to identify alternative, readily available, and cost-effective protein sources that maintain adequate nutritional quality.

South Sumatra, Indonesia, is renowned for its extensive swampland potential and a substantial local feed supply for livestock. *Neptunia oleracea* Lour, more commonly referred to as water mimosa (WM), is a species of legume that is native to swampy environments. It has the potential to serve as a source of protein for livestock, with a crude protein content estimated to be approximately 20.5% (Muhakka et al., 2020). However, to date, the utilization of WM legume has been predominantly focused on ruminants, which are provided with fresh WM legume containing a high proportion of crude fiber (Sumantri et al., 2023; Van Chen et al., 2026). In contrast to ruminants, which are capable of digesting complex carbohydrates, poultry are monogastric animals with limited capabilities for digesting crude fiber (Singh and Kim, 2021). Consequently, in order to utilize WM legumes in poultry feed, it is necessary to employ feed processing techniques to reduce the crude fiber content. One such technique involves the production of juice. The objective of juicing is to obtain nutrients from WM legume in a form that is more easily digestible and absorbable by poultry. In recent years, research on the utilization of forage juice for monogastric livestock has witnessed a surge of interest, particularly in the context of swine, as evidenced by the study conducted by Keto et al. (2021). In contrast, the scope of research in poultry remains considerably limited, underscoring the necessity for further investigation to ascertain its full potential, especially for waterfowl species.

To enhance the benefits of utilizing this WM juice, it is essential to process the WM legume into silage. In addition to reducing crude fiber content, increasing nutritional value, and enhancing feed digestibility, the silage process has the capacity to stimulate microbial growth (Kung et al., 2018). This includes lactic acid bacteria and organic acids, such as acetic, propionic, and butyric acids. Notably, the fermentation process leads to the production of lactic acid. In accordance with the findings of our prior in vitro investigations, lactic acid bacteria isolated from swamp grass silage are classified as *Lactobacillus* sp. and possess the capacity to function as probiotics and acidifiers (Sandi et al., 2018; 2020).

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The ensiling process is significantly influenced by the initial moisture content of the raw material, as moisture content plays a pivotal role in determining microbial activity, anaerobic stability, and the nutritional quality of the silage produced (Zheng et al., 2011). Legumes, by their very nature, contain a high moisture content, which must be taken into consideration during the fermentation process to ensure optimal results. An inadequate moisture content in silage production (below 40%) results in dry silage texture, while a moisture content of more than 70% significantly alters the ensilage process and the resulting phases, leading to undesirable clostridial fermentation instead of the beneficial lactic acid fermentation (Kung et al., 2018). Consequently, further research is necessary to assess the physical, nutritional, phytochemical, and microbial characteristics of WM silage fermented at varying moisture levels.

MATERIALS AND METHODS

Experimental design

This study used a completely randomized design with five treatment groups, each repeated three times. The treatment groups were as follows: a control group consisting of fresh WM legume without ensilage; and WM40, WM50, WM60, and WM70, which were groups of WM silage with moisture contents of 40%, 50%, 60%, and 70%, respectively.

Determination of moisture content of WM legume

The moisture content was determined by analyzing the water content of the WM legume to calculate the dry matter content. This data was then used to determine the amount of water to add to the silage according to the treatment. The procedure for determining the moisture content of the WM legume was as follows: First, a porcelain dish was dried in an oven at 105°C for 1 hour. Then, it was placed in a desiccator for 30 min and weighed. Next, 2 g of the sample was weighed and placed in a dish with a known weight. The sample was dried in an oven at 105°C for 24 hours. Afterward, the dish containing the sample was cooled in a desiccator for 30 min and weighed until a constant weight was achieved. Weighing was carried out 3-4 times to ensure consistent results. The moisture content of the WM legume was calculated according to AOAC (2005).

Determination of WM silage moisture content

The determination of silage moisture content is based on Putri (2024). First, the dry matter content of the sample was determined, as well as the amount of dry matter in the sample (g). Then, the moisture content of the sample was calculated using the following formula: Moisture content of WM (%) = 100% - dry matter content of WM (%)

The next step was to calculate the percentage of water added, using the formula:

Percentage of water added (%) = target moisture content in WM silage (%) - moisture content of WM (%)

To determine the amount of water added (mL), the following formula was used:

Amount of water added (mL) = amount of WM dry matter (g) x percentage of water added (%).

The making process of WM silage

The process of making silage referred to our previous research (Sandi et al., 2018; Yosi et al., 2025) with some modifications. Briefly, the WM leaves were harvested fresh, cleaned of debris, separated from the stems, and air-dried for 24 hours. Then, 7.5 kg of the wilted leaves were placed into 15 double-layered one-kilogram plastic bags (each bag containing 500 g) and mixed with predetermined amounts of distilled water according to the treatment group. Each bag received an addition of molasses amounting to 3% of the dry weight. The mixture was homogenized until thoroughly mixed and then compacted to create anaerobic conditions. The bags were vacuum-sealed, labeled, and stored at room temperature for 21 days.

Determination of the physical quality of WM silage

Physical quality was tested by a panel of 10 moderately trained individuals. The silage assessment used an ordinal scale with standard silage assessment criteria referring to Al Biruni et al. (2024). The standard silage assessment criteria for color are as follows: greenish-yellow = 25, brownish-yellow = 10, and blackish, close to compost color = 0. The odor assessment is as follows: fermented sour smell = 25, sour smell not too strong = 20, no smell in silage = 10, and mold growth and compost causing an unpleasant smell = 0. The assessment based on cleanliness was as follows: no mold = 25, mold and slime = 0. The assessment based on texture was as follows: dry, soft to the touch, crumbly, fermented aroma = 25, moist but not wet = 20, and watery with high water content = 0.

Determination of the nutritional quality of WM silage

The moisture content of WM silage was determined using the oven method (AOAC, 2005). The crude protein and crude lipid content of WM silage were analyzed using the Kjeldahl and Soxhlet methods, respectively (AOAC, 2005). Then, the crude fiber content was measured using the Weende method. The ash content was measured by placing the WM

silage sample in a muffle furnace at 550 °C for 6 h. To measure nitrogen-free extract (NFE), the following formula was used: % NFE = % DM - (% EE + % CP + % ash + % CF)

where: NFE = nitrogen free extract, DM = dry matter, EE = ether extract or crude lipid, CP = crude protein, and CF = crude fiber

The fleigh point (Fp) was calculated according to [Ozturk et al. \(2006\)](#) using the following equation:

$Fp = 220 + [2 \times DM\% - 15] - [40 \times pH]$, where % DM denotes the dry matter content of the silage.

Silage quality is characterized as follows: very good (>85), good (60-85), moderate (55-60), satisfactory (20-55), and poor/worthless (<20).

Determination of phytochemical compounds in WM silage

Qualitative testing of phytochemical compounds refers to the research conducted by [Ramli et al. \(2020\)](#). Alkaloid compounds were analyzed using the Dragendroff test. A total of 100 mg of the sample was dissolved in 10 mL of solvent. The sample was filtered, and then 2 mL of the filtrate was added to 1 mL of Dragendroff reagent. The formation of a red precipitate indicated the presence of alkaloids ([Tiwari et al., 2011](#)). Flavonoid compounds were analyzed using the alkaline reagent test. A total of 100 mg of the sample was dissolved in 10 mL of solvent. The sample was filtered, and several drops of NaOH solution were added to the filtrate (2 mL). If a yellow color formed and faded after the addition of acid, the sample tested positive for flavonoids ([Tiwari et al., 2011](#)). For the saponin compound test, 1 g of sample extract was placed in a test tube, then distilled water was added until the entire sample was submerged. The mixture was boiled for 2-3 min, cooled, and shaken vigorously. A positive result is indicated by the formation of stable foam. For the steroid test, 1 mL of the extract is dissolved in 10 mL of chloroform, and an equal volume of sulfuric acid is added. A positive result is indicated by a red upper layer and a yellow sulfuric acid layer with green fluorescence ([Trease and Evans, 1983](#)). The carotenoid test used the Liberman-Burchard reagent. A total of 2 mL of n-hexane extract solution was added to the reagent and H₂SO₄. The sample showed a positive result when a brown ring formed. To test for phenolic compounds, 2 mL of the sample was placed in a test tube, to which 3 drops of 1% FeCl₃ were added. A positive result is indicated by the appearance of a blue or blackish-green color.

Determination of the microbial parameters of WM silage

A total of 5 g of the sample was weighed and dissolved in 50 mL of distilled water. Then, the pH was measured using a calibrated pH meter (pH meter EZ-9908, China). Total acid content was analyzed by titration according to [AOAC \(2005\)](#). The total bacteria and lactic acid bacteria (LAB) were calculated using the total plate count (TPC) method ([AOAC, 2005](#)).

Statistical analysis

The SPSS statistical software package (IBM SPSS, version 26) was used to analyze all experimental data. A one-way analysis of variance (ANOVA) was performed on the nutritional and microbial data of WM silage. Duncan's multiple range test was used to determine the differences between the treatment groups. The physical properties of the WM silage, including color, odor, cleanliness, and texture, were tested using the Kruskal–Wallis test. If a significant effect was found, the analysis continued with Mann–Whitney testing. A statistical difference was defined as $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect on the physical properties of WM silage

According to the ANOVA results, differences in the moisture content of WM silage significantly affected color, aroma, and texture scores ($P < 0.05$, Table 1), but did not affect cleanliness or pH scores. WM silage with 50% and 60% moisture content had higher color scores than WM silage with 60% and 70% moisture content ($P < 0.05$).

Table 1 - Differences in pH, color, odor, cleanliness, and texture score of WM silage with different moisture contents.

Variable	WM40	WM50	WM60	WM70	SEM	P-value
Color	25.0 ^a	25.0 ^a	23.5 ^b	22.4 ^b	0.30	0.004
Odor	24.5 ^a	24.9 ^a	23.4 ^b	21.5 ^c	0.18	<0.001
Texture	24.9 ^a	24.5 ^a	21.4 ^b	19.5 ^c	0.24	<0.001
Cleanliness	25	25	25	25	0	1.0
pH	4.3	4.2	4.3	4.3	0.03	0.16

WM40, WM50, WM60, and WM70: WM silage groups with moisture content levels of 40%, 50%, 60%, and 70%, respectively. ^{a,b,c} Means within a column with different superscripts differ significantly ($P < 0.05$). SEM, standard error of the mean. The standard silage criteria for color are as follows: greenish-yellow = 25, brownish-yellow = 10, and blackish, close to compost color = 0. For odor: fermented sour smell = 25, sour smell not too strong = 20, no smell in silage = 10, and mold growth and compost causing an unpleasant smell = 0. For texture: dry, soft to the touch, crumbly, fermented aroma = 25, moist but not wet = 20, and watery with high water content = 0. For cleanliness: no mold = 25, mold and slime = 0.

A good silage color is a bright light green, yellow, or brownish green. These colors are normal and desirable for well-fermented silage. The color change from green to brownish green is caused by the formation of the pheophytin pigment during anaerobic preservation. This color indicates high dry matter content and optimal fermentation, producing the desired lactic acid (Hidayat, 2014). Similar results were observed in the aroma and texture scores of WM silage at moisture contents between 40 and 50%, but these scores gradually decreased at 60% and 70% moisture content ($P < 0.05$). A good odor in silage is characterized by a distinctive sour smell, pleasant aroma, and slightly sweet taste. This odor indicates successful lactic acid fermentation and minimal activity of spoilage bacteria (Lendrawati et al., 2012). Good silage texture is moist but not dry or slimy. This texture indicates that the raw material was effectively compacted during ensiling, which removes trapped oxygen and creates an anaerobic environment necessary for lactic acid fermentation (Lendrawati et al., 2012).

Effect on the nutritional value of WM silage

The ANOVA showed that differences in moisture content significantly affected the dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), carbohydrates (CH) and Fleigh point (FP) ($P < 0.05$, Table 2). However, it did not affect the crude lipid content or the NFE of WM silage. Further testing indicated a significant increase in the DM content of WM silage fermented with a water content ranging from 40 to 70%, compared to the control ($P < 0.05$). The DM content of WM silage with a moisture content of 40% was higher than that of other moisture contents (50%-70%). The optimal DM content in silage ranges from 35% to 40% (equivalent to a moisture content of 60% to 65%) (Ohmono et al., 2002). This range is ideal because it enables effective compaction, minimizes oxygen entry, and promotes the desired lactic acid fermentation. The OM content of WM silage with various moisture levels (40%-70%) was higher than the unfermented control ($P < 0.05$; Table 2). However, the OM content of WM silage with moisture contents ranging from 40 to 70% yielded similar results. Favorable OM in silage is characterized by high retention of original biomass and low ash content (Kung et al., 2018). The CF content of WM silage with moisture content of 50% or higher was lower than the 40% moisture treatment and the control group ($P < 0.05$; Table 2). Generally, the main anaerobic fermentation process in silos does not significantly alter the total CF concentration. This is because the primary structural carbohydrates (cellulose, hemicellulose, and lignin) are relatively resistant to degradation by the dominant lactic acid bacteria (Ulger and Kaplan, 2017). CH content significantly increased in the control treatment up to WM silage with 40% and 50% moisture content, but stable values were observed above 50% to 70% ($P < 0.05$; Table 2). CP content increased in silage fermented with 40%-70% moisture content compared to the control group ($P < 0.05$). WM silage fermented with a moisture content of 40% or 50% had a higher FP than WM silage fermented with a moisture content of 60% or 70% ($P < 0.05$; Table 2). This indicates that the ensilage process with a moisture content of up to 50% produces the highest quality silage. Higher Fleigh points indicate better silage fermentation quality (Wang et al., 2022).

Table 2 - Differences in nutritional value and Fleigh point of WM silage with different moisture contents

Variable	Control	WM40	WM50	WM60	WM70	SEM	P-value
Dry matter (%)	23.95 ^c	33.54 ^a	29.06 ^b	28.48 ^b	28.05 ^b	1.45	0.001
Organic matter (%)	97.71 ^b	98.21 ^a	98.14 ^a	98.18 ^a	98.18 ^a	0.10	0.002
Crude lipid (%)	1.22	1.31	1.35	1.55	1.40	0.13	0.227
Crude protein (%)	10.38 ^b	11.28 ^{ab}	13.24 ^a	10.31 ^b	11.64 ^{ab}	0.87	0.040
Carbohydrates (%)	7.70 ^c	19.59 ^a	14.58 ^b	14.80 ^b	13.19 ^b	1.80	0.001
Crude fiber (%)	12.20 ^{ab}	13.20 ^a	7.81 ^b	8.14 ^b	7.20 ^b	1.77	0.019
Nitrogen free extract (%)	5.98	6.37	6.76	6.65	5.98	1.44	0.968
Fleigh point	-	101.4 ^a	93.78 ^{ab}	88.63 ^b	87.76 ^b	3.91	0.029

Control group (fresh WM legume), WM40, WM50, WM60, and WM70: WM silage groups with moisture content levels of 40%, 50%, 60%, and 70%, respectively. ^{a,b,c,d}: Means within a column with different superscripts differ significantly ($P < 0.05$). SEM, standard error of the mean

Effect on the phytochemical compounds in WM silage

A qualitative analysis of WM silage demonstrated that silage fermented at a moisture content ranging from 40 to 70% contained several phytochemical and antibacterial compounds, including flavonoids, steroids, saponins, phenols, and carotenoids. However, it did not contain alkaloid compounds (Table 3). Flavonoids inhibit the growth of unwanted microorganisms, such as fungi (Kiczorowski et al., 2022). Their antioxidant properties help reduce the loss of important nutrients, such as beta-carotene and tocopherol (vitamin E), during storage. Previous studies have shown that the ensilage process affected steroid compounds through microbial degradation and transformation (Bolsen et al., 1996). It was found that the loss of saponins ranged from 12% to 100%, depending on the type of saponin and the ensiling conditions (Kholif, 2023). Phenolic compounds, which are often antinutrients, such as tannins, may decrease in concentration during anaerobic fermentation (Jeyakumar and Lawrence, 2022). Carotenoids in forage are an important source of provitamin A (beta-carotene), a strong antioxidant that boosts the immune system, thereby improving the health and performance of livestock (Miao et al., 2024).

Effect on the microbial count in WM silage

The ANOVA results showed that the total acid content, total bacterial count, and lactic acid bacteria in WM silage fermented with different moisture contents increased compared to the WM group without ensiling ($P < 0.05$; Table 4), but similar results were observed among the groups with 40% to 70% moisture content. The ensiling process increases the total acidity of feed materials by breaking down water-soluble carbohydrates into organic acids through the action of microorganisms, primarily lactic acid bacteria (LAB) (Abedi and Hashemi, 2020). Once anaerobic conditions are achieved in a tightly sealed silo, LAB begins to multiply exponentially. These bacteria ferment carbohydrates into lactic acid, rapidly lowering the pH to 3.8–4.5 (Zhang et al., 2023).

Table 3 - Differences in phytochemical compounds of WM silage with different moisture contents.

Variable	WM40	WM50	WM60	WM70
Alkaloids	Negative (-)	Negative (-)	Negative (-)	Negative (-)
Flavonoids	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Steroids	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Saponins	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Phenols	Positive (+)	Positive (+)	Positive (+)	Positive (+)
Carotenoids	Positive (+)	Positive (+)	Positive (+)	Positive (+)

WM40, WM50, WM60, and WM70: WM silage groups with moisture content levels of 40%, 50%, 60%, and 70%, respectively. ^{a,b,c,d}: Means within a column with different superscripts differ significantly ($P < 0.05$). SEM, standard error of the mean.

Table 4 - Differences in total acids, total bacteria count, and lactic acid bacteria count of WM silage with different moisture contents.

Variable	Control	WM40	WM50	WM60	WM70	SEM	P-value
Total acids (%)	1.29 ^b	2.86 ^a	2.24 ^a	2.03 ^{ab}	2.40 ^a	0.36	0.018
Total bacteria count (log ₁₀ CFU/g)	4.99 ^b	7.86 ^a	7.92 ^a	8.03 ^a	7.97 ^a	0.31	<0.001
Lactic acid bacteria count (log ₁₀ CFU/g)	2.17 ^b	6.98 ^a	6.73 ^a	6.79 ^a	6.61 ^a	0.33	<0.001

control group (fresh WM legume), WM40, WM50, WM60, and WM70: WM silage groups with moisture content levels of 40%, 50%, 60%, and 70%, respectively. ^{a,b,c}: Means within a column with different superscripts differ significantly ($P < 0.05$). SEM, standard error of the mean

CONCLUSION

In conclusion, irrespective of the moisture content level, the ensiling process improved the physical, phytochemical, and nutritional properties of WM compared to the control, fresh WM legumes that were not processed into silage. Furthermore, using 50% moisture content in the ensiling process yielded the optimal results in terms of the physical, microbiological, nutritional, and phytochemical characteristics of the WM silage.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Fitra Yosi.; E-mail: fitrayosi@unsri.ac.id

Data availability

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

Authors' contribution

Conceptualization, F. Yosi; Methodology, F. Yosi and N. Gofar; Investigation, F. Yosi, Muhakka, N. Gofar, N. Hasanah, A. Nababan, I. Lestari; formal analysis, validation, F. Yosi, Muhakka; writing-original draft preparation, F. Yosi; Writing-review and editing, Muhakka, N. Gofar, N. Hasanah, A. Nababan, I. Lestari; Supervision, F.Yosi

Consent to publish

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

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Ethical considerations

All related to ethical issues, consisting of plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by the authors.

Competing interests

All authors involved in this research work declare having no competing interests.

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NILE TILAPIA (*Oreochromis niloticus*) SEED PRODUCTION AND GROWTH PERFORMANCE OF FRY AND GROWER BROODSTOCKS IN NORTHWEST ETHIOPIA

Yibeletal AYNALEM , Alayu YALEW , and Gashaw TILAHUN 

Department of Fisheries and Aquatic Sciences, School of Fisheries and Wildlife, College of Agriculture and Environmental Sciences, Bahir Dar University, Bahir Dar P.O. Box 79, Ethiopia

Email: yibeletalaynaem3@gmail.com

Supporting Information



ABSTRACT: Small-scale Nile Tilapia (*Oreochromis niloticus*) fry production is essential for improving seed availability and supporting sustainable aquaculture. This study evaluated the biological performance and economic feasibility of Nile tilapia fry production in earthen ponds using a locally formulated diet under small-scale farming conditions in Ethiopia. Thirty-three broodstock fish (11 males and 22 females) collected from the Geray Reservoir region were stocked in a 100 m² earthen pond at a male-to-female ratio of 1:2 and fed a locally formulated diet containing 35% crude protein at 3% of body weight per day. The diet consisted of fish meal (38.1%), soybean meal (38.1%), and wheat bran (24.0%), offered twice daily. Male brood stocks showed significantly greater growth performance than females ($P < 0.001$). Each spawning cycle produced approximately 3,300 fry, and broodstocks spawned an average of eight times annually, yielding about 1,200 fry per female and an estimated annual production of 26,400 fingerlings. Fry reared in the best-performing on-farm pond (researcher) achieved significantly greater final weight, final length, and daily growth rate than those reared at the other sites (farmers) ($P < 0.001$), while survival remained consistently high (98.75–100%) with no significant differences among sites. In water quality, no significant differences among sites for temperature, dissolved oxygen, pH, total dissolved solids and ammonia ($P > 0.05$), although electrical conductivity differed significantly ($P < 0.001$). Total feed consumption was 251.16 kg, corresponding to a total feed cost of US\$105.94. The selling price was US\$0.079 per fingerling. The production system generated positive economic returns. These findings demonstrate that earthen-pond-based Nile tilapia fry production using locally formulated feeds is a technically feasible and economically viable approach for increasing the availability of quality fingerlings. The technology has strong potential for dissemination through Farmers Training Centers to support sustainable aquaculture development, fisheries enhancement, and improved rural livelihoods in Ethiopia.

Keywords: Economic feasibility, Ethiopia, Formulated feed, Fry production, Growth performance, *Oreochromis niloticus*

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the most important freshwater fish species in global aquaculture owing to its rapid growth, adaptability to diverse environmental conditions, efficient feed utilization, high consumer acceptance, and excellent reproductive capacity. It is the second most widely farmed aquatic species after carps and is cultured in more than 135 countries, accounting for over 70% of global tilapia production (El-Sayed, 2020; FAO, 2023; Fitzsimmons and Feldman, 2025). Despite being the native home of Nile tilapia, Africa contributes only a small proportion of global aquaculture production, indicating substantial opportunities to improve fish production through enhanced seed availability and better hatchery management. The reproductive characteristics of Nile tilapia make the species particularly suitable for hatchery production. Female Nile tilapias are maternal mouth brooders that incubate fertilized eggs and larvae in the buccal cavity until the fry are sufficiently developed to survive independently (Mansour, 2001; El-Sayed and Fitzsimmons, 2023). Under favorable environmental conditions, females can spawn approximately every 30 days, enabling continuous fry production throughout the year. Nevertheless, realizing this biological potential depends on effective brood stock management, including the selection of healthy broodstocks, balanced nutrition, appropriate stocking density, and suitable male-to-female ratios (El-Sayed, 2006).

Despite the high reproductive capacity of Nile tilapia, the availability of quality fingerlings remains one of the major constraints limiting aquaculture expansion in many African countries. Poor broodstocks management, inadequate hatchery infrastructure, inconsistent seed quality, and low fry survival continue to reduce hatchery productivity (Bhujel, 2011; Hasimuna et al., 2019). Consequently, many fish farmers still rely on fry collected from natural water bodies, a practice that is unreliable, threatens genetic quality, poses biosecurity risks, and is environmentally unsustainable (Bondad-Reantaso, 2007). Strengthening small-scale hatchery production is therefore essential for ensuring a reliable and sustainable supply of quality seed. Feed is another major factor influencing aquaculture productivity and profitability. Feed

commonly accounts for more than half of total production costs, while commercial diets remain expensive and inaccessible to many small-scale farmers (Hasimuna et al., 2019; Das and Govindasamy, 2021). Consequently, locally formulated feeds prepared from readily available ingredients have attracted increasing interest as cost-effective alternatives capable of supporting satisfactory growth and reproductive performance. Providing nutritionally balanced diets with adequate protein and energy is fundamental for improving broodstocks condition, fry survival, and fingerling growth (Spinelli 2014; Engdaw and Geremew, 2024). Across sub-Saharan Africa, earthen ponds remain the dominant aquaculture production system because they require relatively low investment and are well suited to smallholder farming systems (Satia, 2017, MafwilaKinkelaet al., 2019; Kwikiriza et al., 2026). Semi-intensive pond culture offers an effective balance between affordability and productivity by providing better environmental management, improved feeding efficiency, and higher fry survival than extensive systems (Bhujel, 2011; Li and Yakupitiyage, 2023).

Ethiopia has abundant freshwater resources, including lakes, reservoirs, rivers, and dams, providing considerable potential for aquaculture development. Nevertheless, the country's aquaculture sector remains underdeveloped compared with neighboring East African countries because of limited hatchery capacity, inadequate availability of quality fingerlings, high feed costs, and shortages of technical expertise (MOLF, 2018; FAO, 2023). Although government hatchery centers have been established in several regions, current seed production remains insufficient to satisfy the increasing demand from fish farmers. Regional production records further demonstrate the limited capacity of existing hatcheries to supply quality tilapia seed across the country (Table 1).

Table 1 - Regional Nile tilapia seed production in Ethiopia during three consecutive production years (2020/21–2022/23).

Year	Amhara	Oromia	Sidama	SNNP	Sebeta	Sub-total
2020/21	380,000	76,550	49,840	36,000	1,029,150	1,571,540
2021/22	383,262	41,082	53,900	60,000	1,016,850	1,555,094
2022/23	406,225	72,300	69,000	68,000	1,049,800	1,665,325
Annual total	1,169,487	189,932	172,740	164,000	3,095,800	4,791,959

Source: (FAMP, 2024). SNNP= Southern Nations, Nationalities, and Peoples

Improving seed quality remains a major constraint to aquaculture development in Africa. Despite increasing seed production, the availability of genetically improved broodstock and high-quality fingerlings remains limited due to poor broodstocks management, genetic deterioration, inadequate hatchery practices, and weak quality-control systems (Brummett, 2007; Adewumi, 2015; Kajungiro et al., 2019). In Ethiopia, aquaculture still depends largely on fry and fingerlings from natural water bodies, which often have unknown genetic backgrounds and variable growth performance. The use of mixed-sex fingerlings further reduces productivity through uncontrolled reproduction, feed competition, and overcrowding. Reliable hatchery technologies are therefore essential for producing healthy, genetically sound fingerlings and supporting both aquaculture development and fisheries enhancement. However, limited information is available on the biological performance and economic feasibility of small-scale Nile tilapia seed production using locally formulated feeds under Ethiopian conditions. This study evaluated the effects of locally formulated diets on Nile tilapia broodstocks and fingerling performance, assessed fry production in earthen ponds, and examined the economic feasibility of small-scale seed production systems. The findings aim to support affordable hatchery development, improve quality seed availability, and promote sustainable aquaculture growth in Ethiopia.

MATERIALS AND METHODS

Study Area and Experimental Design

The study area was found at JabiTehnan Districts in the West and North Gojjam Zone of the Amhara Region, Northwest Ethiopia. A map of the study area is presented (Figure 1). West Gojjam zone has a total population of 2,789,106 people, of whom 1,400,635 are men and 1,388,471 women. Source (CSA, 2008). This Zone has a total surface area of 13,311.94 square kilometers (Figure 1).

Experimental design

Figure 1 showing the main research activities and experimental procedures carried out in the study. The experiment was conducted in BakeleKebele, a rural area within the FinoteSelam Town Administration, located in the JabiTehnan District of northwestern Ethiopia. The area is characterized by a moderately warm climate, with a mean annual temperature of approximately 20 °C. The experimental pond was manually excavated and disinfected with calcium oxide (CaO) to provide a safe culture environment and maintain water quality. Following a 15-days stabilization period after CaO application, the pond was prepared for the experiment. Soybean grains were soaked in water for 5–7 h at a water temperature of 25 °C before grinding into soybean milk. One kilogram of soybeans was mixed with 15–20 kg of water. After the soybean milk was prepared, it was sprayed onto the pond water to maintain stable water quality and promote

good fish survival and growth. Feed processing and formulation were completed before the bloodstocks were stocked in the experimental pond.

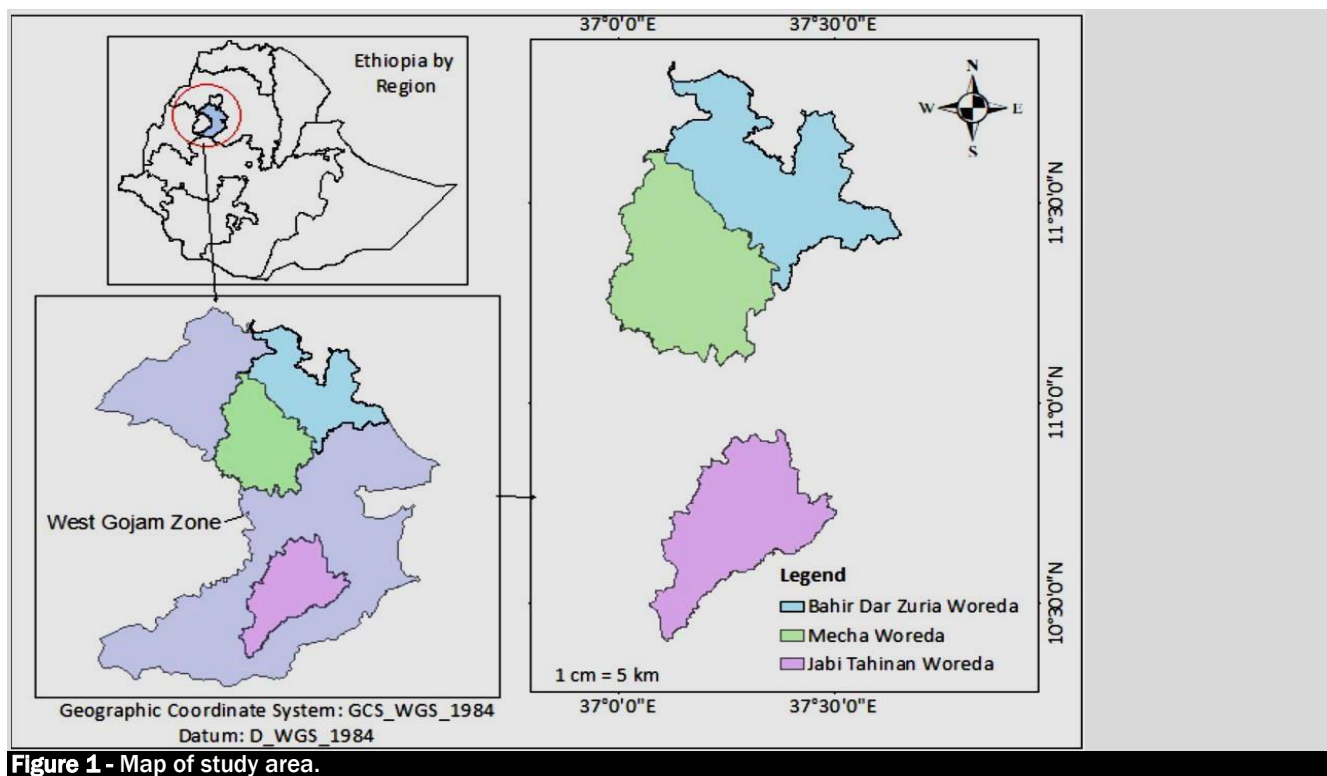


Figure 1 - Map of study area.

Brood stocks collection and management

Permission to collect Nile tilapia broodstock was obtained from the Geray Reservoir Fishers' Cooperative. Healthy broodstock were captured by experienced local fishers from Geray Reservoir, located approximately 3 km from the experimental site. Sex identification was performed by examining the genital papilla and secondary sexual characteristics following (Zaijie and Jianjun, 2016). Males were identified by a single urogenital opening, longer, dorsal and anal fins extending toward the middle of the caudal fin, and generally larger body size than females of similar age (Basiao, 1981). Although manual sex identification may result in an error rate of 3–10% (Hickling, 1963), it remains the standard procedure for broodstocks selection. A total of 33 healthy broodstocks, comprising 11 males (216–300g) and 22 females (166–254 g), were selected based on body weight, total length, and overall health condition. Fish were stocked in the earthen pond at a male-to-female ratio of 1:2, which is widely recommended for efficient spawning and fry production.

Feed formulation and feeding management

A locally formulated diet containing approximately 35% crude protein was prepared using fish meal, soybean meal, and wheat bran (Table 2). Fish meal was produced from fish-processing by products collected from Geray Reservoir, whereas soybean meal and wheat bran were purchased from local agro-processing factories. The proximate composition of individual ingredients was determined according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2019). Diet formulation was performed using the Pearson square method to satisfy the nutritional requirements of Nile tilapia broodstocks and fingerlings. Feed ingredients were finely ground, thoroughly mixed, lightly moistened with water, and pellet using a meat grinder. Pellets measuring 3–4 mm were prepared for broodstocks, whereas pellets of 1.0–1.5 mm were produced for fry during the first month and 1.5–2.0 mm thereafter. Pellets were shade-dried and stored in a cool, dry place until use. Broodstocks and fingerlings were fed daily at 3% of their live body weight for 90 days. The daily feed ration was divided into two meals, with 60% provided at 8:30 AM and 40% provided between 9:30 and 10:30 PM. Feed allowances were adjusted every two weeks according to changes in fish biomass.

Table 2 - Ingredient composition and estimated crude protein contribution of the on-farm formulated feed

Feed Ingredients	Inclusion level (%)	Contribution to crude protein (%)
Fish meal	38.1	22.3
Soybean meal	38.1	10.8
Wheat bran	24	1.9
Total	100	35

Fish sampling of growth and water quality measurements

Fish were sampled at two-week intervals using a scoop net. Handling time was minimized to reduce stress. Individual body weight was measured to the nearest 0.1 g using a digital balance, while total length was recorded to the nearest 0.1 cm using a measuring board. After measurement, all fish were immediately returned to the culture pond. Water quality was monitored weekly throughout the experimental period. Temperature, dissolved oxygen, pH, electrical conductivity, and total dissolved solids were measured in both the morning and afternoon using a portable multi-parameter water-quality meter. Total ammonia concentration was determined separately using standard analytical procedures to ensure that water quality remained within the acceptable range for Nile tilapia culture.

Quality brood stocks and fingerlings remain major constraints to aquaculture development in Africa because of poor broodstock management, genetic deterioration, inconsistent hatchery practices, and inadequate quality-control systems (Brummett, 2007; Adewumi, 2015; Kajungiro et al., 2019). In Ethiopia, fish farming still depends largely on fry collected from natural water bodies, which often have unknown genetic backgrounds and variable growth performance. Furthermore, mixed-sex fingerlings frequently result in uncontrolled reproduction and reduced production efficiency. This study was therefore designed to evaluate the biological performance and economic feasibility of small-scale Nile tilapia fry production in earthen ponds using a locally formulated feed under Ethiopian farming conditions.

Growth parameters

Fish growth performance was evaluated using weight gain (WG), daily growth rate (DGR) specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR) were calculated as standard equations (Adebayo et al., 2004). Growth parameters were calculated by the following formula:

Weight Gain (WG) is the difference between the final body weight and the initial body weight of fish over a period of time expressed in percentage.

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Daily weight gain (g/fish/day)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{culture period (days)}}$$

$$\text{Survival rate (\%)} = \frac{\text{Number of fish harvested}}{\text{Number of fish stocked}} \times 100$$

Specific growth rate: It refers to the instantaneous change in fish weight expressed as the percentage increase in body weight per day over any given time interval. It was determined by taking natural logarithms of body weight and expressing growth as % per day⁻¹ (Ricker, 1979; Márquez et al., 2024)

$$\text{SRG \%} = \frac{(\ln w_f - \ln w_i) \times 100}{\text{Number of day (t)}}$$

Feed conversion ratio (FCR)

The feed conversion ratio is defined as the ratio of dry feed fed per unit live weight gain and serves as a measure of the diet's efficiency. FCR will be calculated by the following formula (Hepher, 1988; Williams, 2009).

$$\text{FCR} = \frac{\text{Total feed fed for fish}}{\text{Live weight gain by fish}}$$

The main problem here is that FCR is usually given in wet weights of both food and weight gain. Some foods, such as plants and or natural food, contain much more moisture than others, such as grains or dry pellets. This may cause bias to be unnecessarily related to the nutrient content (Shoko et al., 2016; FAO, 2024).

$$\text{Survival rate (SR, \%)} = \left(\frac{\text{Number of live fish}}{\text{Number of hatched fish}} \right) \times 100$$

Protein efficiency ratio

The protein efficiency ratio is defined as the ratio between the weight gain of fish and the amount of protein fed (De Silva and Anders, 1995; Webster and Lim, 2002).

$$\text{Protein efficiency Ratio (PER)} = \frac{\text{Weight gain of fish (g)}}{\text{Total crude protein intake (g)}}$$

Data analysis

The data were first subjected to a test of homogeneity and normality of variance, followed by a one-way analysis of variance (ANOVA) to evaluate differences in growth response and survival rates stocked with experimental fish feed formulated with different diets. To determine the pair wise comparison between formulated diets, the Tukey-HSD post-hoc test ($P < 0.05$) was used. The mean value of each water quality parameter was taken and compared between seasons. All statistical analyses were conducted using Microsoft Excel and IBM SPSS Statistics. Results are presented as mean $\pm$ standard error (SE).

RESULTS AND DISCUSSION

Fry production, feed management, and growth performance

Small-scale Nile tilapia (*Oreochromis niloticus*) fry production was successfully carried out in a 100 m² earthen pond. During a single 90-day spawning cycle, 22 female and 11 male broodstocks produced approximately 3,300 fries. The fries were subsequently reared to the fingerling stage over an additional 90 days, reaching a mean final body weight of 39.13 ± 0.02 g and a mean total length of 14.6 cm (Table 3). A total of 50 model farmers participated in the fry production activities and received practical training in pond-based seed production. Feed pellet size was adjusted according to the developmental stage of the fish. Pellets measuring 1–2 mm in diameter were used during the early fry stage, followed by 2–3 mm pellets during the fingerling stage and 3–4 mm pellets for brood stocks. The ingredient composition, inclusion levels, and protein contribution of the on-farm formulated diets are presented in Table 2. The growth performance of brood stocks and fingerlings is summarized in Tables 3 and 4, respectively. Male brood stocks showed greater final body weight and higher daily growth rates than female brood stocks throughout the experimental period (Table 3). Fingerlings reared under the on-farm feeding regime revealed consistent growth, attaining a mean final body weight of 39.13 ± 0.02 g and a mean total length of 14.6 after 90 days of culture (Table 4). Survival and seed production were successfully maintained throughout the study using the on-farm formulated diets under earthen pond conditions.

The production potential and estimated annual revenue of Nile tilapia fry under earthen pond conditions using a male-to-female broodstocks ratio of 1:2. A total of 33 broodstocks, consisting of 11 males and 22 females, were organized into 11 breeding groups. On average, each female broodstock produced 150 fry per spawning, and with eight spawning cycles per year, the system generated approximately 1,200 fry per group. This resulted in a total annual production of 26,400 fry/fingerlings. Assuming a market price of US\$0.079 per fingerling, the estimated annual revenue US\$2085.9, indicating the strong economic potential of Nile tilapia seed production under the studied management conditions (Table5)

Table 3 - Growth performance of male and female grower brood stock of Nile tilapia (*Oreochromis niloticus*) during the experimental period.

Parameters	Males	Females	P-value
Mean initial weight (g)	215.55 ^a	166.41 ^b	0.001
Mean final weight (g)	413.00 ^a	257.05 ^b	0.001
Mean initial length (cm)	16.00 ^a	15.20 ^a	0.001
Mean final length (cm)	30.00 ^a	24.60 ^b	0.001
Body weight gain (g)	271.90 ^a	90.64 ^b	0.001
Daily growth rate	1.65 ^a	0.76 ^b	0.001
Specific growth rate	4.41	3.76 ^b	0.001
Feed conversion ratio	0.14 ^a	0.80 ^b	0.001
Survival rate %	100	100	1.000

Table 4 - Growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared under on-farm conditions mean ±SE

Parameters	Site1	Site2	Site3	P-value
Mean initial length (cm)	0.02±0.00	0.02±0.00	0.02±0.00	1.000
Mean final length (cm)	14.60±0.0 ^a	8.17±0.01 ^b	8.17±0.01 ^b	0.0001
Stocking density (fish m ⁻²)	8	8	17	
Mean initial weight (g)	0.02±0.0	0.02±0.0	1.12±0.0	1.000
Mean final weight (g)	39.13±0.05 ^a	27.01±0.03 ^b	27.01±0.03 ^b	0.0001
Body weight gain(g)	39.11 ^a	26.99 ^b	26.99 ^b	0.001
Daily growth rates	0.43 ^a	0.30 ^b	0.30 ^b	0.001
Specific growth rates (%)	1.20 ^a	0.80 ^b	0.80 ^b	0.001
Survival rates (%)	98.75	99.25	100	

Site 1 and Site 2 = Farmer's pond 1 and pond 2; and Site 3 = Research pond

Table 5 - Nile tilapia fry production potential and estimated annual revenue under earthen pond conditions

MFSR	NG	NoFBS	TLBS	MFPB	SP/Y	TFP	TLFPY	PPF US\$	TR (US\$)
1:2	11	22	33	150	8	1,200	26400	0.079	2085.6

MFSR= Male and Female sex ratio, NG = Number of groups, NO FBS = Number of females brood stocks, TLBS =Total brood stocks, MFPB = Mean fry production per brood stock, SP Y=Spawning per year, TFP = Total fry/fingerlings production, TLFPY = Total fry production per year, PPF =Price per fingerling in US\$, and TR = Total revenue in fry selling per year.

During the experimental period, water temperature ranged from 20.8–27.4 °C, and the optimum temperature for best hatching and survival rates ranged from 25–32 °C. The decrease in water temperature below 22 °C in subtropical areas can lead to a delay or decrease in seed production, as had been reported for Nile tilapia in Vietnam (Dan and Little, 2000), and Egypt (Nasr-Allah et al., 2014). The water quality parameters were aligned with international standards and suitable for the growth of fish (Table 6). The temperature remained almost the same across all sites (around 24 °C), showing that the ponds were exposed to similar environmental and climatic conditions. This similarity suggests that temperature was not a major factor causing differences in water quality among the sites. The dissolved oxygen (DO) level was slightly lower in S3 (5.88 mg/L) compared with S1 and S2 (6.18 mg/L). This small reduction may be explained by the higher fish stocking density and the use of supplementary feed in S3. As more feed enters the pond, more organic waste is produced, encouraging microbial activity and decomposition processes that consume oxygen from the water. However, the DO level in S3 was still within a suitable range for Nile tilapia growth. The pH of S3 (8.82) was slightly lower than that of S1 and S2 (9.12). This change may be related to the greater biological activity in S3. Higher fish numbers and additional feed respiration, organic matter breakdown, and nitrification processes, which release carbon dioxide and hydrogen ions into the water, causing a slight reduction in pH.

The highest total dissolved solids (TDS: 185.5 mg/L and electrical conductivity (EC: 349.5 µS/cm) were recorded in S3, suggesting a greater accumulation of dissolved substances in the pond water. This was likely due to the combined effects of higher stocking density and formulated feed, which increased the input of nutrients, minerals, and metabolic wastes into the aquatic environment. Ammonia concentration was also slightly higher in S3 (0.16 mg/L) compared with S1 and S2 (0.12 mg/L). This increase is expected because a greater number of fish produce more metabolic waste, and uneaten feed contributes additional organic material that breaks down into ammonia. Although ammonia levels increased, they remained within an acceptable range for Nile tilapia culture, indicating that the pond environment was still suitable for fish survival. Overall, S3 represented a more productive but more demanding culture environment. The higher stocking density and feeding strategy improved nutrient availability for fish growth but also increased the pressure on water quality. In contrast, S1 and S2 maintained more stable water conditions because they received lower nutrient inputs and had a lighter biological load. These results highlight the importance of careful management, especially in intensive systems like S3, to maintain a healthy balance between fish production and water quality. Water temperature ranged from 20.8 to 27.4 °C throughout the study. Dissolved oxygen varied between 5.88 and 6.18 mg L⁻¹, while ammonia remained below 0.16 mg L⁻¹ (Table 6).

Table 6 - Physicochemical characteristics of hatchery water during fry production at different sites (mean ± SE)

Sites	TO	Do	pH	TDS	EC	NH ₃	P-value
S1	24.13±1.94 ^a	6.18±0.87 ^a	9.12±0.18 ^a	135.5±46.48 ^{ab}	257.67±65.24 ^b	0.12±0.00 ^a	0.001
S2	24.10±1.89 ^a	6.18±0.87 ^a	9.12±0.18 ^a	135.50±46.48 ^{ab}	257.67±65.24 ^b	0.12±0.00 ^a	0.001
S3	24.11±1.91 ^a	5.88±0.87 ^a	8.82±0.18 ^a	185.5±46.48 ^{ab}	349.50±66.68 ^c	0.16±0.00 ^a	0.001

TO: Temperature (°C); DO: Dissolved oxygen (mg/L); pH: hydrogen ion (H); TDS: Total dissolved solid(mg/L); EC: Electric conductivity (µS) and NH₃ (Ammonia); S1= Site 1, S2= Site 2 and S3= Site 3. Different superscript letters show significant difference.

DISCUSSION

The locally formulated diet used in this study, comprising of fish meal, soybean meal, and wheat bran, supported satisfactory brood stock performance, fry production, and fingerling growth under earthen pond conditions. Fish meal likely contributed highly digestible protein and essential amino acids required for reproduction and tissue growth, while soybean meal provided an accessible plant-based protein source that reduced dependence on expensive commercial ingredients (Hekmatpouret al., 2021; Nakamori, 2022). Wheat bran contributed energy and improved feed texture. It is an

important energy-contributing ingredient through its residual starch and digestible carbohydrates while also improving the physical characteristics of the diet, including pellet integrity and feed texture, thereby enhancing feed handling and stability (Sztupecki et al., 2023; Staset al., 2024).

These results demonstrate that locally available ingredients can be effectively combined to formulate nutritionally suitable diets for small-scale Nile tilapia seed production. Feed cost remains one of the greatest constraints limiting aquaculture development in developing countries. The use of locally formulated feeds in the present study reduced dependence on commercial diets while maintaining acceptable growth performance. However, economic evaluation should consider all production costs, including ingredient preparation, labor, and management inputs. The positive production output obtained under the present system indicates that on-farm feed formulation has potential to improve the economic feasibility of decentralized hatchery operations.

The reproductive performance observed in this study confirms the suitability of Nile tilapia for small-scale seed production under earthen pond conditions. The production of approximately 3,300 fry per spawning cycle from 22 female broodstock demonstrates the potential of pond-based hatchery systems to increase local seed availability. Such systems may reduce dependence on wild-caught fry, which often have uncertain genetic backgrounds and inconsistent production performance. Fingerling growth performance observed in the present study was comparable with previous studies conducted under semi-intensive Nile tilapia production systems (Yimer and Tigabu, 2016; Oumer et al., 2016; Mohammed and Tessem, 2017). The growth response may be attributed to the adequate dietary protein level, appropriate feeding management, and suitable pond environmental conditions maintained throughout the culture period.

Male broodstocks showed significantly higher growth performance than females, which agrees with previous observations in Nile tilapia. This difference is likely related to sex-specific energy allocation, where females invest considerable metabolic energy into egg production and mouth brooding, whereas males can allocate greater portion of available energy toward somatic growth (Engdaw and Geremew, 2024). Furthermore, endocrine regulation, feeding behavior, nutrient utilization efficiency, and reproductive physiology contribute to the enhanced growth efficiency observed in male broodstocks (Wonand Borski, 2013).

The success of production of fingerlings using locally available feed resources provides an important opportunity to demonstrate a considerable potential of low-cost, on-farm hatchery technologies to strengthen aquaculture development in Ethiopia. Limited hatchery capacity, inadequate access to quality broodstocks, and high feed costs remain major barriers to expansion of small-scale fish farming. The production approach evaluated in this study offers a practical alternative for improving seed availability through affordable pond-based hatchery systems (Yimer and Tadesse, 2022) reported that the efficient utilization of locally available feed resources and improved feed formulation are among the most effective approaches for enhancing Nile tilapia growth performance and reducing production costs in Ethiopian aquaculture. Nevertheless, further research is required to evaluate long-term broodstocks reproductive performance, improve feed formulations using locally available ingredients, assess genetic quality of produced seed, and validate the economic performance of this technology under different farming conditions. Such studies will support wider application of sustainable small-scale Nile tilapia seed production systems.

This study demonstrated the feasibility of small-scale Nile tilapia (*Oreochromis niloticus*) seed production using earthen ponds and locally formulated feeds under Ethiopian farming conditions. The application of an on-farm formulated diet significantly improved fish growth performance compared with extensive production conditions, indicating the importance of appropriate feed management in improving productivity within smallholder aquaculture systems (Onomu and Okuthe, 2024; Irabor et al., 2026). The locally prepared diet based on fish meal, soybean meal, and wheat bran provided adequate nutritional support for broodstocks growth, reproduction, and fingerling development. The successful production of fry and the growth performance of both broodstocks and fingerlings indicate that affordable, locally available feed resources can support decentralized seed production without complete dependence on commercial feeds. The study further demonstrated that a small earthen pond system can contribute to improving the availability of quality Nile tilapia seed which is in agreement with El-Sayed et al. (2023), Echessa, (2024) and Wasso et al. (2024). The production of approximately 3,300 fry per spawning cycle from managed broodstocks suggests considerable potential for increasing fingerling supply at the farmer level and reducing dependence on wild-collected seed, which often has uncertain quality and productivity. Male broodstocks showed superior growth performance compared with females, while fingerlings achieved estimated growth under the formulated feeding regime which is in consistence with (Furuya et al., 2023; Engdaw and Geremew, 2024; Sayouhet al., 2024) These findings confirm that appropriate broodstocks management combined with locally formulated feeds can enhance biological performance and production efficiency in small-scale hatchery systems. Overall, the integration of improved broodstocks management, locally available feed ingredients, and farmer-oriented fry production technologies provides a practical pathway for sustainable Nile tilapia aquaculture development in Ethiopia. Promotion of such low-cost seed production approaches through extension services and farmer training platforms could improve access to quality fingerlings, strengthen rural aquaculture enterprises, and contribute to food security and livelihood improvement. Further studies should evaluate long-term brood stocks reproductive performance, genetic quality of produced seed, and economic performance under different farming environments.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Yibelet Aynalem: E-mail: yibeletaynale3@gmail.com: ORCID: [0000-0002-8919-4731](https://orcid.org/0000-0002-8919-4731)

Data availability

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

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Author's contribution

Y. Aynalem: Conceptualization, methodology design, experimentation, data collection and analysis, manuscript drafting and editing.

A. Yalew: Assist with the concept and design of the experimental setup, follow-up, and supervision, providing critical comments on the manuscript, reviewing the manuscript, and editing

G. Tilahun: Conceptualization, supervision, visualization, original draft preparation, critical review, and editing

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Ethical approval

This study strictly address to established ethical guidelines for animal welfare, ensuring the humane handling of Nile tilapia throughout the research. All experimental procedures were carried out with utmost care to minimize stress on the fish. Data collection, analysis, and reporting were conducted with full transparency, and complete datasets are available to ensure verification and reproducibility. The experimental protocol was reviewed and approved by the Institutional Review Board (IRB) of Bahir Dar University, Bahir Dar, Ethiopia, under protocol number 02/IRB/22.

Competing interests

The authors declare no competing interest in this research and publication

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HUSBANDRY AND NUTRITIONAL MANAGEMENT OF HOBBY AND BACKYARD POULTRY FLOCKS: GENOTYPE, NATURAL FEED RESOURCES, HEALTH, AND PERFORMANCE

Ercan MEVLİYAĞULLARI¹✉, Fatma YENİLMEZ², and Arda YILDIRIM³

¹Mevli Pet Food and Animal Nutrition Ltd., Mahfesiğmaz No: 1/1B, Çukurova, 01170, Adana, Türkiye

²Çukurova University, Department of Animal Science, Division of Animal Breeding and Husbandry, Adana, Türkiye

³Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Animal Science, Tokat, Türkiye

✉Email: emevliya@gmail.com



ABSTRACT: Hobby and backyard poultry keeping encompasses systems ranging from a few urban laying hens kept for companionship to low-input rural flocks that generate income and provide animal-source protein. This review examines how flock purpose, genotype, life stage, feed resources, housing, and health considerations inform husbandry and feeding decisions in these diverse systems. Egg production, companionship, and the use of household scraps predominate in urban hobby flocks, whereas income, household nutrition, and low-cost production are central to rural systems. Indigenous breeds are important for adaptation to low-input conditions and genetic resource conservation, although small, dispersed breeding populations are vulnerable to inbreeding and genetic loss. The amount and nutritional value of scavenged feed and separately supplied household scraps vary with season, region, genotype, and flock size; these resources do not guarantee a balanced diet. Balanced feeding is particularly important during chick rearing, while adequate energy, amino acids, minerals, and clean water are required during lay. Evidence on feeding behavior, gut microbiota, stress, biosecurity, and soil lead exposure indicates that nutritional management extends beyond ration composition. Geographic and methodological differences among studies limit generalization. For practical flock management, the review emphasizes defining flock purpose, selecting a suitable genotype, assessing local feed resources, providing life-stage-specific supplementation, ensuring secure housing and feed access, and managing health and environmental risks. Future studies should address genotype-specific nutrient requirements, seasonal feed resources, and the effects of feeding and management interventions. Existing evidence does not establish a universal feed formula for all hobby and backyard poultry.

Keywords: Animal welfare, Backyard poultry keeping, Hobby poultry, Indigenous breeds, Scavenging feed resource, Supplementary feeding.

INTRODUCTION

Small household flocks serve several purposes, including egg production, use of household scraps, gardening, education, and companionship (Elkhoraibi et al., 2014; Buddle et al., 2025). Fortunel (2024) examined the social and spatial circumstances of amateur chicken keeping in France, where the position of chickens between food-producing and companion animals is changing. These differing purposes affect how keepers judge the value of a flock and make management decisions.

For clarity, this review uses three working categories: hobby systems are small, non-commercial flocks kept mainly for personal interest or household use; rural backyard systems are small household flocks managed with limited external inputs and linked to household food or income; and commercial systems are market-oriented flocks managed primarily for meat or egg production. These categories are descriptive rather than rigid, because their boundaries may overlap.

In rural and resource-poor settings, backyard poultry commonly contributes to household income and access to eggs and meat with relatively few purchased inputs (Kumar et al., 2021; Singh et al., 2022). Reviews from India also discuss its contribution to rural employment and household nutrition (Lal et al., 2021; Rajkumar et al., 2021).

These settings require different husbandry and feeding approaches. A few urban hens kept for eggs and companionship may differ substantially from a rural flock that depends on scavenging and generates household income, particularly in genotype, purchased inputs, and access to veterinary services (Özdemir, 2020; Kumar et al., 2021; Singh et al., 2022). Field surveys also need careful interpretation. Kitchen scraps were commonly offered alongside commercial feed in the US survey by Elkhoraibi et al. (2014) and in the eight-household study in Seattle and Portland by Blecha and Leitner (2014). Neither study established that scraps could replace a balanced ration.

This narrative review examines how flock purpose, genotype, life stage, feed resources, housing, health, and welfare inform husbandry and feeding decisions in hobby and backyard poultry. The evidence concerns mainly domestic chickens and includes surveys, qualitative studies, experimental work, and earlier reviews. Findings from commercial flocks are considered where they address relevant biological or management questions. The aim is to identify practical implications and research needs while distinguishing reported practices from demonstrated effects.

REVIEW

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EFFECTS OF FLOCK-KEEPING OBJECTIVES ON MANAGEMENT AND FEEDING DECISIONS

Flock purpose influences genotype choice, feeding expenditure, and the outcomes that keepers consider important (Kumar et al., 2021; Singh et al., 2022). In the US survey, 95.2% of respondents kept chickens for household food production, with gardening and companionship also common motivations (Elkhoraibi et al., 2014). Australian interview participants valued eggs, the use of food scraps, and companionship, while meat production was not a reported benefit (Buddle et al., 2025). Most of the 18 participants interviewed in central North Carolina kept small flocks of several breeds for eggs and companionship. Temperament, appearance, and eggshell color also influenced breed selection (Faulkner et al., 2026).

Respondents in Türkiye reported keeping poultry as pets and enjoying their behavior as well as producing food (Özdemir, 2020). Attachment to birds does not necessarily lead to veterinary consultation: Australian participants described reluctance to seek veterinary advice for sick chickens (Buddle et al., 2025). In North Carolina, keepers often managed health problems at home using online advice and reported limited access to veterinarians experienced in poultry medicine (Faulkner et al., 2026). Husbandry plans should therefore address body condition, chronic disease, and access to treatment alongside egg production.

In rural subsistence systems, supplementary feeding must be considered in relation to purchasing power, local availability, and the expected economic return. Low-input production still requires balanced supplementation and preventive health measures: malnutrition, disease, predation, limited biosecurity, and restricted access to animal-health support remain recurring constraints (Kumar et al., 2021; Singh et al., 2022).

Performance criteria should reflect the purpose of the flock, the resources available, and the health and welfare of the birds. Financial returns alone cannot capture the objectives of every hobby flock.

GENOTYPE SELECTION, BREEDING-STOCK MANAGEMENT, AND GENETIC RESOURCE CONSERVATION

Genotype choice should reflect production goals, available feed resources, and management capacity, because improved germplasm can realize its potential only when nutrition, housing, health care, and local ecological conditions are considered together (Kumar et al., 2021; Singh et al., 2022). Growth rate, egg production, body size, resilience to environmental conditions, and foraging ability can alter the productive response to the same feed resource. Rajkumar et al. (2021) discussed two approaches in India: within-breed selection that preserves the characteristic traits of indigenous breeds and improved lines containing indigenous genetic material. Greater productive potential must be weighed against dependence on externally sourced breeding stock, segregation in subsequent generations, and genetic erosion.

Interviews with Finnish stakeholders identified non-commercial hobby keeping as one option for conserving local livestock breeds, although views differed on its adequacy (Ovaska and Soini, 2017). Eight of ten indigenous chicken breeds in Bulgaria were maintained exclusively by amateur breeders, and all of the breeds evaluated except Bregovska dzhinka were classified as endangered or critical. In this Bulgarian sample, adjusted effective population size was 64.8–1,163.5, and the rate of increase in inbreeding per generation was estimated at 0.04–0.77%. The concentration of some breeds at a small number of breeding sites and within a narrow geographic area constrained their long-term conservation (Pavlova and Lukanov, 2024). Monitoring breeding numbers, sex ratio, effective population size, and the distribution of birds among sites can help identify vulnerable populations. Pedigree recording and planned exchange of breeding stock are practical options, but their effectiveness was not tested in this survey.

In a study of 121 breeders and 21 indigenous breeds in Italy, 38% of participants were classified as backyard/fancy breeders. Of the 37 backyard/fancy breeders who answered the question on production cycles, 30 (81%) operated a closed production cycle. Among all breeders, 73% reported that they did not collect data on the production performance of their flocks (Franzoni et al., 2021). Although backyard/fancy breeders contribute to the maintenance of indigenous breeds, limited performance recording may hinder flock evaluation. Closed production cycles also make the source and relatedness of replacement breeding stock relevant to conservation planning.

Compatibility between genotype and feeding is important in two respects. First, a fast-growing or highly productive laying genotype may have energy, amino acid, and mineral requirements that cannot be met by the scavenging feed resource base. Second, the robustness of an indigenous genotype under low-input conditions does not mean that it can tolerate inadequate nutrition without limit. Data from India compiled by Rajkumar et al. (2021) show that deficits in the scavenging feed resource base vary widely among regions. Expected production level, the capacity to provide balanced feed during chick rearing, the supplementary feeds available, and the breeding objective should therefore be considered together when selecting a genotype.

CHICK REARING AND FLOCK INTEGRATION

Rajkumar et al. (2021) described backyard systems in India in which chicks receive controlled rearing for 4–6 weeks before access to free-range scavenging. Balanced feed, suitable brooding temperatures, clean water, and protection from predators are priorities during this period. Lal et al. (2021) likewise emphasized brooding facilities and preventive health

care, although their short communication provides practical guidance rather than experimental estimates of nutrient requirements.

Mixed-age flocks require particular attention to feed allocation because chicks, growing birds, and laying hens have different nutrient needs, especially for protein and calcium. [Faulkner et al. \(2026\)](#) recorded the use of commercial pellets, chick starter, cracked maize, and scraps, as well as feeds described by participants as medicated or fortified. These reports identify a range of feeding practices but do not verify feed composition or nutritional adequacy. Studies that measure the intake of individual age groups would help determine whether all birds receive an appropriate diet.

Housing, outdoor areas, and access to feed

Housing does more than provide protection at night; its design and management may influence feed intake, drinking-water hygiene, resting behavior, oviposition, disease transmission, and predator losses. In the United States survey, approximately half of the participants reported allowing their chickens to range freely during the day, whereas more than one-third used an enclosed run. Although most respondents provided perches and nest boxes, predation, reported by 48.8% of respondents, was the most common problem ([Elkhoraibi et al., 2014](#)). All eight households in Seattle and Portland had enclosed coops, and most had a predator-protected run. Access to outdoor areas was restricted at times because the chickens damaged garden plants ([Blecha and Leitner, 2014](#)). Participants in North Carolina likewise reported providing dedicated housing for protection against weather and predators and modifying the design of outdoor areas according to local predation pressure ([Faulkner et al., 2026](#)).

Space allowances reported for particular systems should not be treated as universal welfare thresholds. For night shelters in the Indian systems reviewed, [Rajkumar et al. \(2021\)](#) specified approximately 0.09 m² (1 ft²) per bird during the growing period and 0.19–0.23 m² (2–2.5 ft²) during the laying period. These are indoor shelter allowances, not estimates of total outdoor space requirements. Ventilation, dry-litter management, feeder and drinker distribution, access to perches and nest boxes, and simultaneous access to resources should be considered alongside the nominal space allowance.

Feeder provision involves more than linear space per bird. Using video recordings from commercial broiler houses with chain feeders, [Collins and Sumpter \(2007\)](#) found that birds were more likely to join, and less likely to leave, a feeding group as local feeder occupancy increased. This social facilitation produced synchronized feeding bouts. The study did not measure individual feed intake or compare feeder layouts in hobby flocks, so it cannot define an optimal number or arrangement of feeders for those systems. The value of multiple feeding stations in small, mixed-age flocks remains a question for direct investigation.

Outdoor areas should be evaluated as both foraging environments and exposure routes: in a survey in Türkiye, wild-bird and rodent access to feeders and drinkers was associated with owner-reported internal and external parasitism, *Escherichia coli* infection, and chronic respiratory disease ([Özdemir, 2020](#)). Vegetation and invertebrates provide nutritional diversity, whereas wet soil, fecal accumulation, wild birds, and rodents may increase exposure to parasites and pathogens. [Pollock et al. \(2012\)](#) recommended a properly constructed coop and feed-storage area that exclude rodents, removal of loose feed from the coop at night, and keeping the surrounding area clean. The authors also emphasized the limited evidence available to quantify risks specifically in urban flocks.

Scavenging feed resource base and supplementary feeding

Nutritional contribution of scavenging

Scavenging chickens consume variable amounts of invertebrates, spilled grain, and plant material; keepers may also supply household scraps. Their nutritional contribution depends on season, rainfall, vegetation, household practices, flock density, and access to the range. [Rajkumar et al. \(2021\)](#) cited daytime scavenging periods of 6–11 hours in Zimbabwe and daily intakes of 45–54 g per bird from scavenging resources in Central Tanzania. These observations describe different populations and should not be combined into a single feeding standard. Assessments of crop and gizzard contents reviewed by the same authors indicate that nutrient shortages can extend beyond energy.

Across four agroclimatic regions in India, nutrient deficits summarized by [Rajkumar et al. \(2021\)](#) ranged from 9% to 20% for energy, 40% to 50% for protein, 42% to 66% for calcium, and 72% to 83% for phosphorus. The review also noted that protein may be less limiting where insects are abundant. This variation supports regional and seasonal assessment of feed resources rather than assuming that the same nutrient limits production in every scavenging system.

Feeding practices reported in field studies vary considerably. In the US survey, 75.4% of respondents combined purchased feed with kitchen scraps, whereas 21.2% provided only a commercial ration. Feeding based exclusively on scraps or grazing was reported only rarely ([Elkhoraibi et al., 2014](#)). The qualitative study from North Carolina reported commercial pellets, cracked maize, fruit and vegetable scraps, meat scraps, insects, and garden-derived feed resources ([Faulkner et al., 2026](#)). These studies describe feeding practices; they do not establish whether the diets supplied adequate energy, amino acids, minerals, or vitamins.

Keepers perceive kitchen and garden scraps as having economic and environmental value. Participants in the study by [Blecha and Leitner \(2014\)](#) described a cycle in which scraps were fed to chickens, chicken manure was returned to the garden, and waste reduction was an explicit objective. In Australia, the use of food scraps was likewise reported as one reason for keeping chickens ([Buddle et al., 2025](#)). These reported motivations do not establish the nutritional adequacy or net environmental benefit of the practice. [Pollock et al. \(2012\)](#) stated that kitchen scraps should not be used to the exclusion of commercially prepared feed. Scraps should therefore be treated as a supplementary feed resource of controlled quantity and nutritional quality, not as a complete ration.

Life-stage-specific feeding

Nutrient requirements vary with genotype, age, body size, production purpose, physical activity, ambient temperature, and stress. The quantitative specifications considered here largely concern genotypes developed for backyard production in India. Drawing on results from earlier studies, [Rajkumar et al. \(2021\)](#) summarized dietary nutrient concentrations for these genotypes from 1 to 42 days of age, providing approximately 2,400–2,500 kcal/kg metabolizable energy, 16% crude protein, 0.77% lysine, 0.36% methionine, 0.35% available phosphorus, and 0.70% calcium. These values were reported for specific genotypes and a defined age period; they should not be applied as universal requirements for adult laying hens, ornamental breeds, or all hobby chickens.

During the laying period, calcium, phosphorus, and water need to be considered alongside energy and amino acid supply. In the United States survey, approximately half of the keepers reported routinely providing laying hens with a calcium source ([Elkhoraibi et al., 2014](#)). [Rajkumar et al. \(2021\)](#) recommended supplementary calcium sources to support eggshell quality and skeletal health in free-range laying hens. The cited sources do not provide controlled calcium dose–response data for different hobby breeds. Mineral supplementation should therefore be planned around a complete feed appropriate to age and production stage, with attention to the intake of free-choice calcium sources and eggshell quality.

Local feed resources, cost, and record-keeping

The economic viability of supplementary feeding depends not only on the use of inexpensive ingredients, but also on knowledge of the nutritional value of local resources and their effects on production. [Rajkumar et al. \(2021\)](#) considered maize, broken rice, sorghum, and millets as energy sources and various oilseed meals as protein sources, and recommended using a vitamin–mineral premix with local ingredients. This approach is based on resource availability in India. Because the composition, processing method, availability, and price of the same ingredients may differ in other regions, local ingredient analysis and appropriate formulation are needed before using a similar ration.

Feed costs can become a concern as flock size increases. [Elkhoraibi et al. \(2014\)](#) found that owners of larger flocks more often reported difficulty obtaining sufficient quantities of feed at a reasonable cost, although the study did not measure actual expenditure.

Routine records can help keepers recognize changes in feed use, production, and health. In the Italian survey of indigenous breeds, 73% of breeders did not collect production performance data ([Franzoni et al., 2021](#)). Useful records may include feed offered and refused, body weight, egg number, shell defects, hatchability, illness, mortality, and costs. Priorities should reflect flock purpose: pedigree and hatching outcomes for breeding flocks, egg traits for layers, and body condition and health events for birds kept over several years. Records can guide investigation, although concurrent changes in season, age, and management complicate attribution to a particular feed.

FEED, ENVIRONMENT, AND GUT MICROBIOTA

Feeding management extends beyond feed formulation. The manner in which birds access feed may influence feeding behavior ([Collins and Sumpter, 2007](#)), while stress and nutritional conditions may influence host–pathogen interactions ([Humphrey, 2006](#)). [Seidlerova et al. \(2020\)](#) compared the cecal microbiota of 20 indoor laying hens from three commercial farms with that of 17 outdoor hens from six backyard flocks. The relative abundances of Bacteroidetes and Proteobacteria were higher and that of Firmicutes was lower in the outdoor group. In addition, some horizontally transferable antibiotic resistance genes were less abundant in the outdoor group than in the commercial group. However, the indoor hens were 30–50 weeks old and the outdoor hens 2–4 years old, and the groups also differed in genetic background, diet, and environment. Individual antibiotic exposure was not measured, which also limits interpretation of the resistance-gene findings.

Diet was one possible explanation for the microbiota differences. [Seidlerova et al. \(2020\)](#) discussed the contribution of complex plant polysaccharides outdoors and more readily digestible commercial diets indoors, but the study did not isolate these dietary effects. Differences in microbial composition alone do not establish better health, and the results do not identify a probiotic or feed additive suitable for hobby flocks.

Studies in hobby flocks are needed to separate the effects of diet, feeder access, age, and genotype on intake and gut microbiota. Health and production measurements should accompany microbiota analyses so that biological differences can be interpreted in terms of flock outcomes.

FEED AND ENVIRONMENTAL SAFETY

Scavenging can expose birds to contaminants in soil as well as to useful feed resources. Yazdanparast et al. (2022) sampled 69 chickens and their eggs from 55 urban gardens in Sydney and found that soil lead concentration predicted lead in blood and eggs. Blood lead exceeded 20 µg/dL in 31 of the 69 chickens. Their models indicated soil concentrations below 166 mg/kg to keep blood lead below 20 µg/dL, and below 117 mg/kg to keep egg lead below the study benchmark of 100 µg/kg.

These model estimates concern the Sydney sample and are not legal or universal safety limits. They indicate that assessing purchased feed alone can miss an important exposure route: birds may ingest contaminated soil while foraging. The findings support investigation of soil contamination where previous land use or old paint creates concern, alongside checks of feed and water quality (Yazdanparast et al., 2022).

Proper feed storage and assessment of the water sources used are important components of biosecurity (Pollock et al., 2012; Faulkner et al., 2026). Pollock et al. (2012) recommended providing a rodent-proof coop and feed-storage area, leaving no spilled feed overnight, and disposing of manure and carcasses appropriately. In the survey conducted by Elkhoraibi et al. (2014), 81.8% of keepers reported providing fresh water to their chickens every day; however, this self-reported practice does not establish the microbiological quality of the water. Participants in North Carolina used various sources, including well water, the municipal supply, and rainwater, and reported that wild birds sometimes accessed the flock's feed and water resources (Faulkner et al., 2026). These findings indicate that the type of water source and wild-bird access to feed and water should be included in flock assessments.

Biosecurity guidance may be tailored according to whether keepers regard their chickens as production animals or companion animals. Pollock et al. (2012) noted that the potential benefits and risks of urban poultry keeping could not be quantified using direct data and recommended caution when extrapolating from commercial flocks. Faulkner et al. (2026) identified a need to investigate whether biosecurity messages framed from a pet-owner perspective would be more effective for keepers who regard chickens as companion animals. Such messages could clearly describe practical measures, including handwashing, designated yard shoes, coop cleaning, and reduced contact with wild birds.

FEEDING, STRESS, FLOCK HEALTH, AND WELFARE

Nutrition, housing, and stress can interact with the host response to infection. Humphrey (2006) reviewed evidence linking adverse environments, poor diets, and stress with susceptibility to *Salmonella* and *Campylobacter*, including interactions between immune or neuroendocrine responses and pathogen behavior. Much of this evidence concerns commercial poultry, so it does not quantify disease risk in hobby flocks. It nevertheless provides a biological basis for considering feeding and environmental management together when assessing flock health.

The sources from which backyard-flock keepers obtain health information are a major concern. In the United States survey, 59% of respondents reported experiencing no health problems during the previous 12 months. Nevertheless, 61% did not vaccinate against Marek's disease, and keepers most frequently identified the recognition and treatment of health problems as areas in which they needed further information. The internet was the most frequently consulted source (Elkhoraibi et al., 2014). In the qualitative North Carolina study, online groups likewise emerged as rapid and accessible sources of information, and some keepers treated health problems at home without specialist veterinary support (Faulkner et al., 2026). Both studies point to a need for accessible, reliable guidance suited to small-flock owners.

Reproductive and neoplastic disorders also require attention in hobby hens. Beecroft et al. (2022) described a diagnostic case with chronic yolk impaction and oviductal dilation, egg yolk coelomitis (peritonitis), and an associated oviduct/ovary adenocarcinoma with carcinomatosis. The case illustrates the value of veterinary investigation and pathological examination; it does not establish a nutritional cause or the frequency of these disorders in backyard flocks.

A large outdoor area alone does not ensure good welfare. Predation, extreme weather, parasites, contaminated water, and untreated disease may still affect the flock. Rajkumar et al. (2021) emphasized suitable night housing, balanced feed, fresh drinking water, a calcium source, and routine health monitoring. Elkhoraibi et al. (2014) found that most respondents provided fresh water daily and used perches and nest boxes, while predation remained the most frequently reported problem. Pollock et al. (2012) recommended keeping coops clean, providing additional nest bedding to reduce egg breakage, and securing feed-storage areas against rodents. Outdoor access therefore needs to be accompanied by appropriate shelter, preventive care, and regular observation.

INTERPRETATION OF PRODUCTIVITY AND ECONOMIC OUTCOMES

Calik and Obrzut (2023) evaluated three conserved hen strains over five generations under standardized feeding and housing. The strains differed in body and egg weights, age at first lay, and laying performance; several egg-quality traits also changed with hen age. These findings support accounting for genotype and age when comparing flock outcomes. They do not establish that one ration is suitable for every backyard genotype.

Lal et al. (2021) and Rajkumar et al. (2021) described the potential of backyard poultry to supplement rural incomes in India with relatively low initial investment. Profitability still depends on local feed and housing costs, losses, and the value of the products obtained.

A different approach may be required when evaluating productivity in hobby flocks. Blecha and Leitner (2014) noted that the costs of housing and maintaining chickens could exceed savings from home-produced eggs. Participants in Seattle and Portland reported spending money not primarily to reduce costs, but to obtain eggs they considered healthier, support animal welfare, contribute to their children's education, and reduce waste (Blecha and Leitner, 2014). Australian participants likewise considered egg production and the use of scraps alongside the companionship provided by chickens (Buddle et al., 2025). In such a system, success should be evaluated not only by cost per egg, but also by flock health, reduced losses, keeper satisfaction, and the environmental safety of the system.

Integrated husbandry and feeding framework

The following six steps summarize practical implications of the reviewed evidence. Their combined effectiveness has not been tested as a management intervention.

Defining the purpose and flock profile

Management begins with the purpose of the flock. Urban keepers commonly value eggs, gardening, the use of scraps, and companionship (Elkhoraibi et al., 2014; Buddle et al., 2025). These priorities affect suitable flock size, retention of roosters and older hens, breeding plans, and the amount the keeper is willing to spend on feed and care.

Aligning genotype and the breeding-stock plan with the intended objective

Conservation-oriented flocks need regular assessment of effective population size, inbreeding, and genetic diversity, particularly when birds are concentrated at a small number of sites (Pavlova and Lukanov, 2024). The Italian survey identified both closed production cycles and limited routine performance recording (Franzoni et al., 2021). Recording the origin of breeding birds and planning matings can help make conservation decisions explicit. In production-oriented flocks, expected body weight and egg production should be considered alongside the available scavenging resources and capacity to provide supplementary feed (Rajkumar et al., 2021).

Preliminary assessment of space, soil, water, and the scavenging feed resource base

When assessing the outdoor area, not only its size but also vegetative cover, drainage, predation pressure, contact with wild birds, and previous contaminant exposure should be considered. Where historical lead contamination is suspected, soil testing consistent with local regulations and expert guidance may be considered. In the single Sydney study reviewed here, ingested soil was identified as a potential contributor to lead exposure in chickens and eggs (Yazdanparast et al., 2022). Water-source type and wild-bird access to feed and water should also be included in the initial assessment; keepers in North Carolina reported using well water, municipal water, and rainwater, and stated that wild birds occasionally accessed feed and water sources (Faulkner et al., 2026).

Life-stage-specific basal and supplementary feeding

During controlled chick rearing, chicks should be provided with a balanced starter feed and clean water; after transition to outdoor access, supplementary feeding should continue, taking into account the quantity and quality of the scavenging feed resource base (Rajkumar et al., 2021). The quantity and composition of supplementary feed should be adjusted in accordance with genotype, age, egg production, season, body condition, and the available scavenging feed resources. Household scraps should remain supplementary to an appropriate complete diet (Pollock et al., 2012). The need for additional calcium and other minerals in laying hens should be assessed in relation to the basal diet (Rajkumar et al., 2021).

Monitoring feed access, hygiene, and daily observations

Regular observation should establish whether every bird can reach feed and clean water. The socially facilitated feeding described in commercial broilers provides a reason to investigate access within groups (Collins and Sumpter, 2007). Keepers in North Carolina reported handwashing, designated yard shoes, variable cleaning schedules, and occasional wild-bird access to feed and water (Faulkner et al., 2026). Pollock et al. (2012) recommended secure feed storage, removal of spilled feed at night, and a clean area around the coop to reduce rodent access.

Evaluating outcomes through record-keeping

After a change in feeding, keepers should record egg production, shell defects, body weight and condition, disease, mortality, and costs. Records may also include fertility and hatchability in breeding flocks, and pedigree and mating information where breed conservation is an objective. Franzoni et al. (2021) found limited routine recording of production performance. Such records can document changes within a flock and inform veterinary assessment, with allowance for changes in age, season, and other management practices.

EVIDENCE GAPS AND RESEARCH PRIORITIES

Among the sources reviewed, controlled evidence on feeding specifically for hobby chickens was limited. Quantitative nutrient specifications came mainly from [Rajkumar et al. \(2021\)](#), who focused on improved backyard genotypes in India. Studies by [Blecha and Leitner \(2014\)](#), [Elkhoraibi et al. \(2014\)](#), and [Faulkner et al. \(2026\)](#) describe owner practices but do not measure their nutritional adequacy or treatment effects.

Research should first establish life-stage-specific requirements for different hobby and indigenous genotypes. Energy, digestible amino acids, calcium, and available phosphorus should be evaluated against growth and egg production, as well as bone health, eggshell quality, body condition, behavior, and longevity. Seasonal and regional assessments of scavenged feed resources are also needed. These should measure outdoor area, stocking density, plant and invertebrate biomass, actual feed intake, and the extent to which scavenged feed replaces supplementary feed.

Feeding behavior and resource distribution also warrant investigation. The social facilitation observed in commercial broilers by [Collins and Sumpter \(2007\)](#) should be examined in small laying flocks, indigenous genotypes, and mixed-age groups. Studies of diet and microbiota need designs that separate dietary effects from those of age, genotype, and environment. These factors differed simultaneously in the comparison by [Seidlerova et al. \(2020\)](#), preventing attribution to any one factor. Future work should use age- and genotype-matched groups, quantify feed intake, and relate microbiota findings to health and performance.

Lead-exposure models should be tested across urban and rural settings before the thresholds reported for Sydney are applied elsewhere ([Yazdanparast et al., 2022](#)). Keeper education also warrants study. [Elkhoraibi et al. \(2014\)](#) and [Faulkner et al. \(2026\)](#) both identified the internet as an important information source, but their different samples and study periods do not establish a temporal trend. Research should assess the accuracy of feeding and biosecurity messages, their relevance to keepers' companion-animal or production goals, and their effects on behavior.

Finally, terminology and outcome measures need to be standardized. The terms "hobby," "backyard," "small-scale," "rural," and "free-range" denote different flock sizes and input levels across sources. This heterogeneity complicates the integrated assessment of economic outcomes, feed use, and health risks. Future studies should report, at a minimum, flock type and size, age structure, genotype, housing, outdoor area, feed quantity, water source, how poultry products are used, and access to animal health services.

CONCLUSION

Husbandry and feeding plans for hobby and backyard flocks should reflect their purpose, genotype, age structure, local feed resources, and access to veterinary care. Outcomes extend beyond growth and egg output to body condition, longevity, keeper objectives, and the conservation of small breeding populations. Outdoor access supplies foraging opportunities, but scavenged resources, grain, and household scraps do not necessarily meet nutrient requirements. An appropriate basal diet and targeted supplementation need to be accompanied by secure housing, reliable feed and water access, hygiene, and regular flock records. Soil contamination and other environmental hazards also require attention. The available evidence supports this integrated approach, while its geographic and methodological limits preclude a universal ration for all hobby and backyard poultry. Feeding trials in defined genotypes and seasonal assessments of local feed resources remain priorities.

DECLARATIONS

Corresponding author

Correspondence should be addressed to Ercan Mevliyaogullari; E-mail: emevliya@gmail.com; ORCID: <https://orcid.org/0000-0003-3333-1490>.

Data availability

This narrative review used information from the published sources cited in the reference list. No new datasets were generated or analyzed.

Authors' contribution

Ercan Mevliyaogullari, Fatma Yenilmez, and Arda Yildirim: Conceptualization, Investigation (literature review), Writing – original draft, and Writing – review and editing.

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Ethics approval and consent to participate

Not applicable. This narrative review uses published literature and reports no new studies involving animals or human participants. Animal ethics approval and ARRIVE reporting requirements for original animal experiments therefore do not apply.

Competing interests

Ercan Mevliyaoğulları is the founder and owner of Mevli Pet Food and Animal Nutrition Ltd. This affiliation is disclosed as a potential competing interest. The other authors declare no competing interests.

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DYNAMICS OF DAIRY COW MILK PRODUCTIVITY AND FREQUENCY OF VISITS TO THE MILKING ROBOT UNDER A VOLUNTARY MILKING SYSTEM

Inna KEPKALO¹, Maryna KHOMENKO², Mykola SEBA², Nataliya SVYRYDENKO¹, Tamara LYTVYENKO², Nataliia SLOBODYANYUK³, Oksana PYLYPCHUK³, Mykhailo SOPIHA⁴, and Tatyana NAUMENKO⁵

¹ Department of Technology of Production and Processing of Livestock Products National University of Life and Environmental Science of Ukraine "Nizhyn Agrotechnical Institute", Ukraine

² Department of Applied Biology, Animal Breeding and Genetics, Faculty of Livestock Raising and Water Bioresources, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

³ Department of Technology of meat, fish and marine products, Faculty of Food Sciences, Nutrition and Quality Management, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

⁴ Department of English for Technical and Agrobiological Specialities, Faculty of Humanities and Pedagogy, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

⁵ Department of Standardization and Certification of Agricultural Products, Faculty of Food Sciences, Nutrition and Quality Management, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

✉ Email: pilipchuk_os@ukr.net

➤ Supporting Information



ABSTRACT: The study analyzed the frequency of voluntary visits to an automated milking system (AMS) by Holstein cows, along with milk yield indicators, functional characteristics of the udder, and the duration of intervals between milkings. The research was conducted at a farm in the Kyiv region using a loose-housing system and robotic milking. Trends in average daily milk yield and the frequency of visits to the milking robot were analyzed using data from the VMS-2012 automated control system, while milk yield was recorded using "Fullflow" electronic meters. Milk flow rate was determined based on the amount of milk obtained from individual udder quarters and the duration of milking. To evaluate the relationship between productivity and milking frequency, cows were categorized into five groups according to lactation milk yield (5000–6000; 6000–7000; 7000–8000; 8000–9000; and 9000–10000 kg) and according to lactation number (I, II, III, IV or higher). Animals belonging to the Chifa, Starbuck, and Montfrecht lines were evaluated separately, taking into account udder conformation, milk flow rate, and the proportion of milk obtained from individual udder quarters. It was found that cows with a lactation yield of 9000–10000 kg visited the milking robot more frequently—2.8 times per day—compared with animals yielding 6000–8000 kg, which visited it 2.6 times per day. As lactation number increased, the frequency of visits decreased, reaching 2.5 visits per day in cows in their fourth and subsequent lactations. The highest 305-day milk yield was recorded in cows in their third lactation, which visited the robot 2.6 times per day. First-lactation cows, despite having the highest visit frequency of 2.7 times per day, produced 9551 kg of milk during the 305-day lactation. Milk flow rates from individual udder quarters were similar, with slightly higher values observed in the rear quarters.

Keywords: Average daily milk yield, Cows, Lactation, Mammary gland, Milking robots, Udder.

INTRODUCTION

Modern innovative technologies in dairy farming in Ukraine are being implemented very slowly, despite their existing advantages and the new prospects they offer for this industry. The emergence of robotic systems, which are successfully used in dairy farming, has contributed to a technical and technological breakthrough (Heema et al., 2022; Sangode, 2025).

The use of milking robots has changed the approach to milking because the focus is now on the animal and its physiological and ethological needs (Brouček and Tongel, 2015; Legkoduikh and Lutsenko, 2018). The main task of robotic milking is to increase milk yield by creating conditions that are as close as possible to natural ones. The use of this system contributes to the emergence of a practically new technology based on animal self-service that allows cows to freely choose the time and frequency of visits to the milking robot (Tousova, 2014; Simoes Filho et al., 2020).

When using the "voluntary" milking system, a stable milking routine is established by training cows to visit the robot independently. This allows each cow to decide when cow needs to be milked and when and for how long to rest (Kammann et al., 2024). Experts have questions regarding this problem, but the main one is to determine the optimal technological parameters for the development of the mammary gland and the frequency of cows' visits to the milking robot without compromising their health, since untimely milking can lead to disruption of the milk secretion process, and ultimately to a decrease in milk yield (Beggs et al, 2015; Simoes Filho et al, 2020; Chikurtev et al., 2024). In addition, many studies show that the frequency of milking affects the quantity and quality of milk (Mačuhová et al., 2020;

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Meijering et al., 2023). It was found that milking three times increased milk yield by 1.72 kg compared with milking twice (Sánchez-Duarte et al., 2020). Robotic milking ensures complete milking of cows according to their physiological state and the accumulation of milk in the udder, which also help prevents the occurrence of mastitis (Legkoduikh and Lutsenko, 2018; Chikurtev et al., 2024).

The pattern of cows' visits to the milking robot, depending on milk yield and age at lactation, remains a relevant topic for study. Since voluntary milking systems are not widespread in Ukraine, in our opinion, the principles of operation of automatic milking systems are of scientific interest and require in-depth research. Based on the above, the goal of our work was to investigate the dynamics of milk yield and the frequency of cows' visits to the milking robot under a voluntary milking system.

MATERIALS AND METHODS

Animals

The study was conducted at the Terezine cattle farm, Bila Tserkva district, Kyiv region, Ukraine. The farm breeds Holstein cattle and is the first farm in Ukraine to install a robotic milking system. The farm uses a loose housing system for cows.

Feeding

Feeding cattle is an important aspect of production and management; the farm selects only high-quality feed and provides the animals with feed produced on the farm. Feed samples are sent to the laboratory, where their quality is determined. To obtain high-quality products from cows, a nutritious and balanced diet containing several types of feed is essential. The feed ration consisted of 9 kg of compound feed, 28 kg of silage, 8 kg of haylage, 1 kg of straw, and 0.3 kg of hay.

Experimental design

The dynamics of average daily milk yields and the frequency of visits to the milking robot by Holstein cows of different lactations and milk yield levels were studied using the automatic milking control system installed in the VMS-2012 unit. The milk flow rate from the udder quarters of cows was determined by dividing the amount of milk obtained in the morning by the duration of milking, expressed in kg/min. Milk yield indicators for each animal were assessed using an automated information collection system based on electronic "Fullflow" flow meters.

To assess the relationship between milk production and the frequency of visits to the milking robot, the cows were divided into five groups according to total milk yield per lactation: 5,000–6,000 kg ($n = 64$), 6,000–7,000 kg ($n = 68$), 7,000–8,000 kg ($n = 84$), 8,000–9,000 kg ($n = 76$), and 9,000–10,000 kg ($n = 61$). Within each group, the average number of visits to the milking robot per day and the average daily milk yield were determined.

To identify lactation-related differences in the use of the voluntary milking system, the cows were grouped according to lactation number: first lactation ($n = 145$), second lactation ($n = 92$), third lactation ($n = 71$), and fourth and subsequent lactations ($n = 44$). For each group, the average number of visits to the milking robot per day, average daily milk yield, and total milk yield per lactation were determined.

To assess the effects of genetic line and udder morphology on milk production, cows belonging to the Chifa, Starbuck, and Montfrech lines were analyzed. For each line, udder shape (cup-shaped, bath-shaped, and round), milk yield during the first, second, and third lactations, and milk flow rate were evaluated. In addition, the productivity of cows from different lines was compared according to lactation number and the two most common udder shapes, cup-shaped and bath-shaped. Functional characteristics of the udder under voluntary robotic milking conditions were evaluated in 120 Holstein cows. The analysis was performed according to lactation number, taking into account the proportion of milk obtained from each udder quarter.

Statistics

Statistical analysis of the research results was carried out using Microsoft Excel 2021 and Statistica 10.0 software. The arithmetic mean (M) and standard error of the mean (m) were determined. The significance of differences between the indicators was assessed using Student's t -test. A difference was considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

One of the main criteria for the suitability of cows for robotic milking is udder shape. The most suitable are cup-shaped, tub-shaped, while round-shaped udders are less desirable. The assessment of the udders of cows on the farm was carried out across three lines: Chief, Starbuck and Montfrecht (Table 1). Analysing udder shape, it was found that the largest number of animals on the farm had cup-shaped udders. The highest percentage of animals with this udder shape belonged to the Chief line (76.2%), while in cows of the Starbuck and Montfrecht lines, this figure was 6.7% and 18.8% lower, respectively. Tub-shaped udders prevailed in cows belonging to the Montfrecht line (36.2%). It should also be noted

that the smallest proportion of animals in all three studied groups had round-shaped udders: 3.2% in cows of the Chief line, 5.5% in Starbuck, and 6.4% in Montfrecht.

Analysis of milk yield showed that the highest milk yield during the first and third lactations were recorded in animals of the Chief line, at 8308.9 kg and 10112.69 kg, respectively. These yields exceeded those of cows of the Starbuck line by 428.6 kg and 70.19 kg, and those of the Montfrecht by 902.5 kg ($P < 0.001$) and 454.09 kg, respectively. Milk yield during the second lactation was higher in cows of the Starbuck line reaching 9379.2 kg. The difference between the groups was 44 kg compared with the Chief line and 573.08 kg compared with the Montfrecht line. The average milk flow rate was highest in animals of the Chief line (2.53 kg/min); however, the difference compared with the other two groups was not statistically significant. The next task was to analyze the influence of udder shape on milk production in cows. The results of the study are shown in Figure 1.

Table 1 - Udder and teat shape depend on lineage

Lines	Udder shape			Milk yield during lactation			Milk flow rate, kg/min
	cup-shaped %	bath-shaped %	rounded %	I	II	III	
Chief	76.2	20.6	3.2	8308.9±164.4	9334.3±333.4	10112.69±225.4	2.53±0.04
Starbuck	69.5	25	5.5	7880.3±186.3	9379.2±279.8	10042.5±223.2	2.48±1.11
Montfrecht	57.4	36.2	6.4	7406.4±186.3***	8806.12±591.8	9658.6±285.3	2.45±0.10

***: $P < 0.001$

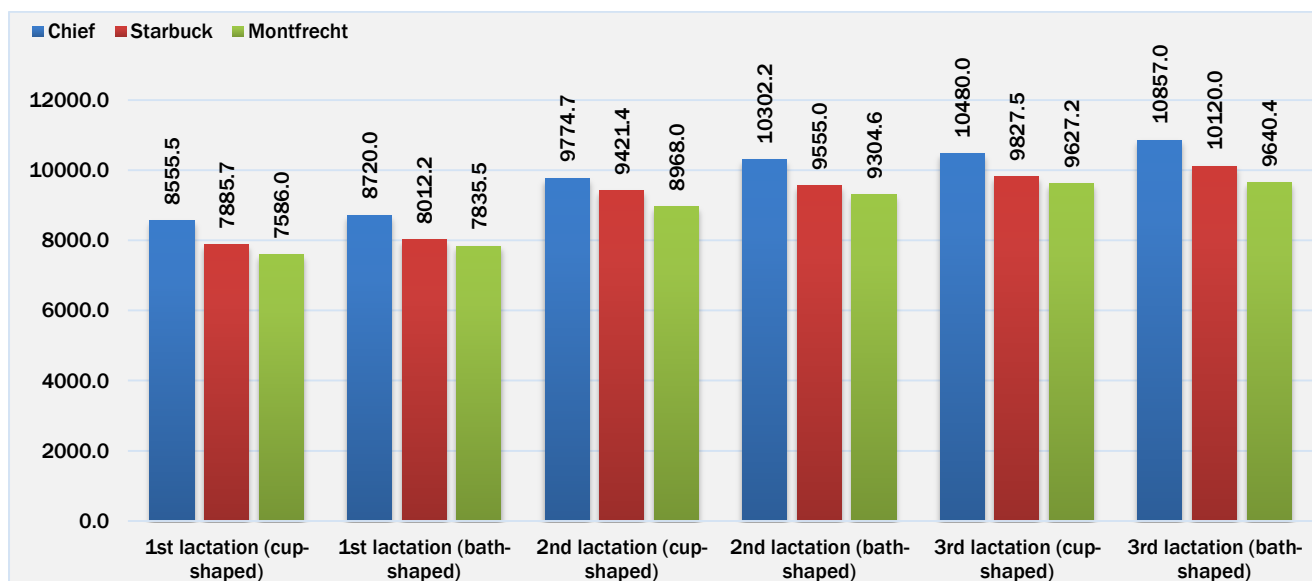


Figure 1 - Milk yield per lactation depending on udder shape and linearity.

The study showed that in animals with tub-shaped udders, milk yield was slightly higher than in animals with cup-shaped udders. The differences between the indicators within the Chief line were as follows: 165 kg in the first lactation - 527.5 kg in the second lactation, and 177.8 kg in the third lactation. In animals belonging to the Starbuck and Montfrecht lines, there was also a tendency toward higher yields in cows with tub-shaped udders. In the first lactation, the differences were 126.5 kg and 249.5 kg; in the second lactation 133.6 kg and 366.6 kg; and in the third lactation, 292.5 kg and 13.2 kg, respectively. Analysis of the milk yield of cows from the studied lines according to udder shape showed that animals belonging to the Chief line had higher milk yields throughout lactation, regardless of udder shape. Offspring of the Chief line with cup-shaped udders in the first lactation produced 669.8 kg and 969.5 kg more milk than cows of the Starbuck and Montfrecht lines, respectively. Among animals with tub-shaped udders, the corresponding differences were 707.8 kg and 884.5 kg, respectively. In the second lactation, the differences for cup-shaped udders were 353.3 kg and 806.7 kg, while for tub-shaped udders, they were 652.5 kg and 1175.6 kg, respectively. In the third lactation, the differences for cup-shaped udders were 625.5 kg and 852.8 kg, while for tub-shaped udders, they were 737 kg and 1216.6 kg, respectively. In the next stage of the research, milk yields per lactation were analyzed, and the results are presented in Table 2. It should be noted that there was a certain pattern of increase in this indicator with increasing milk yield, both on a daily basis under per lactation. As milk yield increased from 5000 to 7000 kg per lactation, average daily milk yield increased from 14.9 to 17.7 kg ($P < 0.001$). At a yield of 7000–8000 kg of milk per lactation, the frequency of visits decreased, similarly to the pattern observed in cows producing 5000–7000 kg of milk per lactation.

From the results obtained, it can be assumed that cows with a milk yield of 7000–8000 kg per lactation have the most optimal mammary gland size and development. Animals with lower milk yields may have an underdeveloped mammary gland, which requires more frequent emptying to optimize its functioning. A similar response is observed with milk yields exceeding 8000 kg per lactation (average daily milk yield above 23 kg; $P < 0.001$), which is also quite natural.

Analysis of changes in the frequency of visits to the milking robot by cows of different lactations (Table 3) indicates a relationship between the age of the animals and, consequently, the size and development of the mammary gland and the frequency of its emptying. Under the voluntary milking system, cows in their first and second lactations, with average lactation milk yields of 9551.0 and 10156.3 kg, respectively, visited the milking robot an average of 2.7 times per day. Mature and older cows in the third, fourth, and subsequent lactations visited the milking robot slightly less frequently - 2.6 and 2.5 times per day, respectively—while maintaining practically the same level of milk production (10224.4 and 10002.1 kg, respectively).

Table 2 - Number of visits to the milking robot per day by cows of different yield level breeds

Milk yield	N	Frequency of visits, times	Average daily milk yield, kg
		M $\pm$ m	M $\pm$ m
5000–6000	64	2.6 $\pm$ 0.05	14.9 $\pm$ 0.13
6000–7000	68	2.6 $\pm$ 0.04	17.7 $\pm$ 0.07***
7000–8000	84	2.5 $\pm$ 0.01	20.5 $\pm$ 0.10***
8000–9000	76	2.7 $\pm$ 0.04	23.3 $\pm$ 0.09***
9000–10000	61	2.8 $\pm$ 0.07	26.1 $\pm$ 0.10***

***: $P < 0.001$ compared with the 5000–6000 kg milk-yield group

Table 3 - Number of visits to the milking robot per day by cows of different ages in lactation and their yield

Age of cows in lactation	N	Frequency of visits, times	Daily milk yield, kg	Lactation milk yield, kg
		M $\pm$ m	M $\pm$ m	M $\pm$ m
I	145	2.7 $\pm$ 0.02	26.2 $\pm$ 0.43	9551 $\pm$ 158
II	92	2.7 $\pm$ 0.01	27.8 $\pm$ 0.93	10156.3 $\pm$ 339.6***
III	71	2.6 $\pm$ 0.0	28 $\pm$ 0.88	10224.4 $\pm$ 322.8***
IV and older	44	2.5 $\pm$ 0.02	27.3 $\pm$ 1.09	10002.1 $\pm$ 406.0***

***: $P < 0.001$ compared with the first-lactation cows

In the studied cows, average daily milk yield increased from the first to the third lactation. Compared with first-lactation cows, average daily milk yield was 1.6 kg higher in second-lactation cows and 1.8 kg higher in third-lactation cows. In cows in their fourth and subsequent lactations, average daily milk yield exceeded that of first-lactation cows by only 1.1 kg. The highest average daily milk yield was recorded in third-lactation cows (28.0 kg) after which it slightly decreased to 27.3 kg in cows in their fourth and subsequent lactations.

It was also important to investigate how average daily milk yield and the frequency of visits to the milking robot by cows of different lactation ages changed during lactation. The dynamics of changes in milk yield throughout lactation can be assessed using the indicators of the lactation curve. Thus, in primiparous cows (Figure 1), milk yield increased fairly evenly, reaching a maximum in the fifth month of lactation, when it amounted to 30.3 kg. The frequency of visits to the milking robot was highest in both the fifth and sixth months of lactation, amounting to 3.3 visits per day.

Analysing changes in average daily milk yields in cows in their second lactation (Figure 2), the researchers see that the curve reaches a maximum in the third month of lactation (32.2 kg), while the peak frequency of visits to the milking robot occurs in the fifth month, at 3.2 visits per day. Therefore, the level of average daily milk yields in individual months of lactation does not reflect the frequency of visits to the milking robot.

Analysing the nature of the lactation curve in cows in their third lactation (Figures 3–5), the researchers see that milk yields increase steadily until the third month of lactation (34 kg). At the same time, the frequency of visits to the milking robot increases in parallel with milk yields, reaching 3.3 visits per day, followed by a gradual decrease in both milk yields and the frequency of visits. This indicates that in cows in their third lactation, which have sufficiently high milk yields, the lactation curve is more stable, and the frequency of visits reflects the level of daily milk yields.

In cows in their fourth lactation (Figure 4), there is a rapid increase in milk yields until the second or third month of lactation, followed by a significant decrease. The frequency of visits to the milking robot is initially somewhat lower than the increase in milk yield and then significantly exceeds it. By the end of lactation, it does not correspond to the milk yield values. In our opinion, in this case, a reflex component may be involved, namely, the receipt of a portion of compound feed during milking. Therefore, such cows can be milked twice a day starting from the fifth month of lactation.

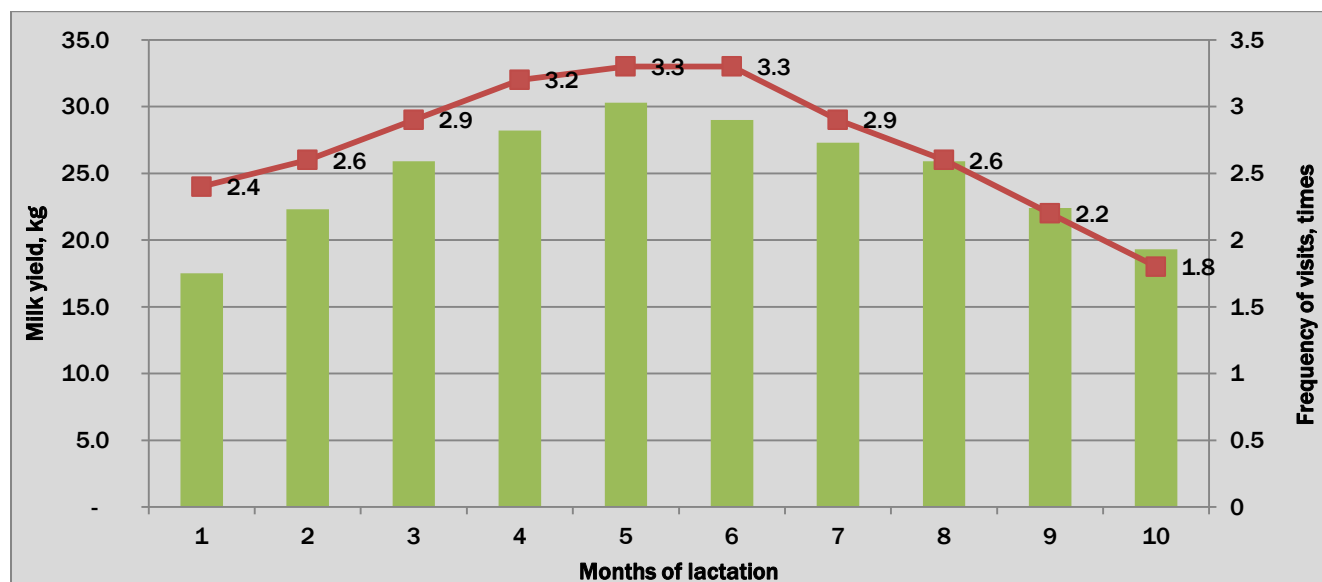


Figure 2 - Dynamics of average daily milk yields and frequency of visits to the milking robot by primiparous cows.

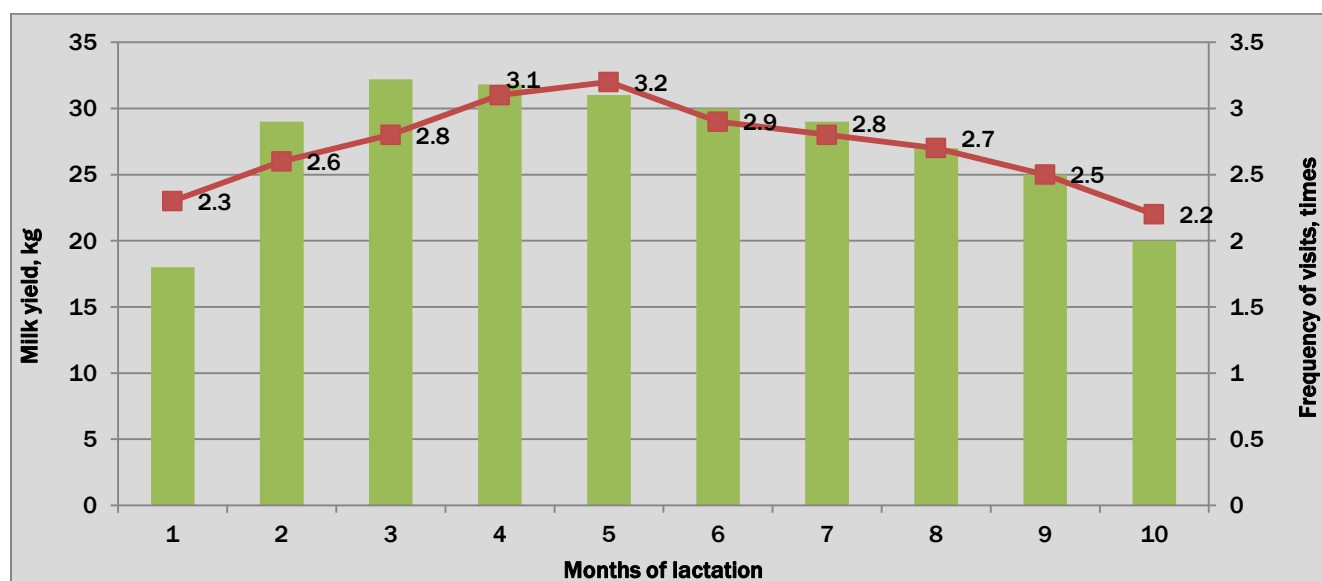


Figure 3 - Dynamics of average daily milk yields and frequency of visits to the milking robot by cows in the second lactation.

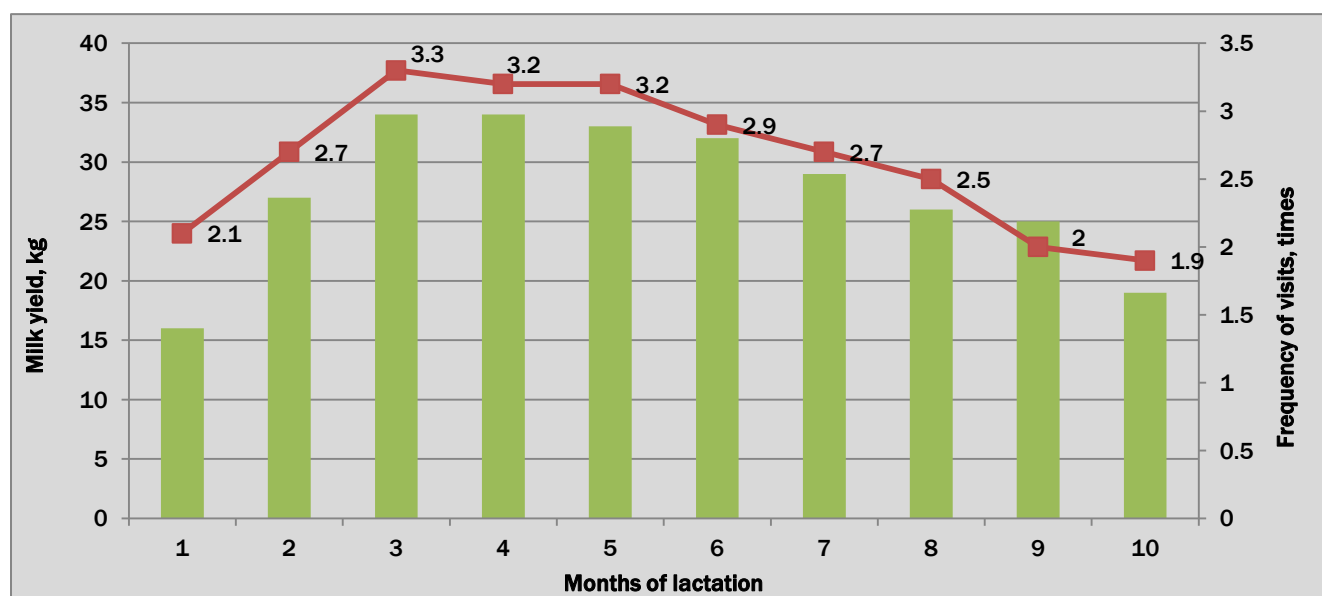


Figure 4 - Dynamics of average daily milk yields and frequency of visits to the milking robot by cows in the third lactation.

For quite a long time, scientists have been interested in studying the development of individual parts of the udder in cows of different breeds. However, even today, due to the limitations of existing devices and methods, this issue has not been sufficiently studied, and the results obtained are not readily comparable. Therefore, we conducted a study to determine the capacity of individual parts of the udder in Holstein cows. The results of the study are presented in Table 4.

When assessing the capacity of individual udder quarters in cows, it was found that, regardless of age and lactation number, the smallest amount of milk was concentrated in the left anterior quarters. Thus, in primiparous cows, the capacity of the right anterior quarters, compared with the left anterior quarters, was higher by 3.4%, that of the left posterior quarters (LP) by 11.7%, and that of the right posterior quarters (RP) by 10.4%. In cows in their second lactation, the differences were 5.0% for the right anterior quarters, 14.9% for the left posterior quarters, and 17.7% for the right posterior quarters. In cows in their third lactation, the corresponding differences were 3.0%, 12.4%, and 13.4%, respectively, while in cows in their fourth and subsequent lactations, they were 4.1%, 10.2%, and 11.3%, respectively. Thus, the largest difference in the capacity of individual udder quarters was observed in cows in their second lactation.

Cows in their third, fourth, and subsequent lactations had indicators close to those of primiparous cows. In this regard, it can be argued that, even under conditions of voluntary milking, a significant difference in the capacity of individual parts of the udder does not allow for increasing the yield of the milking robot.

Evaluating the milk production intensity index in Holstein cows under conditions of “voluntary” milking, it was established (Table 5) that there was no statistically significant difference in milk yields between cows of different lactation numbers (12.5–13.5 kg). Cows in all lactations were milked, on average, within the optimal period of oxytocin activity (up to 6 min). At the same time, cows in their third lactation were milked the fastest, while primiparous cows required the longest milking time. Regarding primiparous cows, it can be assumed that they were still poorly adapted to robotic milking, since the assessment was carried out in the second month of lactation, which may have affected the duration of milking.

Regarding milk flow rate, the highest value was recorded in cows in the second lactation. This value was higher than that of primiparous cows by 0.3 kg/min, that of cows in their third lactation by 0.1 kg/min, and that of cows in their fourth and subsequent lactations by 0.2 kg/min. As observed by other researchers, there was a positive correlation between milk flow rate and single milk yield, and a negative correlation between milk flow rate and milking duration. At the same time, the milk flow rate in Holstein cows under conditions of “voluntary” milking significantly exceeded the minimum requirement of 1.3 kg/min for this indicator, indicating the possibility of further improvement.

Analysing changes in the time intervals between milking in cows of different lactation numbers under conditions of voluntary milking (Table 6), it can be noted that the longest interval was observed at night, that is, between evening and morning milking.

Thus, in primiparous cows, its duration was 45.8% of the daily period, in second-lactation cows, 41.6%; and in third- and fourth-lactation cows, only 37.5%. Therefore, even under conditions of voluntary milking, the duration of the intervals between milking's tends to become more equal with increasing lactation number. However, an interval of 11 hours or more between milking's in primiparous cows indicates the possibility of milking them twice a day, which would not negatively affect milk yield or the processes of milk formation. The duration of the interval between morning and afternoon milking in cows of all lactations was almost the same, accounting for 29.2–33.3% of the daily period. Regarding the duration of the interval between afternoon and evening milking, some leveling was observed with increasing lactation number. Thus, in cows in their first and second lactations, the interval accounted for only 25% of the daily period, whereas in cows in their third lactation, it increased to 29.2%, and in cows in their fourth and subsequent lactations, to 33.3%.

Evaluating the pattern of visits to the milking robot by cows in the technological group (Figure 5), the researchers observed two major peaks: from 6 to 10 a.m. and from 6 to 10 p.m. A slight peak is also observed from 1 to 2 p.m. The lowest frequency of visits to the milking robot, as in the control group, was observed from 1 to 6 a.m. From 6 a.m. to 7 p.m., the milking robot was visited hourly by 3.5–5.5% of the cows in the technological group. The studies also showed that during the day, each cow, depending on its lactation number, visited the milking robot between one and three times.

Table 4 - Capacity of individual udder segments in Holstein cows under “voluntary” milking, (% of total udder milk yield) (n = 120)

Lactation	Udder parts			
	Left front	Right front	Left rear	Right rear
I	18.7	15.6	17.8	18.6
II	22.1	20.6	20.8	22.7
III	30.1	30.5	30.2	28.8
IV and older	29.1	33.3	31.2	29.9

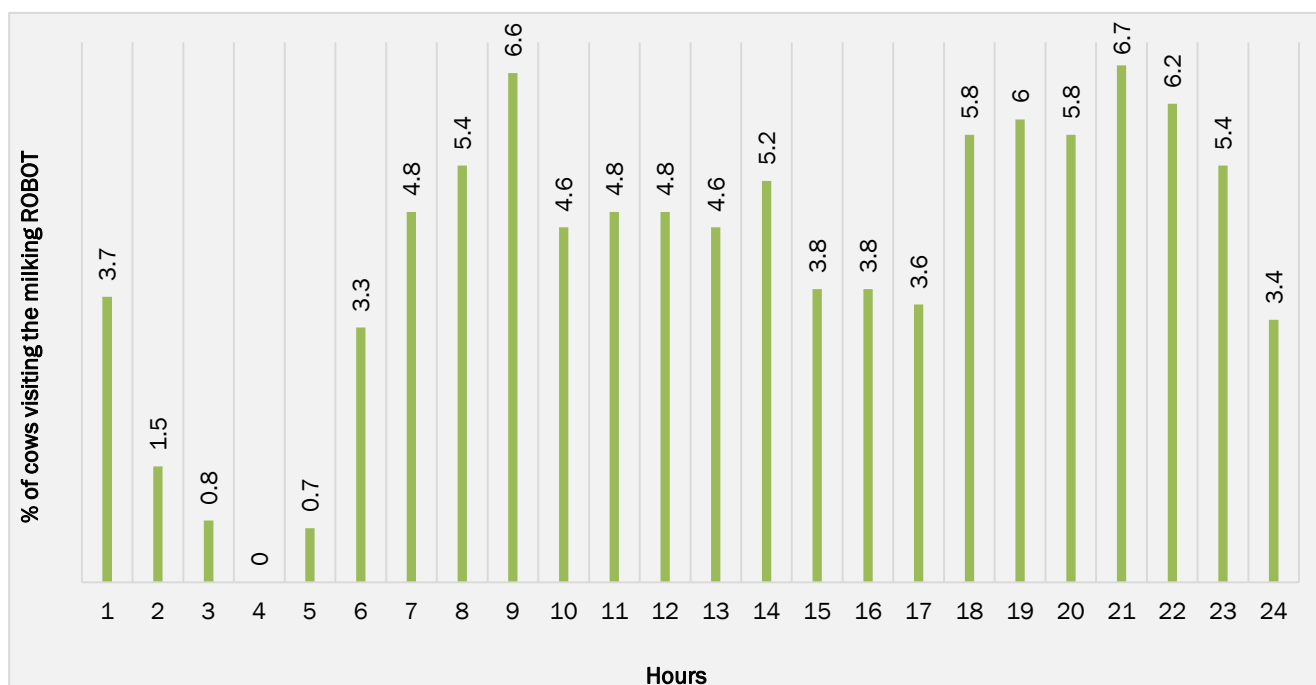


Figure 5 - Frequency of visits to the milking robot by cows

Table 5 - Milk production rates in “voluntary” milking cows

Lactation	One-time milk yield for morning milking, kg	Milking duration, min	Milk output intensity, kg/min
I	12.5±0.85	5.3±0.02	2.4±0.2
II	13.5±0.92	5.1±0.02	2.7±0.5
III	13.2±0.89	5.0±0.03	2.6±0.4
IV and older	12.9±0.9	5.1±0.02	2.5±0.4

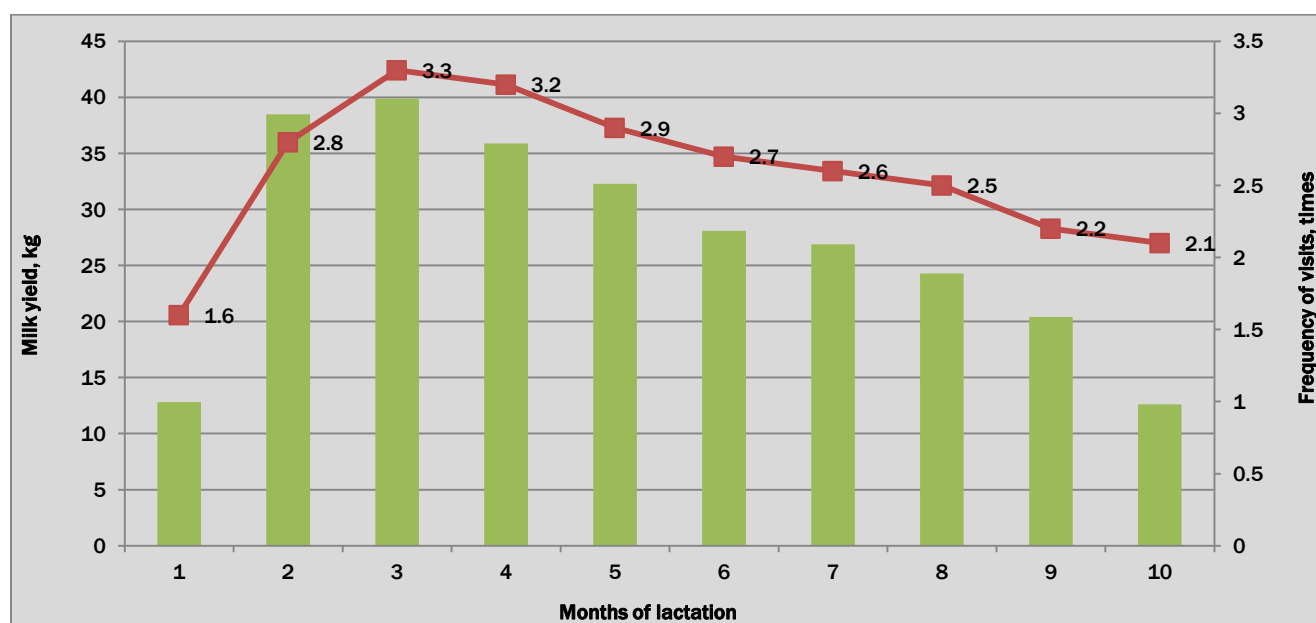


Figure 5 - Dynamics of average daily milk yields and frequency of visits to the milking robot by cows in the fourth lactation

Table 6 - Duration of time intervals between milkings during the day

Lactation	Time interval between milkings, hours (M±m)		
	Evening morning	Morning lunch	Lunch evening
I	11±0.22	7±0.21	6±0.21
II	10±0.15	8±0.22	6±0.23
III	9±0.10***	8±0.11	7±0.13
IV and older	9.1±0.13**	7.1±0.18	8±0.12

***: $P < 0.001$; **: $P < 0.01$.

Milk yield depends significantly on the size, morphological structure and shape of the udder (Klymkovetskyi and Nosevych, 2020). Analysis of animals belonging to three different lines showed that cup-shaped udders predominated in animals of each line. A significant proportion of the animals also had tub-shaped udders, while a small proportion had round-shaped udders. Analysis of milk yield depending on udder shape showed that, regardless of genetic line, milk yield was higher in cows with tub-shaped udders. This is also confirmed by the research results of Vedmedenko et al. (2019). According to his data, cows with cup-shaped udders produced 124.8 kg less milk during the first lactation, 129.7 kg less during the second, and 254.2 kg less during the third.

As a result of the conducted research, it was found that in cows in their first to third lactations, a general trend was observed: as average daily milk yield increased the frequency of visits to the milking robot also increased. In cows in their fourth and subsequent lactations, a significant decrease was observed. In our opinion, this indicates that at this age, udder size reaches its maximum, ensuring optimal functioning of the mammary gland without negatively affecting the pattern of milk formation.

There is no consensus in the literature on this issue. Some authors (Borshch and Ruban, 2015) believe that the higher a cow's daily milk yield, the more frequently it is milked. Thus, cows producing 25.2 kg of milk per day visited the milking robot an average of twice, whereas cows producing 29.05–29.39 kg visited it three and sometimes four times.

Conflicting data have also been obtained regarding the frequency of milking cows. Studies by De Koning (2010) and Habibi et al. (2021) found that when the frequency of milking was reduced from four to two times per day, while maintaining the same housing and feeding conditions, milk yield decreased by 4.4–13.7%. The magnitude of these changes largely depended on the animals' milk yield. We conducted a study to assess the relationship between the frequency of visits to the milking robot per day and milk yield. The results showed that milking frequency had a certain effect on cow milk yield. The highest milk yield over 305 days of lactation (10,224.4 kg of milk) was achieved by cows in their third lactation, which were milked 2.6 times per day. Their yield exceeded that of cows in their first and second lactations by 0.6% and 6.6%, respectively. The lowest yield (9,551 kg of milk) was achieved by primiparous cows, which visited the milking robot 2.7 times per day.

The question of the frequency of milking cows of different lactation numbers and milk yield levels is constantly being raised and addressed by many researchers (Jensen et al., 2018; Sanchez-Duarte et al., 2020). They found that intensive milk production occurs when the udder is sufficiently filled with milk. At the same time, studies by Kosior et al. (2009) and Mangalis et al. (2021) established that reducing the frequency of milking after calving does not reduce milk yield. Folley (1961) also stated in his work that cows produce milk more efficiently at the beginning of lactation than at the end. As milk secretion decreases, the intensity of the milk ejection reflex also decreases, and the relative amount of residual milk increases. From a physiological point of view, more frequent milking (three times per day) allows more milk to be obtained from cows than twice-daily milking. However, the difference in milk yield depends on the age of the animal, its milk yield, and the stage of lactation. In addition, switching to a twice-daily milking regime also has certain advantages (Hogeveen et al., 2001; Borshch, 2016). Nevertheless, all authors agree that with increasing age, the milk yield of animals increases, then stabilizes at a certain maximum, and subsequently decreases until the end of their productive lives (Kostenko, 2005; Solano et al., 2022; Kepkalo et al., 2024).

The results of the present study showed that the milk flow rate from the udder quarters was almost the same but slightly higher in the hind quarters. With increasing age, certain changes were observed in the capacity of individual udder quarters. Regarding milk flow rate in primiparous cows, the lowest value was recorded in the left quarters, where it was 3.4% lower than in the right quarters. Depending on lactation number, the difference in milk yield between the left posterior (LP) and right posterior (RP) quarters was 4.2%. From the first to the fourth lactation, milk yields in the LP quarters remained unchanged, while those in the RP quarters increased slightly. In cows, regardless of lactation number, only 15.8–18.7% of the daily milk yield accumulated in the LP quarters.

The results of the present research confirm the findings of Kostenko (2005), who reported that, regardless of breed, breed combination, lactation number, and milking frequency, the left anterior quarter of the udder is the least developed in cows. The duration of milking in this quarter is shorter by 1.4–96.6%, milking frequency is lower by 4.6–95.6%, and the milking intensity is lower by 2.6–68.7% than in the other quarters of the udder. The difference between the optimal value (25%) and the established milking index in the udder quarters of cows of different breeds and breed combinations

averages 0.2–11.9%. Therefore, the functional development of individual udder quarters is uneven, even under a “voluntary” milking system.

CONCLUSION

According to the results of the study, it was found that, regardless of lactation number, the frequency of visits to the milking robot increases with an increase in average daily milk yield. The maximum udder capacity of Holstein cows is reached by the fourth and subsequent lactations, as evidenced by a decrease in the frequency of visits to the milking robot at the same daily milk yield. It was also found that cows visit the milking robot due to physiological needs. In addition, the intervals between milkings are regulated by the functional state of the mammary gland and become more uniform with increasing lactation number.

DECLARATIONS

Corresponding Author

Correspondence and requests for materials should be addressed to Oksana Pylypchuk; E-mail: pilipchuk_os@ukr.net, <https://orcid.org/0000-0002-2757-6232>

Ethical regulation

The study followed the rules for the treatment of animals in accordance with the European Convention for the Protection of Vertebrate Animals used for Research and Other Scientific Purposes (1986). The research protocol was approved by the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine (Protocol No. 049/2025, dated November 4, 2025). The study was conducted and reported in accordance with the ARRIVE guidelines and the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education of the New York Academy of Sciences, Ad Hoc Animal Research Committee.

Data availability

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

Authors' contribution

I. Kepkalo: Formal analysis, funding acquisition, writing – original draft;
M. Khomenko: Software, visualization writing original draft, review & editing;
M. Seba: Funding acquisition, methodology, validation;
N. Svyrydenko: Formal analysis, conceptualization, investigation, supervision, visualization;
T. Lytvynenko: Investigation;
N. Slobodyanyuk: Resources, supervision, validation;
O. Pylypchuk: Methodology, project administration, original draft, writing – review & editing;
M. Sopiha: Methodology, project administration, software;
T. Naumenko: Resources, software, writing – original draft.

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Competing interests

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

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PHYSIOLOGICAL RESPONSE AND HEAT SHOCK EXPRESSION IN THE NIGERIAN INDIGENOUS GOAT BREEDS RAISED UNDER THERMAL STRESS IN SOUTHWEST NIGERIA

Deborah Olufunke FATOKI¹ , Mathew Oluwaseyi AYoola^{1,2} , and Godwin Ayebaitari BERENA³ 

¹College of Agriculture, Engineering and Science, Agriculture Programme, Bowen University, P. M. B. 284, Iwo, Nigeria

²Smart and Sustainable Agriculture for Food Security Research group, Bowen University, Nigeria. P. M. B. 284, Iwo, Nigeria

³College of Agriculture, Engineering and Science, Biochemistry Programme, Bowen University, P. M. B. 284, Iwo, Nigeria

✉ Email: mathew.ayoola@bowen.edu.ng

➤ Supporting Information



ABSTRACT: Environmental stress remains a major threat to optimum productivity of livestock in tropical climates. Generally, goats often develop adaptive mechanisms to thrive in their native environment. However, little is known about the adaptation of the Nigerian indigenous goat breeds to different environmental conditions. The influence of varying periods of heat exposure on physiological response and HSP70 gene expression in the Nigerian indigenous goat breeds was therefore examined in this study. A total of 45 goats comprising West African dwarf (WAD), Red Sokoto (RS), and Sahel (SH) breeds were assigned to three treatments; 0, 4, and 8 hours of daily exposure to environmental temperature. Each treatment had two males and 3 females per breed of goats. Environmental variables were monitored while Temperature-Humidity-Index (THI) was calculated. Also, body temperature (BST), rectal temperature (RT), respiratory rate (RR), pulse rate (PR) were monitored while serum HSP70 level was determined. Average temperature (29.86 °C), relative humidity (73.57%) and THI (79.34) were recorded. Also, there was no significant effect of breed on all the physiological parameters, hours of exposure to heat had significant influence on BST, RT, RR and PR. Also, physiological indices monitored significantly ($P < 0.05$) increased with days of exposure while effects of sex on physiological responses of the various breeds exposed to environmental stress was not significant. For HSP70 expression, Red Sokoto goats had the highest level (30.08 ± 0.75 ng/mL), compared to WAD goats (22.66 ± 0.53 ng/mL) and Sahel goats (18.01 ± 0.55 ng/mL) while male goats (35.34 ± 0.50) had higher HSP level compared to female goats (14.49 ± 0.44). In conclusion, duration of exposure to heat influenced physiological responses and serum HSP expression in the Nigerian indigenous goats.

Keywords: Adaptation, Heat stress, HSP70 expression, Indigenous goat breeds, Physiological response.

INTRODUCTION

Small ruminants play a pivotal role in global livelihoods due to their economic, agricultural, and cultural importance (Mohamed et al., 2023). In Nigeria, goats represent the most dominant group of small ruminants, with an estimated population of five million (FAO, 2019). Indigenous goat breeds not only serve as financial assets and cultural symbols but also exhibit high productivity, strong disease resistance, hardiness, versatility, and adaptability, which makes them to be well-suited for hot and humid environments (Ofori et al., 2021).

The adaptability of the Nigerian indigenous goat breeds has been demonstrated by their geographical dispersion (Tella et al., 2024) over the last few decades. This adaptability to diverse environments is supported by specific anatomical and physiological features, such as smaller body size and effective thermoregulation (Nair et al., 2021; AL-Ramamneh, 2023). Nevertheless, environmental stressors in various forms have limited them from reaching their maximum productivity (Bertolini et al., 2018). In particular, high temperature and humidity in tropical regions significantly hampers goats from attaining their full genetic potentials (Bertolini et al., 2018) by inducing physiological and biochemical changes that adversely affect their development, reproduction, and overall health (Danso et al., 2024; Parsad et al., 2025).

According to Sejian et al. (2021), despite the ability of goats to adapt to diverse geographical space, they are still vulnerable to climate-induced heat stress, which impairs productivity, immunity, and overall health. Similar sentiments have been raised by other authors especially on the indigenous breeds of goats in Nigeria. Ayoola et al. (2025), reported that thermal stress, marked by increased body temperature and disruption in physiological functions negatively affects goat wellbeing. The author noticed that when goats are stressed, they experience elevated respiratory rates, reduced metabolic and physical activities, sweating and altered endocrine functions which threatens the sustainability of goat farming in tropical and subtropical regions.

Elsewhere in colder climates, goats increase metabolic heat production but experience reduced energy efficiency, affecting performance and production (García et al., 2020). The three Nigerian indigenous goat breeds (Sahel, Red

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Sokoto, and WAD) have distinct and unique traits which help them to adapt to their native regions. Very little is known about the adaptive nature of these breeds when raised outside their natural environment. A better understanding of this adaptive capacity and response to heat exposure is crucial to improve goat productivity and welfare. Also, genetic traits play a significant role in adaptation to environmental stress. HSPs, particularly HSP70, are produced in response to heat stress to protect cellular proteins and maintain homeostasis (Kaushik et al., 2023).

An adequate knowledge of the heat tolerance attributes of the indigenous breeds of goat could guide producers on the best management practices or strategies to adopt in order to maximize productivity in this breed of goats. This study was therefore designed to examine the influence of duration of exposure to heat stress on the physiological response and serum HSP70 expression in the Nigerian indigenous breeds of goats.

MATERIALS AND METHODS

Experimental site

This study was conducted at Bowen University farm, Iwo, Nigeria, within a derived savanna agro-ecological zone characterized by a tropical climate with distinct wet and dry seasons. Bowen University is situated in Iwo, one of the major cities in South West Nigeria on a 640 ha site. It lies on coordinates 7° 38' 6.97" N and 40° 10' 53.62" E at an altitude of 322 m above sea level. The study area is located in a derived savanna agro-ecological zone with a tropical climate characterized by double maximum rainfall and average annual temperatures of 29.66°C and vegetation interphase between the rainforest and savanna grassland with a mixture of deciduous trees and tall grasses (Oguntunji et al. 2019). The Wet (March–September) and dry (October–March) seasons are the principal seasons in the area (Oguntunji et al. 2015).

Experimental animals and design

A total of 45 goats comprising three indigenous breeds in Nigeria; West African dwarf (WAD) goats, Red Sokoto goats, and Sahel goats with average age between 3-4 months were purchased at Kaara Market, Iwo town, Osun state Nigeria and used for this study. The market is about 3-5 km from the main campus with estimated travel time to be 15-20 mins. Prior to the commencement of the study, the goats were subjected to a 5 day acclimatization period for the animals to adjust to the prevailing environmental condition of the study area. The goats (6 males and 9 females per breed) were assigned to three treatment groups based on duration of heat exposure. Each treatment comprised of 15 goats of two males and three females of each breed per treatment. Goats in T1 were assigned to 0 hours of heat exposure (control/ungrazed), T2 had 4 hours of exposure to heat (10 am–2 pm grazing), and T3 had 8 hours of exposure to heat (9 am–5 pm). All animals were offered access to roughages, concentrate feed, and water.

Data collection

Data were collected from each animal on days 0, 3, 6, 9, and 12. Ambient temperature (AT, °C) and relative humidity (RH%) were measured three times daily at 9.00am (morning), 1.00pm (afternoon) and 5.00pm (evening) using a digital thermohygrometer while Temperature-humidity index (THI) was calculated according to Helal et al. (2010) using the formula: $THI = 0.8 \times AT + RH \times (AT - 14.4) + 46.4$. Physiological parameters like respiratory rate (RR), panting rate (PR), body surface temperature (BST), and rectal temperature (RT) were also measured on days, 0, 3, 6, 9 and 12. The measured physiological parameters were as described by Oguntunji et al. (2019). The PR was monitored by placing the hand on the rib cage of the goats for 15 seconds and the number of pulse/heart beats was multiplied by 4 to obtain pulse rate per minute. BST measurement was monitored using a digital infrared thermometer (Model AR330, China) positioned at a distance of 12 inches from the animal while RT was measured using a digital clinical thermometer (COCET®, Kangfu Medical Equipment, China) inserted into the rectum of the goats until a beep is heard and readings recorded. Also, RR was measured by counting the number of breaths per minute.

HSP70 determination

100 µL of each standard (Goat HSP-70 ELISA Kit EG0033) and properly diluted sample are added to the pre-coated microplate wells. It is therefore sealed and incubated at 37 °C for 90 minutes, after which the plate is washed twice (no immersion). To the mixture, 100 µL of biotinylated detection antibody working solution was added and incubated at 37 °C for 60 minutes, and then washed thrice at one minute per wash. 100 µL of HRP-Streptavidin Conjugate was added and incubated at 37 °C for another 30 minutes, and then washed five times at intervals of 1 min. 90 µL of TMB substrate was added, and the mixture incubated 37 °C in the dark for another 10–20 minutes. 50 µL of stop solution was added as the color changed from blue to yellow. Absorbance was read at 450 nm the standard curve was plotted against known concentrations while HSP-70 concentration in serum samples was calculated via interpolation.

Statistical analyses

$$Y_{ijkl} = \mu + G_i + S_j + P_k + (GS)_{ij} + (GP)_{ik} + e$$

Where Y_{ijkl} = Individual observation; μ = fixed overall mean; i = fixed effect of genotype (WAD, RS, and SH); j = fixed effect of sex (males and females); k = fixed effect of duration of exposure period (0, 3, 6, 9); ij = effect of interaction of breed and sex; ik = effect of interaction of breed and exposure period; ijk = effect of interaction of breed, sex, and exposure period; e = experimental error. Data were subjected to descriptive analysis and ANOVA using SPSS 2020 while significant means were separated using NDMRT option of the same software.

RESULTS

The mean climatic parameters during the study period are presented in Table 1. Average environmental temperature ranged from 25.56 °C in the evening to 29.26 °C in the morning while relative humidity ranged from 61.0 %, in the afternoon to 84.12% in the evening. Temperature Humidity Index (THI) followed similar pattern as temperature as THI value ranged from 73.94 in the evening to 85.50 in the afternoon.

Table 1 - Average environmental parameters during the study period

Parameters	Morning	Mid-day	Evening	Mean	SEM $\pm$
Temperature (°C)	29.26	34.76	25.56	29.86	1.07
Relative Humidity (%)	75.60	61.0	84.12	73.57	2.73
Temperature-Humidity Index	78.60	85.50	73.94	79.34	1.45

Physiological response of the indigenous breeds of Nigerian goats to heat stress is shown in Table 2. There were no significant differences ($P > 0.05$) in the physiological parameters monitored among all the goat breeds. Body temperature ranged from 37.69 ± 2.21 °C in Sahel goats to 38.63 ± 2.38 °C in WAD. Rectal temperature ranged from 39.57 ± 1.14 °C in Sahel goats to 39.84 ± 1.42 °C in WAD goats. Pulse rate ranged from 73.13 ± 24.89 beats/min in Red Sokoto goats to 74.23 ± 26.23 beats/min in WAD goats while for respiratory rate, it ranged from 24.83 ± 5.01 breaths/min in Red Sokoto goats to 25.70 ± 5.63 breaths/min in Sahel goats.

The effects of hours of exposure to heat on physiological parameters of Nigerian indigenous goat breeds is shown in Table 3. The BST in goats subjected to T3 was significantly higher ($P < 0.05$) than BST in goats on T2 (38.35 ± 2.38 °C) and T1 (36.79 ± 0.53 °C). Similarly, goats on T3 (40.21 ± 1.38 °C) and T2 (39.93 ± 1.48 °C) had similar ($P > 0.05$) RT but differ significantly ($P < 0.05$) from those in T1 (38.84 ± 0.53 °C). Respiratory rate followed the same pattern as RT, as T2 (24.90 breaths/min) and T3 (29.87 ± 5.90 breaths/min) had significantly similar ($P > 0.05$) but higher ($P < 0.05$) value compared to T1 (21.00 ± 1.14 breaths/min). However, pulse rate in T1 (53.70 ± 2.67 bpm) was significantly lower ($P < 0.05$) than PR in T2 (77.90 ± 15.63 bpm) and T3 (93.50 ± 27.01 bpm) while PR in T3 was higher ($P < 0.05$) than in T2.

Table 4 shows the effect of days of exposure to heat on physiological parameters of Nigerian indigenous goat breeds. Significant effect ($P < 0.05$) of heat exposure was observed on all parameters monitored. Average body temperature at days 0 (36.57 °C) and 3 (37.38 °C) were similar ($P > 0.05$) across breed but significantly lower ($P < 0.05$) than average body temperatures of 39.21 , 38.79 and 39.12 °C recorded at days 6, 9 and 12, respectively. The least ($P < 0.05$) average breed rectal temperature (RT) was obtained at day 0 (38.55 °C), followed by day 3 (39.32 °C). The RT at day 6 (40.27 °C) was not significantly different ($P > 0.05$) from those of days 9 (39.68 °C) and 12 (40.68 °C), though RT at day 12 was significantly higher ($P < 0.05$) than RT at day 9. Pooled pulse rate across breed was significantly lower ($P < 0.05$) at day 0 (53.78) compared to days 3, 6, 9, and 12 with PR of 74.56 , 78.94 , 82.83 , and 85.06 bpm, respectively. However, RR at day 0 (21.94 ± 1.70 brpm) was significantly lower ($P < 0.05$) than RR at day 6 (25.44 ± 4.72 brpm), day 9 (26.39 ± 5.57 brpm) and day 12 (27.89 ± 6.57 brpm) but was not significantly different ($P > 0.05$) from day 3 (24.61 ± 4.65 brpm).

Effects of environmental stress on the physiological parameters of Nigerian Indigenous breeds of goat according to their sex are shown in Table 5. There was no significant influence of environmental stress on the physiological responses observed according to sex of the various breeds monitored. The BST ranged from 37.15 ± 2.36 in Sahel male to 38.17 in WAD male while RT ranged from 39.61 ± 0.94 in Sahel male goats to 39.58 ± 1.40 in Red Sokoto goats.

Also, PR ranged from 67.13 ± 21.76 in Red Sokoto male goats to 73.20 ± 26.88 in WAD male goats while for RR the value ranged from 23.67 ± 4.13 in Red Sokoto male goats to 25.00 ± 5.4 in WAD male goats. However, for females BST ranged from 38.38 ± 2.42 in WAD female goats to 38.22 ± 1.99 and 38.06 ± 1.83 in Sahel and Red Sokoto female goats, respectively. The RT ranged from 39.39 ± 1.53 in Red Sokoto female goats to 39.91 ± 1.52 in WAD female goats and PR was from 70.40 ± 22.84 in Red Sokoto female goats to 76.73 ± 27.12 in WAD female goats while it was within 25.47 ± 6.13 for RR.

The heat shock protein (HSP) responses to environmental stress in Nigerian goat breeds according to breed, exposure duration, and sex are shown in Table 6. Red Sokoto goats had the highest HSP level (30.08 ± 0.75 ng/mL), followed by WAD goats (22.66 ± 0.53 ng/mL) and Sahel goats (18.01 ± 0.55 ng/mL). Duration of exposure showed that goats exposed 4 hours (30.08 ± 0.75 ng/mL) of daily environmental stress had the highest HSP level followed by those exposed to 8 hours (24.83 ± 0.52 ng/mL) while the least HSP level was obtained in goats with no exposure (15.24 ± 0.53 ng/mL) to environmental stress. For the days of exposure, HSP level of the goats at day 0 (22.06 ± 0.53 ng/mL) was relatively lower than 24.08 ± 0.71 ng/mL obtained at day 12 while male goats (35.34 ± 0.50) had higher HSP level compared to female goats (14.49 ± 0.44) in this study.

Table 2 - Effects of breed on the physiological parameters of Nigerian Indigenous breeds of goat exposed to environmental stress

Parameters	Sahel	Red Sokoto	WAD
Body surface temperature (°C)	37.69 ± 2.21	38.32 ± 1.93	38.63 ± 2.38
Rectal temperature (°C)	39.57 ± 1.14	39.58 ± 1.44	39.84 ± 1.42
Pulse rate (bpm)	74.73 ± 22.29	73.13 ± 24.89	77.23 ± 26.23
Respiratory rate (bpm)	25.70 ± 5.63	24.83 ± 5.01	25.23 ± 5.09

^{abc} values along the same row with different superscripts are significantly different ($P < 0.05$). WAD = West African Dwarf

Table 3 - Effects of hours of exposure to environmental stress on the physiological parameters of Nigerian Indigenous breeds of goat across the treatments

Parameters	T1	T2	T3
Body surface temperature (°C)	36.79 ± 0.53^c	38.35 ± 2.38^b	39.50 ± 2.23^a
Rectal temperature (°C)	38.84 ± 0.53^b	39.93 ± 1.48^a	40.21 ± 1.38^a
Pulse rate (bpm)	53.70 ± 2.67^c	77.90 ± 15.63^b	93.50 ± 27.01^a
Respiratory rate (bpm)	21.00 ± 1.14^c	24.90 ± 2.48^b	29.87 ± 5.90^a

^{abc} values along the same row with different superscripts are significantly different ($P < 0.05$). T1 = 0, T2 = 4, T3 = 8 (hours)

Table 4 - Pooled effects of days of exposure to environmental stress on physiological response in the Nigerian Indigenous breeds of goat

Parameters \ Day	0	3	6	9	12
Body surface temperature (°C)	36.57 ± 0.57^b	37.38 ± 1.47^b	39.21 ± 2.36^a	38.79 ± 2.06^a	39.12 ± 2.65^a
Rectal temperature (°C)	38.35 ± 0.61^d	39.32 ± 1.03^c	40.27 ± 1.24^{ab}	39.68 ± 1.04^b	40.68 ± 1.35^a
Pulse rate (bpm)	53.78 ± 1.59^b	74.56 ± 24.37^a	78.94 ± 25.57^a	82.83 ± 23.62^a	85.06 ± 24.88^a
Respiratory rate (bpm)	21.94 ± 1.70^b	24.61 ± 4.65^{ab}	25.44 ± 4.72^a	26.39 ± 5.57^a	27.89 ± 6.57^a

^{abc} values along the same row with different superscripts are significantly different ($P < 0.05$) 226

Table 5 - Effects of breed and sex on the physiological parameters of Nigerian Indigenous breeds of goat exposed to environmental stress

Parameter	Sahel		Red Sokoto		WAD	
	Male	Female	Male	Female	Male	Female
Body temperature (°C)	37.15 ± 2.36	38.22 ± 1.99	37.66 ± 1.46	38.06 ± 1.83	38.17 ± 2.09	38.38 ± 2.42
Rectal temperature (°C)	39.61 ± 0.94	39.52 ± 1.34	39.32 ± 1.46	39.39 ± 1.53	39.58 ± 1.40	39.91 ± 1.52
Pulse rate (bpm)	74.13 ± 20.72	75.33 ± 24.48	67.13 ± 21.76	70.40 ± 22.84	73.20 ± 26.88	76.73 ± 27.12
Respiratory rate (bpm)	25.93 ± 5.30	25.47 ± 6.13	23.67 ± 4.13	24.47 ± 4.85	25.00 ± 5.45	24.73 ± 5.16

^{abc} values along the same row with different superscripts are significantly different ($P < 0.05$)

Table 6 - Heat shock Protein in Nigerian indigenous breeds of goats exposed to environmental stress

Variables		HSP (ng/mL)
Goat breed	Sahel	18.01 ± 0.55 ^b
	Red Sokoto	30.08 ± 0.75 ^a
	WAD	22.66 ± 0.53 ^b
Period of exposure (hrs)	0	15.24 ± 0.53 ^c
	4	30.08 ± 0.75 ^a
	8	24.83 ± 0.52 ^b
Days of exposure	0	22.06 ± 0.53
	12	24.08 ± 0.71
Sex	Male	35.34 ± 0.50 ^a
	Female	14.49 ± 0.44 ^b

^{abc} values along the same row with different superscripts are significantly different (P < 0.05)

DISCUSSION

Relatively higher temperature and low relative humidity were recorded in the afternoon compared to other periods of the day during the trial while average environmental temperature of 29.86 °C and relative humidity of 73.57% were recorded for the study period. The high average temperature, and low RH values recorded during the mid-day compared to other period of the days were indication of stressful conditions livestock generally are exposed to in tropical climates. Although goats are able to thrive within relative humidity of 60- 80%, however when coupled with high temperature, the resultant effect is discomfort in animals. The comfort or thermo-neutral zones in animals is the range of temperature under which an animal can thrive without distress (Adedeji and Ogunwole, 2017). Mean average temperature recorded in this study was higher than the thermo-neutral zone of 12 – 27 °C adjudged comfortable for goats (Mason and Rushen, 2006; Helal et al., 2010). From our study, high mean average temperature in addition to high mean average relative humidity recorded in this study suggest that the goats were exposed to heat stress. Reports by Bhawa et al. (2023) and Onagbesan et al. (2023) have shown that an increase in environmental temperature with a concurrent increase in relative humidity constitutes stress.

Also, high average THI were recorded in the afternoon compared to the other periods of the day while the mean average THI recorded for the period were higher than those recorded either in the morning or evening. Temperature Humidity Index is a metric used to evaluate the level of environmental discomfort and heat stress experienced by animals. Zhou et al. (2023) in their study noted that a THI of 74.80 – 76.51 is the comfortable threshold for goats while a THI of 85.24 is the maximum tolerable limit in goats. Also, according to Helal et al. (2010), a temperature-humidity index of ≤ 74 is regarded as normal, 75 - 78 = alert level, 79 - 83 = danger, and ≥ 84 is emergency. Going by the study of Zhou et al. (2023), THI below 74.8 observed in the evening was below the thermoneutral zone for goats which may be the onset of cold stress. This when coupled with the lower average temperature recorded in the evening period may also suggest that goats experience less stress in the evening compared to other periods of the day while in the noon, goats are in a high state of distress. Nevertheless, mean average THI recorded in this study surpassed the comfort threshold for goats which is suggestive of stress. This result is consistent with reports in literature (Salama et al., 2013; Das et al., 2025). For instance, a study by Salama et al. (2014) suggested that THI above 75 is indicative of stress in goats while recent findings by Das (2025) noted that temperatures of 38°C and THI above 75, predispose goats to thermal discomfort. According to Gupta and Mondal (2021) in a study on heat stress and thermoregulatory responses in goat, the authors noted that heat stress triggers a variety of complex reactions which are essential for cell survival. Elevated heart and/or pulse rate, respiration rate, and rectal temperature are frequently employed in this case as markers of physiological adaptation to heat stress in small ruminant (Danso et al., 2024). Body temperature/rectal temperature have been reported to be directly influenced by ambient or environmental temperature (Ayo et al., 1999; Tucker et al., 2023). Body surface temperature recorded in this study was higher than 34.3 ± 0.2 - 36.8 ± 0.4 °C reported by Adeiza et al. (2024) in Red Sokoto goats. The observed variation in the results might be due to geographical location and different mean average temperature of 25.6 ± 0.26 °C recorded in their study compared to 29.8 °C in this study. Similarly, values reported by Habibu et al. (2021) in Sahel, Red Sokoto and WAD goats exposed to harmattan season in Nigeria were lower than those obtained in this study. This is due to the variations in seasonal environmental conditions in which the goats from the two studies were exposed. The RT of 38.58 – 40.35, 39.23 – 41.52, and 38.95 – 40.13 °C in Sahel, WAD and, Red Sokoto goats respectively reported by Oguntade et al. (2024) were within the range observed in this study and also within 39 - 40 °C specified in literature (Ayo et al., 1999; Minka and Ayo, 2013) as normal in goats. When the ambient temperature increases beyond the threshold level, there is a consequent increase in core body temperature (Das, 2025). Goats like other homeotherms constantly attempt to maintain their core body temperature around 39 °C. This they achieve

by transferring heat load from the core body to the periphery like the rectum (Battini et al., 2015a, 2015b). According to Sophia et al. (2016), native breeds evolve special behavioral and physiological responses to deal with environmental challenges, such as heat stress. Perhaps this may be the reason; the physiological indices of the Nigerian indigenous breed of goats were similar. Also, comparable genetic composition among native breeds and their ability to adapt to the hot-humid climate of southern Nigeria may also be the reason there was no notable variations in physiological indices monitored (Caroprese et al., 2015). This observation is contrary to reports elsewhere on similarity in rectal temperatures in indigenous goat breeds (Das, 2025).

Banerjee et al. (2015) observed differences in RT in among breeds of Indian goats. Nevertheless, RR in this study was lower than 47.98 and 51.20 reported by Shittu et al. (2023) in WAD and Kalahari Red raised in Southwest Nigeria while PR in this study were higher than 60.2 and 61.5 bpm in their study. Oseni and Ajayi (2014) noted that native goats in Nigeria had developed adaptive mechanism through higher respiration rates and sweat gland activity to control body temperature. This study found that hours of exposure to heat stress resulted in elevated physiological responses as goats exposed to T3 had the highest indices, thus suggesting that beyond elevated environmental temperature, the extent of time spent outdoor also determines the level of stress they are exposed to. In support of these findings, other authors have also found a direct relationship between exposure to prolonged period of sunshine and higher physiological indices (Garba et al., 2024; Deori et al., 2024) in goats. For example, in a study conducted by Aleena et al. (2018), in Osmanabadi, Malabari and Salem Black goat breeds of Southern India, and by Dangi et al. (2015) in Barbari goats of Northern India, the authors noted that there were significant differences in RT of goats exposed to 0, 4 and, 8 hours of heat stress. In addition, further observation from Naga et al. (2021) showed notable alterations in physiological markers from Day 3 which may also suggest that heat stress in native goats from Nigeria begins within this period. Becker and Stone (2020) in their study noted that body surface temperature was directly correlated with ambient solar radiation level, thus implying that length of exposure to solar radiation will increase body temperature. Elevated RT for instance has been identified as a reliable predictor of stress in livestock (Fadare et al., 2012), therefore high RT in goats placed in T3 compared to those housed indoor and in T2 suggest heat stress. These values are comparable to those found in heat-stressed WAD sheep by Fadare et al. (2012). Respiration rate is also used as a measure of heat stress to assess the negative impacts of ambient temperature (Al-Haidary et al., 2021). According to Okoruwa (2014), respiratory rate exceeding 12 to 20 breaths per minute is a sign of heat stress. This further buttressed the narrative that length of exposure of goats to heat/ solar radiation increases their RR as evident in goats subjected to T2 and T3 compared to T1 that had normal RR.

Also, it was observed that the physiological parameters monitored initially increased with days of exposure to environmental stress and then remain constant at certain periods. This observation may suggest that as the goats were stressed, they responded with increment in these physiological indices up to the point when they begin to adapt to the stressor thus resulting to similar values observed within some period. Although the effect of breed and sex were not significant on physiological response in Nigerian indigenous goats, there seems to be sex specific variations with relative reduction in these responses in male breeds compared to the females. This observation is supported by Timveh et al. (2022) who also reported no significant influence of breed and sex on these traits. Contrarily, Oguntade et al. (2024) in a study on the genetic variation in indigenous breeds of goats in Nigeria noted that there were significant variations in the thermotolerant traits monitored. Also, Mallam et al. (2025) in their study revealed that breed $\times$ sex significantly influenced rectal temperature (RT) as WAD male and Red Sokoto male had higher RT than the female counterparts. However, in their study, breed and sex did not influence PR, and RR. Observations of relative increase in responses from female goats in this study found support from previous works (Akinyemi et al., 2019; Sejian et al., 2019). Adeyemi et al. (2019) in their study noted that RT, PR and RR in female sheep were higher than in the male sheep while Sejian et al. (2019) also observed elevated RR and RT in female sheep compared to male sheep in their study. Differences noted in physiological responses due to sex by these authors were attributed to many probable factors. Oseni et al. (2017) noted that hormonal changes and reproductive demands in females may have resulted to such variations. Also, lower cortisol levels in bucks, scrotal temperature regulation, and the size and structure of male bodies may have contributed to less stress experienced in bucks (Perumal et al., 2022).

Heat shock protein 70 (Hsp70), is a highly conserved protein, induced in organisms by elevated temperatures (Ross et al., 2003). They are important to thermal stress adaptation (Kumar et al., 2018) and finds expression in cells when animals are exposed to high environmental temperature. Red Sokoto goats had the highest HSP levels among the indigenous breeds, suggesting experience or respond more to stress than WAD and Sahel goats. This however did not translate to higher physiological indices as observed in Table 2. This may probably be that the Red Sokoto breed though highly stressed as observed from the HSP expression than its contemporary (Sahel goats) with similar origin from the northern part of Nigeria was able to develop better adaptive mechanism in coping with stress. According to Akinyemi et al. (2019), high HSP production in Red Sokoto may imply that the breed is more sensitive to stressors or engage a stronger protective response through HSP

production. For period of exposure, the observed difference in the secretion of HSP during the hours of exposure is consistent with report of increase in the expression of HSP when animals are exposed to hot conditions (Aleena et al. 2018; Syafiqa et al., 2023; Tella et al., 2024). The high HSP expression at 4-hour period of exposure coincides with the peak of heat exposure for the goats. This confirms the reason HSP level was elevated at this period due to increase in ambient temperature associated with the noon. Hours of exposure beyond the noon, HSP levels begin to drop suggesting that there is a threshold period during which the goats experience the maximum stress, after which the HSP response stabilizes. This finding is consistent with studies on livestock stress responses, which often peak in the early exposure period and stabilizes as the animals acclimate. Earlier report suggests that HSP showed higher expression levels during heat stress in goats (Dangi et al., 2015), in order to protect cells from heat stress (Hassan et al., 2019). HSP expression at days 0 and 8 were similar. This may suggest that the goats were able to acclimatize better over this period. Experiments conducted in cattle showed that HSP expression under chronic heat stress showed 2 peaks on the 2nd and 17th day of heat stress which was similar to the observation from this study. Although male goats had relatively lower responses to heat stress compared to the females, HSP expression in males however was significantly higher than in the females. High HSP genes in the male serum dictates the extent of expression of stress specific genes in the male goats implying that they were more stressed. However, the relatively lower physiological responses in the males may be due to their more effective adaptive mechanism developed in mitigating heat stress.

CONCLUSION AND RECOMMENDATION

This study showed that breed had no influence on physiological responses in goats while 8 hours duration of heat exposure, 6-12 days of exposure, and sex influenced physiological response. Also, breed, duration of exposure to heat, and sex influenced HSP70 expression in Nigerian indigenous goat breeds. Future studies should investigate how breed-specific HSP70 variations in WAD, Red Sokoto, and Sahel goats correlate with genetic polymorphisms, endocrine profiles, and long-term production traits. Additionally, research should transition to longitudinal seasonal field trials to evaluate the efficacy of sex- and breed-specific management and nutritional interventions against chronic thermal stress.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to mathew.ayoola@bowen.edu.ng; ORCID: 0000-0003-2511-308X.

Ethical approval

The authors confirm that the research reported herein conforms to the ethical policies of the journal, as noted on the journal's author guidelines page. The methods/procedures used in this study were in concomitant with those outlined in the Animals ARRIVE guidelines and were carried out by the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines; EU Directive 2010/63/EU for animal experiments; or the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The study was conducted with the approval of the Bowen University Ethics Committee with reference number BUREC/COAES/AGR/0015

Authors' contributions

M.O. AYoola and D.O. FATOKI contributed to conception and design of study; D.O. FATOKI and G.A. BERENA contributed towards acquisition of data, analysis and interpretation of data; D.O. FATOKI was involved in drafting the manuscript G.A. BERENA revised it critically for important intellectual content; M.O. AYoola supervised the conduct of the research; All authors read and agreed to the version to be published.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

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Competing interest

The authors declare no conflict of interest.

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POLYMORPHISM OF *POU1F1* GENES AND ITS RELATIONSHIP WITH GROWTH TRAITS OF BACH THAO GOAT BREED

Nguyen Duc DIEN¹ , Ngo Thi Kim CHI¹ , Van Tien DUNG¹ , Nguyen Hoang THINH² , Do Duc LUC² , and Pham The HUE¹  

¹ Tay Nguyen University, Dak Lak, Viet Nam

² Vietnam National University of Agriculture, Hanoi, Vietnam

Email: huephamthe58608587@gmail.com

Supporting Information



ABSTRACT: This study aimed to evaluate the polymorphism of the *POU1F1* gene and its association with body weight and weight gain in Bach Thao goats. A total of 110 animals (64 males and 46 females) were used, and ear tissue samples were collected for DNA extraction and PCR-RFLP analysis to determine *POU1F1* genotypes. Allele and genotype frequencies were subsequently calculated. Three genotypes (TT, TC, and CC) were identified, with the T allele and TT genotype showing the highest frequencies. Body weight at birth, and at 3, 6, and 9 months of age, as well as average daily gain (ADG) during the corresponding growth period were recorded. Goats with the TT genotype had significant higher body weight at birth, and at 3, 6, and 9 months of age, together with higher ADG during the corresponding period, than those with the CC genotypes ($P < 0.05$). These findings indicate that *POU1F1* polymorphism is associated with growth performance in Bach Thao goats and may have potential as a molecular marker for marker-assisted selection to improve growth traits in this breed.

Keywords: *POU1F1*, Bach Thao goat, Weight, Gene polymorphism, PCR-RFLP.

INTRODUCTION

The Bach Thao goat, an indigenous goat breed of Vietnam with good adaptability to local production conditions, characterized by its ability to tolerate relatively low-quality diets, maintain growth under adverse climatic conditions, and adapt well to the local natural environment. In addition to its adaptability, Bach Thao goats are valued for their meat quality, high lean meat proportion, nutritional value, and relatively low cholesterol content, making them a potentially important genetic resource for sustainable goat production (Van Binh et al., 2017; Thuan et al., 2021). Therefore, improving growth performance while maintaining the adaptive characteristics of this breed is an important consideration for increasing production efficiency.

Growth performance is influenced by genetic and environmental factors, and identifying genes associated with growth traits can support genetic improvement. The *POU1F1* gene, which encodes the pituitary-specific transcription factor PIT-1, plays an important role in pituitary development and the regulation of growth, milk production, and thyroid hormone secretion (Cohen et al., 1996; Herman et al., 2012). Genetic variation in *POU1F1* may therefore contribute to phenotypic differences in growth and production traits. In goats, Işık and Bilgen (2019) reported associations between *POU1F1* variants and birth weight and growth parameters in Saanen goats. However, the effects of genetic polymorphisms may vary with breed, genetic background, and environmental conditions, indicating that findings from one goat population may not be directly applicable to others. Further investigation of *POU1F1* polymorphisms in locally adapted breeds, such as Bach Thao goats, is therefore warranted.

Several studies have reported associations between *POU1F1* polymorphisms and economically important traits in goats (Aboelenin et al., 2022; Pawar et al., 2024). These polymorphisms have been associated with milk yield and quality (Gündüz and Biçer, 2023), growth characteristics (Lan et al., 2007b), and body-size measurements in Jining gray goats (Feng et al., 2012). Li et al. (2016) further reported associations between polymorphisms in the *POU1F1-PROX1-PITX1-SIX3* gene pathway and growth traits in Guangzhou dairy and Hainan black goats. Similarly, Shaikh (2022) found that SNP rs670830003 significantly affected weaning weight and body weight gain, with heterozygous (TG) goats showing the highest values. More recently, Ayele et al. (2024) identified polymorphisms in the *GH*, *IGF-I*, and *PIT-I* genes associated with growth traits in Saanen, Alpine, and Boer goats. Collectively, these findings support the potential role of *POU1F1* and related growth-regulatory genes as candidate markers for improving growth performance in goats.

Despite these findings, information on the association between *POU1F1* polymorphisms and growth performance remains limited in indigenous and locally adapted goat breeds, including Bach Thao goats. In developing countries, limited systematic genetic selection highlights the need to identify genetic variants associated with economically

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important traits to support more effective breeding programs. Genetic markers associated with body weight and weight gain could facilitate the early identification of animals with superior growth potential and contribute to genetic improvement.

Based on the biological role of PIT-1 and previous evidence linking *POU1F1* polymorphisms with growth traits in goats, we hypothesized that *POU1F1* genetic variation is associated with body weight and weight gain in Bach Thao goats. Therefore, the objective of this study was to identify the polymorphism of *POU1F1* gene and its association with body weight and weight gain in Bach Thao goats.

MATERIALS AND METHODS

Ethical considerations

All animals in our study were conducted in accordance with the laws, guidelines of the relevant organizations and were approved by the Scientific Council of Tay Nguyen University under Decision No. B2024-TTN-03 and Contract No. 55/HĐ-ĐHTN of Tay Nguyen University dated 16 January 2024.

Animals and data collection

A total of 110 Bach Thao goats (64 males and 46 females), an indigenous goat breed of Vietnam, were included in the study. They were born to does aged 2–3 years, corresponding to parity 3–4, selected from five farms belonging to the Dak Lak Goat Cooperative in Dak Lak Province, Vietnam, and reared at their respective farms until 3 months of age. Thereafter, all goats were transferred to a single farm and reared together until the end of the experiment. From approximately 3 to 9 months of age, all experimental goats were maintained under the same housing and management conditions and offered the same total mixed ration (TMR) containing 13% crude protein. Each animal was identified by ear tag. Ear tissues were collected to analysis genotypes of *POU1F1* gene. The study parameters were body weight at birth (BWb) at 3 (BW3), 6 (BW6), 9 months of age (BW9), and average daily gain (ADG) at different growth stages. BWb was recorded within 24 hours from birth. BW3, BW6 and BW9 were determined using an electronic scale at 3, 6, and 9 months of age, respectively. ADG was calculated for the periods, including from birth to 3 months (ADG03), 3 to 6 months (ADG36), and 6 to 9 months (ADG69), and from birth to 9 months of age (ADG09).

DNA extraction

Ear tissue were collected from each goat using sterile ear forceps and preserved in 95% ethanol solution at 4 °C until DNA extraction. Total DNA was extracted from ear tissue samples using a modified phenol-chloroform method as described by Green and Sambrook (2012), with minor modifications to accommodate laboratory conditions. Tissue samples were digested with proteinase K, and the resulting DNA was purified by phenol-chloroform extraction followed by ethanol precipitation. DNA concentration and purity were quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), with samples showing an A260/A280 ratio between 1.8 and 2.0 and a concentration higher than 50 ng/μL considered acceptable for downstream analysis. DNA integrity was further evaluated by electrophoresis on a 1% agarose gel, in which good-quality DNA showed a single, clear band with no smearing, indicating that the DNA was intact and not degraded. Samples meeting both purity and integrity criteria were diluted to working concentrations and stored at -20 °C until PCR reaction.

Genotype Identification

The *POU1F1* gene was amplified using primers designed by Lan et al. (2007b): downstream 5'-AGGCTGTGAGGAAGTGAGAG-3' and reverse 5'-GGAGGAGATGAGGAGGAAAG-3', with an expected product size of approximately 450 bp. Genotyping was carried out by PCR-RFLP with *AluI* according to Lan et al. (2007a). Briefly, 20 μL of PCR product was digested with 10 U of *AluI* enzyme at 37 °C for 5 hours. The digestion DNA fragments were separated by electrophoresis on a 2.0% agarose gel and visualized under UV light. Based on the characteristic electrophoretic banding patterns, the goats were classified into three genotypes (TT, TC, and CC).

Statistical analysis

The Hardy-Weinberg equilibrium of *POU1F1* gene was performed by comparing the expected and observed genotype counts using chi-squared test (χ^2). Allele frequency was defined as the number of copies of a given allele divided by the total number of allele copies in the sampled population. For the two alleles T and C, the frequencies were calculated as follows:

$$p(T) = \frac{2N_{TT} + N_{TC}}{2N} ; \quad q(C) = \frac{2N_{CC} + N_{TC}}{2N}$$

where N_{TT} , N_{TC} , and N_{CC} are the numbers of individuals with the TT, TC, and CC genotypes, respectively; $N = N_{TT} + N_{TC} + N_{CC}$ is the total number of individuals analyzed; and $p(T) + q(C) = 1$.

Genotype frequencies were calculated as follows:

$$f(TT) = \frac{N_{TT}}{N}; \quad f(TC) = \frac{N_{TC}}{N}; \quad f(CC) = \frac{N_{CC}}{N}$$

The effect of *POU1F1* genotype on body weights and average daily gain was analyzed using the statistical model below: $y_{ijk} = \mu + G_i + S_j + \varepsilon_{ijk}$

where: y_{ijk} = study parameters (body weights and average daily gain), μ = overall mean, G_i = effect of genotype i ($i=3$: TT, TC and CC), S_j = effect of sex j ($j=2$: male and female), ε_{ijk} = residual error. The Tukey test was used for the pairwise comparison. A generalized linear model (GLM) was applied using Minitab 16.0 software, with statistical significance determined at $P < 0.05$. The statistical parameters in the tables are number of observation (n), arithmetic mean (mean) $\pm$ standard deviation (SD).

RESULT

Polymorphisms of *POU1F1*

Frequency results were obtained based on the *POU1F1* gene band pattern of exon 6 using the restriction enzyme *AluI*, as shown in Figure 1. PCR amplification of DNA extracted from goat ear tissue samples produced a single band specific to the gene location. The designed primers annealed at 37°C and generated a 450 bp amplification fragment using a specific primer combination, cut with *AluI* of the *POU1F1* gene in all samples PCR-RFLP *AluI* of the *POU1F1* gene. PCR-enhanced fragments cut with *AluI* showed three different restriction digestion patterns, indicating the presence of *POU1F1* gene variation in Bach Thao goats. PCR-RFLP *AluI* showed three genotypes, 'TT' (340 and 110 bp), 'CC' (216, 124 and 110 bp) and 'TC' (340, 216, 124 and 110 bp) (Figure 1).

Genetic variation, genotype and allele frequencies, and genetic diversity of genes belonging to the *POU1F1* pathway. Three expected genotypes (TT, TC, and CC) at *POU1F1* were detected in Bach Thao goat population (Table 1). The TT genotype was the most frequent (0.745), followed by TC (0.145) and CC (0.109). The frequencies of the T and C alleles were 0.818 and 0.182, respectively, with the CC genotype being the least frequent among the studied goats. The observed genotype distribution at the *POU1F1* locus deviated significantly from Hardy-Weinberg equilibrium at SNP6 ($\chi^2 = 28.69$, $P < 0.001$).

The effect of the *POU1F1* gene polymorphism on body weight at different growth of Bach Thao goats is presented in Table 2. Significant differences in body weight between *POU1F1* genotypes were observed at birth, 3 months, and 6 months of age ($P < 0.05$). Goats with the TT genotype had the highest body weight at all evaluated stages, followed by the TC genotype, while the CC genotype showed the lowest body weight values.

At birth, goats with the TT genotype had significantly higher body weight than those with the CC genotype, while individuals with the TC genotype showed intermediate values. A similar trend was observed at 3 and 6 months of age, suggesting a consistent effect of the *POU1F1* gene polymorphism on early growth performance. At 9 months of age, goats with the TT genotype showed significantly higher body weight than those with the CC genotype ($P < 0.05$).

The ADG of Bach Thao goats according to *POU1F1* genotype is presented in Table 3. Significant differences in ADG among genotypes were observed during the period from birth to 3 months and from 3 to 6 months of age ($P < 0.05$). Goats with the TT genotype showed the highest ADG during both periods, whereas those with the CC genotype exhibited the lowest values. Goat with the TC genotype generally showed intermediate ADG values.

During 6 to 9 month, there were no significant differences in ADG among *POU1F1* genotypes ($P > 0.05$), although goats with the TT genotype still tended to have higher ADG compared to those with other genotypes. These results suggest that the impact of the *POU1F1* polymorphism on growth performance is more pronounced during the early growth period.

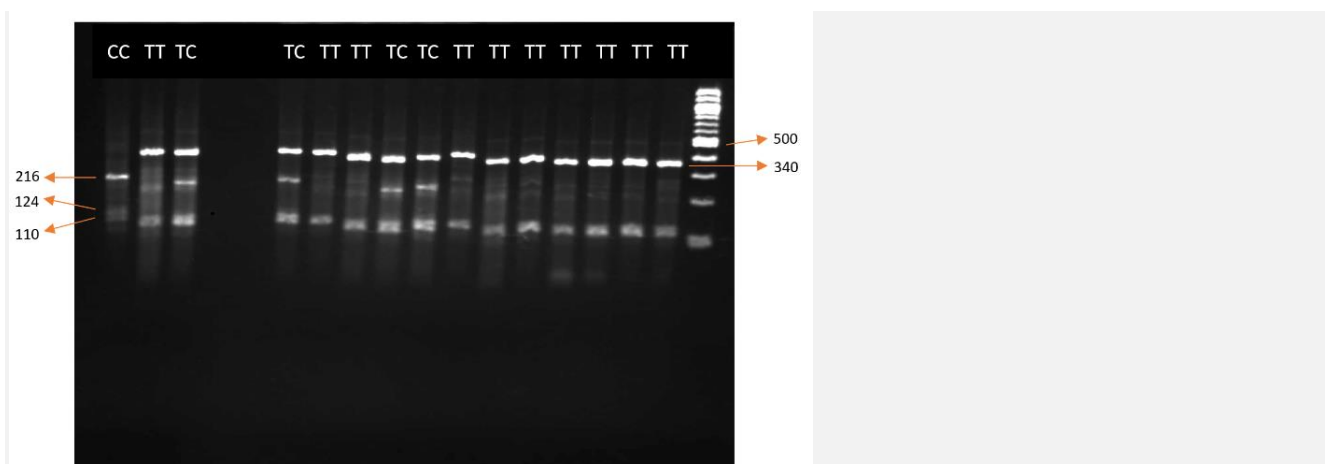


Figure 1 - Electrophoretic image of *POU1F1* products on a 2% agarose gel with *AluI* restriction enzyme. Genotype TT (340 and 110 bp); Genotype TC (340, 216, 124 and 110 bp); Genotype CC (216, 124 and 110 bp).

Table 1 - Genotypic and allelic frequencies of *POU1F1* gene

Item	Genotype				Allele		χ^2
	TT	TC	CC	Total	T	C	
Observed count	82	16	12	110			
Expected count	73.63	32.73	3.64				
Observed frequency	0.745	0.145	0.109	1	0.818	0.182	28.69
Expected frequency	0.669	0.297	0.034				

Table 2 - Body weight (kg) of Bach Thao goats at different ages according to *POU1F1* genotype

Trait	TT			TC			CC			P-value
	n	Mean	SD	n	Mean	SD	n	Mean	SD	
BWB	82	2.54 ^a	0.46	16	2.43 ^{ab}	0.34	12	2.20 ^b	0.49	0.046
BW3	79	12.42 ^a	1.05	16	11.96 ^{ab}	1.23	12	11.47 ^b	1.53	0.017
BW6	77	25.49 ^a	1.94	16	24.96 ^{ab}	1.50	12	23.82 ^b	2.71	0.024
BW9	67	33.94 ^a	2.61	13	32.39 ^{ab}	3.89	10	31.31 ^b	2.61	0.004

The means in the same row with different superscripts are significant different ($P < 0.05$)

Table 3 - Average daily gain of Bach Thao goats according to genotype *POU1F1* (g/day)

Trait	TT			TC			CC			P-value
	n	Mean	SD	n	Mean	SD	n	Mean	SD	
ADG03	79	109.80 ^a	11.94	16	105.96 ^a	12.71	12	103.01 ^b	115.16	0.031
ADG36	77	145.50 ^a	12.33	16	144.39 ^a	10.97	12	137.27 ^b	17.78	0.022
ADG69	67	95.70	4	13	85.30	27.9	10	91.33	22.80	0.118
ADG09	67	116.3 ^a	7.83	13	108.2 ^b	14.11	10	109.5 ^b	8.73	0.001

The means in the same row with different superscripts are significant different ($P < 0.05$)

DISCUSSION

The genotype distribution of the *POU1F1* gene in the Bach Thao goat population showed a significant departure from Hardy-Weinberg equilibrium at SNP6 ($P < 0.05$). Similar deviations from Hardy-Weinberg equilibrium have been reported for *POU1F1* polymorphisms in other goat populations. Ma et al. (2017) reported significant deviations at SNP3, SNP5, and SNP6 in Guanzhong goats and at SNP2 and SNP5 in Hainan black goats. Polymorphisms in the *POU1F1* gene have also been identified in indigenous Chinese goat breeds (Lan et al., 2007a), Sarda goats (Daga et al., 2013), and Osmanabadi goats (Pawar et al., 2024), indicating that genetic variation at this locus occurs across diverse goat populations.

The observed departure from Hardy-Weinberg equilibrium in the present study should be interpreted cautiously because several factors can contribute to deviations from the expected genotype distribution. These include non-random mating, population substructure, genetic drift, selection, and other demographic processes. Although the relatively high frequency of the TT genotype, which was also associated with superior growth performance in the present study, may be compatible with selection at or near the *POU1F1* locus, the present data are insufficient to establish directional selection as the cause of the observed deviation. In particular, information on pedigree structure, genetic relatedness, population stratification, and the history of sire use was not available. Therefore, further studies incorporating population genetic analyses, pedigree information, and a larger and more genetically structured sample are required to determine the underlying causes of the Hardy-Weinberg disequilibrium.

Despite this limitation, the association between *POU1F1* genotype and growth performance observed in the present study provides evidence supporting the potential role of this locus as a candidate molecular marker for growth-related traits in Bach Thao goats. However, the potential application of *POU1F1* in marker-assisted selection should be validated in independent populations and under different production environments before being incorporated into practical breeding programs.

The present study showed significant associations between *POU1F1* genotype and several growth traits in Bach Thao goats. Goats carrying the TT genotype generally showed higher body weights at birth, 3, 6, and 9 months of age, as well as higher ADG during the birth-to-3-month and 3-to-6-month periods, compared with goats carrying the TC and CC genotypes. The significant effects observed during the early growth stages are consistent with the biological role of *POU1F1*, which encodes the pituitary-specific transcription factor *PIT-1* and regulates the expression of growth-related hormones, including growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone β (TSH β) (Cohen et al., 1997; Hu et al., 2026).

Previous studies have also reported associations between *POU1F1* polymorphisms and growth-related traits in goats. Lan et al., (2007a, b) reported associations between *POU1F1* polymorphisms and weight-related traits in Chinese goats, while Nguyen et al. (2023) observed higher body weights in Nàn goats carrying the D1D1 genotype. Similarly, Dorjay and Abraham (2021) reported significant associations between *POU1F1* polymorphism and body weight in indigenous goat populations. These findings, together with the present results, support the potential relevance of *POU1F1* variation to growth performance in goats.

The association observed in the present study should not, however, be interpreted as evidence that the SNP6 variant itself is necessarily the causal mutation. The *POU1F1* gene is part of a broader regulatory network involving genes such as *PROP1*, *PITX1*, and *SIX3*, which have also been implicated in growth-related processes (Ma et al., 2017). Therefore, the observed genotype–phenotype association may reflect linkage disequilibrium between the investigated *POU1F1* variant and other functional variants within or near this regulatory pathway. Previous reports of associations involving the *POU1F1-PROP1-PITX1-SIX3* pathway (Ma et al., 2017) and variants in *GH*, *IGF-I*, and *PIT1* (Ayele et al., 2024) further indicate that growth is controlled by a complex genetic network rather than by a single locus. Because linkage disequilibrium and functional interactions among these loci were not evaluated in the present study, the biological mechanism underlying the association between the *POU1F1* SNP6 genotypes and growth performance remains to be determined.

The stronger genotype effects observed during early growth, particularly for ADG from birth to 3 months and from 3 to 6 months, may also reflect the greater contribution of genetically regulated endocrine processes during early development. In contrast, the absence of significant genotype differences in ADG from 6 to 9 months suggests that environmental and management-related factors may increasingly contribute to variation in growth as animals mature. Growth performance is influenced by multiple non-genetic factors, including nutrition, feed availability, health status, housing, environmental conditions, and management practices. Although the goats were transferred to a common farm at 3 months of age and subsequently maintained under the same management conditions, these factors were not included as separate covariates in the statistical models. Consequently, their potential contributions to the observed variation cannot be completely excluded. The observed association between *POU1F1* genotype and growth should therefore be interpreted as a genotype–phenotype association rather than as an isolated genetic effect independent of environmental influences. In addition, the relatively small number of goats carrying the CC genotype may have limited the statistical power and precision of comparisons involving this less frequent genotype. Also, the present study was conducted in a relatively small population from a single geographical region, and the genotype effect was evaluated within this specific population. Therefore, the results, particularly those involving the CC genotype, should be interpreted cautiously and validated in larger, independent Bach Thao goat populations. Future studies incorporating larger sample sizes, pedigree information, population structure, additional growth-related genes, and genome-wide or targeted linkage disequilibrium analyses would help clarify whether the *POU1F1* SNP6 variant itself contributes functionally to growth or serves primarily as a marker linked to causal genetic variation.

Overall, the present findings provide breed-specific evidence of an association between *POU1F1* polymorphism and growth performance in Bach Thao goats. The consistent performance of TT goats across several growth traits suggests that *POU1F1* may have potential as a candidate molecular marker for growth-related traits. However, further validation in larger and genetically diverse populations is necessary before this locus can be recommended for routine marker-assisted selection in Bach Thao goat breeding programs.

CONCLUSION

In conclusion, three *POU1F1* genotypes (TT, TC, and CC) were identified in Bach Thao goats, with the TT genotype being the most frequent. The *POU1F1* polymorphism was significantly associated with body weight and average daily gain at several growth stages, with TT goats consistently showing superior growth performance compared with TC and CC goats. These findings suggest that the *POU1F1* gene polymorphism is a potential candidate for use as a molecular marker in breeding to improve the growth performance of Bach Thao goats.

DECLARATIONS

Corresponding author:

Correspondence and requests for materials should be addressed to Pham The HUE;
Email: huephamthe58608587@gmail.com; ORCID: <https://Orcid.org/009-0001-1600-0975>

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Author's contribution statement

All authors had equal involvement in the design, experimentation, analysis, and manuscript writing.

Ethical approval

All experimental procedures involving animals were performed in accordance with the guiding principles for the use of animals in scientific research and with relevant animal welfare regulations under supervision of the Scientific Council of Tay Nguyen University.

Availability of data and materials

Available and can be provided upon journal request.

The sustainable development goals mentioned

Eradicating hunger, creating decent jobs, and promoting economic growth.

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Competing interests

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

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POTENTIAL RISK FACTORS ASSOCIATED WITH LARGE INTESTINAL IMPACTION IN WORKING DONKEYS AT EGYPT

Wafi AL-ANTAIF¹ , Mohamed MARZOK¹ , Yaser HAMAD² , Adel ALMUBARAK¹ , and Sabry EL-KHODERY³ 

¹By-product Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, 31982, Saudi Arabia

²Department of Internal Medicine, Faculty of Veterinary Medicine, Omar Al-mukhtar University, Libya

³Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

✉Emails: mmarzok@kfu.edu.sa; mmarzok1968@gmail.com

➤Supporting Information



ABSTRACT: Large intestinal impaction is a principal cause of abdominal pain in donkeys, associated with high morbidity and mortality. The objective of this study was to investigate potential risk factors associated with large intestinal impaction in working donkeys in northern Egypt. A case-control study was conducted on 75 donkeys (4–12 years, weighing 150–290 kg) between September 2020 and August 2024. Clinical, parasitological, and biochemical examinations were performed. Logistic regression was applied to identify independent predictors of impaction. Fifty-three donkeys were diagnosed with large intestinal impaction, confirmed by clinical signs and elevated serum triglycerides (224.6 ± 36.0 vs. 196.0 ± 23.0 mg/dl, $P < 0.05$). Significant risk factors included older age (>10 years; OR = 16.8, 95% CI: 8.17–33.64, $P = 0.001$), female sex ($P < 0.05$), poor body condition ($P < 0.01$), heavy workload ($P < 0.05$), dental disease ($P < 0.05$), orthopedic problems ($P < 0.05$), and feeding rice husk (OR = 39.5, 95% CI: 1.1–14.13, $P = 0.044$). Seasonal variation also increased risk (OR = 5.8, 95% CI: 1.2–27.45, $P = 0.026$). Clinically, constipation (OR = 277.8, $P < 0.0001$), anorexia (OR = 92.16, $P < 0.0001$), and absent gut sounds (OR = 61.36, $P < 0.0001$) were the strongest diagnostic indicators. Poor nutritional management, particularly rice husk feeding, advanced age, and seasonal variations are key predictors of large intestinal impaction in working donkeys in northern Egypt. Clinical vigilance, especially in geriatric animals, is essential to reduce morbidity and improve welfare.

Keywords: Colic; Epidemiology; Equine; Forages; Risk factors

INTRODUCTION

Large intestinal impaction is the dominant cause of colic in donkeys in several settings and is associated with high mortality. At a large UK sanctuary, impaction colic constituted ~55% of all colic episodes; older age, extra rations, prior colic, low weight, musculoskeletal and dental disease, and farm of origin were the significant risk factors (Cox et al., 2007). Another UK case-control study in over 2000 donkeys found increased risk with paper bedding, concentrates, reduced pasture, more careers, weight loss, recent vaccination, and dental pathology (Cox et al., 2009). Similarly, it was noted that musculoskeletal problems may reduce access to water, and cite work showing water deprivation increases large colon impaction risk in horses (Reeves et al., 1996). At Ethiopian clinics, 78.6% of donkey colic was impaction colic, strongly associated with feed change, poor-quality/coarse feed, excessive feeding without water, parasites, heavy work, age, and dental abnormalities (Tadesse and Abera, 2018).

In mixed equid cohorts, large colon impaction and cecal bloat were often attributed to erroneous feeding management (Miranda et al., 2015). In horses, it was found that increased stabling, reduced exercise, dietary changes and hay feeding are linked to large colon impaction/volvulus, paralleling donkey risk patterns (Hallowell, 2017; Giusto et al., 2021; Mathewos et al., 2021). In Brazilian equids including donkeys, feeding low-quality chopped tropical forages markedly increased colic risk (Pessoa et al., 2012). Another equine reviews and prospective studies identified high concentrate intake, low hygienic quality hay, and frequent diet changes as important colic risks (Gonçalves et al., 2002; Kaya et al., 2009; Curtis et al., 2019;).

Recent studies show that donkeys digest acid detergent fiber (ADF) efficiently, but poor quality, high neutral detergent fiber (NDF) have the lowest digestibility (Liu et al., 2020a,b and Yang et al., 2020). Thus NDF, grain rich, low fiber diets increase the risk of colic and other disorders (Liu et al., 2020a,b). In addition, when poorly digestible and coarse fibers are fed without adequate water before/after work, the risk of impaction colic increases (Tadesse and Abera, 2018).

Seasonal incidence of impaction colic indicated that the dry season increases the risk of occurrence (Pessoa et al., 2012). In working horse populations, limited access to water and the summer/dry season substantially increase the incidence of colic, including impaction (Pessoa et al., 2012; Fikri et al., 2024).

In Egypt, limited studies have been conducted on the clinical and clinicopathological features of large intestinal impaction in donkeys (Attia et al., 2018; Bohassan et al., 2025). However, the epidemiological studies on such disease conditions are scarce. Therefore, the objective of this case-control study was to investigate the risk factors associated with large intestinal impaction in donkeys at Dakahlia governorate, Egypt.

MATERIALS AND METHODS

Donkeys

A total of 75 donkeys (*Equus asinus*) were included in a case-control study. The age of the examined donkeys ranged from 4-12 years, and their weight ranged from 150-290 kg. This study was carried out from September 2020 to August 2024. As this was a case-control study, the source of diseased donkeys was admitted cases at the Veterinary Teaching Hospital of Mansoura University, cases referred by veterinarians, and cases identified through calls from owners requesting examination of their donkeys. Control donkeys were selected randomly after identifying donkeys and determining their distribution across the administrative localities of Dakahlia Governorate. The case and control donkeys were finally selected based on the clinical criteria accompanied by elevated triglyceride levels (> 200mg/dl).

Study area

The present study was carried out in Dakahlia Governorate, which is located in the northern part of Egypt, and was selected for this epidemiological investigation. Dakahlia Governorate is located northeast of Cairo. Its area is approximately 3,500 square kilometers (1,400 mi²).

Questionnaire

A questionnaire was constructed to include data concerned with age, sex, feeding practice, management system, and presence of other diseases. The hypothesized risk factors were categorized and fully described (Table 1). The construction of the questionnaire was completed before initiation of the study based on the knowledge about the disease in previous reports and the corresponding management practice in the study area. The questionnaire was approved by all authors, and two authors were responsible for data curation.

Table 1 - Categorization and description of the proposed risk factors associated with large intestinal impaction in working donkeys

Factor	Level
Age	1-5 years =1; >5-10 years=2; > 10 years=3
Season	Winter = 1; Spring = 2; Summer = 3; Fall = 4
Sex	Male = 1; Female = 2
Type of roughage	Wheat hay = 0; Horse bean hay = 1
Feeding on green fodders	Feed on green fodder = 1; Doesn't feed = 0
Presence of gastrointestinal parasites	No = 0; Yes = 1
Dental diseases	Present = 1; Absent = 0
Previous colic	Present = 1; Absent = 0
Work (heavy load)	light = 0; Hard = 1
Body condition	>2 (Good) = 1; ≤ Poor = 0
Adding rice husk to feed stuff	No = 0; Yes = 1
Access to water per day	One time = 0; Free access= 1
Orthopedic diseases	Present = 0; Absent = 1

Clinical examination

Competent clinical history and physical examination of each donkey under investigation was carried out according to the previous standard protocols to reach the correct diagnosis (Constable et al., 2016). Objective of the study was to specify the large intestinal impaction via rectal examination and localization of the abnormalities. Impaction of the colon and or cecum was the final inclusion criteria for the clinical cases. The clinical examination of all cases was conducted by the same author to avoid subjective variation in the clinical findings.

Parasitological examination

Fecal examination was conducted to explore gastrointestinal nematodes following the standard techniques. Briefly, a thorough gross inspection was performed on each sample to identify the parasite. Meanwhile, the same sample was subjected to examination by microscopy to identify parasite stages. A portion of every fecal sample was subjected to conventional concentration floatation and sedimentation procedures (Soulsby, 1982).

Blood samples

Blood samples were collected from the jugular vein aseptically using disposable needles and vacutainer tubes. Blood samples were kept on ice and transported directly to the laboratory. Approximately 5 ml of blood was collected into plain vacutainer tubes before treatment. Samples were centrifuged within 30 minutes of collection. Serum was stored at -80 °C until analysis. Triglycerides were measured at the Mansoura University Veterinary Clinical Laboratory using a commercial enzymatic assay (Spinreact, Spain) on a spectrophotometer. Triglycerides levels were measured according to the standard technique (Pongratz et al., 2016).

Statistical analysis

All data analyses were carried out using a commercial statistical software program (IBM, SPSS for Windows, Version 21.0, USA). Association between the occurrence of large intestinal impaction and the assumed risk factors were studied using logistic regression. Before starting logistic regression, descriptive statistics were applied to declare the frequency of occurrence of large intestinal impaction with frequency of associated risk factors. A bivariate logistic regression model was carried out. In this method, the dependent dichotomous variable was the status of the donkeys (with large intestinal impaction or without). But the independent variables were the postulated risk factors. Factors with significance at $P < 0.2$ were chosen for further multivariate logistic regression model. The results were each expressed as p-value and odds ratio (OR) with a 95% confidence interval (CI 95%). A chi-square (χ^2) of linear analysis was applied to identify the possible association between large intestinal impaction and the variables of clinical findings. When the variables had more than two categories, Chi-square of linear trend with Mantel-Haenszel test was used and the results were significant at $P < 0.05$.

RESULTS

A total of 53 donkeys diagnosed with large intestinal impaction were identified based on the clinical findings and evidence of associated hyperlipidemia. The diagnosis was based on clinical signs and an increase in serum triglyceride levels. The serum triglycerides level was significantly increased in donkeys with large intestinal impaction compared with controls (224.6 ± 36.0 vs. 196.0 ± 23.0 mg/dl, $P < 0.05$).

Analysis of risk factors showed that age, sex, diet composition, body condition, and workload were significantly associated with large intestinal impaction in donkeys. Donkeys older than 10 years were at markedly higher risk ($\chi^2 = 49.91$, $P < 0.001$). Females were more frequently affected than males ($P < 0.05$). Feeding rice husk ($P < 0.05$), poor-quality roughage ($P < 0.05$), dental disease ($P < 0.05$), orthopedic problems ($P < 0.05$), heavy work ($P < 0.05$), and poor body condition ($P < 0.01$) were all significant risk factors. Other factors such as season, previous colic, water access, and parasitic infection showed weaker or borderline associations (Table 2).

Table 2 - Classification of individual donkeys as affected with large intestinal impaction or normal in respect to different risk factors

Risk factor	Category	Impaction	None Impaction	P-value	Chi square
Age (Year)	< 5	3	20	< 0.001	49.91
	>5-10	7	2		
	> 10	43	0		
Sex	Male	17	13	0.03	4.72
	Female	36	9		
Season	Winter	7	4	0.17	4.69
	Spring	10	8		
	Summer	10	5		
	Fall	26	5		
Add rice husk to feed	No	17	18	0.012	6.38
	Add	36	8		
Roughage	Wheat hay (good)	19	14	0.027	4.87
	Horse bean hay (Bad)	34	8		
Green fodders	Yes	21	14	0.058	3.6
	No	32	8		
Dental Diseases	No	20	13	0.030	4.8
	Yes	33	9		
Orthopedic disease	No	20	14	0.040	4.2
	Yes	33	8		
Heavy work	No	17	15	0.012	6.38
	Yes	36	7		
Previous colic	No	21	14	0.058	3.6
	Yes	32	8		
Body condition	Good	18	15	0.007	7.38
	Poor	35	7		
Water	Frequent (Free access)	20	14	0.40	4.2
	Limited (once /day)	33	8		
Gastrointestinal parasites (Nematodes)	Negative	22	14	0.081	3.0
	Positive	31	8		

The final logistic regression model identified age, rice husk in diet, and season as independent predictors of impaction (Table 3). Older donkeys had an odds ratio (OR) of 16.8 (95% CI: 8.17–33.64, $P = 0.001$). Feeding rice husk increased the odds of impaction by 39.5 (95% CI: 1.1–14.13, $p=0.044$). Seasonal variation also played a role, with an OR of 5.8 (95% CI: 1.2–27.45, $P = 0.026$).

Clinically, Table 4 shows the association between large intestinal impaction and clinical signs. All affected donkeys showed depression (100%) and most exhibited abdominal distension (92.45%), anorexia (90.65%), constipation (94.33%), absent gut sounds (88.67%), tachypnea (79.24%), and tachycardia (81.13%). Constipation was the strongest diagnostic indicator (OR = 277.8, 95% CI: 49.15–1080, $P < 0.0001$), followed by anorexia (OR=92.16, 95% CI: 23.27–317.6, $P < 0.0001$) and absent gut sounds (OR = 61.36, 95% CI: 17.99–179.7, $P < 0.0001$). Mucous membrane changes were observed but not statistically significant ($P = 0.24$).

Table 3 - Final logistic regression model for the potential risk factors associated with large intestinal impaction in donkeys

Variable	β	S.E.	Wald	P-value	OR	CI at 95%
Age	5.111	1.536	11.078	0.001	16.8	8.17-33.64
Add rice husk to diet	3.677	1.825	4.060	0.044	39.52	1.1-14.13
Season	1.759	0.793	4.926	0.026	5.8	1.2-27.45
Constant	-18.607	6.506	8.179	0.004	0	–

B: Regression coefficient; S.E.: standard error; OR: Odds ratio; CI at 95%: Confidence interval

Table 4 - Clinical findings associated with large intestinal impaction in donkeys.

Clinical signs	Frequency (n=53)	%	P-value	OR	CI at 95%
Depression	53	100	< 0.0001		
Recumbency	43	81.13	< 0.0001	12.84	24.28-
Abdominal distension	49	92.45	<0.0001		
Appetite					
Anorexia	48	90.65	<0.0001	92.16	23.27 - 317.6
Inappetant	5	9.35			
Gut sound					
Absent	47	88.67	<0.0001	61.36	17.99 to 179.7
Present (weak)	6	11.33			
Defecation					
Constipation	50	94.33	<0.0001	277.8	49.15 - 1080
Normal fecal balls	3	5.67			
Respiratory rate (cycle/min)					
Tachypnea (23 ± 7)	42	79.24	0.001	10.44	4.187 - 25.41
Normal (14±4.0)	13	20.76			
Heart rate (Beat/min)					
Tachycardia (95 ± 8)	43	81.13	0.001	12.84	4.94 - 29.85
Normal (60 ± 11)	12	18.87			
Mucous membrane					
Pale	30	56.6	0.24	1.7	0.79 - 3.74
Brick red	23	33.4			

DISCUSSION

Large intestinal impaction in equids has received considerable attention because the disease adversely affects performance and may be life-threatening. Various investigations have indicated numerous risk factors associated with large intestinal impaction in donkeys worldwide (Cox et al., 2007; Worku et al., 2017). However, additional risk factors may be identified due to differences in management practices. Therefore, our investigation provides a case-control study of the factors associated with large intestinal impaction in donkeys in northern Egypt.

The final multivariate logistic regression model of this study identified age, rice husk supplementation, and season as independent predictors of large intestinal impaction in working donkeys in Dakahlia Governorate, Egypt. The strong effect of older age in this study agrees with earlier donkey studies from the UK and Egypt, where older animals were consistently at higher risk of impaction colic (Cox et al., 2007, 2009; Fikri et al., 2024). Age likely reflects several converging processes rather than a single mechanism. Older donkeys more often have chronic dental disease, lower body weight or poorer body condition, and reduced musculoskeletal function, all of which can impair feed comminution, reduce grazing or drinking access, and predispose animals to reduced gut motility (Constable et al., 2016). More generally, age-related reductions in

intestinal motility and barrier function have been demonstrated in other species, supporting the biological plausibility of increased impaction susceptibility in older working equids.

The addition of rice husk to the feed of donkeys showed a significant association with large intestinal impaction. Rice husk, a by-product of rice milling, is characterized by low crude protein (2–4%) and high lignocellulosic fiber (35–45%) with substantial silica content (15–22%), which severely limits its digestibility and nutritional value in animal feed (Vadiveloo et al., 2009; Jung et al., 2026). Its high fiber and silica content make it unsuitable for monogastric animals, while ruminants may utilize it only after chemical or biological treatment (Kuan et al., 2012). Reviews of dietary fiber in monogastric emphasize that physicochemical fiber traits (solubility, fermentability, and hydration) determine water holding, digesta consistency, transit, and microbial fermentation (Ermmers et al., 2023). Therefore, these compositional constraints make untreated rice husk unsuitable as a primary feedstuff, particularly for monogastric animals, due to poor palatability and potential gastrointestinal irritation (Tan and Norhaizan, 2020). Even in ruminants, its feeding value remains inferior to other rice by-products such as rice bran, which provides higher protein and energy levels (Jung et al., 2026).

Interestingly, no previous studies have reported beneficial effects of rice husk supplementation in monogastric animals. However, Chemical treatments have demonstrated improvements in digestibility by disrupting lignin and enhancing nitrogen availability for rumen microbes (Wei et al., 2025). Biological treatments have also shown promise in degrading lignin and increasing in vitro digestibility (Wang et al., 2020).

The final model retained season as an independent predictor, and autumn recorded the highest prevalence of the disease. This finding may be due to the combined effect of hard work, supplementation with poor-quality roughage and reduced water intake. Previous studies in working equids have shown higher colic frequency during hotter or drier periods, when water intake may be inadequate and available forage becomes drier or less digestible (Cox et al., 2009).

In semiarid equids including donkeys, colic frequency was higher in the dry season linked to poorer forage, more concentrate feeding, and more foreign-body exposure (Pessoa et al., 2012). In UK donkeys, limited pasture access and concentrate feeding were associated with higher impaction risk (Cox et al., 2009). Interestingly, normal horses show seasonal microbiota shifts associated with season forage supplementation, and weather, which may provide plausible gut-level pathway (Salem et al., 2018).

Although the final multivariate model included only three potential factors, univariate analysis showed several predictors including poor-quality roughage, dental disease, orthopedic problems, heavy work, poor body condition, and female sex. This finding is consistent with previous studies (Pessoa et al., 2012; Blikslager, 2019). The associations with dental disease, orthopedic problems, heavy work, and poor body condition are also plausible and align with previous evidence, even though they did not persist in the final model. Dental disease has repeatedly been associated with donkey impaction colic in UK populations and has also been identified as a colic risk marker in working horses (Fikri et al., 2023).

Orthopedic or musculoskeletal problems may reduce exercise regularity or hinder access to water and feed, while heavy work can exacerbate dehydration and feeding irregularity in working animals (Hassen et al., 2022). Poor body condition is especially notable because it is linked not only to colic risk but also to parasitic infestation and poorer general management in donkeys (Beriso et al., 2023; Kebede et al., 2024).

Parasitism showed only a weak or borderline association with impaction in this study. Unfortunately, in the present study, gastrointestinal parasite infection was assessed only as positive or negative. Therefore, that result should be interpreted cautiously rather than dismissed, because gastrointestinal parasites are extremely common in working donkeys across Africa, with reported prevalence ranging from 71.8% to 100% in recent Ethiopian studies and a 99% prevalence reported in a large earlier survey (Getachew et al., 2009; Fesseha et al., 2022).

Water limitation itself was not a significant independent predictor in this dataset, but that should not be interpreted as evidence of no effect. Other equine studies have linked reduced water availability to increased colic risk, including a multicenter case-control study showing higher risk when horses lacked continuous outdoor water access, and field studies of working horses identifying limited daily water access as a major risk factor (Kaneene et al., 1997). The lack of statistical significance here may reflect sample size, exposure misclassification, or collinearity with season, workload, and feed type rather than a true absence of association.

Regarding clinical findings, the results of the present study are typical of large intestinal impaction in donkeys. These findings are in accordance with reduced intestinal transit and accumulation of dry ingesta (Mendoza et al., 2018). Colic in donkeys associated with large intestinal impaction is often underdiagnosed because they show subtle and less dramatic signs compared with those observed in horses. Their stoic nature means careful observation is essential (Mendoza et al., 2018).

Affected donkeys typically presented with depression, dullness, reduced appetite, and prolonged periods of recumbency rather than overt rolling or violent behavior. Gastrointestinal indicators include decreased fecal output, small or dry fecal balls, abdominal distension, and reduced or absent gut sounds. In the present study, pain-related behaviors such as flank watching, mild pawing, or intermittent kicking at the belly were not observed, which is consistent with the findings of previous reports (Constable et al., 2016). Systemic changes, including tachycardia, and dehydration, were common in the present study, while anorexia predisposes donkeys to hyperlipemia, which complicates the clinical picture and worsens the prognosis (Bohassan et al., 2025). Because donkeys mask pain, subtle changes in demeanor, appetite, and fecal output

are often the most reliable early indicators of colic, underscoring the importance of vigilant monitoring and prompt veterinary intervention.

Several limitations may temper the conclusion of this study. First, the sample size was modest, and the case-control design is efficient for risk-factor screening but vulnerable to selection bias and residual confounding (Hillyer et al., 2010). Second, some exposures such as water access, feed quality, and workload are difficult to standardize in owner-reported field studies, and retrospective or observational colic studies often have incomplete recording of potentially important management variables (Molla, 2017; Miklavcic et al., 2024). Finally, donkey production systems differ markedly by ecology, purpose, and husbandry; therefore, risk estimates from northern Egypt should not be assumed to generalize unchanged to pastoral, commercial, or sanctuary populations (Asteraye et al., 2025). However, in Egypt, the donkey management system is closely similar to wide areas in the world. Accordingly, the findings are feasible and acceptable, especially in the MENA region.

CONCLUSION

The results of the present case-control study underscore that poor nutritional management, age, and seasonal variation are potential risk factors for large intestinal impaction in working donkeys. Adding poorly digestible nutrients as rice husk to the diet of donkeys may substantially increase the risk of impaction, especially in geriatric animals. Clinical vigilance, particularly in geriatric donkeys, is important to reduce morbidity and improve welfare in working donkey populations. Equine practitioners should consider nutritional risk factors when confronted with cases with large intestinal impaction.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Mohamed Marzok: mmarzok@kfu.edu.sa; ORCID: 0000-0002-7961-1247

Data availability

Data are available on request.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1038/s41598-023-29360-y>.

Authors' contribution

W. Al-Antaif: Conceptualization, methodology, visualization, data curation, writing—original draft.

M. Marzok: Methodology, formal analysis, investigation, writing—review & editing. yaser hamad: software, data curation, investigation, visualization.

A. Almubarak: Software, formal analysis, visualization, funding acquisition, supervision.

S. El-khodery: Conceptualization, methodology, supervision, writing—review & editing.

All authors have read and agreed to the published version of the manuscript.

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Ethical regulations

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 Mansoura University Animal Care and Use Committee (MU-ACUC-Vet.Med.14.07.2020).

Competing Interests

Authors declare that there is no conflict of interest.

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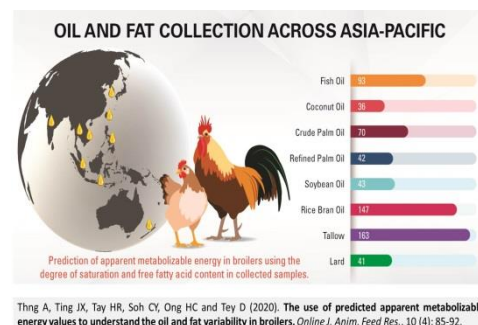
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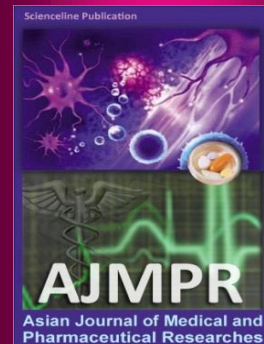
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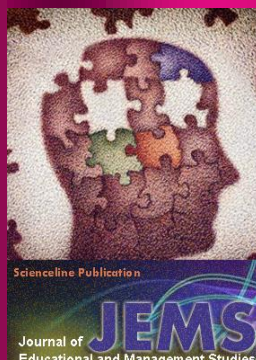
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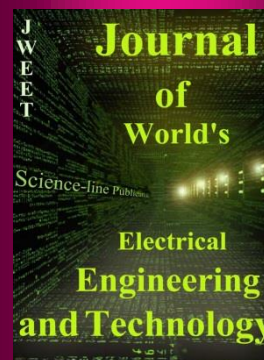
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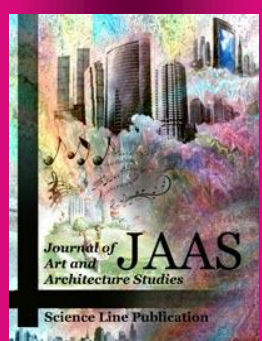
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