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BOOKLET

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*Online J. Anim. Feed Res.*, 15 (5): 246-309; September 2025

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# Volume 15 (5); September 2025

**Research Paper**

## Feedlot performance and carcass characteristics of indigenous cattle breeds in the Amhara region of Ethiopia

Tegegne F, Taye M, Kebede D, Getaneh M, Adimasu E, Asmare B, Tamrat H, Beyero N, and Tassew A.
*Online J. Anim. Feed Res.,* 15(5): 246-256, 2025; pii: S222877012500028-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.28

Abstract
Evaluating the feedlot potential and carcass traits of beef cattle breeds is crucial for identifying breeds suited to meat production and for guiding fattening enterprises. This study was conducted to assess the performance of cattle breeds sourced from selected districts in northwest Amhara, Ethiopia, under controlled feeding conditions. A total of 40 mature (2 pairs of permanent incisors intact bulls were purchased from four purposively selected local markets: Adet (Yilmana Densa), Merawi (Mecha), Dembecha (Dembecha), and Yifag (Libokemkem). The animals were transported to the Bahir Dar University beef farm and randomly allocated to two feeding treatments: 60:40 and 70:30 ratios of concentrate:roughage (Treatments 1 and 2, respectively) of the animals' daily dry matter intake. The experiment was conducted over 95 days via a randomized complete block design (RCBD) with a factorial arrangement. Data collected included body weight, morphological traits, carcass yield, and edible and non-edible offal, analyzed using the general linear model (GLM) procedure of SAS 9.0. Breed significantly influenced initial and final body weights ($P < 0.01$), slaughter weight, hot and cold carcass weights, weight-to-bone thickness ratio, and the weights of tail, head, and skin ($P < 0.05$). Cattle from Yilmana Densa consistently outperformed others, with a mean slaughter weight of 339.35±10.90 kg, hot carcass weight of 196.49±6.50 kg, and cold carcass weight of 193.51±6.07 kg. In contrast, feeding treatments had no significant effect on the evaluated traits. Overall, indigenous cattle breeds in northwest Amhara exhibited promising feedlot potential and acceptable carcass yields. Further studies incorporating meat quality parameters, age effects, and alternative dietary supplements are recommended to optimize production and market value.
Keywords: Beef, Carcass characteristics, Carcass weight, Local cattle breeds, Yilmana Densa.
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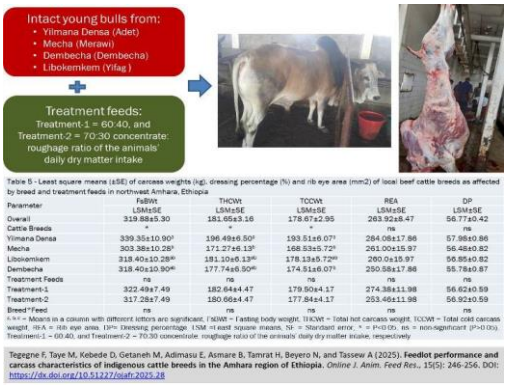



Table 5 - Least square means (±SE) of carcass weights (kg), dressing percentage (%) and rib eye area (mm2) of local beef cattle breeds as affected by breed and treatment feeds in northwest Amhara, Ethiopia

| Parameter | FsBWt LSM±SE | THCWt LSM±SE | TCCWt LSM±SE | REA LSM±SE | DP LSM±SE |
|---|---|---|---|---|---|
| Overall | 319.88±5.30 | 181.65±3.16 | 178.67±2.95 | 263.92±8.47 | 56.77±0.42 |
| Cattle Breeds | * | * | * | ns | ns |
| Yilmana Densa | 339.35±10.90[a] | 196.49±6.50[a] | 193.51±6.07[a] | 284.08±17.86 | 57.96±0.86 |
| Mecha | 303.38±10.28[b] | 171.27±6.13[b] | 168.53±5.72[b] | 261.00±15.97 | 56.48±0.82 |
| Libokemkem | 318.40±10.28[ab] | 181.10±6.13[ab] | 178.13±5.72[ab] | 260.0±15.97 | 56.85±0.82 |
| Dembecha | 318.40±10.90[ab] | 177.74±6.50[ab] | 174.51±6.07[b] | 250.58±17.86 | 55.78±0.87 |
| Treatment Feeds | ns | ns | ns | ns | ns |
| Treatment-1 | 322.49±7.49 | 182.64±4.47 | 179.50±4.17 | 274.38±11.98 | 56.62±0.59 |
| Treatment-2 | 317.28±7.49 | 180.66±4.47 | 177.84±4.17 | 253.46±11.98 | 56.92±0.59 |
| Breed*Feed | ns | ns | ns | ns | ns |

a,b,ab = Means in a column with different letters are significant, FsBWt = Fasting body weight, THCWt = Total hot carcass weight, TCCWt = Total cold carcass weight, REA = rib eye area, DP= Dressing percentage, LSM =Least square means, SE = Standard error, * = P<0.05, ns = non-significant (P>0.05). Treatment-1 = 60:40, and Treatment-2 = 70:30 concentrate: roughage ratio of the animals' daily dry matter intake, respectively

Tegegne F, Taye M, Kebede D, Getaneh M, Adimasu E, Asmare B, Tamrat H, Beyero N, and Tassew A (2025). **Feedlot performance and carcass characteristics of indigenous cattle breeds in the Amhara region of Ethiopia**. *Online J. Anim. Feed Res.*, 15(5): 246-256. DOI: https://dx.doi.org/10.51227/ojafr.2025.28

**Research Paper**

## Dietary leucaena leaves improve growth performance and carcass quality of Vietnamese goats

Liem TN, Dung NM, Duc LM, Hai DT, Tam VTM, An LV, Anh LTQ, Chotchutima S, and Boonsaen P.
*Online J. Anim. Feed Res.,* 15(5): 257-263, 2025; pii: S222877012500029-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.29

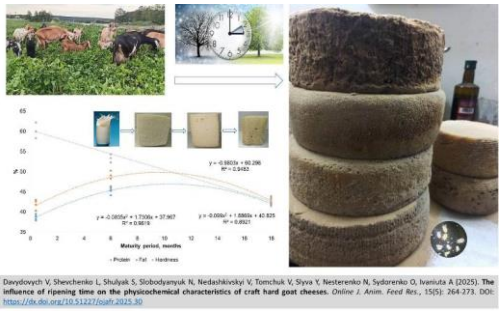
Abstract
The experiment was conducted at a research farm for sixteen male goats, with an average body weight of 12.32 ± 0.14 kg. They were randomly allocated into 4 groups corresponding to 4 diets and fed individually. The diets were formulated to consist of 90% of Guinea grass (Panicum maximum) and 10% of concentrated feed as basal (in DM). Leucaena leaves were substituted at 0%, 10%, 20% and 30% of Guinea grass in four respective diets. A 2-week adaptation period was provided for the goats to the diets and feeding system before data collection. Feed intake, weight gain, feed conversion ratio, and carcass traits of goats differed significantly among the four diets ($P < 0.05$). The inclusion of leucaena leaves in the diets increased feed intake. As the levels of leucaena leaves in diets increased up to 30%, there were corresponding improvements in weight gain. Daily weight gain increased from 45 to 61 g/day and feed conversion ratio (FCR) decreased from 8.43 to 6.62 kg feed/kg gain. Higher leucaena inclusion improved carcass traits but did not affect loin meat quality. Economic analysis also indicated that including up to 30% leucaena leaves in the goats' diet provides a profitable outcome for farmers. The economic impact increased with the rising levels of leucaena leaves in the goats' diet. It is recommended that leucaena leaves be utilized for goat raising in smallholder farming systems in Vietnam.
Keywords: Carcass, Feed conversion ratio, Goats, Growth, Leucaena.
[Full text-PDF] [Scopus] [Crossref Metadata] [Export from ePrints]


Liem T N, Dung N M, Duc L M, Hai D T, Tam V T M, An L V, Anh L T Q, Chotchutima S, Boonsaen P (2025). **Dietary leucaena leaves improve growth performance and carcass quality of Vietnamese goats**. *Online J. Anim. Feed Res.* 15(5): 257-263. DOI: https://dx.doi.org/10.51227/ojafr.2025.29

**Research Paper**

## The influence of ripening time on the physicochemical characteristics of craft hard goat cheeses

Davydovych V, Shevchenko L, Shulyak S, Slobodyanyuk N, Nedashkivskyi V, Tomchuk V, Slyva Y, Nesterenko N, Sydorenko O, Ivaniuta A.
*Online J. Anim. Feed Res.,* 15(5): 264-273, 2025; pii: S222877012500030-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.30



Davydovych V, Shevchenko L, Shulyak S, Slobodyanyuk N, Nedashkivskyi V, Tomchuk V, Slyva Y, Nesterenko N, Sydorenko O, Ivaniuta A (2025). **The influence of ripening time on the physicochemical characteristics of craft hard goat cheeses.** *Online J. Anim. Feed Res.*, 15(5): 264-273. DOI: https://dx.doi.org/10.51227/ojafr.2025.30

Abstract
The unique taste characteristics of craft hard cheeses made from raw goat milk, ripened using mites *Acarus siro* L., have contributed to increasing consumer demand enable the assessment of their quality and authenticity. In this study, 15 heads of Alpine and Yoghurt cheeses each weighing 4.5-5.0 kg were produced from raw goat milk and allowed to ripen for 12 and 18 months, respectively. Both cheeses were ripened with natural surface colonization by the mites *Acarus siro* L. It was found that the moisture content of Alpine cheese decreased from 43.31 on day 7 to 28.99% at 12 months of age, and the moisture content of Yoghurt cheese decreased from 46.90% on day 7 to 29.99% at 18 months. Moisture loss in both cheeses was strongly dependent on ripening time. The protein content in craft hard cheeses increased with age: from 21.45% to 28.68% in Alpine cheese and from 20.52% to 29.52% in Yoghurt cheese. Corresponding to the increase in dry matter content, fat content also increased in both varieties: from 24.45% to 31.50% in Alpine cheese and from 22.06% to 29.91% in Yoghurt cheese. A characteristic feature of both cheeses was the formation of holes, the size and distribution of varied with ripening duration. The hardness of Alpine and Yoghurt cheeses decreased with age, while the fracturability increased, reaching a minimum in the oldest cheeses, a change closely related to moisture loss. The rind of old-ripened Alpine and Yoghurt cheeses exhibited an amber color of varying intensity, with small verrucae due to the activity of the mite *Acarus siro* L. The observed changes in the physicochemical characteristics of young, mature, and old-ripened artisanal cheeses made from raw goat milk can serve as criteria for assessing their quality, age, and authenticity. Production of such cheeses contributes to diversifying the product range and enhancing the market competitiveness of premium goat cheeses.
Keywords: Alpine cheese, Dry matter, Mite *Acarus siro* L, Rind, Yoghurt cheese.

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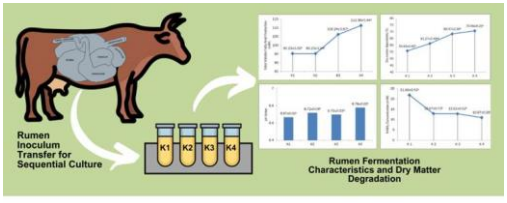
**Research Paper**

## Sequential culture of rumen fluid as a sustainable inoculant for *in vitro* ruminants feed evaluation

Rifai A, Syahrir S, and Natsir A.
*Online J. Anim. Feed Res.,* 15(5): 274-282, 2025; pii: S222877012500031-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.31



Rifai A, Syahrir S, and Natsir A (2025). **Sequential culture of rumen fluid as a sustainable inoculant for *in vitro* ruminants feed evaluation.** *Online J. Anim. Feed Res.*, 15(5): 274-282. DOI: https://dx.doi.org/10.51227/ojafr.2025.31

Abstract
Rumen fluid plays a crucial role in *in vitro* studies for evaluating ruminant feed. Maintaining microbial activity in rumen fluid can serve as a breakthrough approach to reducing dependence on fresh rumen fluid collection by utilizing sequential culture techniques. This study aimed to assess the effectiveness of rumen microbial inoculants through sequential cultures with a 48-hour incubation period. A completely randomized design was applied with four treatments: K1 = Culture 1 (inoculant derived from fresh rumen fluid), K2 = Culture 2 (inoculant derived from Culture 1), K3 = Culture 3 (inoculant derived from Culture 2), and K4 = Culture 4 (inoculant derived from Culture 3). The test substrates included dwarf elephant grass and *Indigofera zollingeriana* leaves using analysis *in vitro* sequential cultures adapted from Tilley and Terry (1963) and the Consecutive Batch Culture (CBC) method. Parameters measured included rumen fermentation characteristics such as pH, ammonia nitrogen (N-$NH_3$) concentration, total volatile fatty acid (VFA) production, and dry matter digestibility. Data were analyzed using analysis of variance (ANOVA) followed by Tukey's HSD (Honest Significant Difference) test. The results showed that the sequential culture process significantly affected *in vitro* rumen fermentation characteristics. The pH remained stable within the optimal range (6.67–6.78). Increased culture sequences enhanced N-$NH_3$ concentration, total VFA production, and dry matter digestibility. It can be concluded that rumen microbial inoculants remain effective up to the fourth sequential culture for *in vitro* evaluation of ruminant feeds.
Keywords: Digestibility**,** Dry matte, Inoculant, Microbial viability, Sequential culture

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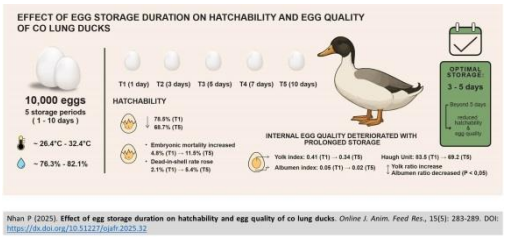
**Research Paper**

## Effect of egg storage duration on hatchability and egg quality of Co Lung ducks

Nhan P.
*Online J. Anim. Feed Res.,* 15(5): 283-289, 2025; pii: S222877012500032-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.32




Abstract
This study aimed to evaluate the effects of different egg storage durations on hatchability and internal egg quality of Co Lung duck eggs. A total of 10,000 eggs were incubated across five treatments representing different storage periods (T1: 1 day, T2: 3 days, T3: 5 days, T4: 7 days, T5: 10 days). Environmental data recorded at the storage site showed daily temperature variations from 26.4°C to 32.4°C and humidity ranging from 76.3% to 82.1%. Storage time significantly affected embryonic mortality, which increased from 4.8% (T1) to 11.5% (T5), and dead-in-shell rate, which rose from 2.1% to 5.4% ($P < 0.01$). Hatchability significantly declined from 78.5% (T1) to 68.7% (T5). Internal egg quality also deteriorated with prolonged storage (more than 5 days). The yolk index decreased from 0.41 to 0.34, albumen index from 0.05 to 0.02, and Haugh Unit from 83.5 to 69.2, indicating significant loss of freshness. Meanwhile, yolk ratio increased while albumen ratio decreased significantly ($P < 0.05$), suggesting moisture redistribution. No significant changes were observed in egg weight, shell thickness, or shell ratio. Overall, storage beyond 5 days led to reduced hatchability and poorer internal egg quality. Therefore, the optimal storage duration for Co Lung duck eggs is 3 to 5 days. Farmers and hatchery managers can incubate eggs within this period to maximize hatchability and freshness.
Keywords: Co Lung duck, Egg quality, Embryonic mortality, Hatchability, Indigenous poultry breeds.

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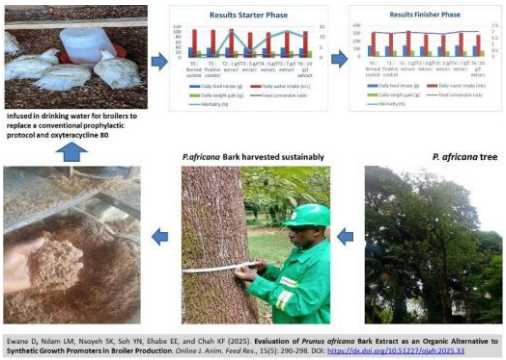
**Research Paper**

## Evaluation of *Prunus africana* bark extract as an organic alternative to synthetic growth promoters in broiler production

Ewane D, Ndam LM, Nsoyeh SK, Soh YN, Ehabe EE, and Chah KF.
*Online J. Anim. Feed Res.,* 15(5): 290-298, 2025; pii: S222877012500033-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.33




Abstract
Concerns over synthetic inputs in organic poultry production systems prompted an evaluation of aqueous *Prunus africana* bark extracts as natural feed additive via drinking water. Using 210 unsexed Cobb 500 day old broiler chicks, a 42 day trial was conducted to compare graded levels of ground *P. africana* bark infused in drinking water to oxy-tetracycline 80 and a conventional prophylactic calendar on growth, hematology and economic response in chickens. The feed efficiency, weight gain and final weights of birds fed *P. africana* did not differ significantly ($P > 0.05$) from those in the control groups. Carcass yields between the control and prunus groups did not vary significantly ($P < 0.05$) except the oxyterracycline control that had significantly ($P < 0.05$) higher slaughter weight (1913.3 g vs. 1681.7 g), carcass weight (1681.7 g vs. 1468.3 g) and drumstick weight (233.3 g vs. 198.3 g) compared to T4 (5 g/L). Significant differences ($P < 0.05$) were observed in hematological and serum biochemistry at the starter phase (day 21) but not ($P > 0.05$) during the finisher phase (day 42). The unit total expenses were significantly lower ($P < 0.05$) for treatments with inclusions of bark extract, thereby improving their gross margins, cost-to-benefit ratios, and economic efficiency. However, a progressive increase in the concentration of bark extracts did not significantly ($P > 0.05$) affect the profitability of the farm enterprises. Although metabolic challenges were observed in young chicks *P. africana* bark extracts improved their growth, and carcass quality thereby confirming their potential use as a natural growth promoter in broiler production in replacement of the synthetic conventional prophylactic protocols.
Keywords: Chickens, Economic efficiency, Feed-additive, Natural products, Prophylactic.

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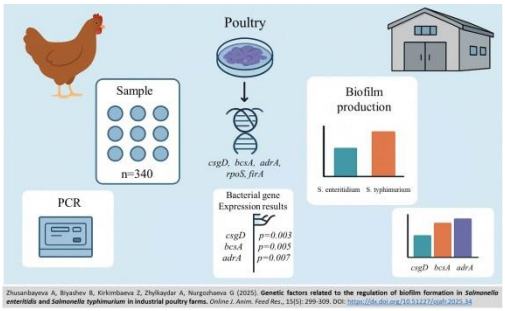
**Research Paper**

## Genetic factors related to the regulation of biofilm formation in Salmonella enteritidis and Salmonella typhimurium in industrial poultry farms

Zhusanbayeva A, Biyashev B, Kirkimbaeva Z, Zhylkaydar A, Nurgozhaeva G.

*Online J. Anim. Feed Res.,* 15(5): 299-309, 2025; pii: S222877012500034-15
DOI: https://dx.doi.org/10.51227/ojafr.2025.34

Abstract
The purpose of the study was to examine the genetic mechanisms and regulation of biofilm formation in *Salmonella enteritidis* and *Salmonella typhimurium* isolated at industrial poultry farms. The methodology included the collection of 340 samples from industrial poultry production facilities in Kazakhstan, Latvia, and Turkey between 2022 and 2025. The isolated strains were serotyped, genomic deoxyribonucleic acid was extracted, and the presence of the *csgD*, *bcsA*, *adrA*, *rpoS*, and *fimA* genes was detected using polymerase chain reaction (PCR). The level of gene expression was determined using quantitative polymerase chain reaction, and the phenotypic ability to form biofilms was evaluated by crystal violet staining. The results showed the isolation of 238 *Salmonella* strains, including 124 *S. enteritidis* and 114 *S. typhimurium*. The highest contamination was recorded in slaughter lines, accounting for 43.3% of the total positive samples. The analysis showed varying *Salmonella* serotype prevalence across countries, with *S. enteritidis* dominant in Kazakhstan (45.9%), *S. typhimurium* in Latvia (64.7%), and a balanced distribution in Turkey. The analysis revealed a high prevalence of biofilm formation genes, particularly *fimA* (94.1%), while *rpoS* ranged from 66.7% to 85.5%. According to quantitative polymerase chain reaction data, the expression of *csgD* (P = 0.003), *bcsA* (P = 0.005), and *adrA* (P = 0.007) was significantly higher in *S. typhimurium*, indicating its greater potential for biofilm formation. The phenotypic assessment confirmed this: 65% of *S. typhimurium* were strong or very strong biofilm producers, compared with 45% in *S. enteritidis* ($OD_{570}$: 1.42±0.15 and 0.97±0.12, respectively). The results can be practically applied in the development of sanitary control programmes, including the implementation of protocols for periodic assessment of biofilm-forming strains using qPCR screening. Based on the expression profile of key genes, criteria can be developed for selecting effective disinfectants (e.g., peroxide compounds or quaternary ammonium compounds) and for creating regulations targeting critical areas, such as poultry slaughterhouses and evisceration equipment.




Keywords: Phenotypic variability, Poultry farming, Regulatory mechanisms, Serotypes, Stress response.



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# FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF INDIGENOUS CATTLE BREEDS IN THE AMHARA REGION OF ETHIOPIA

Firew TEGEGNE[1], Mengistie TAYE[1,2]✉, Damitie KEBEDE[1], Mezgebu GETANEH[1,3], Esubalew ADIMASU[1], Bimrew ASMARE[1], Habtamu TAMRAT[4], Netsanet BEYERO[1], and Asaminew TASSEW[1]

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✉*Supporting Information*

RESEARCH ARTICLE

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**ABSTRACT**: Evaluating the feedlot potential and carcass traits of beef cattle breeds is crucial for identifying breeds suited to meat production and for guiding fattening enterprises. This study was conducted to assess the performance of cattle breeds sourced from selected districts in northwest Amhara, Ethiopia, under controlled feeding conditions. A total of 40 mature (2 pairs of permanent incisors intact bulls were purchased from four purposively selected local markets: Adet (Yilmana Densa), Merawi (Mecha), Dembecha (Dembecha), and Yifag (Libokemkem). The animals were transported to the Bahir Dar University beef farm and randomly allocated to two feeding treatments: 60:40 and 70:30 ratios of concentrate:roughage (Treatments 1 and 2, respectively) of the animals' daily dry matter intake. The experiment was conducted over 95 days via a randomized complete block design (RCBD) with a factorial arrangement. Data collected included body weight, morphological traits, carcass yield, and edible and non-edible offal, analyzed using the general linear model (GLM) procedure of SAS 9.0. Breed significantly influenced initial and final body weights ($P < 0.01$), slaughter weight, hot and cold carcass weights, weight-to-bone thickness ratio, and the weights of tail, head, and skin ($P < 0.05$). Cattle from Yilmana Densa consistently outperformed others, with a mean slaughter weight of 339.35±10.90 kg, hot carcass weight of 196.49±6.50 kg, and cold carcass weight of 193.51±6.07 kg. In contrast, feeding treatments had no significant effect on the evaluated traits. Overall, indigenous cattle breeds in northwest Amhara exhibited promising feedlot potential and acceptable carcass yields. Further studies incorporating meat quality parameters, age effects, and alternative dietary supplements are recommended to optimize production and market value.

**Keywords:** Beef, Carcass characteristics, Carcass weight, Local cattle breeds, Yilmana Densa.

## INTRODUCTION

Ethiopia possesses the largest livestock population in Africa, with an estimated 70 million cattle, 52.5 million goats, 42.9 million sheep, 57 million poultry, 8.1 million camels, 2.1 million horses, 10.8 million donkeys, 0.38 million mules, and 6.99 million beehives (CSA, 2021). Among the national cattle herd, indigenous breeds account for 97.4%, while hybrid and exotic breeds represent only 2.3% and 0.31%, respectively.

The livestock sector is a cornerstone of Ethiopia's economy (Alemneh and Getabalew, 2019; Abebe et al., 2022; Aragie and Thurlow, 2024), contributing about 16.5% to the national gross domestic product (GDP), 35.6% of the agricultural GDP, 15% of export earnings, and 30% of agricultural employment (Eshetu & Abraham, 2016). Beyond its economic contribution, livestock provides households with food (milk, meat, and blood), hides, draft power, wealth accumulation, and a form of insurance against shocks (Dinku, 2019). Cattle also hold important cultural and social value, particularly among pastoralist and agro-pastoralist communities.

In Ethiopia, cattle are managed for multiple purposes, including meat, milk, and draft power. Unlike countries with specialized beef breeds, Ethiopia does not maintain cattle exclusively bred for beef production (Alemneh and Getabalew, 2019). Instead, beef is often sourced from old oxen that have already served for draft purposes, which limits both yield and quality. Despite this practice, indigenous cattle possess untapped potential for beef production, yet their growth performance and carcass quality remain poorly characterized. Efforts to improve the beef potential of local breeds have been minimal (Tucho et al., 2021), with most research and development programs focusing on dairy traits. This lack of attention has slowed progress in developing efficient beef production systems. Even though there are no specialized beef cattle breeds, in Ethiopia, approximately 1.2% of the total cattle population is raised exclusively for meat (CSA, 2021).

Cattle fattening is a newly emerging business sector in Ethiopia due to its sizable role in creating employment opportunities and income generation for urban and peri-urban inhabitants (Ayalew et al., 2018; Belayneh et al., 2021; Erge et al., 2022; Lire Gibore, 2022). Despite this growth, it faces numerous challenges, including limited genetic



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improvement programs, scarcity of quality feed resources, high disease burden, weak livestock policies, and socioeconomic constraints (Abebe et al., 2022; Milikias & Gebre, 2024; Wendimu et al., 2023). Nevertheless, several indigenous cattle breeds such as Harar, Arsi, and Bale (Gadisa et al., 2019), Ogaden (Mekuriaw et al., 2009), and Boran, Arsi, and Harar (Tefera et al., 2019) are recognized for their superior meat yield and carcass quality.

Northwest Amhara also harbors a diverse range of cattle breeds with potential for beef production. However, their fattening performance and carcass traits remain poorly characterized, particularly under the mixed crop–livestock production system. Understanding the growth potential and carcass characteristics of these cattle is essential for breed selection, improved management practices, and the development of a sustainable beef industry.

Therefore, this study was conducted to evaluate the feedlot performance and carcass characteristics of cattle breeds purchased from selected districts in northwest Amhara, Ethiopia, under a natural pasture hay-based diet.

## MATERIALS AND METHODS

### Descriptions of the study area

The study was conducted at the beef cattle farm of the College of Agriculture and Environmental Sciences, Zenzelima campus, Bahir Dar University in Bahir Dar town. The animals were maintained in a slatted-floor barn throughout the experiment. The animals used in the experiment were sourced from four selected districts located in the northwest Amhara region, namely, the Yilmana Densa, Mecha, and Dembecha districts from the West Gojjam zone and the Libokemkem district from the South Gondar zone of the Amhara region. The study districts were purposively selected because of the flourishing potential of cattle fattening activity by rural and peri-urban dwellers, and the dearth of information in the selected areas. Information on the geographical location, agro-ecologies, elevation, and climatic conditions, as well as the land area, livestock population, and human population of the study districts, is presented in Table 1.

**Table 1 -** Geographical location, altitude ranges, climate conditions, agro-ecology, and human and livestock population of the study districts from which the experimental animals were sourced.

| Descriptors | Name of the districts where the experimental animals were sourced | | | |
|---|---|---|---|---|
| | Dembecha | Yilmana Densa | Mecha | Libokemkem |
| **Geographical location** | | | | |
| Latitude | 10°32'59.99"N | 11°10' - 11°15'N | 11°5′ - 11°38′N | 12°39'66" - 12°42'45"N |
| Longitude | 37°28'59.99"E | 37°30' - 37°40'E | 36°58′ - 37°22′E | 37°26'99" - 37°28'42"E |
| **Agro-ecology (%)** | | | | |
| Highland | 11% | 24% | Absent | 18% |
| Midland | 83% | 64% | Absent | 43% |
| Lowland | 6% | 12% | Absent | 39% |
| Altitude (m.a.s.l.) | 1500-2999 | 1552-3535 | 1795-3268 | 1,800-2,000 |
| Annual T° (°C) | 10 °C-20 °C | 15 °C -24 °C | 17 °C-30 °C | 19°C-30°C |
| Annual rainfall (mm) | 1200-1600 | 1200-1600 | 820-1250 | 1300 |
| Land area | 971.29 | 1018.11 | 159,898 | 1081.57 |
| Human population | 151,023 | 214,852 | 375,716: | 226, 958 |
| Cattle | 177375 | 123,440 | 351,844 | 115452 |
| Goat | 11726 | 11,471 | 61,883 | 36448 |
| Sheep | 51820 | 79,217 | 110,834 | 17939 |
| Equines | 26055 | 24,904 | 39,214 | 2,552 |
| Chicken | 14241 | 88,439 | 230,286 | 327403 |

The sources of the information are each district's Agriculture Development Offices.

### *Experimental design, treatments and animal management*

A total of 40 mature (2 pairs of permanent incisors) intact bulls were purchased from four (10 from each) different local markets, namely, Adet, Merawi, Dembecha, and Yifag markets located at Yilmana Densa, Mecha, and Dembecha districts of West Gojjam zone and Libokemkem district of South Gondar zone of the Amhara region, Ethiopia, respectively. The marketplaces in each of the districts were selected based on the assumption that the cattle in each district would be presented to the mentioned markets and that there could be differences in relation to the type of animals available in each marketplace. The cattle breeds distributed in the West Gojjam Zone and presented to the indicated markets (Yilmana Densa, Mecha, and Dembecha) are known to be Gojjam Highland Zebu (Bos Indicus), whereas those cattle presented to the Yifag market are expected to be Fogera cattle (Zenga) (Kebede & Ayalew, 2014). After purchase, the animals were ear-tagged and brought to the College of Agriculture and Environmental Sciences (CAES) animal experimental site for the experiment. At the experimental site, the animals were allowed access to feed and water *ad libitum* and some amount of concentrated feed for 15 days during the acclimatization period. The animals were then systematically (based on initial weight) assigned to two treatment feeds, which were classified as Treatment-1,

comprising a 60:40 concentrate-to-roughage ratio of the daily dry matter intake of the experimental animal, whereas Treatment-2 included a 70:30 concentrate-to-roughage ratio of the daily dry matter intake. The daily dry matter intake was calculated on the basis of the assumption that cattle can consume 3% of their body weight. The dry matter (DM) percentages of the roughage and concentrate feeds used in the experiment were considered to be 92.82% and 91.53%, respectively. The roughage feed used in this experiment was purchased from grass hay harvested from a natural pasture at the 50% blooming stage. The concentrated feed was formulated with 75% maize, 24% *noug* seed (*Guizotia abyssinica*) cake, and a 1% salt mixture. The experimental design used in this experiment was a randomized complete block design (RCBD) with a factorial arrangement. The initial body weights of the experimental animals were estimated via a heart girth meter (SCHWEINE/PORCS), which was used to block the animals into experimental groups. The feeding trial was conducted for 95 days from April to July 2021. Throughout the experimental period, the animals had free access to roughage feed and water.

***Chemical analysis of the treatment feed ingredients***

The proximate analysis of the concentrate and roughage feeds (offered and refused) used in the experiment is presented in Table 2.

**Table 2 -** Proximate analysis of the treatment feed used to evaluate the beef performance of cattle breeds purchased from four selected districts of northwestern Amhara, Ethiopia

| Types of feed | DM% | Ash% | CP% | NDF% | ADF% | ADL% | OM% |
|---|---|---|---|---|---|---|---|
| Concentrate | 91.53 | 2.80 | 9.28 | 35.79 | 7.29 | 2.42 | 97.20 |
| Hay (offered) | 92.82 | 9.85 | 4.96 | 76.00 | 48.60 | 12.59 | 90.15 |
| Hay (refusal) | 92.45 | 11.35 | 3.47 | 80.61 | 54.33 | 15.34 | 88.65 |

The samples were taken in triplicate, and the means were taken; DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; OM = organic matter.

**Data types and methods of data collection**

Data on morphological traits such as initial body weight (IBW), final body weight (FBW), total body weight gain (TBWG), daily body weight gain (DBWG), slaughter weight (SW), carcass characteristics (total hot carcass weight, cold carcass weight, and dressing percentage), and measurements of different edible and nonedible offal components of the experimental animals were collected. Morphological measurements were taken on thirteen traits of the experimental animals at the beginning and end of the feeding experiment, following the trait definition and reference points indicated by ICAR (2017) for conformation recording of beef cattle breeds (Table 3). Similarly, the IBW and FBW of the experimental animals were measured at the beginning and end of the experiment, respectively, while the slaughter weight was measured immediately before the slaughtering of fattened animals. To measure the carcass characteristics (total hot carcass weight) and edible and non-edible characteristics of the evaluated cattle breeds, a total of 24 (three animals from each treatment) were slaughtered at the College of Agriculture and Environmental Sciences mini abattoir following appropriate animal slaughtering procedures and considering animal welfare ethics at the end of the experiment. The animals were stunned via a pistol bolt and slaughtered by cutting the throat via a sharp knife. The weights of the live animals and their morphological traits were measured via a girth meter (SCHWEINE/PORCS), whereas carcass and offal weight measurements were taken via a ground scale and a Salter balance, respectively. The carcass weights of the left and right carcasses were determined by splitting via a saw, and the weights were summed to determine the total carcass weight. The carcass was maintained in a chilling room at 2–4 °C, and the cold carcass weight was measured after 24 hours of chilling. Carcass weight and offal measurements were taken just after slaughter.

**Table 3 -** Definitions and reference points of linear body measurements (cm) and body weights (kg) recorded for experimental local beef cattle breeds in northwestern Amhara, Ethiopia.

| Trait's name | The trait definition, and reference points considered to measure the traits |
|---|---|
| Body weight | Body weight as measured by heart girth |
| Body length | Length from shoulder to pins |
| Back length | Length from shoulder to hips |
| Thurl width | Distance between thurls |
| Body depth | Distance between top of back and bottom of barrel at the deepest point; independent of stature |
| Chest depth | Distance between top of back just behind shoulder and bottom of barrel behind the front leg |
| Flank depth | Distance between top of back just before hips and bottom of barrel just before the rear leg |
| Length of rump | Distance from hips to pins |
| Height at withers | Measured from top of the back in between the shoulders to the ground |
| Height at rump | Measured from the top of the back in between the hips to the ground |
| Width at hips | Distance between the hips |
| Width at pins | Distance between the pins |
| Back width | Width of the back behind the shoulders |
| Thickness of bone | Thickness of the canon bone in the forelegs |

Source: ICAR (2017)

In addition, data on total body weight gain (Negash et al., 2008; Gage et al., 2022), average daily body weight gain (Gage et al., 2022), weight–bone thickness ratio (Musa et al., 2021), and dressing percentage (Erge et al., 2022; Gage et al., 2022; Mummed & Webb, 2019) were derived following the procedures used by previous scholars. The ratio was calculated as follows: Ratio = $\frac{\text{Final body Weight}}{\text{Bone thickness}}$ (Musa et al., 2021)

**Data analysis**

The general linear model (GLM) procedures of the Statistical Analysis System (SAS, version 9.0) were used for data analysis. The feed treatment options and breeds of local beef cattle were fitted as fixed factors, whereas body weight, morphological measurements, and carcass and offal characteristics of the evaluated cattle breeds were considered response variables for the analysis. The statistical model used to analyze quantitative data collected from the investigated cattle breeds was as follows: $Y_{ijk} = \mu + B_i + T_j + BT_{(ij)k} + e_{ijk}$

Where: $Y_{ijk}$ = the recorded values for each quantitative response variable (live body weight, morphological traits, and carcass and offal characteristics) for the evaluated cattle breeds in the $i^{th}$ breed, $j^{th}$ treatment feed, and their interaction effects; $\mu$ = the overall mean; $B_i$ = the $i^{th}$ cattle breed (i = cattle breed from Yilmana Densa, Mecha, Libokemkem, and Dembecha districts); $T_j$ = the $j^{th}$ treatment feed (j = treatment-1, and treatment-2); $BT_{(ij)k}$ = the $k^{th}$ effect of the interaction between cattle breed and treatment feed; $e_{ijk}$ = error term associated with each observation

## RESULTS AND DISCUSSION

**Body weight and morphological traits**

Table 4 shows the overall values of the least square means (LSM±SE) of the initial and final live body weights, average daily weight gain, final measurement of morphological traits, and final body weight-to-bone thickness ratio of the evaluated cattle breeds. As indicated in Table 4, the breed of cattle had a significant influence on the initial and final body weights ($P<0.01$) and ratio (final body weight to the thickness of bone) ($P<0.05$) of the evaluated cattle breeds. However, breed had no significant influence on any of the evaluated morphological measurements or the body weight gain of cattle breeds. Accordingly, from the evaluated cattle breeds, the cattle breed brought from Mecha had the lowest initial (265.20±8.56 kg) and final (359.60±10.99 kg) live body weight compared with other cattle breeds. The highest initial (310.10±8.56 kg) and final (416.70±10.99 kg) live body weights were recorded for cattle breeds from Yilmana Densa district. In addition, cattle breeds from Yilmana Densa presented the highest ratio (23.67±0.87). The variation in the initial and final live body weights and ratios among the evaluated cattle breeds may be due to the differences in muscling ability and agroecology, and/or management dissimilarities of cattle at a younger age before the intervention of the experiment among the sample districts. Conversely, treatment had no significant influence on the body weight and morphological traits of the evaluated cattle breeds. This might be because the nutrient density of diet-2 was beyond the digesting ability of the animals to make use of the nutrients in it, which in turn indicates that there is an optimum roughage concentrate mix in livestock feed (Richardson et al., 2011).

The effects of breed on the initial and final live body weights of different beef cattle breeds have been reported by different scholars in Ethiopia and elsewhere. For example, Xie et al. (2012) reported a significant effect of breed on the initial live body weight of beef cattle breeds, as the Limousin and Simmental breeds had heavier initial body weights than did the Luxi, Jinnan, and Qinchuan cattle breeds in China under village-based management conditions in Liaoning Province, North China, which is consistent with the present findings. Similarly, Pesonen et al. (2012) reported a significantly greater initial body weight for Limousin (325 kg) bulls than for Aberdeen Angus (285 kg) and Angus x Limousin crossbred bulls (276 kg); however, the initial body weight did not differ from the final live body weight, which is not in line with the current results. In addition, similar to the present observation, Pesonen et al. (2012) reported a non-significant effect of breed on daily weight gain ($gd^{-1}$) for the aforementioned beef cattle breeds. Furthermore, Tefera et al. (2019) reported a significant effect of breed on the live body weight of 7–9-year-old Arsi, Boran, and Harar cattle breeds, as the highest value was recorded for Boran (433.00±39.27 kg), followed by Arsi (192.00±9.17 kg) and Harar (155.75±43.84 kg) cattle breeds.

Similar to the present findings, a non-significant ($P>0.05$) influence of treatment feeds on the final body weight, live body weight change, and average daily gain of two-year-old Kereyu bulls was reported at the Adami Tulu Agricultural Research Center (Tesfaye et al., 2018). In addition, Gudeto et al. (2019) reported a non-significant influence of dietary rations on final body weight and total and average daily weight gain of yearling Arsi bulls analyzed at 60 days, 120 days, and 238 days of the fattening period at the Adami Tulu Agricultural Research Center. Furthermore, a non-significant influence of feeding treatment on final body weight was reported for local intact oxen aged approximately 5 years in Wolaita, southern Ethiopia (Bassa et al., 2016). However, inconsistent with the present results, a significant effect of supplementation with different concentrate feeds at various proportions on the final body weight and total body weight gain of beef cattle breeds has been reported in Ethiopia and elsewhere. For instance, Gebremariam (2019) reported a significant effect of treatment feeds on the final body weight and average daily gain of Hararghe highland bulls fed grass

hay as a basal diet in Eastern Ethiopia. Similarly, different from the current observations, a significant influence of varying inclusion levels of groundnut haulms and maize offal on the final weight, weight change, and average daily gain of Bunaji bulls aged 2.5-3 years has been reported in Nigeria, as the highest values of these traits were recorded for treatment feeds containing 20% groundnut haulms: 80% maize offal ratio (Goska et al., 2017).

The overall values of initial body weight (291.25±4.28 kg) and final body weight (389.68±5.49 kg) of cattle breeds in the present study were greater than the values of initial body weight (149±6.36 kg) and final body weight (274.8±7.2 kg) reported for two-year-old Kereyu bulls (Tesfaye et al., 2018). Similarly, the values of initial body weight (249.13±4.15 kg) and final body weight (306.23±5.22 kg) recorded for local intact oxen aged approximately 5 years in Wolaita, southern Ethiopia, were lower than the current observations (Bassa et al., 2016). In addition, compared with the current findings, lower initial body weights (194.03±8.84 kg), final body weights (264.72±19.49 kg), and total body weight gains (70.69±16.86 kg) have been reported for Baggara bulls fed different roughage diets supplemented with molasses in Sudan (Adam et al., 2016). This implies that cattle breeds evaluated in the present study had better fattening performance in a feedlot operation.

**Carcass weights and dressing percentages**

The overall values of the LSM±SE of fasting body weight, hot carcass weight, and cold carcass weight for the evaluated cattle breeds were 319.88±5.30 kg, 181.65±3.16 kg, and 178.67±2.95 kg, respectively, and breed had a significant (P<0.05) effect on all of these traits (Table 5). However, breed had a non-significant (P>0.05) influence on the dressing percentage of the evaluated cattle breeds. Accordingly, cattle breeds from Yilmana Densa presented the highest fasting body weight (339.35±10.90 kg), total hot carcass weight (196.49±6.50 kg), and total cold carcass weight (193.51±6.07 kg) measurements. In contrast, cattle breeds from Dembecha presented the smallest values of fasting body weight (303.38±10.28 kg) and hot carcass weight (171.27±6.13 kg) compared with the other cattle breeds. Conversely, treatment had no significant (P>0.05) effect on the fasting body weight, total hot or cold carcass weight, or dressing percentage of the examined cattle breeds.

Consistent with the current findings, a significant (P<0.001) influence of breed on warm carcass weight and cold carcass weight was reported between the Arado, Boran, Barka, and Raya cattle breeds in Ethiopia (Mummed & Webb, 2019). Similarly, Erge et al. (2022) reported a significant (at least P<0.001) influence of breed on slaughter weight, hot carcass weight, and cold carcass weight for Arsi, Harar, Jersey x Horro F1, and Ogaden cattle breeds fed a corn silage-based finishing diet in Ethiopia. In addition, a significant influence of breed on slaughter/fasting weight was reported for Limousine and Retinta bulls (Avilés et al., 2015). In contrast, Musa et al. (2021) reported a non-significant influence of breed on slaughter weight, hot carcass weight, and cold carcass weight for Arsi, Borana, Harar, and Harar x HF crossbred cattle breeds in Ethiopia. Furthermore, in agreement with the present observations, a non-significant influence of breed on dressing percentage has been reported for Arsi, Boran, and Harar (Tefera et al., 2019), and Arsi, Boran, Harar and Harar x HF (Musa et al., 2021) cattle breeds in Ethiopia. However, in contrast to the current observations, a significant influence of breed on the dressing percentage of different beef cattle breeds has been reported in the literature (Pesonen et al., 2012; Xie et al., 2012; Mummed and Webb, 2019; Coleman et al., 2016; Erge et al., 2022). In contrast to these observations, a significant influence of different feeding regimes using different feed ingredients at various proportions on carcass weights and dressing percentages of beef cattle breeds has been reported around the world (Irshad et al., 2013; Clinquart et al., 2022). For example, a significant effect of replacing hay with maize silage at various rates on the carcass weights and dressing percentages of Harar cattle was reported in Ethiopia (Gage et al., 2022).

Similarly, the carcass yield and hot carcass weight of Hararghe Highland bulls fed grass hay as a basal diet were significantly (at least P<0.01) influenced by supplementation with different concentrate feeds, and the highest values of these carcass traits were observed for treatment feeds prepared with 4 kg $d^{-1}$ maize grain and 4 kg $d^{-1}$ mixtures of maize grain, wheat bran, dried cafeteria leftover and scrambled whole groundnut in equal proportions (Gebremariam, 2019). In addition, a considerable effect of the feeding system on slaughter weight was reported for Limousine and Retinta beef cattle breeds kept under feedlot conditions (Avilés et al., 2015).

The overall values of fasting weight and total hot and cold carcass weight in the present study were higher than the values reported for draught cattle raised for beef in Eastern Ethiopia, which were 247.93±5.27 kg, 90.98±2.11 kg, and 89.16±10.94 kg, respectively (Senbeta & Megersa, 2019). In addition, compared with the present findings, smaller overall values of hot carcass weight (106.93±0.21 kg) and cold carcass weight (101.19±0.18 kg) were reported for Arado, Barka, Boran, Raya, and nondescript cattle breeds slaughtered at Abergelle and Melgawendo abattoirs (Mummed & Webb, 2019). Moreover, the values of slaughter weight (179.1±1.0 kg), hot carcass weight (86.8±3.5 kg), and cold carcass weight (82.7±3.4 kg) reported for the Arsi, Boran, Harar, and Harar x HF cattle breeds (Musa et al., 2021) were lower than the current findings. Furthermore, compared with the present findings, smaller values of slaughter weight (215.58±12.21 kg), hot carcass weight (102.93±6.64 kg), and cold carcass weight (99.56±6.63 kg) were reported for the Arsi, Harar, Jersey x Horro, and Ogaden cattle breeds fed a corn silage-based finishing diet (Erge et al., 2022). These findings indicate that cattle breeds considered in the present study have better beef potential than other Ethiopian cattle breeds do.

**Table 4 -** Least square means (LSM±SE) of initial body weight, final body weight, total body weight (kg), and final morphological measurements (cm) of mature (with 2 pairs of permanent incisors) local intact bulls affected by breed and treatment feeds in selected districts of Northwest Amhara, Ethiopia

| | | Cattle Breeds | | | | | Treatment Feeds | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters** | **Overall** | **Sig.** | **Yilmana Densa** | **Mecha** | **Libokemkem** | **Dembecha** | **Sig.** | **Treatment-1** | **Treatment-2** | **Feed*Breed** |
| Initial body weight | 291.25±4.28 | ** | 310.10±8.56[a] | 265.20±8.56[b] | 297.00±8.56[a] | 292.70±8.56[a] | ns | 291.10±6.05 | 291.40±6.05 | ns |
| Final body weight | 389.68±5.49 | ** | 416.70±10.99[a] | 359.60±10.99[b] | 394.90±10.99[a] | 387.50±10.99[ab] | ns | 393.85±7.77 | 385.50±7.77 | ns |
| Total body weight gain | 98.43±5.30 | ns | 106.60±10.60 | 94.40±10.60 | 97.90±10.60 | 94.80±10.60 | ns | 102.75±7.49 | 94.10±7.49 | ns |
| Daily body weight gain | 1.036±0.056 | ns | 1.122±0.112 | 0.994±0.112 | 1.031±0.112 | 0.998±0.112 | ns | 1.082±0.079 | 0.990±0.079 | ns |
| Body length | 91.73±0.92 | ns | 90.80±1.83 | 90.70±1.83 | 92.60±0.83 | 92.80±1.83 | ns | 91.90±1.30 | 91.55±1.30 | ns |
| Back length | 67.63±0.97 | ns | 66.70±1.95 | 69.70±1.95 | 66.60±1.95 | 67.50±1.95 | ns | 67.85±1.38 | 67.40±1.38 | * |
| Thurl width | 34.33±0.52 | ns | 36.10±1.05 | 34.10±1.05 | 33.80±1.05 | 33.30±1.05 | ns | 34.10±0.74 | 34.55±0.74 | ns |
| Body depth | 72.58±0.70 | ns | 73.20±1.39 | 74.40±1.39 | 71.00±1.39 | 71.70±1.39 | ns | 72.15±0.98 | 73.00±0.98 | ns |
| Chest depth | 63.33±0.35 | ns | 62.70±0.71 | 63.70±0.71 | 63.00±0.71 | 63.90±0.71 | ns | 63.20±0.50 | 63.45±0.50 | ns |
| Flank depth | 55.63±0.55 | ns | 55.00±1.10 | 56.60±1.10 | 56.00±1.10 | 54.90±1.10 | ns | 55.15±0.78 | 56.10±0.78 | ns |
| Length of rump | 38.53±0.42 | ns | 38.40±0.83 | 38.20±0.83 | 38.70±0.83 | 38.80±0.83 | ns | 37.90±0.59 | 39.15±0.59 | ns |
| Height at withers | 127.15±0.37 | ns | 128.40±0.74 | 127.10±0.74 | 126.70±0.74 | 126.40±0.74 | ns | 126.85±0.53 | 127.45±0.53 | ns |
| Height at rump | 123.75±0.49 | ns | 124.20±0.98 | 124.20±0.98 | 123.50±0.98 | 123.10±0.98 | ns | 123.60±0.69 | 123.90±0.69 | ns |
| Width at hips | 32.20±0.71 | ns | 34.20±1.41 | 32.60±1.41 | 30.90±1.41 | 31.10±1.41 | ns | 31.35±1.00 | 33.05±1.00 | ns |
| Width at pins | 17.68±0.36 | ns | 18.30±0.72 | 16.60±0.72 | 18.00±0.72 | 17.80±0.72 | ns | 17.55±0.51 | 17.80±0.51 | ns |
| Back width | 25.65±0.61 | ns | 25.50±1.22 | 24.50±1.22 | 26.00±1.22 | 26.60±1.22 | ns | 25.80±0.86 | 25.50±0.86 | ns |
| Thickness of bone | 17.88±0.23 | ns | 17.70±0.45 | 17.80±0.45 | 18.20±0.45 | 17.80±0.45 | ns | 17.60±0.32 | 18.15±0.32 | ns |
| Ratio | 21.93±0.43 | * | 23.67±0.87[a] | 20.37±0.87[b] | 21.84±0.87[ab] | 21.86±0.87[ab] | ns | 22.44±0.61 | 21.42±0.61 | ns |

[a, b, c] = Means within a column with different subscripts are significantly different (P<0.05), Sig = Significant, ns = non-significant, * = P<0.05; ** = P<0.01, Treatment-1 = 60:40 concentrate: roughage ratio of the animals' daily dry matter intake; Treatment-2 = 70:30 of concentrate: roughage ratio of the animals' daily dry matter intake



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**Table 5 -** Least square means (±SE) of carcass weights (kg), dressing percentage (%), and rib eye area (mm2) of local beef cattle breeds as affected by breed and treatment feeds in northwest Amhara, Ethiopia

| Parameter | FsBWt | THCWt | TCCWt | REA | DP |
|---|---|---|---|---|---|
| | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE |
| Overall | 319.88±5.30 | 181.65±3.16 | 178.67±2.95 | 263.92±8.47 | 56.77±0.42 |
| Cattle Breeds | * | * | * | ns | ns |
| Yilmana Densa | 339.35±10.90[a] | 196.49±6.50[a] | 193.51±6.07[a] | 284.08±17.86 | 57.98±0.86 |
| Mecha | 303.38±10.28[b] | 171.27±6.13[b] | 168.53±5.72[b] | 261.00±15.97 | 56.48±0.82 |
| Libokemkem | 318.40±10.28[ab] | 181.10±6.13[ab] | 178.13±5.72[ab] | 260.0±15.97 | 56.85±0.82 |
| Dembecha | 318.40±10.90[ab] | 177.74±6.50[ab] | 174.51±6.07[b] | 250.58±17.86 | 55.78±0.87 |
| Treatment Feeds | ns | ns | ns | ns | ns |
| Treatment-1 | 322.49±7.49 | 182.64±4.47 | 179.50±4.17 | 274.38±11.98 | 56.62±0.59 |
| Treatment-2 | 317.28±7.49 | 180.66±4.47 | 177.84±4.17 | 253.46±11.98 | 56.92±0.59 |
| Breed*Feed | ns | ns | ns | ns | ns |

[a, b, c] = Means in a column with different letters are significant, FsBWt = Fasting body weight, THCWt = Total hot carcass weight, TCCWt = Total cold carcass weight, REA = Rib eye area, DP= Dressing percentage, LSM =Least square means, SE = Standard error, * = P<0.05, ns = non-significant (P>0.05), Treatment-1 = 60:40, and Treatment-2 = 70:30 concentrate: roughage ratio of the animals' daily dry matter intake, respectively

**Edible and non-edible offal characteristics**

The results of edible and non-edible offal characteristics of the evaluated cattle breeds as affected by breed and treatment feeds are presented in Table 6. Except for tail weight and head and skin weight, the cattle breed had no significant effect on the non-edible carcass characteristics of the evaluated cattle breeds. Similarly, treatment feed had no significant effect on the offal carcass measurements of the evaluated cattle breeds. Similar to the present findings, Musa et al. (2021) reported a non-significant effect of breed on scrotal fat, kidney fat, heart fat, and omental fat for Arsi, Boran, Harar, and Harar x HF crossbred beef cattle breeds in Ethiopia; however, the author reported a significant (P<0.05) influence of breed on the pelvic fat of the evaluated cattle breeds. In addition, a non-significant influence of breed on the weight of the kidney, spleen, and head was reported for Ethiopian cattle breeds, including the Arsi, Harar, Jersey x Horro F1, and Ogaden cattle breeds (Erge et al., 2022), which is consistent with the present findings. In addition, unlike heart fat and omental fat, the weights of kidney fat and pelvic fat of the Arsi, Boran, and Harar cattle breeds were not significantly affected by breed (Tefera et al., 2019). In contrast to the present observations, Erge et al. (2022) reported a significant influence of breed on the weight of the heart, liver, hide, gastrointestinal tract (GIT), empty gut, lung and trachea, and feet of the Arsi, Harar, Jersey x Horro F1 crossbred, and Ogaden cattle breeds. Likewise, inconsistent with the present findings, a significant influence of breed on offal characteristics, including pelvic fat, scrotal fat, kidney fat, and rib eye area, was reported for Borana and Kereyu cattle breeds managed under natural pasture grazing conditions in Ethiopia (Mohammed et al., 2008).

Regarding the treatment feeds, similar to the present findings, a non-significant influence of soybean meal replacement by Crambe crushed at varying levels (0–15%) in the concentrate supplement on carcass characteristics, including liver, pelvic fat, leg length, total meat, loin characteristics, carcass fat thickness, and preslaughter and carcass weights of Nellore cows finished on pasture (*Brachiaria humidicola*), was reported in Brazil (Souza et al., 2015). Additionally, similar to the present findings, the feeding of different dietary rations to Kereyu bulls aged two years did not significantly affect the characteristics of the edible and nonedible organs or carcass, such as the tail, skin, feet, lungs, pancreas, bladder, penis, full gut, empty gut, small and large intestine, tongue, hump, and head, of the evaluated bulls in Ethiopia (Tesfaye et al., 2018). Moreover, a non-significant effect of different roughage sources on the non-edible organs or carcass, including the tail, lung and trachea; the spleen; the heart; the pancreas; the liver; the genitalia; and the empty intestine, has been reported for Baggara bulls in Sudan (Adam et al., 2016).

Instead, unlike the present observations, a significant (P<0.05) effect of dietary changes on the loin eye area of Hararghe Highland bulls (Gebremariam, 2019) and the total edible offal of Harar oxen (Gage et al., 2022) has been reported in Ethiopia. In addition, inconsistent with the present finding, a substantial (P<0.05) effect of treatment feeds on the percentage of nonedible offal components was reported for Aceh cattle fed with forage and concentrate at different levels in Indonesia, as the highest percentage of nonedible offal was recorded for the treatment groups allotted to 15 kg of forage and 2 kg of commercial concentrate (Koesmara et al., 2019). Similarly, noncarcass characteristics, including the heart and liver of Nellore steers, were strongly associated with the feed efficiency of the experimental animals, different from the present findings (Nascimento et al., 2016). Furthermore, a significant (at least P<0.05) influence of treatment feeds on noncarcass characteristics such as head, skin with a tail, hooves, gut fill, plunk, and empty body weight was reported for short horn zebu bulls grazing on natural pastures and supplemented with crude protein at varying levels in Uganda, as the highest values of these traits were recorded for animals supplemented with a formulated ration containing 110 CP $kg^{-1}$ of dry matter and 130 CP $kg^{-1}$ of dry matter compared with the other inclusion levels of crude protein (Nantongo et al., 2021).



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**Table 6 -** Least square means (±SE) of edible and nonedible offal characteristics (kg) of local beef cattle breeds affected by breed and treatment feeds in selected districts of northwest Amhara, Ethiopia

| Parameter | Tail | HS | Head | FH | Tongue | LT | Heart | HF | Pancreas | Kidney | KF |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE |
| Overall | 1.15±0.05 | 14.22±0.29 | 29.73±0.85 | 6.57±0.24 | 1.12±0.07 | 4.09±0.19 | 1.0±0.04 | 0.56±0.05 | 0.89±0.05 | 0.61±0.02 | 3.80±0.27 |
| Cattle Breeds | * | * | ns | ns | ns | ns | ns | ns | ns | * | ns |
| Yilmana Densa | 1.45±0.10[a] | 15.82±0.60[a] | 30.63±1.79 | 7.10±0.50 | 1.23±0.16 | 4.38±0.40 | 1.18±0.09 | 0.58±0.10 | 0.89±0.10 | 0.72±0.05[a] | 4.28±0.57 |
| Mecha | 1.17±0.09[b] | 13.37±0.54[b] | 29.02±1.60 | 6.25±0.45 | 1.03±0.14 | 3.92±0.35 | 0.95±0.08 | 0.50±0.09 | 0.83±0.09 | 0.58±0.04[b] | 3.43±0.51 |
| Libokemkem | 1.00±0.09[b] | 13.80±0.54[b] | 29.17±1.60 | 6.17±0.45 | 1.05±0.14 | 3.88±0.35 | 1.02±0.08 | 0.62±0.09 | 0.90±0.09 | 0.52±0.04[b] | 3.58±0.51 |
| Dembecha | 0.98±0.10[b] | 13.91±0.60[b] | 30.08±1.79 | 6.77±0.50 | 1.18±0.16 | 4.18±0.40 | 1.18±0.09 | 0.55±0.10 | 0.93±0.10 | 0.62±0.05[ab] | 3.92±0.57 |
| Treatment Feeds | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Treatment-1 | 1.16±0.07 | 14.18±0.40 | 29.13±1.20 | 6.75±0.33 | 1.23±0.10 | 3.95±0.27 | 1.08±0.06 | 0.62±0.07 | 0.86±0.06 | 0.62±0.03 | 4.25±0.38 |
| Treatment-2 | 1.14±0.07 | 14.27±0.40 | 30.33±1.20 | 6.39±0.33 | 1.02±0.10 | 4.23±0.27 | 1.08±0.06 | 0.50±0.07 | 0.91±0.06 | 0.60±0.03 | 3.36±0.38 |
| Breed*Feed | ns | ns | ns | ns | ns | ns | ns | ns | ns | * | ns |

**Table 6 -** Continued

| Parameter | Bladder | L+B | PF | SI | LI | OF | Hump | Testicle | Penis | SF | FG | EG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE | LSM±SE |
| Overall | 0.34±0.03 | 4.69±0.14 | 1.26±0.10 | 11.19±0.62 | 7.36±0.74 | 5.54±0.38 | 6.5±0.45 | 0.50±0.03 | 0.53±0.03 | 1.84±0.11 | 35.13±1.49 | 9.28±0.34 |
| Cattle Breeds | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Yilmana Densa | 0.39±0.05 | 4.81±0.30 | 1.34±0.20 | 11.80±1.31 | 6.93±1.55 | 5.66±0.81 | 7.21±0.96 | 0.53±0.06 | 0.59±0.05 | 2.03±0.23 | 34.91±3.15 | 9.53±0.72 |
| Mecha | 0.32±0.05 | 4.72±0.26 | 0.93±0.18 | 10.95±1.17 | 8.03±1.39 | 4.52±0.72 | 6.03±0.86 | 0.48±0.05 | 0.52±0.05 | 1.87±0.21 | 32.70±2.81 | 8.45±0.64 |
| Libokemkem | 0.37±0.05 | 4.38±0.26 | 1.32±0.18 | 10.98±1.17 | 6.77±1.39 | 6.15±0.72 | 6.58±0.86 | 0.48±0.05 | 0.52±0.05 | 1.83±0.21 | 36.22±2.81 | 9.20±0.64 |
| Dembecha | 0.28±0.05 | 4.87±0.30 | 1.44±0.20 | 11.03±1.31 | 7.72±1.55 | 5.83±0.81 | 6.47±0.96 | 0.48±0.06 | 0.48±0.05 | 1.63±0.23 | 36.68±3.15 | 9.94±0.72 |
| Treatment Feeds | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| Treatment-1 | 0.32±0.04 | 4.70±0.20 | 1.37±0.13 | 11.88±0.88 | 6.75±1.04 | 6.13±0.54 | 7.20±0.64 | 0.52±0.04 | 0.54±0.04 | 1.78±0.16 | 35.12±2.11 | 9.48±0.48 |
| Treatment-2 | 0.36±0.04 | 4.68±0.20 | 1.15±0.13 | 10.50±0.88 | 7.97±1.04 | 4.95±0.54 | 5.95±0.64 | 0.48±0.04 | 0.51±0.04 | 1.90±0.16 | 35.13±2.11 | 9.08±0.48 |
| Breed*Feed | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

***Note***: - a, b, c = Means in a column with different subscripts are significantly different (P<0.05), T = Tail, HS = Head and skin, H = Hide, FH = Feet with hooves, Tg = Tongue, LT = Lung and Trachea, Hr = Heart, HF = Heart fat, P = Pancreas, K = Kidney, KF = Kidney fat, B = Bladder, L+B = Liver + Bile, PF = Pelvic fat, SI = Small Intestine, LI = Large Intestine, OF = Omental fat, Hp = Hump, Ts = Testicle, Pn = Penis, SF = Scrotal fat, FG = Full gut, EG = Empty gut, LSM = Least square means, SE = standard error, ns = none –significant (P>0.05), * = P<0.05, Treatment-1. 60:40 concentrate: roughage ratio of the animals' daily dry matter intake; Treatment-2. 70:30 of concentrate: roughage ratio of the animals' daily dry matter intake



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The overall values of heart (1.0±0.04 kg), liver and bile (4.69±0.14 kg), kidney (0.61±0.02 kg), and lung and trachea (4.09±0.19 kg) weights obtained in the present study were lower than the values of heart (1.19 kg), kidney (0.72 kg), liver (5.32 kg), and lung (5.21 kg) weights reported for Charolais x Nelore steers fed ground corn in Santa Maria, Brazil (Freitas et al., 2019). Similarly, the values of the kidney (1.61±0.04 kg), liver (7.53±0.12 kg), heart (2.52±0.05 kg), and pancreas (0.56±0.08 kg) of pure Holstein calves were greater than the values reported in the present study (Rezagholivand et al., 2021). The overall value of the rib eye area (263.92±8.47 $mm^2$) of the evaluated cattle breeds was lower than the value recorded for Nguni heifers aged 24 months (4412.30±978.89 $mm^2$) fed pasture-based grazing and 10% cactus diets (Nyambali et al., 2022). In addition, compared with the present findings, a greater value of the rib eye area (5.791±2.34 $inch^2$) was reported for Arsi, Borana, HF-cross, and Harar bulls in Ethiopia (Musa et al., 2021). However, Musa et al. (2021) reported lower values of scrotal fat (0.52±0.04 kg), kidney fat (0.57±0.04 kg), pelvic fat (0.29±0.02 kg), omental fat (0.88±0.07 kg), and heart fat (0.53±0.03 kg) for Arsi, Borana, HF-cross, and Harar bulls than the present findings. Similarly, compared with the present findings, lower values of kidney fat (1.01±0.09 kg), heart fat (0.30±0.02 kg), omental fat (1.35±0.13 kg), and pelvic fat (1.09±0.05 kg) were reported for the Arsi, Harar, Jersey x Horro, and Ogaden cattle breeds (Erge et al., 2022).

## CONCLUSION

The study demonstrated that beef cattle breeds from northwest Amhara exhibit promising feedlot performance and carcass yield when finished under controlled feeding conditions. Significant differences were observed among breeds, with Yilmana Densa cattle outperforming others in slaughter weight, hot carcass weight, and cold carcass weight, highlighting their superior beef production potential. In contrast, dietary treatment (60:40 vs. 70:30 concentrate: roughage ratios) did not significantly influence growth or carcass traits, indicating that breed factors contributed more strongly than feed ratio in this context. The non-significant effect between the treatment diets indicates that there is an optimum roughage: concentrate ratio. Overall, indigenous cattle breeds in the region can provide acceptable meat yield under smallholder and commercial fattening systems, but their full potential remains underexplored. Further investigations are required to exhaustively quantify the feedlot potential and carcass yield and quality of these cattle breeds under different age groups with varying dietary supplements.

## DECLARATIONS

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**Ethics approval and consent to participate**

The authors complied with the ARRIVE guidelines and or the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education by the New York Academy of Sciences, Ad Hoc Animal Research Committee. The proposal was presented and approved by the Research and Post-graduate Vice Dean's Office of the College of Agriculture and Environmental Sciences of Bahir Dar University.

**Consent for publication**

All authors agree to the publication of this manuscript.

**Availability of data and materials:**

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

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**Authors Contributions**

F.Tegegne and M.Taye contributed to the development of the concept note, animal selection, experimental animal follow-up, data analysis, reviewed and finalized the manuscript; D.Kebede, M.Getaneh, and E.Admasu contributed to animal selection, experimental animal follow-up, data collection, data analysis, drafted the manuscript; B.Asmare, H.Tamrat, N.Beyero, and A.Tassew contributed to the development of the concept notes, formulation of the rations, experimental animal follow-up, reviewed and approved the manuscript.

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**Competing interests**

The authors declare that they have no competing interests.



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# DIETARY LEUCAENA LEAVES IMPROVE GROWTH PERFORMANCE AND CARCASS QUALITY OF VIETNAMESE GOATS

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**ABSTRACT**: The experiment was conducted at a research farm for sixteen male goats, with an average body weight of 12.32 ± 0.14 kg. They were randomly allocated into 4 groups corresponding to 4 diets and fed individually. The diets were formulated to consist of 90% of Guinea grass (*Panicum maximum*) and 10% of concentrated feed as basal (in DM). Leucaena leaves were substituted at 0%, 10%, 20% and 30% of Guinea grass in four respective diets. A 2-week adaptation period was provided for the goats to the diets and feeding system before data collection. Feed intake, weight gain, feed conversion ratio, and carcass traits of goats differed significantly among the four diets ($P < 0.05$). The inclusion of leucaena leaves in the diets increased feed intake. As the levels of leucaena leaves in diets increased up to 30%, there were corresponding improvements in weight gain. Daily weight gain increased from 45 to 61 g/day and feed conversion ratio (FCR) decreased from 8.43 to 6.62 kg feed/kg gain. Higher leucaena inclusion improved carcass traits but did not affect loin meat quality. Economic analysis also indicated that including up to 30% leucaena leaves in the goats' diet provides a profitable outcome for farmers. The economic impact increased with the rising levels of leucaena leaves in the goats' diet. It is recommended that leucaena leaves be utilized for goat raising in smallholder farming systems in Vietnam.

**Keywords:** Carcass, Feed conversion ratio, Goats, Growth, Leucaena.

## INTRODUCTION

Goat production is an important contributor to global meat and dairy supply, particularly in developing countries where smallholders dominate (Hegde, 2020). Goats are valued for their low investment requirements, adaptability to harsh climates, and growing consumer demand for meat. However, productivity remains limited by feed shortages, protein deficiencies, and the decline of natural grazing lands (Mazinani and Rude, 2020; Lohani and Bhandari, 2021; Nguyen et al., 2023). In Vietnam, the goat population has more than doubled in the past decade, with over 417,000 households engaged in small-scale farming, yet feed scarcity continues to restrict production efficiency (Nguyen et al., 2023).

Leucaena (*Leucaena leucocephala*) is a perennial legume that offers a promising solution to these constraints. Rich in crude protein (20–30% DM), it produces over 6 tons DM/ha annually, adapts well to tropical environments, and can be harvested year-round with minimal inputs (Casanova-Lugo et al., 2014). Studies have demonstrated that leucaena improves feed intake, growth, and carcass yield in ruminants (Muinga et al., 1995; Wiyabot, 2022; Marhaeniyanto et al., 2023). Although it contains anti-nutritional compounds such as mimosine, these can be managed when inclusion rates are controlled (De Angelis et al., 2021).

This study aims to investigate the effects of different levels of leucaena leaves in grass-based diets on growth performance, meat quality and economic analysis of goat production in small-scale farming in Vietnam. Co goat production is primarily managed by smallholders, who feed their goats mainly by natural grasses, with leguminous forages rarely included in their diets (Nguyen et al., 2023; Olmo et al., 2024). Therefore, the objective of our experiment is to identify the optimal inclusion rates of leucaena in goat feeds that do not adversely affect animal growth.

## MATERIALS AND METHODS

### Animals

The experiment was conducted on 16 male goats of the local breed Co goat, at the age of 9 months, with an initial average weight of 12.32 ± 0.14 kg per goat. The goats were vaccinated against pasteurella, cholera, and foot-and-mouth disease. They were uniformly dewormed.



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### Diets and feeding

Guinea grass (*Panicum maximum* cv. Mombasa) and leucaena (*Leucaena leucocephala* cv. Taramba) were grown at the Institute for Development Studies, University of Agriculture and Forestry, Hue University. Guinea grass was harvested at a cutting interval of 45 days, and leucaena leaves were harvested at a cutting interval of 4 months. Only edible parts of guinea grass and leucaena leaves were collected daily, chopped, and thoroughly mixed before being fed to the goats as fresh matter. The concentrated feed was BEEF622 from the feed market. The chemical composition of the ingredients is presented in Table 1. Goats were randomly divided into 4 groups and fed according to 4 experimental diets with the levels of leucaena at 0%, 10%, 20%, and 30% on a dry matter basis (Table 2), including: Diet 1 (KP1) consists of 90% Guinea grass and 10% concentrated feed; Diet 2 (KP2) consists of 80% Guinea grass, 10% concentrated feed and 10% leucaena; Diet 3 (KP3) consists of 70% Guinea grass, 10% concentrated feed and 20% leucaena; Diet 4 (KP4) consists of 60% Guinea grass, 10% concentrated feed and 30% leucaena.

Goats were kept individually in separate pen cages, equipped with a water supply and free access to mineral blocks. They were fed twice daily at 9:00 a.m. and 3:00 p.m. The feeding process began with concentrates, which were given separately, followed by mixed green feed. The amount of feed in DM provided to goats per day was calculated at 4% of their body weight. At the end of each day, any leftover feed was collected, dried, and weighed. Every month, the amount of feed supply was adjusted according to each goat's body weight to ensure that the feed supply met their nutritional requirements. An adaptation period of 2 weeks before the feeding experiment, which followed, lasted for 4 months, from August to December 2024.

**Table 1 -** Chemical composition of the ingredients

| Ingredients | DM (%) | Chemical composition (% in DM basic) | | | |
|---|---|---|---|---|---|
| | | CP | NDF | ADF | Ash |
| Guinea grass | 24.3 | 8.6 | 73.0 | 40.6 | 8.0 |
| Leucaena leaves | 33.2 | 25.4 | 33.0 | 19.2 | 7.0 |
| Concentrated feed | 86.4 | 19.0 | 37.6 | 20.2 | 10.0 |

DM is the abbreviation of dry matter, CP is the abbreviation for crude protein, NDF is the abbreviation of neutral detergent fibre, and ADF is the abbreviation of acid detergent fibre.

**Table 2 -** Ingredient and chemical composition of diets

| Ingredient composition (kg/100 kg DM) \ Experimental diets | KP1 | KP2 | KP3 | KP4 |
|---|---|---|---|---|
| Concentrated feed | 10 | 10 | 10 | 10 |
| Guinea grasses | 90 | 80 | 70 | 60 |
| Leucaena leaves | 0 | 10 | 20 | 30 |
| Total in ration | 100 | 100 | 100 | 100 |
| **Chemical composition (g/kg DM)*** | | | | |
| OM | 931 | 930 | 929 | 929 |
| CP | 96 | 113 | 130 | 146 |
| EE | 19 | 22 | 26 | 29 |
| NDF | 694 | 654 | 614 | 574 |
| ADF | 386 | 364 | 342 | 321 |
| ME (MJ/kg DM) | 9.34 | 9.61 | 9.88 | 10.15 |

*Values calculated based on the composition of ingredients. OM is the abbreviation of organic matter, EE is the abbreviation of ether extract, ME is the abbreviation of metabolisable energy. Diet 1 (KP1) consists of 90% Guinea grass and 10% concentrated feed; Diet 2 (KP2) consists of 80% Guinea grass, 10% concentrated feed and 10% leucaena; Diet 3 (KP3) consists of 70% Guinea grass, 10% concentrated feed and 20% leucaena; Diet 4 (KP4) consists of 60% Guinea grass, 10% concentrated feed and 30% leucaena.

### Experiment design

The experiment was conducted at the research farm of the Institute for Development Studies of the University of Agriculture and Forestry, Hue University, Vietnam, from August 2024 to January 2025. Sixteen (16) goats were randomly divided into 4 groups corresponding to 4 diets (Completely Randomized Design), raised and fed individually in 16 pens. Each pen measured 1.0 m in height, 1.5 m in length, and 0.8 m in width, and was located 0.8 m above the ground. The pens were identical in size and environmental conditions. Each pen had a separate feeder and tap water. All pens were set in an animal facility that maintained uniform environmental conditions.

### Data collection

Feed intake was recorded daily for each goat. The body live weight of each goat was measured at 8:00 a.m. before feeding on days 0, 30, 60, 90, and 120 of the experiment. The prices of forages were calculated based on the actual price

of 700 VND/kg of Guinea grass and 1,000 VND/kg of leucaena leaves on the fresh material (Conversion rate: 1 USD ≈ 25,000 VND in December 2024). The price of concentrated feed was determined based on the market price. After 4 months, at the end of the experiment, 12 goats were slaughtered to measure the characteristics of the carcass and the chemical composition of the loin. The measurement of carcass traits in goats was conducted following the Vietnam National Standard on Animal Welfare – Slaughter (TCVN 13905-1:2023). Before slaughter, the goats were fasted for 18 hours, provided free access to water, and measures were taken to minimize stress. The pre-slaughter weight was recorded using the Nhon Hoa Scale 30 kg (model CDH-30) with an error margin of ± 50 g to ± 150 g. The goats were electronically stunned before having their jugular vein and carotid artery cut. Blood was drained into a pre-weighed bucket and weighed. Hair was removed and weighed. The internal organs (digestive tract, lungs, trachea, heart, liver, kidneys, kidney fat, spleen and pancreas) were removed and weighed by Nhon Hoa Scale 10 kg (model PDM 036-2017) with an error margin of ± 5 g to ± 15 g. The empty body weight (excluding blood, hair, and internal organs) was measured. After the skin was removed, the head was separated at the atlas vertebra, and the legs were separated at the carpal and tarsal joints, then weighed. The hot carcass weight was measured without blood, hair, internal organs, head, legs and skin. Finally, loin meat samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), and total ash content.

### Chemical analysis

Chemical analysis of the samples was conducted to measure dry matter (DM), crude protein (CP), ether extract (EE), and total ash according to AOAC (1990). Additionally, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed using the method described by Van Soest et al. (1991).

### Data analysis

Collected data were statistically analyzed using ANOVA with Minitab version 16.2.0 (2010). Comparison of significant differences in mean values was assessed at the probability level of $P<0.05$. The statistical model used is as follows:

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

where: $\mu$ represents the overall mean value; $T_i$ denotes the effect of the diet; and $e_{ij}$ is the error term.

## RESULTS AND DISCUSSION

### Feed intake

The daily feed intake of goats in each month and the average of 4 months differed between the diets ($P<0.05$; Table 3). The lowest feed intake was recorded at 385 g/day for the KP1 without leucaena, while the highest was 427 g/day for the KP2 containing 10% of leucaena in the diet (on a DM basis). The trend of feed intake decreased when leucaena leaf increased to 20 and 30%, although it remained higher than the intake for KP1 without leucaena. Previous studies have shown that substituting leucaena in diets based on grass or maize can lead to increased feed intake (Balogun and Otchere, 1995; Haque et al., 1997; Fasae et al., 2011). Wiyabot (2022) found no significant difference in feed intake when goats were fed diets with leucaena levels at 25%, 50%, 75%, and 100%. However, a diet with 40% leucaena leaves resulted in slight hair loss (Balogun and Otchere, 1995). The leucaena contents of toxicity may contribute to reduced feed intake and productivity (Phaikaew et al., 2012). In our experiment, increasing the level of leucaena leaves in the diets led to higher feed intake compared to goats fed only on guinea grass. A grass-leucaena mix increased feed intake, but excessive leucaena may reduce it (Table 3).

**Table 3 -** Daily feed intake (g DM/head/day) by month of experiment

| Daily feed intake by month | Experiment diet (Mean ± Standard Deviation) | | | | SEM | P value |
|---|---|---|---|---|---|---|
| | KP1 | KP2 | KP3 | KP4 | | |
| First month | 325 ± 22.8$^{b}$ | 402 ± 33.1$^{a}$ | 366 ± 23.5$^{ab}$ | 356 ± 20.9$^{ab}$ | 12.8 | 0.009 |
| Second month | 362 ± 17.4 | 393 ± 30.0 | 379 ± 5.1 | 373 ± 11.8 | 9.25 | 0.191 |
| Third month | 384 ± 24.8 | 418 ± 22.0 | 417 ± 3.1 | 414 ± 10.5 | 8.73 | 0.054 |
| Fourth month | 433 ± 12.1 | 453 ± 20.8 | 460 ± 6.1 | 458 ± 14.6 | 7.20 | 0.080 |
| Average | 385 ± 18.4$^{b}$ | 427 ± 23.4$^{a}$ | 415 ± 7.4$^{ab}$ | 410 ± 13.9$^{ab}$ | 8.42 | 0.028 |
| **Daily feed intake of ingredients** | | | | | | |
| Concentrated feed | 85.28 ± 3.22 | 89.47 ± 5.94 | 91.28 ± 1.58 | 88.36 ± 5.84 | 2.27 | 0.341 |
| Guinea grass | 297 ± 18.16$^{a}$ | 283 ± 18.58$^{a}$ | 239 ± 5.88$^{b}$ | 198 ± 8.70$^{c}$ | 7.01 | 0.001 |
| Leucaena leaves | 0$^{d}$ | 42.8 ± 2.82$^{c}$ | 81.4 ± 2.00$^{b}$ | 115.8 ± 5.08$^{a}$ | 1.54 | 0.001 |

$^{a, b, c}$ values within a row with different letters were significantly different ($P<0.05$). Diet 1 (KP1) consists of 90% Guinea grass and 10% concentrated feed; Diet 2 (KP2) consists of 80% Guinea grass, 10% concentrated feed and 10% leucaena; Diet 3 (KP3) consists of 70% Guinea grass, 10% concentrated feed and 20% leucaena; Diet 4 (KP4) consists of 60% Guinea grass, 10% concentrated feed and 30% leucaena.

**Effects of dietary levels of leucaena on the growth of goats**

Increasing the level of leucaena leaves in diets that include Guinea grass and concentrated feed resulted in improved live weight and daily weight gain, while also decreasing the feed conversion ratio (FCR) (Table 4). In the first two months, there was no significant difference in the live weight of goats across four diets. However, differences in growth performance became noticeable during the third and fourth months ($P<0.05$). Substituting leucaena leaves into the diets led to a positive increase in the body live weight of the goats ($P<0.05$). During the first month of the experiment, there was no difference in daily weight gain among the diets. However, from the second month to the fourth month, differences in daily weight gain of goats emerged between the control group (KP1), which had no leucaena leaves, and the diets that included leucaena leaves (KP2, KP3, and KP4) ($P<0.05$). Increasing the level of leucaena leaves in the diet was associated with higher daily weight gain in the goats, with the highest gain observed at 30% leucaena leaves in KP3. The FCR improved from KP1 to KP4, decreasing from 8.43 to 6.62, respectively.

The inclusion of leucaena leaves in goat rations has been studied in many countries. Our experiment focused on local breeds "*Co* goat", which typically have smaller body weight and daily weight gains compared to breeds such as Back Thao, Boar, or other crossbreeds (Pham and Nguyen, 2015; Pham et al., 2019). We found that incorporating leucaena leaves at levels up to 30% in the diets of these local breeds of goat in Vietnam improved both feed intake and daily weight gain. These findings align with those of Adejumo and Ademosun (1991); Fasae et al. (2011); Marhaeniyanto et al. (2023), who also reported positive effects from including various levels of leucaena in diets. Specifically, Marhaeniyanto et al. (2023) observed that supplementing with 20% *Leucaena leucocephala* leaves in a concentrate containing 15% crude protein resulted in an average daily gain of 99.29 g/head/day. Conversely, Wiyabot (2022) reported no significant difference in daily weight gain among goats when leucaena was used as a roughage substitute at levels exceeding 50%. Additionally, Adejumo and Ademosun (1991) recommended that to promote growth without adverse effects, leucaena should not comprise more than 40% in goat rations. We recommend including 30% of leucaena in the diet to ensure that toxicity thresholds remain manageable for smallholder farming systems and align well with the needs of our goat breed. Additionally, biomass production of leucaena at the smallholder scale in Vietnam is limited. Therefore, our findings suggest that maintaining this 30% leucaena in the diet will optimize and sustain feed supply effectively.

**Table 4** - Weight gain, daily weight gain and feed conversion ratio of goats by month of experiment

| Live weight (kg/head) | KP1 | KP2 | KP3 | KP4 | SEM | P value |
|---|---|---|---|---|---|---|
| Initial body weight | 12.15 | 12.37 | 12.55 | 12.20 | 0.300 | 0.778 |
| First month | 13.05 | 13.45 | 13.68 | 13.42 | 0.294 | 0.530 |
| Second month | 14.20 | 14.90 | 15.22 | 15.08 | 0.304 | 0.138 |
| Third month | 15.95$^{b}$ | 16.98$^{ab}$ | 17.40$^{a}$ | 17.40$^{a}$ | 0.268 | 0.007 |
| Fourth month | 17.52$^{b}$ | 18.73$^{a}$ | 19.33$^{a}$ | 19.48$^{a}$ | 0.258 | 0.001 |
| **Daily weight gain (g/day)** | | | | | | |
| First month | 30 | 36 | 38 | 41 | 2.98 | 0.127 |
| Second month | 38$^{b}$ | 48$^{ab}$ | 52$^{a}$ | 55$^{a}$ | 2.89 | 0.009 |
| Third month | 58$^{b}$ | 69$^{a}$ | 73$^{a}$ | 78$^{a}$ | 2.51 | 0.001 |
| Fourth month | 52$^{c}$ | 58$^{bc}$ | 64$^{ab}$ | 69$^{a}$ | 2.00 | 0.001 |
| Average | 45$^{c}$ | 53$^{b}$ | 56$^{ab}$ | 61$^{a}$ | 1.59 | 0.001 |
| FCR (kg feed/kg gain) | 8.43$^{a}$ | 7.88$^{ab}$ | 7.20$^{bc}$ | 6.62$^{c}$ | 0.246 | 0.001 |

$^{a, b, c}$ values within a row with different letters were significantly different ($P<0.05$). Diet 1 (KP1) consists of 90% Guinea grass and 10% concentrated feed; Diet 2 (KP2) consists of 80% Guinea grass, 10% concentrated feed and 10% leucaena; Diet 3 (KP3) consists of 70% Guinea grass, 10% concentrated feed and 20% leucaena; Diet 4 (KP4) consists of 60% Guinea grass, 10% concentrated feed and 30% leucaena.

**Carcass quality of goats**

There were differences in slaughter body weight, hot carcass weight, and percentage of carcass among goats on different diets (Table 5). Increasing the level of leucaena leaves in their diets resulted in varying growth performance in the goats. The hot carcass weight was found to be higher in the rations containing 20% and 30% leucaena leaves, while the lowest hot carcass weight was observed in the rations without leucaena inclusion ($P<0.05$) (Table 5). The carcass weight, body meat, and their ratios in KP3 and KP4 were higher than those in KP1. Increasing the level of leucaena leaves in the diet markedly improved the carcass characteristics of slaughter goats. However, there were no differences in the meat quality of the loin based on DM, CP, EE, and total ash among diet treatments. The study concluded that incorporating leucaena leaves up to 30% in a basal goat diet of guinea grass and concentrated feed improved meat production, but had no effects on the loin meat quality in local goats. Leucaena inclusion improved carcass traits compared to traditional local goat diets in Vietnam (Pham et al., 2019). This finding aligns with the carcass characteristics observed in Afar goats fed with leucaena in Ethiopia (Terefe et al., 2013; Gebrehiwot et al., 2017) and in indigenous Anglo-Nubian hybrid goats in Thailand (Wiyabot, 2022).

**Economic analysis**

The cost-benefit analysis of investing in this experiment was calculated based on the prices of animals, feed, and veterinary services for goats during their feeding period. Table 6 provides a summary of the economic impact data on sixteen male goats raised on four different rations. The lowest economic impact was found in KP1. As the inclusion of leucaena leaves in the diets increased from 10%, 20%, to 30%, the economic analysis results increased by 28%, 43% and 62%, respectively, compared to the baseline without leucaena leaves. In the condition of market price fluctuations, we conducted a sensitivity analysis to evaluate how the profit changed in response to a 10% increase or 10% decrease in the sale price. Our findings indicate that, in all scenarios, KP2, KP3, and KP4 consistently generate higher profits than KP1 (Table 6). The cost-benefit analysis of goat farming in this experiment indicates that goat farming can be a profitable business for smallholders in Vietnam when incorporating leucaena leaves at up to 30% dry matter (DM) in their forage. Leucaena is primarily fed to goats as fresh material through cut-and-carry feeding systems, which are both flexible and labor-efficient (Palmer et al., 2010). Under tropical conditions, leucaena can provide over 4 tonnes of foliage per hectare per year (Casanova-Lugo et al., 2014; Cowley and Roschinsky, 2019), making it a valuable feed source for small-scale goat production.

**Table 5 -** Carcass characteristics and loin meat quality of goats (n=3)

| Category \ Experimental diets | KP1 | KP2 | KP3 | KP4 | SEM | P value |
|---|---|---|---|---|---|---|
| Pre-slaughter weight (kg) | 17.53$^{b}$ | 18.57$^{ab}$ | 19.20$^{a}$ | 19.60$^{a}$ | 0.324 | 0.009 |
| Blood (kg) | 0.60 | 0.61 | 0.61 | 0.61 | 0.027 | 0.992 |
| Hair (kg) | 0.03 | 0.05 | 0.04 | 0.04 | 0.006 | 0.122 |
| Internal organs (kg) | 5.80 | 5.45 | 5.54 | 5.41 | 0.092 | 0.059 |
| Empty body weight (kg) | 10.54$^{c}$ | 11.48$^{bc}$ | 12.22$^{ab}$ | 12.89$^{a}$ | 0.279 | 0.002 |
| Head (kg) | 1.28$^{b}$ | 1.43$^{ab}$ | 1.48$^{a}$ | 1.54$^{a}$ | 0.036 | 0.006 |
| Legs (kg) | 0.50 | 0.51 | 0.53 | 0.60 | 0.026 | 0.102 |
| Skin (kg) | 1.29$^{b}$ | 1.38$^{ab}$ | 1.54$^{ab}$ | 1.62$^{a}$ | 0.062 | 0.021 |
| Hot carcass weight (kg) | 7.47$^{c}$ | 8.15$^{bc}$ | 8.66$^{ab}$ | 9.11$^{a}$ | 0.205 | 0.003 |
| **Dressing percentage (%)** | | | | | | |
| Pre-slaughter weight bases (%) | 42,59$^{c}$ | 43.88$^{bc}$ | 45.12$^{ab}$ | 46.45$^{a}$ | 0.529 | 0.005 |
| Empty body weight bases (%) | 70.85 | 71.02 | 70.91 | 70.76 | 0.470 | 0.982 |
| Chemical composition of loin | | | | | | |
| DM (%) | 22.55 | 23.19 | 23.27 | 23.23 | 1.74 | 0.989 |
| CP (%) | 84.72 | 85.31 | 86.31 | 85.70 | 0.587 | 0.388 |
| EE (%) | 3.78 | 4.35 | 3.05 | 4.02 | 0.434 | 0.320 |
| Total ash (%) | 4.64 | 4.59 | 4.73 | 5.58 | 0.210 | 0.951 |

$^{a, b, c}$ values within a row with different letters were significantly different (P<0.05). Diet 1 (KP1) consists of 90% Guinea grass and 10% concentrated feed; Diet 2 (KP2) consists of 80% Guinea grass, 10% concentrated feed and 10% leucaena; Diet 3 (KP3) consists of 70% Guinea grass, 10% concentrated feed and 20% leucaena; Diet 4 (KP4) consists of 60% Guinea grass, 10% concentrated feed and 30% leucaena.

**Table 6 -** Economic analysis of goat raising by diets

| Item | Unit | Price (VND)* | Experimental diets | | | |
|---|---|---|---|---|---|---|
| | | | KP1 | KP2 | KP3 | KP4 |
| **Investment costs** | | | | | | |
| Animal breed | kg | 120,000 | 1,458,000 | 1,485,000 | 1,506,000 | 1,464,000 |
| Concentrated feed | kg | 13,000 | 133,032 | 139,571 | 142,392 | 137,846 |
| Guinea grass | kg | 700 | 113,466 | 107,872 | 91,164 | 75,647 |
| Leucaena leaves | kg | 1,000 | - | 17,123 | 32,559 | 46,315 |
| Vaccine | head | 50,000 | 50,000 | 50,000 | 50,000 | 50,000 |
| Total | | | 1,754,498 | 1,799,566 | 1,822,115 | 1,773,808 |
| **Sale income** | | | | | | |
| Fixed sale price | kg | 120,000 | | | | |
| Income | VND | | 2,103,000 | 2,247,000 | 2,319,000 | 2,337,000 |
| Profit | VND | | 348,502 | 447,435 | 496,885 | 563,192 |
| % compared to KP1 | | | 100 | 128 | 143 | 162 |
| **Sensitivity analysis** | | | | | | |
| Sale price increases by 10% | kg | 132,000 | | | | |
| Income | VND | | 2,313,000 | 2,472,000 | 2,551,000 | 2,571,000 |
| Balance/Profit | VND | | 558,502 | 672,434 | 728,885 | 797,192 |
| % compared to KP1 | | | 100 | 120 | 131 | 143 |
| Sale price decreases by 10% | kg | 108,000 | | | | |
| Income | VND | | 1,893,000 | 2,022,000 | 2,087,000 | 2,103,000 |
| Balance/Profit | VND | | 138,502 | 222,434 | 264,885 | 329,192 |
| % compared to KP1 | | | 100 | 161 | 191 | 238 |

*Conversion rate: 1 USD ≈ 25,000 VND in December 2024. Diet 1 (KP1) consists of 90% Guinea grass and 10% concentrated feed; Diet 2 (KP2) consists of 80% Guinea grass, 10% concentrated feed and 10% leucaena; Diet 3 (KP3) consists of 70% Guinea grass, 10% concentrated feed and 20% leucaena; Diet 4 (KP4) consists of 60% Guinea grass, 10% concentrated feed and 30% leucaena.

## CONCLUSIONS

This study demonstrated that feed intake, weight gain, feed conversion ratio, and carcass traits of goats differed significantly among the four dietary treatments. The inclusion of leucaena leaves in the diets increased feed intake and improved growth performance as the proportion of leucaena rose to 30%. Daily weight gain increased from 45 g/day in the control diet to 61 g/day in the KP4 diet, while the feed conversion ratio improved from 8.43 to 6.62 kg feed/kg gain. Higher levels of leucaena also enhanced carcass traits, although loin meat quality remained unaffected. Economic analysis confirmed that supplementing up to 30% leucaena leaves in goat diets yields profitable outcomes for smallholder farmers, with greater economic benefits at higher inclusion levels. Overall, the findings support the recommendation that leucaena leaves be incorporated into goat feeding strategies to improve productivity and profitability in smallholder farming systems in Vietnam.

## DECLARATIONS

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### Data availability

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

### Authors' contributions

TN.Liem, NM.Dung, and LM.Duc were responsible for implementing the research activities. DT.Hai and VTM.Tam carried out the laboratory work, while LTQ.Anh conducted the economic analysis. S.Chotchutima and P.Boonsaen collaborated as partners in developing the research plan and provided funding through the project "Improving Smallholder Goat Fattening Systems Based on Fodder from Grasses and Legumes in Thailand, Laos, and Vietnam", supported by the Lancang-Mekong Cooperation Special Fund.

### Ethical regulations

All experimental procedures involving animals were conducted in accordance with Vietnamese regulations on animal welfare and research ethics and were approved by the Animal Ethics Committee of Hue University (Approval No. HUVN0055, dated 20 February 2025). The authors also complied 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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# THE INFLUENCE OF RIPENING TIME ON THE PHYSICOCHEMICAL CHARACTERISTICS OF CRAFT HARD GOAT CHEESES

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RESEARCH ARTICLE

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**ABSTRACT**: The unique taste characteristics of craft hard cheeses made from raw goat milk, ripened using mites *Acarus siro* L., have contributed to increasing consumer demand enable the assessment of their quality and authenticity. In this study, 15 heads of Alpine and Yoghurt cheeses each weighing 4.5-5.0 kg were produced from raw goat milk and allowed to ripen for 12 and 18 months, respectively. Both cheeses were ripened with natural surface colonization by the mites *Acarus siro* L. It was found that the moisture content of Alpine cheese decreased from 43.31 on day 7 to 28.99% at 12 months of age, and the moisture content of Yoghurt cheese decreased from 46.90% on day 7 to 29.99% at 18 months. Moisture loss in both cheeses was strongly dependent on ripening time. The protein content in craft hard cheeses increased with age: from 21.45% to 28.68% in Alpine cheese and from 20.52% to 29.52% in Yoghurt cheese. Corresponding to the increase in dry matter content, fat content also increased in both varieties: from 24.45% to 31.50% in Alpine cheese and from 22.06% to 29.91% in Yoghurt cheese. A characteristic feature of both cheeses was the formation of holes, the size and distribution of varied with ripening duration. The hardness of Alpine and Yoghurt cheeses decreased with age, while the fracturability increased, reaching a minimum in the oldest cheeses, a change closely related to moisture loss. The rind of old-ripened Alpine and Yoghurt cheeses exhibited an amber color of varying intensity, with small verrucae due to the activity of the mite *Acarus siro* L. The observed changes in the physicochemical characteristics of young, mature, and old-ripened artisanal cheeses made from raw goat milk can serve as criteria for assessing their quality, age, and authenticity. Production of such cheeses contributes to diversifying the product range and enhancing the market competitiveness of premium goat cheeses.

**Keywords:** Alpine cheese, Dry matter, Mite *Acarus siro* L, Rind, Yoghurt cheese.

## INTRODUCTION

Cheese is a food product with a technology history spanning over 8,000 years. The raw material for cheese production is milk, sourced from various mammals. Milk-processing technologies continuously refined are crucial for developing new cheese varieties. By manipulating ripening parameters time, temperature, and humidity and incorporating additives such as fruits, nuts, or spices, producers achieve an extraordinary diversity of cheeses with distinctive textures, flavors, and aromas (Zhang et al., 2021). The growing emphasis on healthy eating has driven the production of raw-milk cheeses that undergo minimal or no processing. This primarily concerns the production of craft hard cheeses small ruminants, especially goats. Such cheeses are not only rich in macro- and micronutrients and bioactive compounds, but also harbor beneficial lactic acid bacteria that may support human health (Hosken et al., 2023). Recent studies report rising production and consumption of hard and semi-hard cheeses across Europe, trends that correlate with increased life expectancy and reduced cardiovascular disease risk in European populations (Nájera et al., 2021).

In the European Union, over 90% of cheese production is derived from cow's milk, with sheep and goat cheeses accounting for only 2%. Although Ukraine is not yet a major global cheese producer or exporter, it has substantial potential to expand goat-milk cheese varieties via smallholder farmers, driven by rising demand for mature and long-ripened cheeses (Mureșan et al., 2021). Despite representing a small proportion of global hard and semi-hard cheese production, goat cheeses are considered premium varieties thanks to their distinctive flavors and hold a place of pride on cheeseboards. Craft raw goat-milk cheeses produced in small batches and characterized by superior sensory qualities



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compared to industrial cheeses are particularly prized. These attributes are attributed to goat cheese's high fat content and elevated levels of free amino acids. These attributes are attributed to goat cheese's high fat content and elevated levels of free amino acids.

The chemical composition of cheese depends not only on the species and blend of milk but also on seasonal factors, herd health (Oliynyk et al., 2024), the initial milk composition (Mylostyvyi et al., 2023), and the specific production and ripening technologies. The structure, appearance, and internal texture of cheese serve as key quality criteria and influence consumer appeal. The rind color, slice shape, and presence, size, and distribution of holes provide visual quality cues that consumers use before tasting. The physicochemical changes in hard goat cheese are strongly influenced by ripening duration and environmental conditions. Ripening transforms fresh curd into cheese with defined appearance, texture, aroma, and flavor profiles. The unique sensory profile of raw goat-milk cheese arises from interactions among microbial communities and arthropods—particularly rind-forming mites—and the balance of chemical constituents. The physicochemical properties of hard cheese serve as indicators of quality, safety, maturity, and authenticity, reflecting the extent of lipolysis, proteolysis, and glycolysis during ripening (Álvarez and Fresno, 2021).

With each passing year, new consumers increasingly prefer cheeses made from raw goat milk due to their more pronounced and piquant taste compared to cheeses made using industrial pasteurization of milk. The main disadvantage of milk pasteurization is the inactivation of beneficial microorganisms together with pathogenic and undesirable ones. This leads to reduced activity of proteases and lipases, which are essential for the unique taste and aroma of cheeses (Sakaridis et al., 2022). In addition to the deteriorated sensory characteristics, pasteurisation affects milk quality, as evidenced by Canestrato Pugliese PDO cheese. Despite the stability of the chemical composition, sensory properties of this cheese were significantly inferior those of raw milk (Natrella et al., 2023). These data highlight the important role of the microbiota indigenous to a given region and responsible for) lipolysis and proteolysis, generating key aromatic compounds (Shulga et al., 2023). Consequently, the use of autochthonous rennet is explored to create a microbial consortium closely resembling that of) raw milk (Vera-Santander et al., 2024).

To date, researchers mostly continue to debate the safety of using of raw versus pasteurized milk in cheese-making. Proponents emphasize compliance with stringent sanitary requirements during milk production and processing, coupled with rigorous hygienic practices during ripening to ensure product safety. Despite recent growing interest in investigating the characteristics of craft cheeses produced from raw goat milk, their physicochemical composition has been inadequately characterized owing to the continuously expanding product range. Therefore, this study aims to determine the physicochemical characteristics of Alpine and Yoghurt craft hard cheeses produced from raw goat milk as a function of ripening period. This approach will enable the establishment of reliable criteria for age, quality, and authenticity.

## MATERIALS AND METHODS

### Animals

Goat milk was used in the study.Milk was sourced from Anglo-Nubian goats at Eco Farm Zhuravka in the Kyiv region.

### Experimental Design

In this study, two batches of Alpine and Yoghurt craft hard cheeses produced from raw goat milk were prepared according to the scheme described by Davydovych et al. (2025). The study period spanned May 2023 to January 2025. Samples were chosen based on age: young (7 days), mature (6 months) and aged (12 months for Alpine cheese and 18 months for Yoghurt cheese).

### Sampling

The study used 15 heads of Alpiyskiy cheese and 15 heads of Yogurtovy cheese. For analysis, 5 heads of Alpiyskiy cheese with a ripening period of 7 days, 6 months and 12 months were selected, as well as 5 heads of Cheese Yogurt aged 7 days, 6 months and 18 months. Average samples of cheese weighing at least 200 g were taken from each head, packed in vacuum packaging and delivered chilled to the analytical laboratory.

### Sample Analysis

Cheese chemical parameters s was analyzed at the State Scientific and Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE), in Kyiv, Ukraine. The SSRILDVSE testing center is accredited by the National Accreditation Agency of Ukraine under DSTU EN ISO/IEC 17025:201 standards. The moisture, dry matter, ash, protein, and fat contents of the goat-milk hard cheeses were determined as follows. Moisture content was measured by gravimetric analysis, drying samples in a VENTICELL oven (BMT, Czech Republic). Dry matter was calculated by difference. Nitrogen content was determined by the Kjeldahl method: samples were digested in an automatic mineralizer (Velp Scientifica DKL 12, Italy), distilled using a semi-automatic steam distiller (UDK 139, Velp Scientifica, Italy), and distilled ammonia quantified with an automatic Kjeldahl steam distillation unit (DKL 12, Velp Scientifica, Italy). Protein content was calculated using the appropriate nitrogen conversion factor. Ash content was assessed by incineration in a SNOL muffle furnace (Utenoselektrotechnika, Germany). Fat content was determined by acid hydrolysis (concentrated sulfuric acid and isoamyl alcohol), followed by centrifugation (Nova Safety centrifuge, Funke-Gerber, Germany) and measurement of the fat layer in a graduated butyrometer. Cheese hardness was expressed as the percentage ratio of moisture weight to the weight difference between the total sample and its fat portion. To identify the mite *Acarus siro*, the rind cuts of Alpine

and Yoghurt cheeses (aged > 6 months and 3–4 mm thick) were taken (Mullen and OConnor, 2019), placed on a glass slide and examined under liquid petrolatum (PJSC "Pharmaceutical Factory "Viola", Ukraine). Observations were performed with a Micromed Evolution ES-4140 microscope equipped with a camera adapter (Ningbo Shenghen Optics & Electronics Co., Ltd., Bulgaria).

**Statistical analysis**

Data were analyzed by one-way ANOVA. Changes in physicochemical parameters of Alpine and Yoghurt hard cheeses as a function of ripening period were evaluated by correlation and regression analyses.) Analyses were conducted using Microsoft Excel 2016 and XLSTAT (Addinsoft, Paris- 2017). Results are presented as mean ± SD. Within each cheese type, differences were considered significant at $P < 0.05$ using Tukey's test with Bonferroni correction.

## RESULTS AND DISCUSSION

The ripening of Alpine hard cheese was characterized by the greatest moisture loss at the 6th month, amounting to 12.47% compared to the young cheese aged 7 days. Moisture content decreased from 43.31% to 30.84% between the 7th day to 6 months. From the 6th to the 12th month of ripening, Alpine cheese showed minimal further moisture loss, with its moisture content stabilizing (Table 1). The proportions of the major dry-matter components of Alpine cheese varied in response to moisture loss. Specifically, fat content increased to 29.99% in mature cheese (6 months) and to 31.50% in aged cheese (12 months), compared to young cheese (7 days). Protein content exhibited similar trends. As expected, increased dry-matter content was comprised by higher ash content до 4.85% at 6 months and до 4.49% at 12 months relative to cheese aged 7 days. A strong inverse correlation relationship was observed between moisture content ($r = -0.904 \pm 0.081$, $P < 0.001$) and hardness ($r = -0.893 \pm 0.085$, $P < 0.001$), with ripening period, while fat content displayed a strong positive correlation ($r = -0.909 \pm 0.078$, $P < 0.001$). In all cases, the data were best fitted by a second-degree polynomial regression curve (Figure 1).

**Table 1 -** Chemical analysis of Alpine craft hard cheese, x ± SD, %

| Parameter \ Ripening period of cheese | 7th days | 6 months | 12 months |
|---|---|---|---|
| Moisture | 43.31 ± 1.22$^a$ | 30.84 ± 0.59$^b$ | 28.99 ± 0.66$^b$ |
| Fat | 24.45 ± 0.86$^b$ | 29.99 ± 0.54$^a$ | 31.50 ± 1.27$^a$ |
| Protein | 21.45 ± 0.61$^b$ | 28.90 ± 0.35$^a$ | 28.67 ± 0.34$^a$ |
| Ash | 3.19 ± 0.13$^b$ | 4.85 ± 0.22$^a$ | 4.49 ± 0.25$^a$ |

*Note: Different letters of superscript indicate the probable differences between the values within the same table row ($P < 0.05$) as determined by Tukey test with Bonferroni correction.

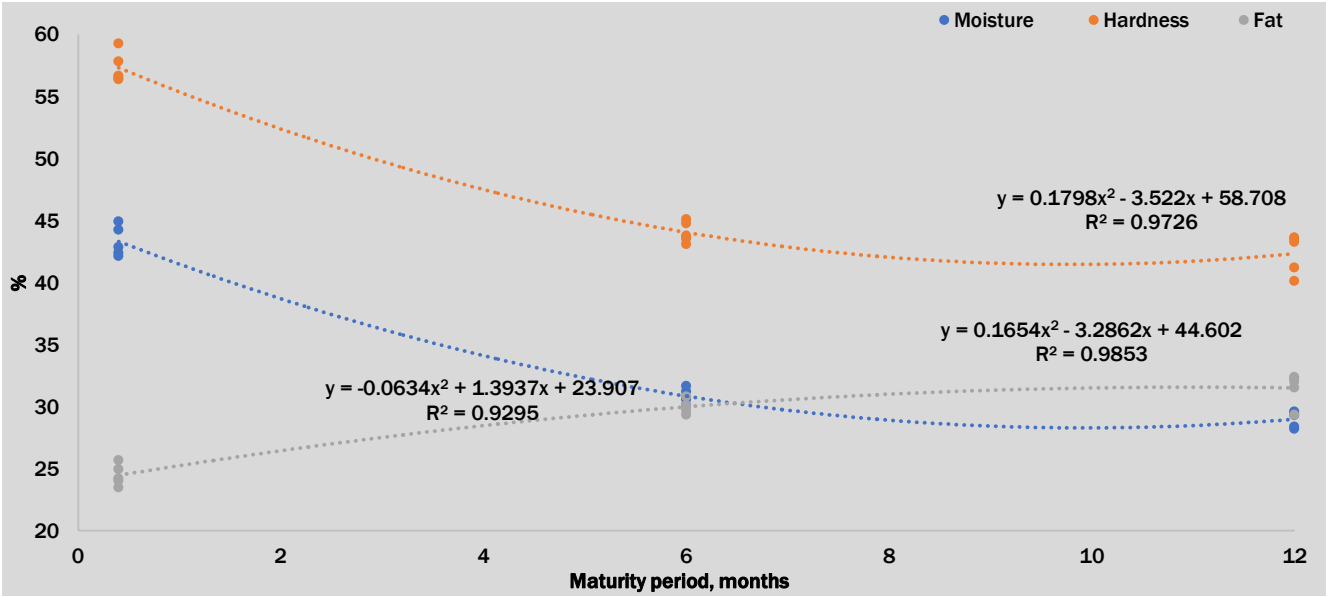


**Figure 1 -** Relationship between moisture, fat, and hardness and the ripening period of Alpine cheese (n = 15).

Fat content in dry matter remained constant with age, whereas protein content increased to 41.80% at 6 months and by to 40.39% at 12 months, respectively, compared to young cheese (Table 2). This, in turn, contributed to a reduction of the fat-to-protein ratio by 0.11 points at 6 months. Cheese hardness decreased with age falling to 44.05 units at 6 months and to 42.34 units at 12 months. Young Alpine cheese (7 days) exhibited a milk-colored rind indistinguishable from paste (Figure 2 a, b). The cheese interior was characterized by a homogeneous, plastic paste with isolated, small, rounded eyes. It was easy to slice and exhibited a rubbery consistency. At 6 months, the Alpine cheese rind displayed a golden hue, was well developed, and contrasted with the paste. The paste was plastic and homogeneous, containing

small, rounded, and irregularly shaped eyes. Some eyes coalesced, particularly in the cheese core) (Figure 2 c, d). At 12 months, the rind was well formed, dark amber, layered, and exhibited localized *A. siro* damage, creating contrast with the paste. The paste remained homogeneous yet slightly brittle, with rounded and irregular eyes, some of which had merged. Small dark spots near the rind (Figure 2 e, f) corresponded to residual *A. siro* activity.

Ripening of Yoghurt hard cheese exhibited characteristics distinct from that Alpine cheese. Moisture content of Yoghurt cheese was strongly inversely correlated with ripening period dependence ($r = -0.935 \pm 0.067$, $P < 0.001$), while ash content showed a strong positive correlation ($r = -0.958 \pm 0.054$, $P < 0.001$). Regression analysis indicated that moisture content varied with age according to a second-degree polynomial, whereas ash content increased linearly throughout ripening (Figure 3).

At the same time, the intensity of moisture loss in this cheese from the 7th day to the 6th month of ripening decreased from 46.91% to 36.54%, while by the 18th month – to 29.93%. Against the background of an increase in the dry matter content, the fat content in Yogurtovy cheese significantly increased from 22.06% to 30.83% by the 6th month and to 29.91% by the 18th month of ripening. Protein concentration exhibited a similar trend during ripening (Table 3).

Fat and protein contents in Yoghurt cheese dry matter peaked at 6 months, whereas levels in young and aged cheeses were significantly lower (Table 4). However, these fluctuations did not alter the fat-to-protein ratio throughout ripening. A strong inverse linear correlation was observed between Yoghurt cheese hardness and ripening period ($r = -0.974 \pm 0.043$, $P < 0.001$). Regression analysis likewise demonstrated that protein and fat contents in dry matter vary with ripening period, fitting a second-degree polynomial (Figure 4). On day 7 of ripening, Yoghurt cheese had a well-formed, continuous rind indistinguishable from the paste. The slice exhibited a rubbery texture with medium and small holes distributed across the surface (Figure 5 a, b). At 6 months, the Yoghurt cheese rind was light amber and contrasted with the paste. The paste contained small and medium irregularly shaped holes throughout, some of which had coalesced in the core. At this stage, the paste was plastic yet slightly brittle (Figure 5 c, d). At 18 months, Yoghurt cheese met the criteria for aged cheese, featuring a hard amber rind that contrasted with the paste. The rind bore layered damage and small lesions attributable to *A. siro* activity. This cheese was difficult to slice, exhibiting a brittle texture with randomly distributed medium-sized holes (Figure 5 e, f).

**Table 2 -** Physicochemical analysis of dry matter of Alpine craft hard cheese in ripening process, x ± SD, %

| Parameter \ Ripening period of cheese | 7 days | 6 months | 12 months |
|---|---|---|---|
| Fat, % | 43.14 ± 1.06 | 43.36 ± 0.81 | 44.37 ± 2.07 |
| Protein, % | 37.87 ± 1.87 [b] | 41.80 ± 0.70 [a] | 40.39 ± 0.49 [a] |
| Fat-to-protein ratio, un. | 1.14 ± 0.07 [a] | 1.03 ± 0.02 [b] | 1.11 ± 0.05 [ab] |
| Hardness, un. | 57.33 ± 1.23 [a] | 44.05 ± 0.85 [b] | 42.34 ± 1.58 [b] |

*Note: Different letters of superscript indicate the probable differences between the values within the same table row ($P < 0.05$) as determined by Tukey test with Bonferroni correction.

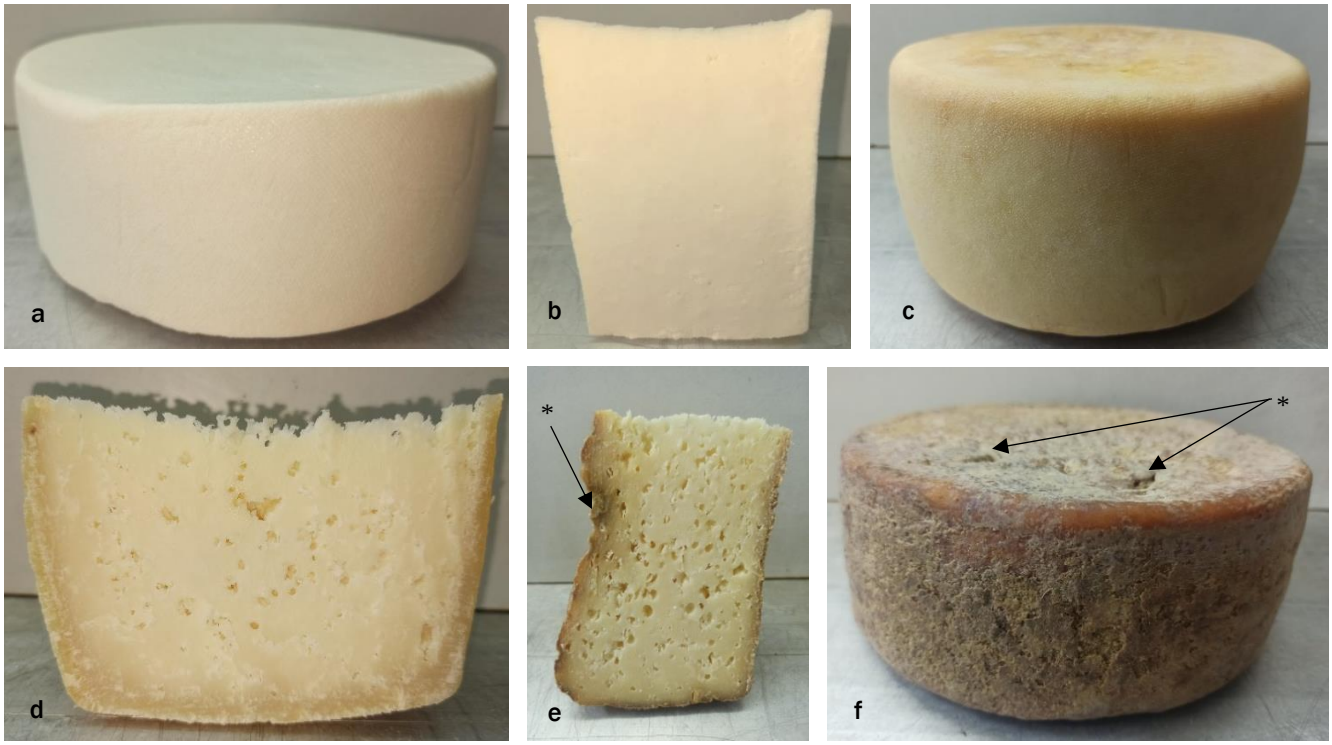


**Figure 2 -** Alpine cheese with ripening period of 7 days (a: head, b: slice); 6 months (c: head, d: slice); 12 months (e: head, f: slice); *: place of damage to cheese rind by mite.

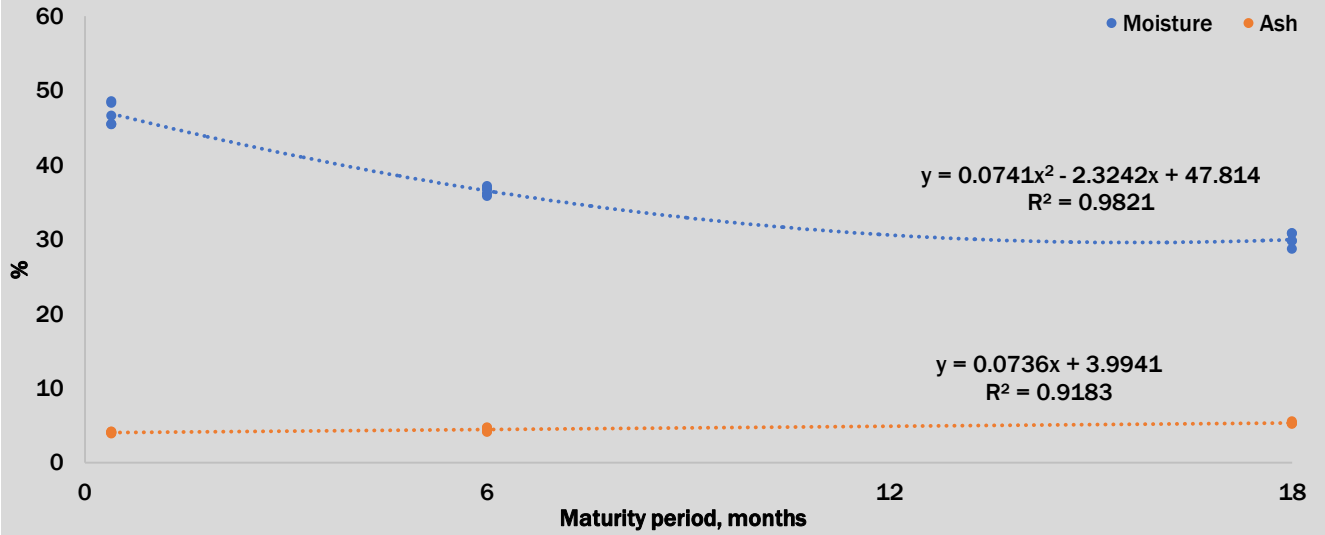


**Figure 3 -** Relationship between moisture and ash contents and ripening period of Yoghurt cheese (n = 15).

**Table 3 -** Chemical composition of Yoghurt craft hard cheese, x ± SD, %.

| Parameter \ Ripening period of cheese | 7 days | 6 months | 12 months |
|---|---|---|---|
| Moisture | 46.91 ± 1.53 [a] | 36.54 ± 0.54 [b] | 29.93 ± 0.87 [c] |
| Fat | 22.06 ± 0.18 [b] | 30.83 ± 0.92 [a] | 29.91 ± 0.81 [a] |
| Protein | 20.52 ± 0.53 [b] | 28.77 ± 0.43 [a] | 29.52 ± 0.23 [a] |
| Ash | 4.13 ± 0.17 [c] | 4.43 ± 0.09 [b] | 5.32 ± 0.13 [a] |

*Note: Different letters of superscript indicate the probable differences between the values within the same table row (P < 0.05) as determined by Tukey test with Bonferroni correction.

**Table 4 -** Physiochemical composition of dry matter of Yoghurt craft hard cheese during ripening period, x ± SD, %.

| Parameter \ Ripening period of cheese | 7 days | 6 months | 12 months |
|---|---|---|---|
| Fat | 41.56 ± 1.27 [b] | 48.58 ± 1.42 [a] | 42.71 ± 0.82 [b] |
| Protein | 38.65 ± 0.64 [c] | 45.34 ± 0.77 [a] | 42.17 ± 0.25 [b] |
| Fat-to-protein-ratio | 1.08 ± 0.03 | 1.07 ± 0.04 | 1.01 ± 0.02 |
| Hardness | 60.17 ± 1.95 [a] | 52.83 ± 0.96 [b] | 42.78 ± 0.94 [c] |

*Note: Different letters of superscript indicate the probable differences between the values within the same table row (P < 0.05) as determined by Tukey test with Bonferroni correction.

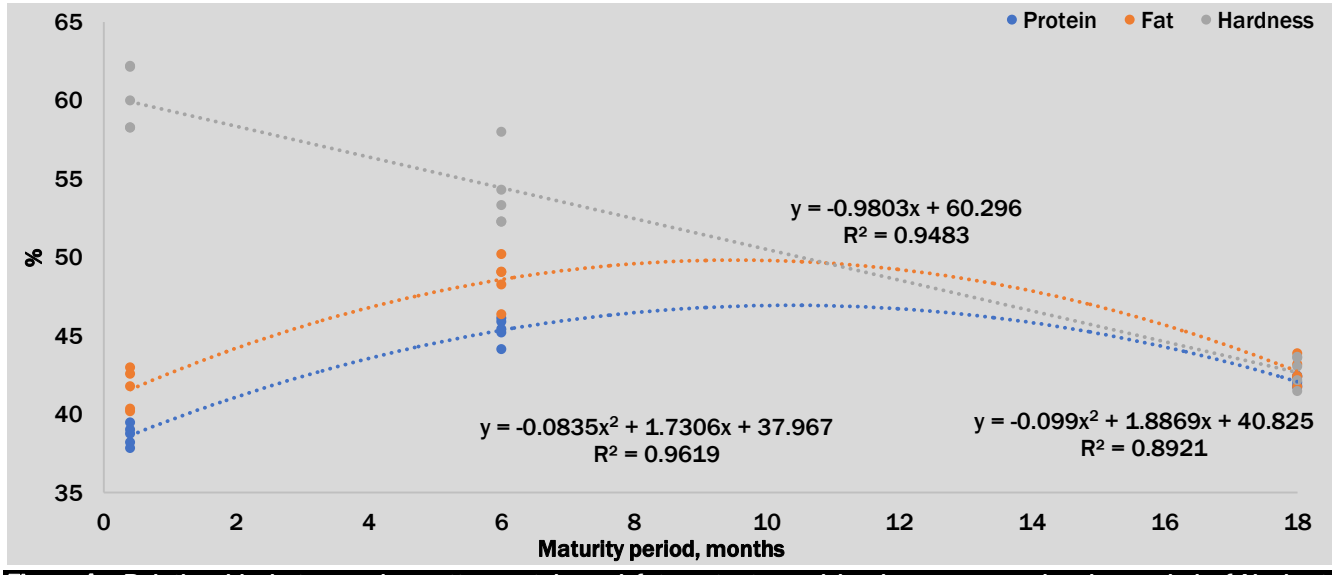


**Figure 4 -** Relationship between dry-matter protein and fat contents, and hardness, versus ripening period of Yoghurt cheese (n = 15).


**Figure 5 -** Yoghurt cheese with ripening period of 7 days (a: head, b: slice); 6 months (a: head, b: slice); 18 months (e: head, f: slice).

Moisture content of hard cheeses, including Alpine and Yoghurt, influences both yield and ripening suitability. According to standard classifications extra-hard cheese should have moisture content of 25-35%, hard - 35-45%, semi-hard - 45-50%, semi-soft - 42-55%, and soft - 55-80% (Zheng et al., 2021). Alpine cheese is hard at 7 days and extra-hard at 6–12 months, whereas Yoghurt cheese is semi-hard at 7 days, hard at 6 months, and extra-hard at 18 months. The results of this study are consistent with the previously obtained data on the chemical composition of Caciotta and Canestrato hard cheeses made from raw goat milk. Moisture-loss patterns vary with production technique and rind development. In Caciotta cheese made from raw goat milk, moisture content decreased from 44.4 to 25.1% over 24 months. Increased dry matter corresponded to rises in fat (27.0 to 36.5%), protein (23.8 to 33.2%), and ash (3.4 to 4.3%), accompanied by greater brittleness and reduced hardness (39.5%).

The highest moisture loss in Canestrato hard cheese occurred between months 6 and 12 (4.4%) (Sadvari et al., 2024a). The observed moisture-loss trends for Alpine and Yoghurt cheeses align with reports on traditional Chinese cheeses (23.2–59.2% moisture; Zhang et al., 2022) and Korycińskie semi-hard varieties, demonstrating dependence on ripening-room temperature and casein matrix water-holding capacity (Kliks et al., 2022; Tulyaganovich et al., 2022).

Ripening duration and conditions govern biochemical processes—glycolysis, lipolysis, and proteolysis—that generate the distinctive sensory profile of goat cheeses (Levak et al., 2023a). Cheese fat content modulates sensory richness, with the fat-to-protein ratio typically ranging from 0.70 to 1.15 (Lipkowitz et al., 2018). In both Alpine and Yoghurt cheeses, this ratio remained within the specified limits throughout ripening. Mineral content in Alpine and Yoghurt hard cheeses slightly increased with age, as indicated by ash content; however, this trend was not strictly linear and reflected concurrent rises in dry-matter levels. Production practices—particularly pasture-based feeding—also influence cheese mineral profiles. Artisanal cheeses exhibit mineral compositions comparable to those of organic varieties (de Oliveira Filho et al., 2022). Salt content is a key determinant of both mineral balance and flavor (Møller et al., 2013). Recently, hard cheese producers have extended ripening to produce premium ultra-hard cheeses for target markets (Levak et al., 2023b). This study also included Alpine and Yoghurt cheeses made from raw goat milk aged 12 and 18 months, respectively. Hard-cheese quality depends not only on milk composition but also on microbial species and abundance (Sadvari et al., 2024b; Iakubchak et al., 2024; Kukhtyn et al., 2025).

Microbial effects on sensory properties and structure are exemplified by rind formation in PDO Pecorino Siciliano, PDO Piacentinu Ennese, and Caciocavallo Palermitano cheeses (Settanni et al., 2021). Hard-cheese texture varies substantially with both type and age. Our observations of Alpine and Yoghurt cheeses align with findings for young and mature Kope cheeses (Esmaeilzadeh et al., 2021). In Kope cheese, initial rubbery consistency at day 7 transitions to increased hardness by day 187; proteolysis under acidic conditions gradually homogenizes texture by weakening the casein network. Aged cheeses exhibit increased hardness and brittleness from casein hydration, and weakened interparticle bonds enhance friability, accounting for the fragile structure of Alpine (12 months) and Yoghurt (18 months) cheeses. Texture is also influenced by production methods—especially pasteurization—and differences between industrial and artisanal technologies markedly affect cheese quality. For example, industrially produced Fiore Sardo PDO (sheep milk) exhibited greater paracasein hydration and water-to-protein proton ratio than artisanal counterparts. In our study, artisanal cheeses displayed more eyes on the slice surface than industrial samples (Anedda et al., 2021).

In Alpine cheese, eye formation intensified with maturity, with minimal coalescence. Conversely, Yoghurt cheese exhibited eye coalescence in the core at 6 months, while aged samples featured discrete medium-sized eyes within a dense matrix—likely due to elevated $CO_2$ partial pressure and softer paste in the core relative to the rind. Carbon dioxide generatedduring ripening initially dissolved in the cheese matrix influenced by microbial community composition (Munch et al., 2023). Within cheese, $CO_2$ is partly irreversibly absorbed into the paste and partly remains in the free phase (Lepilkina et al., 2021). Once saturation occurs, $CO_2$ diffuses to form eyes or escapes through the rind (Auer et al., 2021). Excessive $CO_2$ production and increased partial pressure can coalesce eyes, creating undesirable cracks or fissures (Lamichhane et al., 2021). Although extensive eye formation may compromise the market appearance of long-ripened cheeses like Emmental, Gouda, or Maasdam, they remain suitable for shredding, processing, or inclusion in other dishes (González et al., 2020). Similarly, these secondary uses may apply to craft Alpine and Yoghurt goat-milk cheeses; however, defects often arise from rind damage by *A. siro* rather than excessive eye formation. Rind development critically shapes internal texture and biochemical activity; in this study, cheeses were ripened uncoated.

A debate regarding the safety of arachnid-ripened cheeses, particularly those involving mites. It is believed that *Acarus siro*, the species most frequently associated with cheese ripening, can secrete compounds that induce allergic reactions in humans. Studies have shown that the opisthonotal glands of Astigmata secrete monoterpenes as well as various aromatic, aliphatic, and other compounds possessing pheromonal and fungicidal activities. A study of Cantal vieux showed that the main mite species was *Acarus siro* L. It has been proven that aromatic compounds released by mites do not penetrate the cheese matrix during ripening; instead, they contribute to flavor only upon rind consumption) (Shimizu et al., 2022). In Alpine and Yoghurt hard cheeses, *A. siro* contributes to rind aroma and flavor development, as well as rind formation and detachment from molds. By considering these results, it is advisable to develop rapid detection methods for mites on goat-milk hard-cheese rinds during ripening and to define their maximum permissible levels.

## CONCLUSION

Moisture evaporation intensity is a crucial factor in ripening craft hard cheeses from raw goat milk. Between day 7 and month 6, Alpine cheese experienced its greatest moisture loss with 43.31% to 30.84%. Moisture content and hardness exhibited a strong inverse correlation with age, while fat content correlated positively. Increased dry matter corresponded with higher protein, fat, and ash levels. Cheese hardness declined to 44.05 units at 6 months and to 42.34 units at 12 months, reflecting increased fragility due to moisture loss. Aging also increased eye formation and produced an amber rind bearing mite *A. siro* damage and activity traces. Moisture loss drove changes in Yoghurt cheese physiological parameters, exhibiting a a strong inverse correlation with ripening duration. The highest moisture loss of Yogurtovy cheese was detected in the period from the 7th day to the 6th month of ripening, which decreased from 46.91% to 36.54%. By the 18th month of ripening of Yogurtovy cheese, its moisture reached 29.91%, which is associated with the peculiarity of crust formation.Rising dry matter corresponded with higher protein, fat, and ash contents. Hardness demonstrated a strong inverse relationship with age. Aging produced an amber rind and a brittle paste by 18 months. The rind displayed minor *A. siro* damage. These findings enrich understanding of physicochemical evolution in mite-ripened, raw-goat-milk hard cheeses.

## DECLARATIONS

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

**Authors' contribution**
Viktor Davydovych and Larysa Shevchenko contribute to the research, data analysis, and manuscript writing.
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# SEQUENTIAL CULTURE OF RUMEN FLUID AS A SUSTAINABLE INOCULANT FOR *IN VITRO* RUMINANTS FEED EVALUATION

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**ABSTRACT**: Rumen fluid plays a crucial role in *in vitro* studies for evaluating ruminant feed. Maintaining microbial activity in rumen fluid can serve as a breakthrough approach to reducing dependence on fresh rumen fluid collection by utilizing sequential culture techniques. This study aimed to assess the effectiveness of rumen microbial inoculants through sequential cultures with a 48-hour incubation period. A completely randomized design was applied with four treatments: K1 = Culture 1 (inoculant derived from fresh rumen fluid), K2 = Culture 2 (inoculant derived from Culture 1), K3 = Culture 3 (inoculant derived from Culture 2), and K4 = Culture 4 (inoculant derived from Culture 3). The test substrates included dwarf elephant grass and *Indigofera zollingeriana* leaves using analysis *in vitro* sequential cultures adapted from Tilley and Terry (1963) and the Consecutive Batch Culture (CBC) method. Parameters measured included rumen fermentation characteristics such as pH, ammonia nitrogen (N-$NH_3$) concentration, total volatile fatty acid (VFA) production, and dry matter digestibility. Data were analyzed using analysis of variance (ANOVA) followed by Tukey's HSD (Honest Significant Difference) test. The results showed that the sequential culture process significantly affected *in vitro* rumen fermentation characteristics. The pH remained stable within the optimal range (6.67–6.78). Increased culture sequences enhanced N-$NH_3$ concentration, total VFA production, and dry matter digestibility. It can be concluded that rumen microbial inoculants remain effective up to the fourth sequential culture for *in vitro* evaluation of ruminant feeds.

**Keywords:** Digestibility, Dry matte, Inoculant, Microbial viability, Sequential culture

## INTRODUCTION

Ruminants possess a complex gastric system comprising four compartments: the rumen, reticulum, omasum, and abomasum, with the rumen being the largest and most functionally significant (Palma-Hidalgo et al., 2021). The rumen contains a heterogeneous mixture of feed, water, fermentation by-products, and a dense population of living microorganisms. The rumen microbiota is diverse and dynamic, influenced by geographical region and the type of feed consumed (Silva et al., 2024). The primary microbial groups present in the rumen include bacteria, protozoa, and fungi (Castillo and Hernández, 2021). The adaptability and structural complexity of these microbial communities enable them to play a critical role in breaking down plant biomass into microbial protein, volatile fatty acids (VFAs), and other fermentation end-products that serve as essential nutrients for the host animal's metabolism (Ji et al., 2017).

The *in vitro* method for feed evaluation offers several advantages over *in vivo* techniques. It is cost-effective, time-efficient, and allows greater control over incubation conditions (Getachew et al., 2002). *In vitro* methods have been widely adopted in animal nutrition research as preliminary tools before conducting *in vivo* trials, significantly reducing the reliance on experimental animals and overall research costs (Vinyard and Faciola, 2022). The use of rumen fluid microbes in *in vitro* fermentation systems is essential for simulating rumen fermentation dynamics and estimating feed digestibility with results that closely reflect *in vivo* conditions (Raffrenato et al., 2021).

Despite the critical role of rumen fluid in *in vitro* studies, its acquisition poses several challenges. Lodge-Ivey et al. (2009) noted that obtaining rumen fluid typically involves rumen cannulation, which requires surgically fistulated animals. Alternative methods, such as using esophageal or oral cannulae, are less invasive but can stress the animals and risk contamination with saliva (Fortina et al., 2022). Additionally, ethical considerations arise when using live animals as rumen fluid donors (Spanghero et al., 2019). Logistical constraints, including limited availability of donor animals, long-distance transportation, and timing issues, especially when rumen fluid is sourced from slaughterhouses further complicate its use in routine research.

One promising solution is to culture rumen fluid in laboratory settings while maintaining its microbial viability, thus minimizing dependence on cannulated animals, rumenocentesis, or slaughterhouse sources (Tunkala et al., 2022). Creating optimal conditions for the growth of anaerobic rumen microbes requires controlling key environmental factors such as temperature, pH, buffering capacity, osmotic pressure, and redox potential (Castillo-González et al., 2014).



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Maintaining an active culture of rumen fluid over multiple incubation cycles allows researchers to preserve microbial activity for successive *in vitro* degradation assays of various feed types.

The Tilley and Terry (1963) method is a widely used two-stage *in vitro* digestibility assay involving incubation with rumen fluid followed by enzymatic digestion using HCl-pepsin (Zewdie, 2019). This method has demonstrated high correlation with *in vivo* digestibility and remains a standard technique for evaluating feed quality (Tassone et al., 2020). In parallel, the Consecutive Batch Culture (CBC) method, developed by Gascoyne and Theodorou (1988), mimics the rumen environment through sequential inoculation and incubation of subcultures in fresh buffer under controlled conditions. In this system, microbial communities are transferred to new culture media at defined intervals to maintain active fermentation (Mbiriri et al., 2016).

Integrating the 48-hour rumen incubation phase from the Tilley and Terry method with the principles of the CBC system results in a sequential culture technique. This approach aims to produce stable and reproducible rumen fluid inoculants for *in vitro* testing. Rumen fluid collected from donor animals is cultured under controlled laboratory conditions designed to replicate *in vivo* rumen fermentation. Sequential culturing presents a viable alternative to conventional sourcing of rumen fluid, enabling researchers to maintain microbial stability while customizing nutrient and environmental parameters. Therefore, this study was conducted to evaluate the effectiveness of rumen fluid as an inoculant through sequential culturing, by assessing its impact on fermentation characteristics and *in vitro* dry matter digestibility of selected ruminant feedstuffs.

## MATERIALS AND METHODS

### Ethical considerations

All methodologies and guidelines applied in this experiment were approved by the Animal Ethics Committee for Research and Education at the Faculty of Animal Science, Hasanuddin University, Makassar, prior to the commencement of the study. Ethical approval was granted under the reference number 018/UN4.12/EC/XI/2023, in accordance with the seven WHO ethical standards (2001).

### Tool preparation

Feed bags were made from nylon fabric (Depure) measuring 8 × 4 cm with a pore size of 100 µm, based on the method of Carro et al. (1995). A modified U-shaped press was used to form the curved bottom of each bag. To ensure submersion and containment of the feed sample during incubation, each nylon bag was equipped with a 20 g glass weight and secured using a clamp. Prior to use, the bags were dried in an oven at 65°C for 72 hours to remove residual moisture and then weighed to determine their initial dry mass. The lid of the artificial rumen was constructed from a No. 8 rubber stopper, with an upper diameter of 4.5 cm and a lower diameter of 3.8 cm. Two holes (6 mm in diameter) were drilled into the stopper. The first hole was fitted with a 10 cm silicone hose (3 mm inner diameter, 5 mm outer diameter) connected to a gas valve for releasing fermentation gases. The second hole housed a 19 cm silicone hose equipped with a pinch clamp and a 60 mL syringe for transferring the subculture inoculum. The fermentation chamber consisted of a 250 mL polypropylene Erlenmeyer flask with a 4 cm mouth diameter and a height of 13.7 cm. This setup simulated anaerobic rumen fermentation conditions for *in vitro* culture.

### Feed sample preparation

The feed ingredients used in this study consisted of a mixture of 70% dwarf elephant grass and 30% *Indigofera zollingeriana*, harvested 70 days after uniform pruning during the dry season. The harvested materials were oven-dried at 70°C for 72 hours until completely dry (Memmert Universal Oven UNB 400). The dried samples were then ground using a 14-mesh grinding machine (B-One DM-120 M) to obtain a uniform particle size suitable for *in vitro* fermentation.

### Preparation of artificial saliva

Artificial saliva this solution served as a pH stabilizer and a mineral source during fermentation, providing essential nutrients for sustaining microbial activity in the *in vitro* rumen environment. Artificial saliva, also referred to as McDougall's solution, was prepared according to the formulation described by McDougall, as cited in Close and Karl-Heinz (1986).

### Rumen fluid preparation

Rumen fluid was collected from two cattle slaughtered at the CV Akbar Jaya Sejahtera abattoir, located in Tamangapa, Antang, Makassar (slaughter certificate number 06020013030319), Indonesia. Immediately after slaughter, the warm rumen contents were transferred into a thermos box to maintain temperature and preserve microbial viability during transportation to the laboratory. Upon arrival, the rumen solids were filtered using a nylon cloth with 250 µm porosity (Yáñez-Ruiz et al., 2016) to extract the fluid fraction. The resulting rumen fluid was used as the microbial inoculant for the *in vitro* fermentation process.

### Experiment design

The stability and fermentative activity of rumen microorganisms after repeated incubation were evaluated using feed samples composed of dwarf elephant grass and *Indigofera zollingeriana* leaves. The assessment employed a sequential culture *in vitro* method adapted from Tilley and Terry (1963) and CBC. This approach was designed to determine the extent to which microbial viability and activity could be maintained across multiple incubation cycles, thus offering a potential alternative to the repeated collection of fresh rumen fluid for use as an inoculant.

The study consisted of four culture stages, where the inoculum from the previous stage was used to initiate the next incubation. The treatment groups were as follows: K1= Culture 1 (inoculant derived from fresh rumen fluid), K2= Culture 2 (inoculant derived from Culture 1), K3= Culture 3 (inoculant derived from Culture 2), K4= Culture 4 (inoculant derived from Culture 3).

### Experiment procedure

The first stage of incubation was initiated by weighing 2.5 grams of the feed sample and placing it into a pre-weighed nylon bag. The bag was then equipped with a 20 g glass weight and secured with a clamp to ensure submersion. The bag containing the sample was inserted into an artificial rumen vessel and filled with 250 mL of a 4:1 mixture of freshly prepared rumen fluid and artificial saliva, following the procedure of Tilley and Terry (1963). The vessel was sealed with a ventilated rubber stopper and flushed with $CO_2$ gas to create anaerobic conditions by displacing residual oxygen.

Incubation was conducted in a Memmert WPE 45 water bath at 39°C for 48 hours, with manual shaking performed twice daily to maintain uniform fermentation. At the end of each incubation, 50 mL of the fermentation medium was withdrawn using a 60 mL syringe and transferred into a new artificial rumen flask containing a fresh 2.5 g feed sample and 200 mL of artificial saliva solution. This subculturing process was repeated across four consecutive culture stages (K1 to K4), each with a 48-hour incubation period under identical conditions. At the end of each culture stage, samples of the remaining inoculant were collected and analyzed to determine pH, ammonia nitrogen (N-$NH_3$) concentration, total volatile fatty acid (VFA) production, and dry matter digestibility (DMD).

### Parameter and laboratory analysis

#### *pH value*

The pH of the artificial rumen fluid inoculant was measured immediately after transferring the subculture into the new fermentation medium to assess the stability of the microbial environment. The pH solution analysis of a reference Covington et al. (1985). The electrode was immersed directly into the rumen fluid sample, and the pH value was recorded from the digital display. Following pH measurement, the remaining rumen fluid sample was centrifuged at 10,000 rpm for 15 minutes to separate the supernatant from suspended solids. The resulting supernatant was then stored in a freezer at −20°C for subsequent analysis of ammonia nitrogen (N-$NH_3$) concentration and total volatile fatty acids (VFA).

#### *N-NH3 concentration*

Ammonia nitrogen (N-$NH_3$) concentration was determined using the Conway microdiffusion method, as described by Thirumalaisamy et al. (2022). To ensure an airtight seal, the rim of the Conway dish was coated with petroleum jelly. One milliliter of the fermentation supernatant was pipetted into one of the outer compartments of the dish, and 1 mL of sodium carbonate ($Na_2CO_3$) solution was added to the opposite compartment, taking care to avoid premature mixing. A central well in the dish was filled with 1 mL of boric acid ($H_3BO_3$) solution containing a mixed indicator to absorb the released ammonia. The Conway dish was then sealed and gently tilted to mix the supernatant and $Na_2CO_3$ solution. The setup was incubated at room temperature (25°C) for 24 hours to allow ammonia gas diffusion into the boric acid. After the diffusion period, the boric acid solution was titrated with 0.0103 N sulfuric acid ($H_2SO_4$) until a color change from green to red signified the titration endpoint. The volume of $H_2SO_4$ used was recorded to calculate the N-$NH_3$ concentration, expressed in millimoles per liter (mM), using the following formula: N-$NH_3$ (Mm) = Volume of $H_2SO_4$ x Normality of $H_2SO_4$ x 1000.

#### *Total volatile fatty acids (VFA)*

The total volatile fatty acid (VFA) concentration in the artificial rumen fluid was determined using the steam distillation method, following the procedure outlined by Kromann et al. (1967) and employing a Kjeldahl micro distillation apparatus. This method isolated and quantified the volatile fatty acids produced during microbial fermentation. The procedure began by mixing 5 mL of rumen fluid supernatant with 200 mL of distilled water in a distillation tube. To this mixture, 1 mL of 15% sulfuric acid ($H_2SO_4$) was added to facilitate the release of volatile fatty acids. The distillate was collected in a receiving Erlenmeyer flask pre-filled with 5 mL of 0.5 N sodium hydroxide (NaOH) and 2–3 drops of phenolphthalein (PP) indicator to maintain alkaline conditions. After the completion of the distillation process, the contents of the Erlenmeyer flask were titrated with 0.25 N hydrochloric acid (HCl) until the color changed from red to colorless, indicating the titration endpoint. The volume of HCl used corresponded to the total VFA concentration in the sample and was expressed in mM, using the following formula: Total VFA = (Vb - Vs) x N-HCl x 1000/5 mM.

### Dry matter digestibility

Dry matter digestibility (DMD) of the feed samples was determined following the *in sacco* approach described by Kera et al. (2022), after 48 hours of *in vitro* incubation. Upon completion of incubation, the nylon bags containing the residual feed were carefully removed from the fermentation medium and gently rinsed under running tap water until the rinse water ran clear, indicating the removal of adhering fermentation residues. The washed bags were then oven-dried at a constant temperature of 65°C for 72 hours, or until they reached a stable weight, to ensure complete moisture evaporation. After drying, the bags were transferred to a desiccator to cool and to prevent reabsorption of moisture from the surrounding air. The final weight of each bag was recorded, and the dry matter digestibility was calculated based on the difference between the initial sample weight and the residual weight after incubation, using the following formula: DMD (%) = (Sample weight (g) – Residue weight (g))/ (Sample weight (g))×100%.

### Statistical analysis

The collected data, including pH value, ammonia nitrogen (N-$NH_3$) concentration, total volatile fatty acids (VFA), and dry matter digestibility (DMD), were analyzed using analysis of variance (ANOVA) according to a completely randomized design (CRD) consisting of four treatments and four replications. The significant effects of the treatment were further determined using Tukey's HSD (Honest Significant Difference) test was used for post hoc comparisons.

## RESULTS

### pH value of artificial rumen fluid inoculant

The culture process was carried out four times, each culture was incubated for 48 hours before the sub-culture was transferred to the next culture medium. Measurement of rumen fluid pH was carried out at the end of the incubation period. Based on the results of analysis of variance (ANOVA), it was found that the culture treatment had a significant effect ($P < 0.05$) on the pH value of the artificial rumen fluid. The average pH across cultures ranged from 6.67 ± 0.00 to 6.78 ± 0.02. Culture 4 recorded the highest pH (6.78 ± 0.02), which was significantly different from the other treatments. Meanwhile, Culture 3 (6.70 ± 0.03) was not significantly different from Culture 1 or Culture 2. However, Culture 1 (6.67 ± 0.00) was significantly different from Culture 2 (6.72 ± 0.04), indicating a gradual yet significant increase in pH with each successive culture.

### N-$NH_3$ concentration of artificial rumen fluid inoculant

The concentration of N-$NH_3$ in artificial rumen fluid measured after 48 hours of incubation showed that the culture process had a significant effect ($P<0.05$) on the concentration of N-$NH_3$ in artificial rumen fluid. This can be seen from the graph in Figure 2. The highest average N-$NH_3$ concentration was observed in Culture 1, with a value of 21.88 ± 0.42 mM, which was significantly different ($P < 0.05$) from the other culture treatments. Culture 2 and Culture 3 recorded N-$NH_3$ concentrations of 12.87 ± 0.73 mM and 12.82 ± 0.52 mM, respectively, showed no significant difference between them; however, both were significantly lower than Culture 1 and significantly higher than Culture 4. The lowest N-$NH_3$ value was recorded in Culture 4 at 10.97 ± 0.26 mM, which was significantly different from all other treatments. The progressive decrease in N-$NH_3$ concentration from Culture 1 through Culture 4 suggests that the sequential culture process influences the availability and utilization of ammonia nitrogen in the artificial rumen fluid.

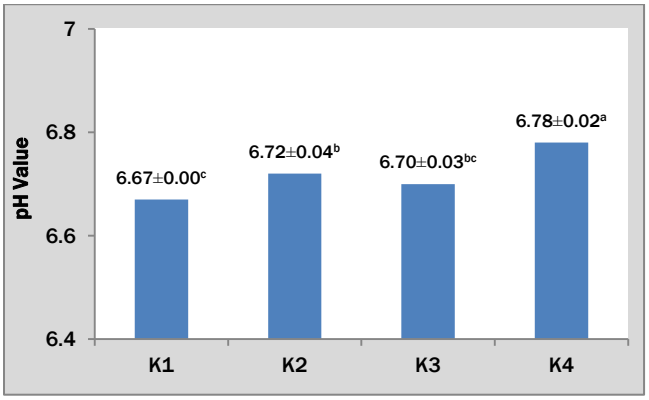


**Figure 1 - Diagram of the effect of the culture process on the pH value of artificial rumen fluid.** Different superscripts a, b and c on the pH value statistically indicate significant differences ($P < 0.05$). K1= Culture 1 (inoculant derived from rumen fluid), K2= Culture 2 (inoculant derived from culture 1), K3= Culture 3 (inoculant derived from culture 2), K4= Culture 4 (inoculant derived from culture 3).

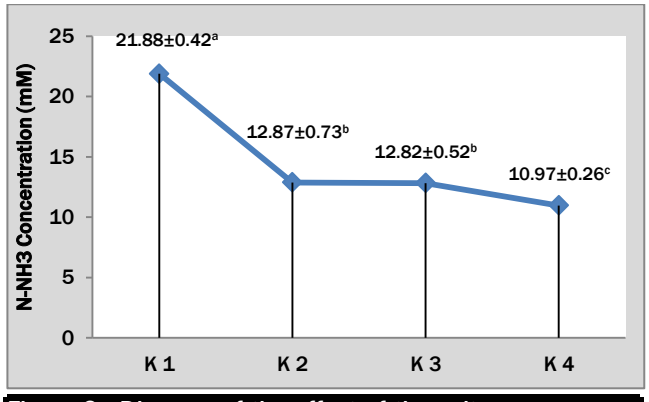


**Figure 2 - Diagram of the effect of the culture process on the N-$NH_3$ concentration of artificial rumen fluid.** Different superscripts a, b and c on N-$NH_3$ values statistically indicate significant differences ($P < 0.05$). K1= Culture 1 (inoculant derived from rumen fluid), K2= Culture 2 (inoculant derived from culture 1), K3= Culture 3 (inoculant derived from culture 2), K4= Culture 4 (inoculant derived from culture 3).

**Total volatile fatty acid (VFA) production**

Based on the results of analysis of variance, the production of total volatile fatty acids (VFA) in artificial rumen fluid cultured four times showed significant differences (P<0.05), as shown in Figure 3. The post hoc test results showed that the average VFA production in Culture 1 and Culture 2 was not significantly different, with values of 95.23 ± 5.02 mM and 95.23 ± 3.24 mM, respectively. However, both cultures produced significantly lower VFA concentrations than Culture 3 and Culture 4. VFA production began to increase in Culture 3 (106.24 ± 3.87 mM) and peaked in Culture 4 (111.36 ± 5.44 mM), although the difference between these two cultures was not statistically significant. Overall, these results indicate that the sequential culture process significantly influenced total VFA production, with a notable increase occurring after the second culture stage.

**Dry matter digestibility of feed samples**

The degradation of dry matter is very influential on the fulfillment of the energy source of microorganisms in the manufacture of artificial rumen fluid. The results of the dry matter degradation analysis can be seen in Figure 4 which presents a graph of dry matter degradation. The graph illustrates that dry matter degradation increased progressively with each stage of the culture process. Culture 1 recorded the lowest degradation value at 55.83 ± 0.92%, which was significantly different (P < 0.05) from Culture 3 and Culture 4, which showed the highest degradation value at 68,47 ± 3,39% and 70.69 ± 9.22%. Culture 2 showed an increase to 61.27 ± 5.49%, though this was not significantly different from all cultures. Culture 3 further increased to 68.47 ± 3.39%, showing a significant difference from Culture 1 but not from Cultures 2 or 4. Overall, the trend indicates that the sequential culture process positively influenced dry matter degradation, with significant improvements observed particularly in Culture 3 and Culture 4.

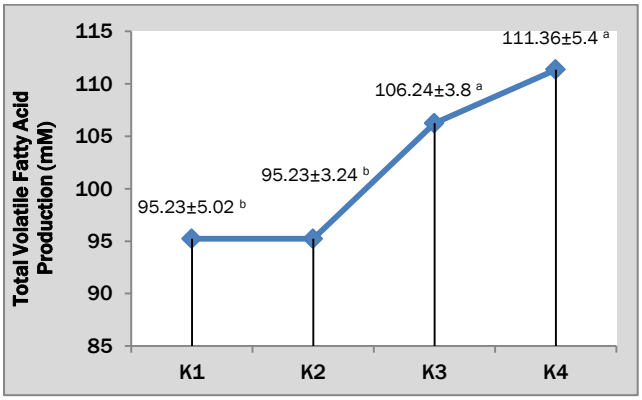


Figure 3 - Diagram of the effect of culture process on the production of total volatile fatty acids (VFA) in artificial rumen fluid. Different superscripts a and b on VFA values statistically showed significant differences (P<0.05). K1= Culture 1 (inoculant derived from rumen fluid), K2= Culture 2 (inoculant derived from culture 1), K3= Culture 3 (inoculant derived from culture 2), K4= Culture 4 (inoculant derived from culture 3).

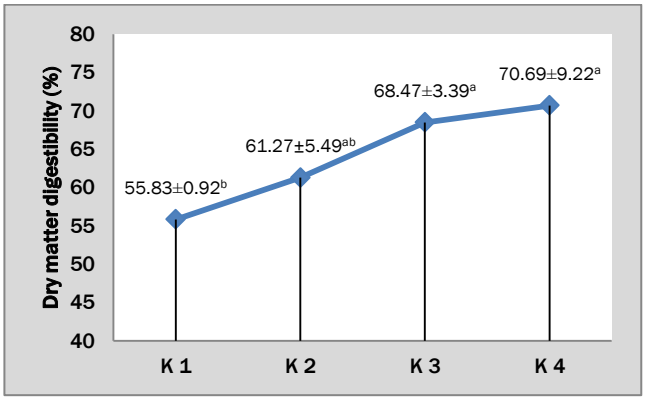


Figure 4 - Diagram of the effect of the culture process on the digestibility of feed dry matter in artificial rumen fluid. Different superscripts a and b on the degradation value of feed dry matter statistically showed significant differences (P < 0.05). K1= Culture 1 (inoculant derived from rumen fluid), K2= Culture 2 (inoculant derived from culture 1), K3= Culture 3 (inoculant derived from culture 2), K4= Culture 4 (inoculant derived from culture 3).

## DISCUSSION

**pH value of artificial rumen fluid inoculant**

pH is a critical parameter in rumen fermentation, as it influences microbial growth, survival, and metabolic activity. According to Lund et al. (2020), pH affects the environmental conditions required for microbial proliferation. Jin and Kirk (2018) further explained that pH can alter microbial metabolic pathways by influencing cell surface interactions and enzyme activity. In this study, the pH values of artificial rumen fluid ranged from 6.67 in Culture 1 to 6.78 in Culture 4. These values fall within the optimal range of 5.5–7.0 for rumen fermentation, as reported by Öztürk and Gur (2021), indicating a suitable environment for microbial degradation of feed throughout the sequential cultures.

An upward trend in pH was observed across the culture stages, with a significant increase noted in the final culture. This suggests that the sequential culturing process influenced acid-base dynamics within the fermentation system, possibly due to shifts in microbial composition and fermentation by-products. Shen et al. (2023) noted that rumen pH is modulated by factors such as forage-to-concentrate ratio and the presence of buffering agents like bicarbonate, calcium carbonate, and magnesium oxide. In this study, artificial saliva based on McDougall's solution was added at each stage to maintain pH stability. This buffer rich in sodium bicarbonate helps sustain near-neutral pH conditions favorable for microbial activity (McDougall, 1948). Camacho et al. (2019) emphasized that the buffering capacity of McDougall's solution depends on both sodium bicarbonate content and $CO_2$ infusion to displace oxygen and maintain anaerobic conditions. The consistent application of this buffer and $CO_2$ flushing in every stage likely contributed to the observed pH

stability and gradual increase.

**N-$NH_3$ concentration of artificial rumen fluid**

Ammonia nitrogen (N-$NH_3$) concentration is a key indicator of nitrogen metabolism and microbial protein synthesis in the rumen. The metabolic activity of rumen microbiota plays a central role in nitrogen recycling, particularly through the utilization of ammonia as a primary nitrogen source (Hartinger et al., 2018). Approximately 80% of rumen bacteria rely on ammonia for their nitrogen requirements (Zurak et al., 2023). In this study, the highest N-$NH_3$ concentration was observed in Culture 1 (21.88 ± 0.42 mM), which can be attributed to the initial microbial adaptation phase. During this stage, residual nitrogenous compounds from the original rumen fluid—such as amino acids and soluble proteins—may have contributed to the elevated ammonia levels. This finding aligns with Zurak et al. (2023), who noted that ammonia in the rumen is produced from the microbial degradation of dietary proteins and amino acids.

As the culture progressed from Culture 2 to Culture 4, a gradual decrease in N-$NH_3$ concentration was observed. This trend suggests that microbes became more efficient in utilizing ammonia for microbial protein synthesis. Sari et al. (2021) stated that decreasing ammonia concentrations in fermentation media are indicative of increased microbial uptake for anabolic processes. Similarly, Silviani et al. (2024) emphasized that microbial protein synthesis is directly influenced by the availability of ammonia and the consumption of digestible dry matter, which supplies the energy needed for microbial growth.

The observed N-$NH_3$ concentrations, ranging from 21.88 mM in Culture 1 to 10.97 mM in Culture 4, remained within the optimal range of 6 to 21 mM reported by Suryani et al. (2020) for supporting rumen microbial activity. This indicates that despite the decreasing trend, the artificial rumen environment remained suitable for sustaining microbial metabolism throughout the sequential cultures.

**Total volatile fatty acid (VFA) production**

Total volatile fatty acids (VFAs), also known as short-chain fatty acids, are the primary end-products of anaerobic microbial fermentation in the rumen (Hasan et al., 2015). These compounds play an essential role in maintaining optimal conditions for microbial growth and contribute significantly to the host animal's energy supply (Jian et al., 2016). In the context of *in vitro* rumen fermentation, the culture process aims to sustain microbial activity to ensure consistent VFA production. The results of this study showed that VFA production in the early stages of culturing (Culture 1 and Culture 2) did not differ significantly. This may be attributed to the microbial community still undergoing adaptation to the *in vitro* rumen environment. During this period, the microbes require time to re-establish their metabolic activity. Hu and Yu (2005) noted that feed must first be hydrolyzed into soluble carbohydrates before fermentation into VFAs can occur, highlighting the lag between inoculation and active fermentation.

As the culture progressed to later stages (Culture 3 and Culture 4), a significant increase in VFA concentration was observed. This suggests that once adapted, microbial populations become more efficient at fermenting substrates. Alabi et al. (2023) reported that anaerobic microbes degrade plant lignocellulosic materials through fermentation, resulting in the production of VFAs. In this study, the increasing trend in VFA concentration was likely also influenced by the closed nature of the *in vitro* system, where VFAs are not absorbed as they would be *in vivo* through the rumen wall. Nozière et al. (2011) stated that in ruminants, VFAs are typically absorbed across the rumen epithelium and utilized as a major energy source. The average VFA concentrations observed in this study ranged from 95.23 mM in Culture 1 and 2, to 111.36 mM in Culture 4. These values fall within the optimal range for efficient microbial fermentation, typically between 70 and 150 mM (McDonald, 2010). Tunkala et al. (2022) similarly reported that fresh rumen fluid produced VFA concentrations ranging from 84.6 to 113.7 mM, which further supports the validity of the values obtained in this study. The stable and adequate VFA production across all cultures may also be supported by the presence of protein-rich feed components that are resistant to rapid degradation and the continuous use of buffering agents to stabilize fermentation conditions.

**Dry matter digestibility of feed samples**

Dry matter digestibility is a key indicator of microbial activity in the rumen and reflects the efficiency of feed degradation in the fermentation system (Moon et al., 2010). In the early stages of culture, rumen microorganisms still adapt to the artificial environment and feed substrate, which may result in suboptimal digestibility. This is consistent with findings by McDermott et al. (2020), who observed that in the first stage of the consecutive batch culture (CBC) method, dry matter digestibility was lower than subsequent cultures as microbial populations gradually adapted and increased their enzymatic activity. Rumen feed digestibility is largely determined by the ability of microbial enzymes to hydrolyze feed components, particularly structural carbohydrates (Castillo and Hernández, 2021). This study observed a progressive increase in dry matter digestibility across the sequential culture stages, indicating improved microbial adaptation and fermentative efficiency. Badarina et al. (2023) noted that feed can be categorized as having good digestibility when it reaches at least 60%. In this context, digestibility values observed after the initial culture stage in this study were in line with or exceeded that threshold, suggesting effective microbial utilization of feed substrates in the later cultures. Dry

matter digestibility reflects microbial activity and plays a critical role in supporting microbial growth and, ultimately, the nutrient availability for the host animal. The trend observed in this study is further supported by the concurrent increase in volatile fatty acid (VFA) production as culture stages progressed.

Dry matter comprises various organic constituents, primarily carbohydrates such as cellulose and hemicellulose (Palangi and Macit, 2019). As rumen microorganisms degrade these complex lignocellulosic structures into simpler polysaccharides, they generate VFAs as primary fermentation end-products (Palmonari et al., 2024). Thus, the positive correlation between increasing dry matter digestibility and VFA production observed in this study suggests that more substrate became available for microbial fermentation in the later culture stages, enhancing energy yield and microbial activity.

## CONCLUSION

Sequential culturing of rumen fluid up to the fourth stage successfully maintained microbial viability and fermentative capacity for *in vitro* feed evaluation. The consistent increase in dry matter digestibility, volatile fatty acid production, stable pH, and optimal ammonia-N concentrations indicate that the microbial ecosystem remains functionally robust across culture cycles. These findings demonstrate that sequentially cultured rumen fluid can be a viable and sustainable inoculant alternative to fresh rumen fluid. The approach reduces dependence on fistulated animals, minimizes ethical concerns, and enhances reproducibility in laboratory-scale fermentation trials.

## DECLARATIONS

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### Data availability

The data generated and/or analyzed in this study can be obtained from the corresponding author upon reasonable request.

### Authors' contribution

All authors contributed equally to the conception, design, data collection, analysis, and writing of the manuscript. All authors read and approved the final manuscript.

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### Consent to publish

All authors agree to the publication of this manuscript.

### Competing interests

The authors have not declared any conflict of interest.

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# EFFECT OF EGG STORAGE DURATION ON HATCHABILITY AND EGG QUALITY OF CO LUNG DUCKS

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**ABSTRACT**: This study aimed to evaluate the effects of different egg storage durations on hatchability and internal egg quality of Co Lung duck eggs. A total of 10,000 eggs were incubated across five treatments representing different storage periods (T1: 1 day, T2: 3 days, T3: 5 days, T4: 7 days, T5: 10 days). Environmental data recorded at the storage site showed daily temperature variations from 26.4°C to 32.4°C and humidity ranging from 76.3% to 82.1%. Storage time significantly affected embryonic mortality, which increased from 4.8% (T1) to 11.5% (T5), and dead-in-shell rate, which rose from 2.1% to 5.4% ($P < 0.01$). Hatchability significantly declined from 78.5% (T1) to 68.7% (T5). Internal egg quality also deteriorated with prolonged storage (more than 5 days). The yolk index decreased from 0.41 to 0.34, albumen index from 0.05 to 0.02, and Haugh Unit from 83.5 to 69.2, indicating significant loss of freshness. Meanwhile, yolk ratio increased while albumen ratio decreased significantly ($P < 0.05$), suggesting moisture redistribution. No significant changes were observed in egg weight, shell thickness, or shell ratio. Overall, storage beyond 5 days led to reduced hatchability and poorer internal egg quality. Therefore, the optimal storage duration for Co Lung duck eggs is 3 to 5 days. Farmers and hatchery managers can incubate eggs within this period to maximize hatchability and freshness.

**Keywords:** Co Lung duck, Egg quality, Embryonic mortality, Hatchability, Indigenous poultry breeds.

## INTRODUCTION

Among Vietnam's many indigenous poultry breeds, the Co Lung duck stands out for its adaptability and reproductive potential. This breed originated from Ba Thuoc District, Thanh Hoa Province, and has become regionally recognized for its quality meat and egg production (Ha and Mui, 2018). In addition to the farming of local duck breeds, many high-yielding poultry breeds, including exotic duck varieties, have been introduced and crossbred in various regions. This trend has led to genetic dilution and degradation of indigenous duck breeds (Cuc, 2010; Pham et al., 2021). Moreover, uncontrolled crossbreeding has contributed to the emergence and spread of infectious diseases. The Co Lung duck, in particular, is at risk of genetic erosion due to a lack of systematic conservation and investment at the local level. Without a clear and effective strategy for conserving, developing, and utilizing this genetic resource, the purebred Co Lung duck may eventually disappear as a distinct indigenous breed (Ha et al., 2020).

Duck eggs are an affordable and nutrient-rich food that play a significant role in the diet of many Asian populations. They contribute approximately 10% to 30% of the world's total egg consumption (Quan and Benjakul, 2019). While duck eggs are traditionally consumed in processed forms such as salted eggs, pidan, and balut, there has been a growing preference for consuming them fresh in recent years (Huang et al., 2007; Quan and Benjakul, 2019). However, studies focusing on the storage-related quality changes in duck eggs remain limited (Lokaewmanee, 2017; Quan and Benjakul, 2018). In contrast, numerous researches have focused on the quality deterioration of chicken eggs during storage (Liu et al., 2016; Brodacki et al., 2019; Yamak et al., 2021). Egg storage is an essential procedure in hatchery operations, allowing synchronization of incubation and flexibility in production scheduling. Effective incubation and hatchery management are critical for achieving high hatchability and ducklings' quality, while recent innovations in incubation systems have created new technological opportunities and raised broader ethical concerns regarding poultry breeding practices (Kasielke, 2020; Adame and Ameha, 2023; Underwood et al., 2021). However, prolonged storage can negatively affect internal egg quality, increase embryonic mortality, and reduce hatchability rates. Egg quality is assessed through several indicators, including egg weight, shape index, Haugh unit, albumen weight, yolk weight, and shell weight (Robert, 2004; Hisasaga et al., 2020; Nasri et al., 2020). According to Curtis et al. (1985), poultry breeds and lines selected for different production purposes exhibit variations in egg quality, which are correlated with both egg yield and weight. Therefore, selecting for egg quality traits may influence other production-related characteristics (Falconer and Mackey, 1996). Despite its importance, limited research has been conducted on how different durations of egg storage affect hatchability and egg quality in indigenous duck breeds under smallholder and non-industrial farm conditions. Therefore,



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this study was designed to investigate the effect of various egg storage durations on hatchability, embryonic mortality, and selected egg quality traits in Co Lung ducks.

## MATERIALS AND METHODS

### Time and place of study

The experiment was conducted on 10,000 eggs of Co Lung ducks, collected from a duck farm located in Phong My commune, Dong Thap province of Vietnam, during the period from January to April 2024.

### Animals and experimental design

Co Lung ducks were raised in open-sided housing with corrugated metal roofs. The floor was covered with a 10 cm-thick layer of sand to enhance drainage and ventilation. The sides of the shed were enclosed with nylon mesh to block wind and insects. The ducks were fed a commercial layer diet containing 18% crude protein (CP) and 2,800 kcal/kg metabolizable energy (ME). Eggs were incubated using a fully automatic Mactech 5000 incubator (Mactech Technology Co., Ltd., Hanoi, Vietnam) with a capacity of 5,000 eggs per batch. The incubation conditions followed standard duck egg protocols: temperature ranged from 37.2 to 37.7°C and relative humidity from 75% to 80%. Eggs were automatically turned six times per day. From day 1 to 14, eggs were only turned; from day 15 to 32, turning was combined with cooling. The incubator was equipped with digital sensors for temperature and humidity to ensure consistent environmental control during the incubation period. The experiment was arranged in a completely randomized design (CRD) with five treatments corresponding to different egg storage durations, including T1 (1 day), T2 (3 days), T3 (5 days), T4 (7 days), and T5 (10 days). Each treatment was replicated five times, and each replicate represented an independent experimental unit. In each unit, 400 eggs were incubated to monitor hatchability parameters, and 50 eggs were sampled to assess egg quality. A total of 10,000 eggs were used for incubation, and 1,250 eggs were used for quality evaluation.

### Data collection

Eggs were collected daily at 7:00 AM and 3:00 PM, and marked by date and treatment. During incubation, candling was conducted on day 6 (stage 1) to identify infertile and early dead embryos, and again on day 18 (stage 2) to record late embryonic mortality. After hatching was completed, the number of successfully hatched and dead-in-shell eggs was recorded for each replicate. Temperature and relative humidity at the egg storage site were measured using a Fluke 971 Temperature-Humidity Meter at five fixed time points: 6:00 AM, 9:00 AM, 2:00 PM, 6:00 PM, and 10:00 PM.

***Infertility rate (%):*** Infertility rate = $\frac{\sum infertile\ eggs}{\sum incubated\ eggs}\ x\ 100$

***Embryonic mortality rate (%):*** Embryonic mortality rate = $\frac{\sum early\ dead\ embryos\ (day\ 6) + \sum late\ dead\ embryos\ (day\ 18)}{\sum fertile\ eggs}\ x\ 100$

***Dead-in-shell rate (%):*** Dead-in-shell rate = $\frac{\sum dead\ in\ shell\ eggs}{\sum fertile\ eggs}\ x\ 100$

***Hatchability rate (%):*** Hatchability rate = $\frac{\sum hatched\ eggs}{\sum fertile\ eggs}\ x\ 100$

***Egg shape index (%):*** Shape index = $\frac{egg\ width}{eggs\ length}\ x\ 100$

***Yolk index:*** Yolk index = $\frac{Yolk\ height}{Yolk\ diameter}\ x\ 100$

***Albumen index:*** Albumen index = $\frac{Albumen\ height}{Albumen\ diameter}\ x\ 100$

***Haugh unit (HU):*** $HU = 100 \times \log(H - 1{,}7 \times W^{0{,}37} + 7{,}57)$

where: H: Albumen height (mm); W: Egg weight (g)

***Yolk color:*** Determined using the Roche color fan (scale 1 to 15)

***Shell thickness (mm):*** Measured at three locations (blunt end, equator, pointed end) using a micrometer; the final value was the average of the three measurements.

### Statistical analysis

The experimental data were initially processed using Microsoft Excel 2016 and then analyzed by analysis of variance (ANOVA) based on the general linear model (GLM) using Minitab version 16.0. Differences among treatment means were compared using Tukey's test at a 95% confidence level.

## RESULTS AND DISCUSSION

### Environmental conditions during egg storage

Environmental conditions at the storage site are presented in Table 1. The recorded temperature showed a typical daily variation pattern, ranging from 26.4°C at 22:00 to a peak of 32.4°C at 14:00. Humidity fluctuated between 76.3% and 82.1%, with the lowest value also observed at 14:00. Excessively high humidity can inhibit proper water loss from the egg, while overly low humidity may lead to excessive evaporation, both of which can negatively impact embryo survival (Ibrahim et al., 2012). Embryonic development may be hindered when relative humidity levels are either too high or too low. Optimal growth is typically achieved when the surrounding humidity approaches a maximum level within the recommended range. These fluctuations in ambient conditions may influence the rate of egg quality deterioration and embryo viability, especially during prolonged storage periods. The range of temperature and humidity observed in this study was within tolerable limits for egg storage, although sustained exposure to temperatures above 30°C during the day might have accelerated water loss and albumen thinning, which can compromise hatchability and internal egg quality.

**Table 1** - Environmental Conditions During Egg Storage

| Time | Temperature (°C) | Humidity (%) |
|---|---|---|
| 6:00 | 26.8 | 82.1 |
| 9:00 | 29.6 | 79.1 |
| 14:00 | 32.4 | 76.3 |
| 18:00 | 29.5 | 79.6 |
| 22:00 | 26.4 | 81.9 |

### Effect of storage time on hatching performance

The results presented in Table 2 indicate that while egg weight remained unaffected by storage time (P = 0.22), the duration of storage exerted a substantial influence on hatching performance and embryo viability in Co Lung ducks. Hatchability decreased significantly from 78.5% in T1 to 68.7% in T5 (P = 0.002), with the highest rates observed in eggs stored for 1 to 3 days (T1 and T2), and a marked decline evident from T3 onward. This reduction of nearly 10 percentage points underscores the negative impact of prolonged storage. Embryonic mortality followed a similar trend, increasing from 4.8% in T1 to 11.5% in T5 (P = 0.004), suggesting that the viability of developing embryos diminishes with longer storage periods. Dead-in-shell rates also rose significantly with time, from 2.1% in T1 to 5.4% in T5 (P = 0.009), possibly due to impaired gas exchange or shell membrane alterations. These results are consistent with previous findings that linked extended storage to declining hatchability and increased embryo loss (Pokhrel et al., 2018). Although the infertile egg rate varied from 9.8% to 15.3%, the difference was not statistically significant (P = 0.36), indicating that infertility may depend more on breeder performance than on storage duration. The observed trends in mortality and hatchability are supported by research showing that prolonged storage alters embryonic morphology and leads to blastodermal degeneration (Arora and Kosin, 1966; Reijrink et al., 2008). Additional physiological mechanisms may include elevated lipid peroxidation, which compromises embryonic development (Cherian et al., 2007), and degradation of the internal albumen environment. Studies have also reported that storing eggs beyond 7 to 10 days increases the risk of early and late embryonic death (Ombansılar et al., 2007; Onbaşılar, 2007), and even short-term storage of more than 3 days may negatively affect certain avian species such as golden pheasants (Kustra et al., 2020). On a cellular level, extensive investigations have identified apoptosis and necrosis as key contributors to reduced embryo survival during storage (Bloom et al., 1998; Fasenko, 2007; Hamidu et al., 2011), though some evidence suggests that these forms of cell death may arise from intrinsic embryonic mechanisms rather than storage duration or temperature. Furthermore, microbial contamination, particularly Salmonella, can affect egg safety and viability, as highlighted by Saitanu et al. (1994), who found that 12.4% of duck eggs in Thai markets carried Salmonella on the shell surface. Preventive measures such as egg washing, refrigeration, and thorough cooking have been recommended to mitigate such risks (Messens et al., 2011). Collectively, these findings reinforce the conclusion that limiting egg storage to less than one week is essential to maintain high hatchability and minimize embryonic loss in Co Lung ducks.

Table 2 - Effect of storage time on hatching performance

| Indicator | T1 | T2 | T3 | T4 | T5 | SEM | P value |
|---|---|---|---|---|---|---|---|
| Egg weight (g) | 80.6 | 81.2 | 83.7 | 82.4 | 82.5 | 0.26 | 0.22 |
| Infertile egg rate (%) | 15.3 | 12.4 | 12.5 | 9.8 | 11.3 | 0.21 | 0.36 |
| Embryonic mortality (%) | $4.8^{c}$ | $6.1^{bc}$ | $7.9^{b}$ | $9.4^{a}$ | $11.5^{a}$ | 0.52 | 0.004 |
| Dead-in-shell rate (%) | $2.1^{c}$ | $2.7^{bc}$ | $3.9^{b}$ | $4.8^{a}$ | $5.4^{a}$ | 0.35 | 0.009 |
| Hatchability (%) | $78.5^{a}$ | $77.1^{a}$ | $73.4^{b}$ | $70.6^{bc}$ | $68.7^{c}$ | 0.61 | 0.002 |

$^{a,b,c:}$ Means within a column with different superscripts differ significantly (P<0.05). Storage periods (T1: 1 day, T2: 3 days, T3: 5 days, T4: 7 days, T5: 10 days)

**Effect of storage time on internal egg quality**

Internal egg quality characteristics were significantly influenced by the duration of storage, as indicated in Table 3. Egg weight ranged from 80.4 g in T1 to 83.8 g in T3 and did not show statistically significant differences among treatments (P = 0.41), suggesting that initial egg mass was consistent regardless of storage time. Similarly, the shape index remained unaffected (P = 0.52), with values fluctuating narrowly between 72.3% and 73.1%, reflecting uniformity in egg dimensions. However, several key quality traits declined with longer storage. The yolk index decreased significantly from 0.41 in T1 to 0.34 in T5 (P = 0.008), indicating weakening of the vitelline membrane, likely due to water migration from the albumen into the yolk during storage. This is consistent with observations by Onbaşilar et al. (2007), who found that dehydration during storage negatively affects albumen consistency and yolk integrity in Pekin ducks. The albumen index followed a similar downward trend, dropping significantly from 0.05 in T1 to 0.02 in T5 (P = 0.001), which suggests structural degradation and thinning of the albumen. The deterioration of albumen is closely related to the increase in pH over time.

Table 3 - Effect of storage time on internal egg quality

| Indicator | T1 | T2 | T3 | T4 | T5 | SEM | P value |
|---|---|---|---|---|---|---|---|
| Egg weight (g) | 80.4 | 81.6 | 83.8 | 80.5 | 82.2 | 0.35 | 0.41 |
| Shape index (%) | 72.8 | 73.1 | 72.4 | 72.3 | 72.9 | 0.45 | 0.52 |
| Yolk index | 0.41$^{a}$ | 0.39$^{a}$ | 0.36$^{b}$ | 0.35$^{b}$ | 0.34$^{c}$ | 0.01 | 0.008 |
| Albumen index | 0.05$^{a}$ | 0.04$^{a}$ | 0.03$^{b}$ | 0.02$^{c}$ | 0.02$^{c}$ | 0.004 | 0.001 |
| Albumen pH | 8.1$^{c}$ | 8.4$^{bc}$ | 8.7$^{b}$ | 8.9$^{a}$ | 9.1$^{a}$ | 0.06 | 0.001 |
| Haugh Unit | 83.5$^{a}$ | 80.4$^{b}$ | 76.3$^{b}$ | 73.1$^{bc}$ | 69.2$^{c}$ | 1.76 | 0.001 |
| Yolk color (Roche scale) | 11.4 | 11.2 | 10.9 | 10.6 | 10.5 | 0.29 | 0.09 |
| Yolk ratio (%) | 31.9 | 32.2 | 33.1 | 34.4 | 35.1 | 0.52 | 0.07 |
| Albumen ratio (%) | 57.3$^{a}$ | 56.5$^{a}$ | 55.4$^{b}$ | 54.1$^{b}$ | 52.8$^{c}$ | 0.61 | 0.005 |
| Shell ratio (%) | 10.8 | 11.3 | 11.6 | 11.5 | 12.1 | 0.41 | 0.11 |
| Shell thickness (mm) | 0.41 | 0.39 | 0.40 | 0.41 | 0.40 | 0.004 | 0.06 |

$^{a,b,c:}$ Means within a column with different superscripts differ significantly (P<0.05). Storage periods (T1: 1 day, T2: 3 days, T3: 5 days, T4: 7 days, T5: 10 days)

In this study, albumen pH rose from 8.1 on day 1 to 9.1 by day 10, consistent with the findings of Pereira et al. (2022), who reported a negative correlation between albumen pH and egg freshness. This pH increase is mainly attributed to the escape of carbon dioxide through eggshell pores, which disrupts the carbonic acid–bicarbonate buffering system within the albumen, making the environment more alkaline (Samli et al., 2005; Ragni et al., 2007; Shin et al., 2012). Yuceer and Caner (2014) explained that this alkalization leads to depolymerization of proteolytic enzymes, destabilizing the ovomucin–lysozyme complex, which causes the thick albumen to lose its gel-like consistency and become thinner. Brake et al. (1997) further emphasized that elevated storage temperatures can accelerate protein denaturation and moisture transfer from the albumen to the yolk, contributing to faster deterioration. These biochemical changes were reflected in the Haugh Unit (HU), a primary indicator of egg freshness, which declined sharply and significantly from 83.5 in T1 to 69.2 in T5 (P = 0.001). This substantial reduction of over 14 points supports the findings of Dassidi et al. (2022), who noted that eggs stored for 14 days exhibited significantly lower HU values, indicating compromised internal quality. Although yolk color, measured on the Roche scale, decreased slightly from 11.4 in T1 to 10.5 in T5, the change was not statistically significant (P = 0.09). Nonetheless, this trend may suggest pigment fading due to oxidative degradation or breakdown of carotenoids. Notably, the yolk color of Co Lung duck eggs in this study was markedly higher than the 5.1 reported for Beijing ducks by Denley et al. (2005), likely due to differences in dietary pigment intake between breeds. In terms of component proportions, the yolk ratio increased from 31.9% in T1 to 35.1% in T5, while the albumen ratio declined from 57.3% to 52.8%, with both showing significant differences (P = 0.005), indicating a redistribution of internal contents likely driven by moisture loss from the albumen and swelling of the yolk. The shell ratio varied between 10.8% and 12.1% but did not show a significant effect from storage time (P = 0.11), and shell thickness remained relatively stable between 0.39 and 0.41 mm (P = 0.06), indicating that external shell traits were not altered. The presence of a calcified eggshell serves as a protective barrier, shielding the egg from mechanical injury and reducing the risk of microbial infiltration (Hincke et al., 2011). Collectively, these findings confirm that internal egg quality progressively deteriorates with longer storage duration, especially under ambient or elevated temperatures. Similar observations were reported in multiple studies, which found that prolonged storage and higher temperatures significantly affect egg integrity (Huang and Lin, 2011; Pandian et al., 2012; Lokaewmanee, 2017; Quan and Benjakul, 2018, and 2019). Interestingly, Jones et al. (2018) demonstrated that refrigeration was more effective in preserving egg quality than washing or applying oil coatings. These patterns emphasize the importance of controlled storage conditions to maintain internal egg quality and extend shelf life.

## CONCLUSION

This study demonstrated that prolonged storage of Co Lung duck eggs adversely affects hatching performance and internal egg quality. When eggs were stored for more than 5 days, embryonic mortality increased significantly from 4.8% (T1) to 11.5% (T5), and hatchability declined sharply from 78.5% to 68.7%. In terms of internal quality, the yolk index dropped from 0.41 to 0.34, and Haugh Unit decreased from 83.5 to 69.2 with longer storage duration. These results highlight that storage beyond 5 days leads to notable declines in egg viability and freshness. Therefore, to maintain optimal hatchability and internal quality, Co Lung duck eggs should be incubated within 3 to 5 days after laying. This finding provides practical guidelines for duck farmers and hatchery managers in Vietnam to improve productivity and conserve the genetic value of indigenous Co Lung ducks.

## DECLARATIONS

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### Data availability

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

### Ethical regulations

Formal ethical approval was not required for this study because no invasive procedures were performed. All animal care, handling, and sample collection complied with the Law on Animal Husbandry (No. 32/2018/QH14) of the National Assembly of the Socialist Republic of Vietnam. Animal welfare was carefully monitored and maintained throughout the experimental period. In addition, the authors confirm that the study was conducted 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.

### Authors' contribution

Phan Nhan was solely responsible for the conceptualization and design of the study, experimental execution, data collection and analysis, as well as drafting and revising the manuscript. All aspects of this work were conducted independently by the author.

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### Competing interests

The authors have not declared any competing interests.

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# EVALUATION OF *Prunus africana* BARK EXTRACT AS AN ORGANIC ALTERNATIVE TO SYNTHETIC GROWTH PROMOTERS IN BROILER PRODUCTION

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**ABSTRACT**: Concerns over synthetic inputs in organic poultry production systems prompted an evaluation of aqueous *Prunus africana* bark extracts as natural feed additive via drinking water. Using 210 unsexed Cobb 500 day old broiler chicks, a 42 day trial was conducted to compare graded levels of ground *P. africana* bark infused in drinking water to oxy-tetracycline 80 and a conventional prophylactic calendar on growth, hematology and economic response in chickens. The feed efficiency, weight gain and final weights of birds fed *P. africana* did not differ significantly ($P > 0.05$) from those in the control groups. Carcass yields between the control and prunus groups did not vary significantly ($P < 0.05$) except the oxyterracycline control that had significantly ($P < 0.05$) higher slaughter weight (1913.3 g vs. 1681.7 g), carcass weight (1681.7 g vs. 1468.3 g) and drumstick weight (233.3 g vs. 198.3 g) compared to T4 (5 g/L). Significant differences ($P < 0.05$) were observed in hematological and serum biochemistry at the starter phase (day 21) but not ($P > 0.05$) during the finisher phase (day 42). The unit total expenses were significantly lower ($P < 0.05$) for treatments with inclusions of bark extract, thereby improving their gross margins, cost-to-benefit ratios, and economic efficiency. However, a progressive increase in the concentration of bark extracts did not significantly ($P > 0.05$) affect the profitability of the farm enterprises. Although metabolic challenges were observed in young chicks *P. aficana* bark extracts improved their growth, and carcass quality thereby confirming their potential use as a natural growth promoter in broiler production in replacement of the synthetic conventional prophylactic protocols.

**Keywords:** Chickens, Economic efficiency, Feed-additive, Natural products, Prophylactic.

## INTRODUCTION

Global chicken and eggs consumption surpasses that of other meats (OECD-FAO, 2021). The high demand for chicken and its products has led to extensive use of synthetic inputs for growth promotion (Alshemani et al., 2021). This scenario contributes to the emergence of antimicrobial resistant bacteria and other ailments in the consumers. To meet up with postwar demands in the 1950s, the Food and Drug Administration of the United States approved the use of antibiotics as growth promoters for poultry hitherto, limited solely to human health. Today, there is increase demand for foods devoid of synthetic supplements because of health concerns often associated with them (Amarachukwu, 2022; Rashidinejad, 2024). To address the increasing demand for organic foods, the use of plant-based health supplements is encouraged (Kairalla et al., 2023). The bark extract of the *Prunus africana*, an afro-montane tree species, is widely used in human medicine to treat common fungal and bacterial infections in the urino-genital, digestive, reproductive and respiratory organs (Bii et al., 2010). *Prunus africana* extracts exhibit antimicrobial, anti-inflamatory, antiagiogenic, antiandrogenic, antioxidant, analgestic, antidipeptidyl peptidase-4 activity, and astringent properties which have been documented by many authors (Ndung'u et al., 2024). This is because the extracts contain phytochemicals that act in synergy (Stewart, 2003). The high demand for *P. africana* bark as an export commodity, has led to its overexploitation thereby prompting its protection via Appendix II of the Convention on International Trade in Endangered Species (CITES, 2022). The reliance on wild-collections from forest stands is responsible for this threat, therefore conservation practices including enrichment plantings have been recommended.

Unfortunately, motivation among local farmers is weak. However, the promotion of local uses such as the replacement of expensive prophylactic protocols in livestock production could boosts efforts at local conservation through cultivation of the species. This is because local farmers can easily identify with such local uses rather than exports. There is paucity of research that backs locally viable economic alternatives to the export of *P. africana* bark from producing countries. This study is therefore necessary to begin filling that research gap. The opportunities of using prunus a natural growth promoter in broiler production are therefore, more compelling in the present world, when healthy chickens produced with less synthetics and antibiotics are high in demand.



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## MATERIALS AND METHODS

### Ethical issues related to the experimental animals

Unsexed Cobb 500 broiler chickens were used as experimental animals for this study. The chickens were raised in standard pens and temperature and humidity were closely monitored using a thermo-hydrometer (model 288-ATH, SL Technologies). The experiments were carried out following the National Ethical Committee Guidelines (No. FWA-IRB00001954) and International (European Committee Council Directive of November 24, 1986(86/69/EEC); Guide for the Care and Use of Laboratory Animals (U.S. National Research Council, 1996) for the care and use of laboratory animals. All efforts were made to minimize the suffering and stress of chickens used at each stage of the study. Ethical approval was given by the University of Buea Institutional Animal Care and Use Committee (UB-IACUC) via permit No.UB-IACUC No.25/2023, signed by Committee Chair Prof Jane Francis Akoachere and Committee Secretary Dr Rene B Ayiseh.

### Sample collection, preparation and experimental design

Stem bark from mature *Prunus africana* plant was collected in April 2023 from NSEH village of Bui division in North West Cameroon, with approximate coordinates of 6.0000 $^0$N and 10.5000 $^0$E; The plant material was chopped into small pieces, dried under shade for 8 weeks, ground to fine powder on a hammer mill, and sent to the Teaching & Research Farm of the University of Buea for eventual use. Average ambient diurnal temperature and humidity during drying ranged from 16.6$^o$C to 28$^o$C and 65% to 85% respectively. Daily, varying quantities of the powder (1g, 3g, 5g, 7g and 10g) were weighed, soaked separately overnight in one litre of potable water and the infusions filtered through a muslin cloth the following day to obtain aqueous bark extracts, that were used to compare in a conventional prophylactic calendar and oxyterracycline 80.

### Experimental design

The experimental design used was a completely randomized design with the model: $Y=\mu+Tj+\sum eij$

Where Y1= the J$^{th}$ measurement on the I$^{th}$ treatment; μ= the overall mean; Tj= effect of the I$^{th}$ treatment; $\sum eij$ = effect of the random error.

It was designed to have seven treatments sub-divided into 3 replicates of 10 birds each, to give 21 experimental units Two hundred and ten day-old unsexed Cobb 500 breed broiler chicks were obtained from the FECAM SARL Commercial Hatchery in Bafoussam (Cameroon), vaccinated and randomly assigned to the seven treatment groups that were given graded quantities of test ingredients in drinking water. The treatment groups comprised: a conventional prophylactic calendar (Table 1) as negative control (T0), Oxy-tetracycline 80 at 0.5 g/L water as positive control (T1), and five graded levels of aqueous *P. africana* bark extracts [1 g/L (T2), 3 g/L (T3), 5 g/L (T4), 7 g/L (T5) and 10 g/L (T6)].

At the start, the entire pens and their easily accessible feeders and drinkers, were washed with detergent and water, disinfected (using tetrahydrofuran and Virunet), and wood shavings spread out as deep litter. Charcoal pots and 100-Watt electric bulbs were centrally placed in each pen and the chicks were brooded at slightly decreasing temperatures (at 34°C, 32°C and 30°C in the first, second and third weeks, respectively) with the temperature monitored using a thermo-hydrometer (model 288-ATH, SL Technologies). A corn/soybean basal diet was formulated for the two growth phases; starter and finisher (Table 2), and water was offered *ad libitum* to the birds. Daily feed intake and weekly weight gains were measured using a digital electronic balance, (WANT WT-GF 0.1 g from WANT Balance Instrument Co Ltd- China).

**Table 1 -** Conventional prophylactic calendar for disease prevention for broilers in Buea used for birds in treatment 0 (T0)

| Day/Age | Type of medication | Mode of administration | Dosage | Function |
|---|---|---|---|---|
| 1 | Avinew (A), Bioral (B) and Galivac (G) | Beak dipping or Intra ocular | 1000D in 10L | Prevention of NCD, IB and Gumboro |
| 1-5 | Anti-stress and vitamin | Drinking water | 5g in 5L | Against stress |
| 6-8 | Antibiotic(oxy) | Drinking water | 5g in 2.5L | Disease prevention |
| 8 | Vaccine; A, B, G | Drinking water | 1000D in 10L | Booster against viral infection |
| 8-10 | Vitamin (Amin total) | Drinking water | 5g in 10L | Growth promoter |
| 11-13 | Anti-coccidiosis | Drinking water | 5g in 10L | Prevention of coccidiosis |
| 14-16 | Vitamin (Amin total) | Drinking water | 5g in 10L | Growth promoter |
| 17-19 | Antibiotic(oxy) | Drinking water | 5g in 10L | Anti-infectious |
| 20-22 | Vitamin | Drinking water | 5g in 10L | Growth promoter |
| 21 | Vaccine; A, B, G | Drinking water | 1000D in 10L | Booster against viral infection |
| 23-25 | Anti-coccidiosis | Drinking water | 5g in 10L | Prevention of coccidiosis |
| 26-29 | Vitamin | Drinking water | 5g in 10L | Growth promoter |
| 30 | Dewormer(anthelmintic | Drinking water | 5g in 2.5L | Against worms |
| 35-37 | Liver protector | Drinking water | 1ml in 1L | Diuretic |
| 38-42 | Vitamin | Drinking water | 5g in 10L | Growth promoter |

Vaccine: A=Avinew, B=Bioral G=Galivac, NCD= New castle disease, IB= Infectious bronchitis.

**Table 2** - Composition of basal and experimental diet for broiler starter and finisher.

| Ingredients | Starter (%, w/w) | Finisher (%, w/w) |
|---|---|---|
| Maize | 54.00 | 65.0 |
| Soybean meal | 35.35 | 27.50 |
| Fishmeal | 5.00 | 4.00 |
| Premix * | 1.50 | 1.50 |
| Calcium Phosphate | 3.00 | 1.00 |
| Lysine | 0.50 | 0.40 |
| Methionine | 0.40 | 0.35 |
| Salt | 0.25 | 0.25 |
| Total | 100 | 100 |
| **Calculated chemical composition** | | |
| Metabolizeable energy (Kcal/kg) | 2817 | 2964 |
| Crude protein (%) | 24.6 | 21.2 |
| Crude fiber (%) | 2.50 | 2.60 |
| Calcium (%) | 1.40 | 0.90 |
| Phosphorus (%) | 0.90 | 0.50 |
| Methionine (%) | 1.00 | 0.80 |
| Lysine (%) | 1.30 | 1.20 |

*Premix Composition (Vitamins per kg); Vit A 3,000,000 UI; Vit D3 600,000 UI; Vit E 4,000 mg; Vit K3 500 mg; Vit B1 320 mg; Vit B2 1,000 mg; Vit B3 2400 mg; Vit B6 400 mg; Vit B12 7 mg; Vit PP/Ac nicot/niacin 4,800 mg; Biotin 10 mg; Choline chloride 100,000 mg; Folic acid 160 mg; Cupper II sulphate 200 mg; zinc oxide 10,000 mg; manganese oxide 14,000 mg; Calcium iodate 200 mg; Lysine 7800 mg; Meth 200,000 mg; Iron sulphate 8,000 mg, Sulfate 2,000 mg.

**Data collection**

***Analysis of blood lipid, hematological and serum biochemical profiles***

At the end of the starter and finisher phases hematological and serum biochemical profiles were analyzed. Blood lipid profile was additionally analyzed at the end of the finisher phase. Three birds were randomly selected for each replicate and from which 2 mL blood samples were collected (using a syringe) from each birds' wing vein, and kept in sets of tubes. Green topped tubes containing heparin were used for blood lipid analysis. This portion of blood was stored for 30 min, centrifuged at 2,000 rpm on a benchtop centrifuge (model TD4Y, China) and the serum retrieved and deep-frozen at -20°C for 24 h. The serum was later thawed and analyzed on a spectrophotometer (Unico-2400, Japan) for their total cholesterol, triglycerides, high-density and low-density lipoproteins (Aberare et al., 2011). The other portion was poured into EDTA coated-vacuum capillary tubes then analysed, using standard techniques (Abdul Hamid, 2012), for their white blood cell count, red blood cell count, hemoglobin concentration, packed cell volume, mean corpuscular volume and mean corpuscular hemoglobin contents. Blood for the serum biochemical analyses, was collected in dry tubes (without anticoagulant), refrigerated for 24 h at 20°C, centrifuged at 1500 rpm and the supernatant analyzed using the Chronolab kit (Chrono lab Systems, Spain) and the semi-automated spectrophotometer Sanymed kit (Sanymed Sas, Italy) (operating at 37°C) for alanine amino transferase and alkaline phosphatase, respectively.

***Carcass and organ characteristics***

The birds randomly selected for heamatological analysis in starter and finisher phases were sacrificed then characterized for their gut pH. Those at the finisher phase were particularly fasted overnight, then sacrificed, and characterized for their carcass, organ and weights. Organ weights were measured using a digital electronic balance, (WANT WT-GF 0.1 g from WANT Balance Instrument Co Ltd- China), while gut pH was measured a digital pH meter (MODENA, Apluste).

***Costs benefit analysis***

To analyze the economic performance of the broiler production, seven economic parameters were determined using values from the separate costs of feed, medication and other inputs. The unit cost of feed consumed (i) was estimated as the ratio of the cost of feed per unit weight gained while the cost of medication per growth promoter consumed (ii) was estimated as the ratio of the cost of medication to the unit quantity of growth promoter consumed. The total expenses incurred (iii) were estimated as the sum of the costs of feed and antibiotic/medication consumed while the total revenue (iv) was calculated as the product of the live body weight and the unit price (per kg) of the birds. The gross margin (v) on its part was estimated as the difference between the total revenue and total expenses, the benefit cost ratio (vi) as the ratio of the gross margin to the total expenses (Lundholm, 2005) while the economic efficiency (vii) was the ratio of the gross margin to the cost of feed consumed (Omar et al., 2019).

**Statistical analysis**

Data were entered into spreadsheets using Microsoft Excel and analyzed using SPSS version 22 software package. These were then used to estimate descriptive parameters like the means, the standard errors of the mean and statistical

differences between group means, as well as one-way analysis of variance (ANOVA). Shapiro-Wilk test for normality and Levene's test for homogeneity of variances were used to test whether the data meets the assumptions of ANOVA. Duncan Multiple Range Test was used for post-hoc test comparison of group mean values. Significant levels were measured at 95% confidence threshold.

## RESULTS

### Effects of extract inclusions on growth performance of broiler chicken

The growth performance of broiler chicken given varying levels of *P. africana* bark extract in drinking water (Table 3) showed that these effects were slightly different for the starter and finisher phases depending on the parameters measured. The average feed intake, daily weight gain and feed conversion ratio were not significantly different ($P > 0.05$) between the control and prunus groups, although T4 (5 g/L) showed significantly ($P < 0.05$) lower daily gain compared to the controls during the starter phase, and also lower daily feed intake during the finisher phase. Average daily water intake was not significantly different ($P > 0.05$) between the control and the prunus groups. However, chickens receiving higher densities of Prunus bark infusions (T3 (3 g/L); T4 (5 g/L); T5 (7 g/L) and T6 (10 g/L) significantly ($P < 0.05$) consumed smaller amounts of water during the finisher phase compared to T2 (1g/L), which received the lowest density. Also, mortality was recorded for all prunus groups in the starter but not in the finisher phase unlike the normal and positive controls which did not record any mortality.

### Effect of extracts on the carcass, visceral organs and blood lipid profiles

Table 4 which presents the effect of aqueous *P. africana* bark extracts inclusion in drinking water on the weights of the carcass and visceral organs of broiler chicken, shows that the inclusions did not have any significant effect on the birds' live weights ($P > 0.05$). However, the positive control is associated with a significant ($P < 0.05$) increase in slaughter and carcass weights compared to T4, while the negative control (T0) was not significantly different ($P > 0.05$) from all test ingredients in the quantitative carcass parameters. As for the weights of the visceral organs, the aqueous *P. africana* bark extracts inclusions did not show any clear patterns on their evolution, except for a slight progressive decrease of the liver weight as the level of *P. africana* inclusion increased. The liver weight values of T6 were significantly ($P < 0.05$) lower than those of the positive control (T1), and also T2, T3 and T5, which had lower concentrations of the bark extract. Results on the birds' blood lipid profiles showed that the negative control (T0) had significantly ($P < 0.05$) higher levels of low density lipoproteins compared to the treatments with the highest levels of prunus bark inclusion (T5 and T6), while the positive control (T1) significantly ($P < 0.05$) had higher levels of Total triglycerides compared to T3. All other lipoprotein quality parameters did not differ significantly ($P > 0.05$).

### Effect of aqueous *Prunus africana* bark extracts on the hematological parameters and serum biochemistry

The hematological parameters and serum biochemistry of broiler chickens exposed to graded levels of *P. africana* bark extract are presented in Table 5. Significant differences in hematological parameters observed in the starter phase were not significant ($P > 0.05$) during the finisher phase. During the starter phase, hemoglobin (Hb) levels were significantly ($P < 0.05$) higher for birds in the negative control (T0), compared to the oxytertacycline positive control (T1). However, the mean Hb concentration of the birds in the test ingredients and control groups did not vary significantly ($P > 0.05$). Similar trends observed for Hb in the starter phase were also observed for parked cell volume (PCV). Interestingly, birds that received lower levels of the test ingredients (T2, T3, T4, and T5) had a significantly ($P < 0.05$) higher mean white blood cell (WBC) counts compared to the negative control (T0) during the starter phase. However, the mean WBC counts of birds that received the highest level (T6) was not significantly different ($P > 0.05$) from both the positive and negative controls. For this study, the birds' serum was evaluated with respect to the changes in their alanine amino transferase (ALT) and alanine phosphatase (ALP) contents. Significant differences ($P<0.05$) observed during the starter phase were absent in the finisher phase. During the starter phase, the mean ALT concentrations were similar ($P > 0.05$) between negative control (T0) and all the test ingredient treatments. However, the positive control birds (T1) showed significantly ($P < 0.05$) lower mean ALT values compared to birds in T2. On the other hand, ALP did not vary significantly ($P > 0.05$) among the groups during the starter and finisher phases.

### Effects of extracts on gut acidity in broiler guts

The pH of the various segments of the gut of birds exposed to graded levels of *P. africana* bark extract in drinking water is presented in Table 6. The pH of the crop, proventriculus, small intestine and large intestine of birds in the test ingredient and control groups did not vary significantly ($P > 0.05$) during the starter (day 21) and finisher (day 42) phases. However, during the starter phase the crop, small intestine and large intestine all had an alkaline pH which became acidic in the finisher phase. Also, the proventriculus of chickens increased in acidity with increasing levels of aqueous *P. africana* in the finisher phase compared to the normal and positive controls.

**Table 3** - Growth performance of broiler chickens fed varying inclusion levels of aqueous *P. africana* bark extract as additive in drinking water.

| Treatment | T0: Negative control | T1: Positive control | T2: 1 g/L extract | T3: 3 g/L extract | T4: 5 g/L extract | T5: 7 g/L extract | T6: 10 g/L extract | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|
| **Starter** | | | | | | | | | |
| Daily feed intake (g | 39.18 | 38.06 | 38.72 | 37.55 | 35.93 | 37.97 | 36.01 | 0.432 | P=0.092 |
| Daily water intake (mL) | 108.3 | 107.06 | 110.17 | 97.78 | 97.67 | 98.15 | 101.76 | 1.583 | P=0.071 |
| Daily weight gain (g) | 27.08$^{b}$ | 27.07$^{b}$ | 25.74$^{ab}$ | 26.04$^{ab}$ | 24.19$^{a}$ | 26.73$^{b}$ | 26.18$^{ab}$ | 0.271 | P=0.041 |
| Feed conversion ratio | 1.44 | 1.40 | 1.50 | 1.44 | 1.49 | 1.42 | 1.45 | 0.010 | P=0.074 |
| Mortality (%) | 0.00 | 0.00 | 13.20 | 3.30 | 10.00 | 13.30 | 10.00 | 1.971 | P=0.069 |
| **Finisher** | | | | | | | | | |
| Daily feed intake (g | 139.80$^{b}$ | 135.37$^{ab}$ | 144.55$^{b}$ | 132.04$^{ab}$ | 126.22$^{a}$ | 144.60$^{b}$ | 139.23$^{b}$ | 2.352 | P=0.076 |
| Daily water intake (mL) | 310.83$^{ab}$ | 295.21$^{ab}$ | 333.76$^{b}$ | 284.95$^{a}$ | 274.87$^{a}$ | 304.85$^{ab}$ | 278.45$^{a}$ | 6.593 | P=0.036 |
| Daily weight gain (g) | 70.56 | 71.94 | 73.15 | 70.24 | 68.84 | 71.18 | 71.79 | 0.971 | P=0.068 |
| Feed conversion ratio | 1.98 | 1.88 | 1.98 | 1.89 | 1.84 | 2.03 | 2.02 | 0.020 | P=0.079 |
| Mortality (%) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | P=1.000 |

$^{a, b}$= means followed by the same letters in a row, were not significantly different (P > 0.05); SEM= Standard error of the mean.

**Table 4** - Effect of aqueous *P. africana* bark extracts in drinking water on the weights (g) of carcass and visceral organs of broiler chicken.

| Body section | Body part | T0: Negative control | T1: Positive control | T2: 1 g/L extract | T3: 3 g/L extract | T4: 5 g/L extract | T5:7 g/L extract | T6: 10 g/L extract | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|---|
| Carcass weights | Live weight (g) | 1978.3 | 2050 | 1995 | 1921.7 | 1805 | 2008.3 | 1891.7 | 26.69 | P=0.084 |
| | Slaughter weight (g) | 1828.3$^{ab}$ | 1913.3$^{b}$ | 1861.66$^{ab}$ | 1766.7$^{ab}$ | 1681.7$^{a}$ | 1863.3$^{ab}$ | 1760.0$^{ab}$ | 24.68 | P=0.041 |
| | Carcass weight (g) | 1606.7$^{ab}$ | 1681.7$^{b}$ | 1620.0$^{ab}$ | 1590.0$^{ab}$ | 468.3$^{a}$ | 1618.3$^{ab}$ | 1638.3$^{ab}$ | 21.12 | P=0.039 |
| | Breast weight (g) | 550 | 555 | 538.3 | 521.7 | 498.3 | 563.3 | 523.3 | 9.58 | P=0.122 |
| | Back muscle weight (g) | 293.3 | 275 | 270 | 280 | 266.7 | 296.7 | 253.3 | 5.21 | P=0.101 |
| | Drumstick (g) | 233.3$^{b}$ | 233.3$^{b}$ | 220.0$^{ab}$ | 210.0$^{ab}$ | 198.3$^{a}$ | 233.3$^{b}$ | 221.7$^{ab}$ | 3.78 | P=0.045 |
| | Wing (g) | 80 | 88.3 | 76.7 | 83.3 | 75 | 82 | 76.7 | 1.74 | P=0.082 |
| | Intestines (g) | 78.3 | 76.7 | 85,0 | 81.7 | 86.7 | 73.3 | 76.7 | 2.16 | P=0.076 |
| Visceral organs | Liver (g) | 41.7$^{ab}$ | 46.7$^{b}$ | 46.7$^{b}$ | 48.3$^{b}$ | 41.7$^{ab}$ | 46.7$^{b}$ | 31.7$^{a}$ | 1.81 | P=0.035 |
| | Lungs (g) | 13.3 | 13.3 | 15.0 | 18.3 | 21.7 | 15.0 | 13.3 | 1.39 | P=0.078 |
| | Heart (g) | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 0.00 | P=1.000 |
| | Gizzard (g) | 56.7 | 60.0 | 60.0 | 55.0 | 55.0 | 56.7 | 63.3 | 1.44 | P=0.086 |
| | Ilium (g) | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 0.00 | P=1.000 |
| Blood lipid profile | Total cholesterol (%) | 222.3 | 363.5 | 378.3 | 485.2 | 315.3 | 440.8 | 445.3 | 38.47 | P=0.092 |
| | Total triglycerides (%) | 409.0$^{ab}$ | 662.8$^{b}$ | 387.7$^{ab}$ | 257.3$^{a}$ | 540.0$^{ab}$ | 421.3$^{ab}$ | 366.8$^{ab}$ | 38.92 | P=0.024 |
| | High Density lipoprotein (%) | 57.8 | 68 | 59.5 | 58.7 | 55.7 | 51.0 | 47.8 | 2.45 | P=0.073 |
| | Low Density lipoprotein (%) | 12.9c | 11.2$^{abc}$ | 10.6$^{abc}$ | 10.9$^{abc}$ | 12.5$^{bc}$ | 8.80$^{a}$ | 9.50$^{ab}$ | 0.42 | P=0.042 |

$^{a, b}$= means followed by the same letters in a row, were not significantly different (P > 0.05); SEM= Standard error of the mean



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**Table 5 -** Effects of graded levels of *P. africana* bark extract on the hematological parameters and serum biochemistry of broiler chickens on day 21 and day 42.

| Parameters | T0: Negative control | T1: Positive control | T2: 1 g/L extract | T3: 3 g/L extract | T4: 5 g/L extract | T5: 7 g/L extract | T6: 10 g/L extract | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|
| **Starter** | | | | | | | | | |
| Hemoglobin (g/dL) | 15.60$^{b}$ | 14.25$^{a}$ | 14.85$^{ab}$ | 14.35$^{ab}$ | 14.60$^{ab}$ | 14.58$^{ab}$ | 14.30$^{ab}$ | 0.52 | P=0.046 |
| Pack cell volume (%) | 46.80$^{b}$ | 42.75$^{a}$ | 44.55$^{ab}$ | 43.05$^{ab}$ | 43.80$^{ab}$ | 43.75$^{ab}$ | 42.20$^{a}$ | 1.38 | P=0045 |
| Red blood cells (× 106 mm3) | 3.5 | 3.41 | 3.5 | 3.5 | 3.5 | 3.41 | 3.41 | 0.08 | P=0.082 |
| White blood cells (× 106 mm3) | 11.33$^{a}$ | 13.83$^{ab}$ | 14.83$^{b}$ | 15.50$^{b}$ | 16.00$^{b}$ | 19.50$^{c}$ | 13.50$^{ab}$ | 1.5 | P=0.037 |
| Alanine amino transferase (UI | 0.50$^{ab}$ | 0.46a | 0.61$^{b}$ | 0.55$^{ab}$ | 0.51$^{ab}$ | 0.50$^{ab}$ | 0.51$^{ab}$ | 0.02 | P=0.041 |
| Alanine phosphatase (UI) | 7.76 | 8.55 | 8.15 | 8.54 | 6.66 | 7.65 | 7.1 | 1.12 | P=0.072 |
| **Finisher** | | | | | | | | | |
| Hemoglobin (g/dL) | 12.49 | 11.66 | 12.21 | 12.49 | 12.49 | 12.21 | 12.49 | 0.48 | P=0.121 |
| Pack cell volume (%) | 37.48 | 34.98 | 36.65 | 37.49 | 37.48 | 36.65 | 37.48 | 1.44 | P= 0.096 |
| Red blood cells (× 106 mm3) | 3.25 | 3.08 | 3.16 | 3.25 | 3.25 | 3.33 | 3.25 | 0.14 | P=0.153 |
| White blood cells (× 106 mm3) | 18.66 | 18.53 | 18 | 16.4 | 20.25 | 18 | 16.06 | 2.26 | P=0.087 |
| Alanine amino transferase (UI | 27 | 34.16 | 37.16 | 32 | 35 | 37.83 | 32.33 | 2.24 | P=0.084 |
| Alanine phosphatase (UI) | 68.8 | 58.31 | 71.99 | 69.2 | 60.06 | 59.05 | 70.39 | 2.68 | P=0.096 |

$^{a, b}$= means followed by the same letters in a row, were not significantly different (P > 0.05); SEM= Standard error of the mean

**Table 6 -** pH of broiler gut exposed to graded concentrations of *P. africana* bark.

| Treatment | T0: Negative control | T1: Positive control | T2: 1 g/L extract | T3: 3 g/L extract | T4: 5 g/L extract | T5: 7 g/L extract | T6: 10 g/L extract | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|
| **Day 21** | | | | | | | | | |
| Crop | 8.66 | 8.16 | 7.50 | 8.33 | 8.16 | 7.66 | 8.16 | 0.232 | P=0.081 |
| Pro-ventriculus | 5.50 | 5.50 | 6.00 | 6.50 | 5.83 | 5.66 | 5.66 | 0.312 | P=0.094 |
| Small intestine | 8.33 | 8.50 | 8.33 | 7.83 | 8.16 | 8.33 | 8.33 | 0.142 | P=0.076 |
| Large intestine | 8.83 | 9.00 | 8.16 | 8.50 | 8.83 | 8.66 | 8.66 | 0.312 | P=0.087 |
| **Day 42** | | | | | | | | | |
| Crop | 5.66 | 7.00 | 5.16 | 6.50 | 6.00 | 6.33 | 5.66 | 0.142 | P=0.073 |
| Pro-ventriculus | 3.83 | 5.00 | 3.83 | 3.83 | 3.50 | 3.33 | 3.00 | 0.131 | P=0.064 |
| Small intestine | 5.66 | 5.83 | 5.81 | 5.60 | 5.16 | 5.33 | 5.33 | 0.161 | P=0.084 |
| Large intestine | 6.33 | 5.66 | 5.83 | 5.36 | 5.35 | 5.36 | 5.36 | 0.122 | P=0.059 |

SEM = Standard error of the mean

**Cost benefit analysis**

The effects of inclusions of aqueous *P. africana* bark extracts on the economic performance in broiler chickens over the entire experimental period (Table 7) showed that the cost of additive was significantly ($P < 0.05$) higher for the normal and positive control treatments than for all those with *Prunus* while no significant differences ($P > 0.05$) were observed in the cost of feed within the groups. The unit total expenses were significantly higher ($P < 0.05$) for the normal and positive control treatments when compared with those exposed to prunus, leading thereby to a significantly ($P < 0.05$) lower gross margin, cost-to-benefit ratio, and economic efficiency of the former treatments as compared to the latter. Overall, a progressive increase in the concentration of aqueous of bark extracts did not significantly ($P > 0.05$) affect the profitability of the farm enterprises.

**Table 7 -** Effects of aqueous *P. africana* bark extract in drinking water on economic performance, in US dollars, of broiler chicken

| Economic parameters | T0: Negative control | T1: Positive control | T2: 1 g/L extract | T3: 3 g/L extract | T4: 5 g/L extract | T5: 7 g/L extract | T6: 10 g/L extract | SEM | P-value |
|---|---|---|---|---|---|---|---|---|---|
| Cost of additive consumed (USD) | 2.45[a] | 2.14[a] | 0.29[b] | 0.29[b] | 0.27[b] | 0.29[b] | 0.28[b] | 0.22 | P=0.038 |
| Cost of feed consumed (USD) | 1.01 | 0.96 | 1.01 | 0.96 | 0.94 | 1.04 | 1.03 | 0.02 | P=0.064 |
| Total expenses (USD) | 3.76[a] | 3.10[a] | 1.30[b] | 1.26[b] | 1.20[b] | 1.33[b] | 1.21[b] | 0.22 | P=0.028 |
| Total revenue (USD) | 6.05 | 6.27 | 6.10 | 5.88 | 5.52 | 6.15 | 5.79 | 0.08 | P=0.086 |
| Gross margin (USD) | 2.59[a] | 3.17[a] | 4.80[b] | 4.62[b] | 4.32[b] | 4.82[b] | 4.48[b] | 0.21 | P=0.035 |
| Benefit to cost ratio | 0.8[a] | 1.0[a] | 3.7[b] | 3.7[b] | 3.6[b] | 3.6[b] | 3.4[b] | 0.29 | P=0.026 |
| Economic efficiency | 2.4[a] | 3.3[a] | 4.8[b] | 4.8[b] | 4.6[b] | 4.7[b] | 3.4[b] | 0.20 | P=0.024 |

[a, b]= means followed by the same letters in a row, were not significantly different (P>0.05); SEM= Standard error of the mean.

## DISCUSSION

The similarity in growth performance with respect to feed intake, weight gain and feed conversion ratio, of broilers in the controls and *P. africana* bark extracts exposed groups, indicates the plant extract has growth promoter effects similar to oxytetracycline 80 and the conventional prophylactic protocol. Thus, *P. africana* aqueous bark extracts (1-10 g) did not impair growth performance. However, the extract seemed to trigger some metabolic challenges in young chicks during the starter phase, which were seemingly overcome during the finisher phase.

The normal and positive controls (T0 and T1, respectively) had similar carcass yield characteristics to test treatments with the higher levels of aqueous *P. africana* extracts (T5 and T6). However, these higher levels produced better carcass quality characterized by lowering levels of low-density lipoproteins, often associated with increased risks of cardiovascular diseases in humans.

Reduced liver weights in T6 suggest haepatoprotective effects of aqueous *P. africana* bark extracts to the broiler chicken. This is may be confirmed by the similarity ($P > 0.05$) in values of ALT in the finisher phase of the positive control (T1) and lowest *P. africana* inclusion level (T2) which were significantly different ($P < 0.05$) at the starter phase. Here, the continuous intake of the extract could have resolved an initial metabolic challenge in the starter phase, which caused broilers, fed with T2 to record significantly ($P < 0.05$) higher ALT values at the start. Hepatoprotective agents protect liver cells from damage and improve liver function by promoting regeneration of liver cells, thereby leading to small liver size. Mwitari et al. (2013) reported that *P. africana* bark is used for liver problems.

The hematological profile of control and test ingredient groups showed that differences observed at the starter phase were no longer present in the finisher phase, indicating therefore the adaptation of the chicks during their growth and development. The immune systems of younger birds are naturally more sensitive to stressors (pathogen load, diets and diet changes, vaccinations, new environments, etc.) that could provoke fluctuations in red and white blood cell counts (Niu et al., 2022). As the bird's progress to the finisher phase, their development is more complete and their immune systems are stronger. They are then better equipped to handle these stressors; hence their stable blood cell counts.

The determination of digesta pH in broilers serves as a tool to indicate the potential for optimum gut health and maximal nutrient absorption. The lowering of gut pH during the finisher phase following the intake of aqueous *P. africana* extracts can be explained in a similar observation reported by Anugom and Ofongo (2019) following administration of aqueous *Ocimum gratissimum* leaf extract. The increasing acidity (lowering of pH) of the small and large intestine associated the intake of the *P. africana* extract during the finisher phase certainly improved the gut health (Hinton et al., 2000) as increased intestinal acidity could stimulate growth of beneficial bacteria/microbes like *Lactobacillus*, inhibit the growth and colonization of enteropathogens and other harmful microbes like *Salmonellae*, *Enterobacterium* and *Escherichia coli*. At lower digestive pH, the nutrients are better partitioned for optimal growth and nutrient utilization (Lewis et al., 2003), the intestinal absorptive cells proliferate better (Niewold, 2007) and pancreatic secretions are stimulated (Dibner and Buttin, 2002).

A conventional prophylactic protocol is one of the major sources of synthetic inputs in conventional broiler production. This is because it requires the periodic addition of synthetic inputs in the feed or drinking water, to serve as



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anti-stress, antibiotics, anti-coccidia, anti-helminthes, diuretics, growth promoters and immune boosters. *Prunus africana* has been known to serve some of these purposes in literature (Ndung'u et al., 2024). Substitution of such chemicals in broiler production with organic constituents of plants like *P. africana* bark extracts, can go a long way to improve the health quality of broiler meat at the table. *Prunus africana* presents a unique opportunity as an organic feed additive, because it is a forest, species, which is not produced with synthetic inputs, either in natural or cultivated stands.

## CONCLUSION

It is concluded from this study that, the aqueous extracts of *Prunus africana* bark between 1g/L and 10g/L, can be used as a natural growth promoter in broiler chicken production to replace a conventional prophylactic protocol or oxy-tetracycline 80. However, it seems to trigger some metabolic challenges in the young chick that require a further investigation. All the 5 levels tested are biologically and economically promising. This contributes to closing research gaps on alternatives to synthetic growth promoters and the export of *P. africana* bark from producing countries.

## DECLARATIONS

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**Data availability**
Data are available from the corresponding author Email: ewane.divine@ubuea.cm, upon reasonable request

**Author contribution**
ED: Conceptualization; formal analysis; investigation; methodology; project administration; resources, supervision, validation, writing original draft, review and editing; NLM: Conceptualization; formal analysis, investigation; project administration; resources supervision, validation writing original draft; NSK: Conceptualization, Data curation, formal analysis, investigation, methodology, project administration, resources, validation writing original draft; SYN: Data curation, formal analysis, investigation, methodology, project administration, resources, validation writing original draft; EEE: Data curation, formal analysis; resources, supervision, validation, writing original draft, review and editing; CKF: formal analysis; resources, supervision, validation, writing original draft, review and editing

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# GENETIC FACTORS RELATED TO THE REGULATION OF BIOFILM FORMATION IN *Salmonella enteritidis* AND *Salmonella typhimurium* IN INDUSTRIAL POULTRY FARMS

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**ABSTRACT**: The purpose of the study was to examine the genetic mechanisms and regulation of biofilm formation regulation in *Salmonella enteritidis* and *Salmonella typhimurium* isolated at industrial poultry farms. The methodology included the collection of 340 samples from industrial poultry production facilities in Kazakhstan, Latvia, and Turkey between 2022 and 2025. The isolated strains were serotyped, genomic deoxyribonucleic acid was extracted, and the presence of the *csgD*, *bcsA*, *adrA*, *rpoS*, and *fimA* genes was detected using polymerase chain reaction (PCR). The level of gene expression was determined using quantitative polymerase chain reaction, and the phenotypic ability to form biofilms was evaluated by crystal violet staining. The results showed the isolation of 238 *Salmonella* strains, including 124 *S. enteritidis* and 114 *S. typhimurium*. The highest contamination was recorded in slaughter lines, accounting for 43.3% of the total positive samples. The analysis showed varying *Salmonella* serotype prevalence across countries, with *S. enteritidis* dominant in Kazakhstan (45.9%), *S. typhimurium* in Latvia (64.7%), and a balanced distribution in Turkey. The analysis revealed a high prevalence of biofilm formation genes, particularly *fimA* (94.1%), while *rpoS* ranged from 66.7% to 85.5%. According to quantitative polymerase chain reaction data, the expression of *csgD* ($P = 0.003$), *bcsA* ($P = 0.005$), and *adrA* ($P = 0.007$) was significantly higher in *S. typhimurium*, indicating its greater potential for biofilm formation. The phenotypic assessment confirmed this: 65% of *S. typhimurium* were strong or very strong biofilm producers, compared with 45% in *S. enteritidis* ($OD_{570}$: 1.42±0.15 and 0.97±0.12, respectively). The results can be practically applied in the development of sanitary control programmes, including the implementation of protocols for periodic assessment of biofilm-forming strains using qPCR screening. Based on the expression profile of key genes, criteria can be developed for selecting effective disinfectants (e.g., peroxide compounds or quaternary ammonium compounds) and for creating regulations targeting critical areas, such as poultry slaughterhouses and evisceration equipment.

**Keywords:** Phenotypic variability, Poultry farming, Regulatory mechanisms, Serotypes, Stress response.

## INTRODUCTION

The development of poultry farming, as one of the most rapidly expanding branches of the agro-industrial complex, is accompanied by increased biological and sanitary risks associated with microbial contamination of products (Abreu et al., 2023; Sychov et al., 2024). *Salmonella* bacteria, which are highly adaptable and capable of long-term persistence in the environment, pose a particular threat to food chain safety (Turmagambetova et al., 2017; Mussayeva et al., 2023). For example, Ehuwa et al. (2021) emphasize that *Salmonella* remains a persistent public health concern due to its ability to survive under diverse environmental conditions and contaminate a wide range of foods. Similarly, Mkangara (2023) highlights the role of biofilm formation in poultry-processing plants, showing that biofilms protect *Salmonella* from sanitation and antimicrobial measures, thereby facilitating its persistence and transmission through the food chain. Biofilm formation is therefore a crucial factor in the survival of these microorganisms in the production lines of poultry processing plants, providing them with resistance to sanitation, antimicrobials, and physical influences. Biofilm formation is therefore a crucial factor in the survival of these microorganisms in poultry production lines. as it provides resistance to sanitation, antimicrobials, and physical influences (Berezin et al., 2008; Obe et al., 2021). Among the various serotypes, *Salmonella enteritidis* and *Salmonella typhimurium*, which are widespread in industrial poultry farming and possess a pronounced pathogenic potential for humans, are considered particularly dangerous (Demyanyuk et al., 2023; Shaji et al., 2023).

An important feature of the poultry processing plants environment is the constant presence of stress factors, including temperature fluctuations, the use of disinfectants, and the mechanical stress, as noted by Ncho et al. (2024). These conditions contribute to the activation of bacterial stress responses, which in turn can enhance biofilm formation. Mendybayeva et al. (2023) demonstrated in an experimental model that exposure to hypo- and hyperosmotic conditions increases the expression of regulatory genes, including *rpoS* and *csgD*, which are associated with the synthesis of matrix components and greater resistance of *Salmonella* to environmental influences at various stages of the technological and



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industrial process. However, their study did not address differences in gene expression between serotypes, nor did it include an extended molecular genetic analysis to identify specific pathways regulating the stress response.

The formation of a mature biofilm largely depends on the ability of bacteria to produce cellulose and protein components of the matrix (Coutinho et al., 2016; Aipova et al., 2020). It has been established that the *csgD*-dependent regulatory system is the key controller of this process; however, data on the serotype-specifics of mechanisms of its activation under industrial conditions remain limited. Dančová et al. (2024) reviewed the role of the *csgD* gene in the regulation of amyloid fibre production in *Salmonella enterica*, demonstrating its importance for the initial stages of biofilm formation through quantitative PCR and evaluation of colony morphology on Congo Red agar. However, their study did not include a comparative analysis of *csgD* expression levels among different serotypes, leaving open the question of serotype-specific regulatory mechanisms.

A serious challenge in poultry processing is the high variability in the biofilm forming ability of different *Salmonella* strains, which complicates risk forecasting (Pyatkovskyy, 2023; Adamchuk and Voinalovych, 2024). Arkali and Çetinkaya (2020) demonstrated substantial differences in the intensity of biofilm formation among isolates from various industrial facilities (equipment surfaces and cooling baths) using the crystal violet staining method. However, their study did not include parallel molecular genetic analyses (such as the expression of *csgD*, *adrA*, and *bcsA* genes), which could have identified the molecular determinants underlying the observed phenotypic variability.

Intracellular signal transmission systems, particularly, the cyclic di-GMP system, are fundamental to the regulation of biofilm formation. It is well established that an increase in the level of this second messenger stimulates the synthesis of exopolysaccharides and promotes biofilm development. İnce and Akan (2023) investigated the effect of mutations in cyclic di-GMP synthase genes on biofilm production in laboratory *Salmonella* strains, revealing a substantial decrease in biofilm activity. However, such studies have rarely been conducted on isolates obtained directly from industrial poultry facilities.

The regulation of the stress response mediated by the sigma factor *rpoS* is also recognised as a critical element for bacterial survival under harsh conditions (Bouillet et al., 2024; Abutalip et al., 2025). Increased *rpoS* expression enhances resistance to disinfectants and promotes biofilm formation. Rychshanova et al. (2021) demonstrated the major role of this gene under oxidative stress; however,their study was limited to model systems and did not include industrial isolates, which restricts the practical applicability of their findings. An important factor in the initial stages of bacterial attachment to surfaces is the activity of fimbrial structures (Montayev et al., 2023; Ospanov et al., 2024). The *fimA* gene encodes the main subunit protein of type-1 fimbriae, which mediate primary cell adhesion. Liu et al. (2022) showed that type-1 fimbriae gene regulate cell adhesion and amino acid excretion, providing insights into biofilm-based fermentation in *E. coli*. Zhanabayeva et al. (2021) reported the high conservation and prevalence of the *fimA* gene among various *Salmonella* serotypes; however, their study did not examine gene expression under conditions simulating poultry processing environments.

Recent studies, such as Ban-Cucerzan et al. (2025), have also highlighted the role of environmental conditions in modulating bacterial biofilm properties. Commonly, high levels of organic residues and the presence of microdefects on equipment surfaces create favourable conditions for the establishment and development of biofilm communities. Chen et al. (2021) demonstrated that contamination of slaughter lines is directly correlates with the intensity of *Salmonella* biofilm formation; however, their study did not examine the relationship between these observations and molecular and genetic characteristics of the strains.

Insufficient knowledge of the regional specificity of *Salmonella* serotypes in relation to their biofilm-forming activity remains a significant problem. Given the differences in technological processes and sanitary standards across countries, these aspects are particularly important. Sharma et al. (2022) reported that the geographical origin of isolates influences their resistance levels and biofilm-forming ability; however, their conclusions were based primarily on epidemiological data without a detailed molecular interpretation of the underlying mechanisms.

Thus, the studies reviewed above highlight the importance of a comprehensive analysis of the genetic regulators of biofilm formation in *Salmonella enteritidis* and *Salmonella typhimurium* serotypes circulating in industrial poultry farms. The study aimed to identify the molecular mechanisms regulating biofilm formation in *Salmonella enteritidis* and *Salmonella typhimurium* isolated from industrial poultry facilities, thereby determining the characteristics of their biofilm activity and resistance of environmental stresses. The objectives of the study included assessing the prevalence of genes responsible for biofilm formation, analysing their expression levels in different serotypes, and phenotypically evaluating the ability to form biofilms under conditions that simulate poultry production processing environments.

## MATERIALS AND METHODS

### Study design and sampling

The study was conducted between 2022 and 2025 at the Kazakh National Research Agrarian University in cooperation with the industrial poultry farms of Kazakhstan, Latvia, and Turkey. Typical poultry farms with both cage and outdoor systems were selected as sites, differing in production scale (ranging from small to large farms) and biosafety level (from basic to advanced, according to the internal protocols of the enterprises). Sampling was carried out

purposefully, taking into account the technological role of each facility and the potential risk of contamination. The sample included production sites where broiler and laying hens were raised; facilities with incomplete sanitary documentation were excluded. Samples were collected from poultry houses, incubators, and slaughtering lines, including equipment surfaces, watering systems, litter transportation belts, and interior walls. The flushing method with sterile swabs (Copan Diagnostics Inc., USA) placed in a transport medium (Amis Inc., USA) was used, followed by delivery to the laboratory in refrigerated containers at +4 °C within no more than 24 hours. All sampling was conducted in compliance with the sanitary regulations in force in each country to minimize cross-contamination and ensure the representativeness of the results.

### Isolation and identification of *Salmonella* strains

Primary isolation of *Salmonella* spp. was performed on Buffered Peptone Water and Rappaport-Vassiliadis Soy Peptone Broth enrichment media (Oxoid Ltd., UK), followed by planting on selective media (XLD Agar and CHROMagar *Salmonella*, France). The isolates were serotyped using a set of agglutination sera (Denka Seiken, Japan) according to Kaufman-White scheme. All isolated strains of *Salmonella enteritidis* and *Salmonella typhimurium* were stored in a strain collection on Tryptic Soy Broth supplemented with glycerin at -80°C.

### Genomic DNA Extraction and PCR Analysis

Genomic DNA was extracted from isolates using the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, USA) to analyse genetic factors regulating biofilm formation. DNA concentration and purity were determined with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and further verified by electrophoresis in 1% agarose gel stained with SYBR Safe dye (Thermo Fisher Scientific, USA). The *csgD*, *bcsA*, *adrA*, *rpoS*, and *fimA* genes responsible for cellulose synthesis, curl formation, and stress response regulation were detected using PCR on a T100 Thermal cycler (Bio-Rad, USA). Specific oligonucleotide primers were designed based on NCBI data and synthesised by Integrated DNA Technologies (USA). Amplification conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, primer annealing at 56 - 60°C for 30 seconds (depending on the target gene) and elongation at 72°C for 1 minute, with the final extension at 72°C for 7 minutes. Amplification specificity was confirmed by electrophoresis in a 1.5% agarose gel.

### Quantitative Real-Time PCR

Expression level of biofilm-associated genes were analyzed by quantitative real-time PCR (qPCR) using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) with SYBR Green Master Mix (Thermo Fisher Scientific, USA). The 16S rRNA gene was used as the internal control for normalization, and relative expression levels were calculated using the ΔΔCt method.

### Phenotypic Assessment of Biofilm Formation

The biofilm-forming ability of isolates was phenotypically assessed using crystal violet staining on 96-well polystyrene plates (Nunc, Denmark). Each sample was incubated for 48 hours at 28°C in Luria-Bertani broth (Oxoid Ltd., UK) supplemented with 0.2% glucose to stimulate biofilm development. After incubation, biofilms were fixed with methanol, stained with 0.1% crystal violet solution, and solubilized with 95% ethanol. Optical density was measured at 570 nm using a Multiskan FC microplate reader (Thermo Fisher Scientific, USA).

### Statistical analysis

Statistical data processing was performed in GraphPad Prism 9 software, USA (GraphPad Software, 2020). The Shapiro-Wilk criterion was used to verify the normality of the data distribution. Intergroup differences were assessed using the Student's t-test to measure normally of the distributed data and the Mann-Whitney test was performed to detect abnormal samples. The level of statistical significance was considered as $P < 0.05$.

## RESULTS

As a result of the study, 238 strains of *Salmonella* bacteria were isolated in industrial poultry farms in Kazakhstan, Latvia, and Turkey, of which 124 belonged to the *Salmonella enteritidis* serotype, and 114 to *Salmonella typhimurium* (Table 1). The highest number of isolates was recorded in Kazakhstan (98 positive samples), with *S. enteritidis* being the dominant serotype. In Latvia, 76 isolates were obtained, with a predominance of *S. typhimurium*, particularly on slaughter lines. In Turkey, 64 isolates were identified, with an approximately equal distribution between the two serotypes. Among the sampling sites, slaughter lines showed the highest level of contamination (43.3% of the total number of positive samples), while incubators had the lowest occurrence of *Salmonella* spp. (18.9%). These findings highlight the importance of comprehensive monitoring at all stages of poultry production.

**Table 1** - Distribution of isolated strains of *Salmonella* spp. by country and technological facilities

| Country | Object | Number of samples | *S. enteritidis* | *S. typhimurium* | Total positives |
|---|---|---|---|---|---|
| Kazakhstan | Poultry house | 60 | 22 | 14 | 36 |
| Kazakhstan | Incubator | 40 | 9 | 7 | 16 |
| Kazakhstan | Slaughter line | 40 | 20 | 26 | 46 |
| Latvia | Poultry house | 50 | 10 | 15 | 25 |
| Latvia | Incubator | 30 | 5 | 7 | 12 |
| Latvia | Slaughter line | 40 | 7 | 32 | 39 |
| Turkey | Poultry house | 50 | 14 | 10 | 24 |
| Turkey | Incubator | 30 | 8 | 6 | 14 |
| Turkey | Slaughter line | 40 | 9 | 17 | 26 |
| Total | - | 340 | 104 | 134 | 238 |

*Source: compiled by the authors.

The analysis of the obtained data demonstrated clear differences in the prevalence of *Salmonella enteritidis* and *Salmonella typhimurium* serotypes across the countries and technological facilities of poultry enterprises studied. In Kazakhstan, the dominant serotype was *S. enteritidis* (45.9% of all isolates), which corresponds to previously identified trends in the spread of this pathogen in regions with large industrial poultry farms. In Latvia, by contrast, *S. typhimurium* was detected more frequently (64.7%), particularly on slaughter lines, suggesting its possible dissemination through equipment and transport belts. In Turkey, the distribution of serotypes was relatively balanced, which may reflect similar transmission mechanisms or characteristics of local poultry farming practices.

The distribution of *Salmonella* spp. among different sampling sites also revealed substantial differences. The highest number of positive samples was recorded on slaughter lines (43.3% of the total number of isolates), likely due to the high degree of equipment contamination, challenges in disinfection, and cross-contamination of birds during processing. The finding is particularly supported by the high proportion of *S. typhimurium* in Latvia (82.1% of slaughter samples), consistent with the hypothesis that this serotype can persist and spread within the meat processing facilities. In contrast, incubators showed the lowest level of contamination (18.9%), which may be attributed to controlled conditions, regular sanitation, and the absence of faecal pollution.

Additional analysis of geographical differences demonstrated that the higher detectability of *S. enteritidis* in Kazakhstan may be attributed to its historically high prevalence among laying hens and the limited effectiveness of existing biosafety protocols. In contrast, in Latvia, the more frequent detection of *S. typhimurium* on slaughter lines supports the hypothesis of stable reservoirs in meat processing plants, highlighting the need for more rigorous disinfection measures. In Turkey, the approximately equal ratio of serotypes may suggest combined sources of infection, including feed, bedding, and equipment.

Statistical analysis confirmed significant differences in serotype prevalence between countries ($p < 0.05$), particularly in the “slaughter lines” and “poultry houses” groups. The prevalence of *S. enteritidis* in Kazakhstan was statistically higher ($p = 0.03$) compared to other countries, while S. typhimurium dominated in Latvia ($p = 0.02$). In Turkey, no statistically significant differences were observed between serotypes ($p = 0.07$), which may indicate a high level of circulation of both pathogens within the country’s poultry systems.

These results emphasize the need for enhanced monitoring of *Salmonella* spp. in slaughterhouses and processing facilities, as these represent critical points in the pathogen transmission chain. The observed differences between countries may reflect the influence of local factors, such as poultry management systems, disinfection practices, and food safety controls. A molecular study of *S. enteritidis* and *S. typhimurium* isolates further revealed a high prevalence of key genes associated with biofilm formation (Table 2). The analysis focused on five target genes—*csgD, bcsA, adrA, rpoS, and fimA*—each of which plays an essential role in synthesizing structural biofilm components, regulating stress responses, and enhancing bacterial resistance to environmental factors.

**Table 2** - Percentage of positive isolates for detection of *csgD*, *bcsA*, *adrA*, *rpoS*, and *fimA* genes (%)

| Country | Keep condition | *csgD* | *bcsA* | *adrA* | *rpoS* | *fimA* |
|---|---|---|---|---|---|---|
| Kazakhstan | Cage | 91.2% | 88.2% | 85.3% | 80.9% | 94.1% |
| Kazakhstan | Outdoor | 86.3% | 82.4% | 78.4% | 74.5% | 92.2% |
| Latvia | Cage | 85.7% | 80% | 77.1% | 72.9% | 93.3% |
| Latvia | Outdoor | 83.3% | 77.8% | 70.4% | 66.7% | 88.9% |
| Turkey | Cage | 92.1% | 90.8% | 88.2% | 85.5% | 95% |
| Turkey | Outdoor | 90% | 87.5% | 85% | 81.3% | 93.8% |
| Average | - | 88.7% | 85.3% | 81.5% | 77.3% | 94.1% |

*Source: compiled by the authors.

Analysis of the prevalence of key genes regulating biofilm formation in isolates of *Salmonella enteritidis* and *Salmonella typhimurium* revealed clear patterns reflecting the complexity and multi-layered genetic control of this process. All five examined genes showed a high detection rate, which confirms the wide representation of biofilm formation mechanisms in *Salmonella* spp. populations on poultry farms.

The *fimA* gene was identified as the most consistently detected in almost all isolates. This gene encodes a subunit of type 1monomeric fimbriae, which mediates the initial stage of bacterial cell adhesion to abiotic surfaces. The high prevalence of *fimA* highlights the fundamental role of fimbriae in colonising the environment and triggering biofilm formation. Type 1 fimbriae are involved not only in primary attachment but also in subsequent cell aggregation, contributing to the development of dense multilayered structures.

The *csgD* gene showed a slightly lower, though still high detection rate. As a central regulator of biofilm formation, *csgD* controls the synthesis of amyloid fibrils and initiates cellulose production. Its expression product activates a cascade of signalling pathways, including two-component regulatory systems, enabling the bacteria to transition to a biofilm state. The high prevalence of this gene confirms its pivotal role in switching *Salmonella* spp. between planktonic and the attached lifestyle, which is critical for their long-term persistence under the harsh conditions of poultry farms.

The *bcsA* and *adrA* genes, which control the synthesis of cellulose – the main component of the extracellular biofilm matrix, showed high but somewhat variable prevalence. *bcsA* encodes cellulose synthase, which is directly responsible for the polymerisation of β-1,4-glucans, while *adrA* regulates the activity of the Bcs complex by producing cGMP, thereby enhancing cellulose synthesis in response to external signals. The high detection rate of these genes confirms the active involvement of isolates in forming stable three-dimensional biofilm structures with a dense matrix capable of protecting cells from antiseptics and antibiotics.

The *rpoS* gene, which encodes the sigma factor of the general stress response, was characterised by the greatest variability. Its lower detection rate may reflect the dynamic regulation of this element depending on environmental conditions. *rpoS* activates the expression of a wide range of stress-associated genes, including those involved in biofilm formation, and regulates the transition to the stationary growth phase. The observed variability in this gene suggests that not all isolates possess the same capacity to activate universal defence mechanisms under stress, which directly influences their survival in harsh production environments.

Collectively, the high prevalence of the *fimA*, *csgD*, *bcsA*, and *adrA* genes demonstrates the strong genetic potential of most isolates for biofilm formation. The variability in *rpoS* highlights differences in stress tolerance among strains. These findings confirm the complex nature of biofilm formation in *Salmonella* spp., which involves both structural and regulatory components. The presence of such intricate genetic systems indicates that biofilms are essential elements for *Salmonella* in poultry production, protecting bacteria from external factors and increasing the risk of product contamination. Quantitative PCR analysis was used to determine the relative expression levels of the *csgD*, *bcsA*, *adrA*, *rpoS*, and *fimA* genes in isolated strains of *Salmonella enteritidis* and *Salmonella typhimurium*. All genes showed differential activity between the two serotypes, reflecting the serotype-specific of biofilm formation at the transcriptional level (Table 3). The average expression level of the key regulatory gene *csgD* was higher in *S. typhimurium*, which indicates a more active activation of the signalling pathways of biofilm formation in this serotype. The *bcsA* and *adrA* genes, which control cellulose synthesis, also showed higher transcriptional activity in *S. typhimurium*, which confirms its potentially greater ability to form a dense biofilm matrix.

**Table 3 -** The average relative level of gene expression (ΔΔCt) in *Salmonella enteritidis* and *Salmonella typhimurium*

| Gene | *S. enteritidis* | *S. typhimurium* | p-value |
|---|---|---|---|
| *csgD* | 1.85±0.14$^{b}$ | 3.12±0.18$^{a}$ | 0.003 |
| *bcsA* | 1.62±0.1$^{b}$ | 2.75±0.15$^{a}$ | 0.005 |
| *adrA* | 1.51±0.09$^{b}$ | 2.48±0.13$^{a}$ | 0.007 |
| *rpoS* | 1.33±0.12$^{b}$ | 1.89±0.11$^{a}$ | 0.021 |
| *fimA* | 2.05±0.17 | 2.22±0.19 | 0.157 |

Note: Values with different letters (a, b) within the same row indicate statistically significant differences ($P < 0.05$). The highest value in each row is marked with "a". Source: compiled by the authors.

Gene expression analysis of *csgD*, *bcsA*, *adrA*, *rpoS*, and *fimA* showed clear differences between the serotypes of *Salmonella enteritidis* and *Salmonella typhimurium*, presenting the features of the molecular regulation of biofilm formation in each of them. The most pronounced differences were recorded in the *csgD* gene, which acts as a central transcriptional regulator of biofilm formation. Substantially higher *csgD* expression in *S. typhimurium* indicates increased activation of signalling pathways that trigger extracellular matrix synthesis and switch cells to a biofilm lifestyle. This regulator controls the downstream expression of a number of structural genes and thereby determines the ability of the bacterial population for long-term attachment and survival in the harsh environment.

The *bcsA* and *adrA* genes responsible for the synthesis of cellulose - one of the main components of the extracellular matrix - demonstrated similar trends. Substantially higher transcriptional activity of *bcsA* and *adrA* in *S. typhimurium* proves its genetic predisposition to the synthesis of dense and stable biofilms with a developed structural matrix. The high level of *bcsA* expression indicates the active work of the cellulose synthase complex, capable of producing a substantial amount of β-1, 4-glucans, which enhances the mechanical strength of the biofilm. Therewith, *adrA* activates cGMP synthesis and enhances cellulose production, forming a closed regulatory loop that enhances the biofilm properties of the population. The expression of the *rpoS* gene, responsible for activating stress responses and the transition of cells to the stationary growth phase, was also higher in *S. typhimurium*. This result indicates a greater ability of this serotype to activate universal protective mechanisms in response to adverse environmental factors. Increased *rpoS* transcription correlates with potentially higher *S. typhimurium* resistance to environmental conditions, including exposure to disinfectants and other stressors during production. The analysis of *fimA* expression was considered separately. Although the gene is traditionally regarded as the most important factor of initial adhesion, its expression levels were nearly identical in both serotypes. This finding highlights the universality of type 1 fimbriae during the early stages of attachment and their relatively conserved regulation, independent of serotype.

Overall, the results demonstrate that *S. typhimurium* exhibits a higher level of transcriptional activation of the main biofilm formation genes, providing an advantage in the formation of denser, more stable and viable biofilms. This serotype is potentially dangerous in terms of long-term persistence on equipment surfaces and in the environment of poultry farms. *S. enteritidis* showed less pronounced activity in most genes, which may indicate a slightly weaker biofilm potential compared to *S. typhimurium*. Nevertheless, the high activity of *fimA* in both serotypes confirms the presence of a basic level of adhesive properties sufficient to initiate colonisation and subsequent spread.

A phenotypic assessment of biofilm formation by crystal violet staining revealed varying degrees of biofilm production among the isolates of *Salmonella enteritidis* and *Salmonella typhimurium*. The isolates exhibited a wide range of optical density values ($OD_{570}$), reflecting differences in their biofilm-forming potential (Table 4). Based on these measurements all strains were classified into four groups: weak, moderate, strong, and very strong biofilm-forming agents. The largest proportion of strong and very strong biofilm-forming agents was found among *S. typhimurium*, where the number of isolates with high $OD_{570}$ exceeded 65%. *S. enteritidis* showed more moderate activity, with about 45% of the isolates classified as strong and very strong biofilm producers.

**Table 4 -** Distribution of isolates by biofilm formation level (optical density $OD_{570}$, M±m)

| Serotype | Average $OD_{570}$ (M±m) | Weak (%) | Moderate (%) | Strong (%) | Very strong (%) |
|---|---|---|---|---|---|
| *S. enteritidis* | $0.97\pm0.12^{b}$ | 18% | 37% | 28% | 17% |
| *S. typhimurium* | $1.42\pm0.15^{a}$ | 8% | 27% | 35% | 30% |

*Different superscript letters within the same column indicate statistically significant differences ($P < 0.05$). For average $OD_{570}$ values, $P = 0.032$. Source: compiled by the authors.

The phenotypic assessment of the ability of *Salmonella enteritidis* and *Salmonella typhimurium* isolates to form biofilms demonstrated substantial differences in the level of biofilm activity between the serotypes. The classification based on optical density indicators ($OD_{570}$) revealed a clear trend towards a more pronounced biofilm-forming ability in *S. typhimurium*, consistent with previously obtained molecular results.

The proportion of strong and very strong biofilm-forming agents among *S. typhimurium* reached 65%, while *S. enteritidis* had a rate of only 45%. This difference highlights the higher potential of *S. typhimurium* in the formation of mature and dense biofilms capable of providing long-term bacterial persistence on abiotic surfaces. The average value of $OD_{570}$ in *S. typhimurium* was 1.42±0.15, substantially higher than that of *S. enteritidis* (0.97±0.12), thereby confirming a more active accumulation of biomass and the intensity of matrix formation.

The observed high biofilm activity of *S. typhimurium* is primarily explained by the increased expression of regulatory and structural genes that control extracellular matrix synthesis. This ensures not only the strong fixation of bacterial cells on surfaces but also the formation of a protective barrier that can effectively reduce the penetration of antiseptics and other disinfectants. These features lead to conditions for the survival of *S. typhimurium* even during aggressive sanitary treatment of industrial premises. Therewith, *S. enteritidis* showed a more moderate biofilm-forming activity, which may indicate its slightly lower ability to form long-term fixation and persistence. Nevertheless, even with relatively lower $OD_{570}$ values, about 45% of isolates of this serotype were classified as strong and very strong biofilm-forming agents, confirming the potential danger of this pathogen in poultry processing plants. High biofilm activity of individual *S. strains. enteritidis* strains can be explained by the presence of favourable regulatory mutations in them or by the activation of alternative synthesis pathways of extracellular matrix components.

The results indicate that the phenotypic ability to biofilm is the most important characteristic of the virulence and resistance of *Salmonella* spp. The high density and structural organisation of biofilms provide both physical protection of

cells and contribute to the horizontal transfer of resistance genes, which increases the risk of the formation of multidrug-resistant strains in an industrial environment. Consequently, the presence of a large number of strong biofilm-forming agents among *S. typhimurium* isolates poses a serious threat to the sanitary safety of poultry farms and requires constant monitoring and the development of targeted strategies for the destruction of biofilms in technological cycles.

## DISCUSSION

This study established substantial differences in the prevalence of *Salmonella enteritidis* and *Salmonella typhimurium* serotypes in poultry farms in Kazakhstan, Latvia, and Turkey, and to identify critical contamination points at various stages of the production cycle. The findings confirm the importance of comprehensive monitoring of pathogens in the industry, which is consistent with the conclusions of Badie et al. (2021), who emphasized the need to account for regional and technological factors in the spread of *Salmonella* in poultry processing complexes.

The revealed predominance of *S. enteritidis* is particularly notable in Kazakhstan, which reflects the steady consolidation of this serotype in the poultry practice of the region. Similar patterns were observed in a study by Pradhan et al. (2023), which underlined the relationship between the characteristics of poultry keeping and the dominance of *S. enteritidis*. However, in contrast to these data, Chen et al. (2023) reported the predominance of *S. typhimurium* in similar conditions. This discrepancy may be explained by differences in the genetic composition of poultry herds and the biosafety programmes used, which confirms the more objective nature of this study (large number of samples).

High frequency of *S. typhimurium* detection in Latvian slaughterhouses demonstrates the presence of stable contamination reservoirs in processing plants, which underlines the importance of this stage of the production process as a critical control point. The highest number of positive samples on slaughter lines indicates a high probability of accumulation of pathogens on equipment surfaces, transport belts and in hard-to-reach areas where disinfection is difficult. Similar conclusions are presented in the work of Holden et al. (2022), underscoring the importance of equipment in the transmission of pathogens and pointing to the role of micro-lesions of the surface and biofilms in the accumulation of bacteria. Ma et al. (2025), on the contrary, argued the minimal role of slaughter lines in the spread of *Salmonella* spp., which is not confirmed in this study, while the processing stage was uncovered to be the most problematic and required special attention from sanitary control. This highlights the need for enhanced monitoring of equipment conditions and a review of standard sanitation procedures at slaughterhouses to prevent further spread of infection.

The relatively uniform distribution of serotypes in Turkey deserves special attention, which is likely due to the specific features of the industrial environment and the combined sources of infection. This finding may reflect the influence of various factors, including heterogeneous sanitary practices, the use of feed from different origins, and the possible introduction of pathogens from the environment. Such conditions promote the simultaneous circulation of several serotypes, thereby complicating the development of targeted prevention and control measures. Kao et al. (2023) reported similar results, emphasizing the complex role of feed, litter, and equipment in maintaining the circulation of multiple serotypes at the same time. In contrast, a study by Siddique et al. (2021) highlighted the dominance of a single serotype under similar conditions, a finding not supported by the present study. Furthermore, the observed uniformity in serotype distribution may indicate the need for more detailed monitoring programmes that account for the variety of possible contamination sources and their contributions to the epidemiological situation in enterprises.

Analysis of the molecular data revealed a high prevalence of genes responsible for biofilm formation in isolates of both serotypes. Fimbriae provide the basic level of adhesion required to initiate biofilm formation, while the subsequent development of structural and protective mechanisms depends on other regulatory systems (Butsenko et al., 2020; Umitzhanov et al., 2023). The *fimA* gene was particularly stable, consistent with the conclusions of Dlamini et al. (2024), who identified its key role in initiating cell attachment. The frequent detection of this gene across all examined samples underscores its conservative nature and functional significance in ensuring the primary stage of bacterial adhesion to abiotic surfaces, such as equipment and transport belts. By contrast, Ćwiek et al. (2020) reported a more variable expression of *fimA*, while the results obtained in this study confirm its universality under poultry production conditions. This emphasizes its potential contribution to the resistance of bacterial populations against physical and chemical stressors at various stages of production. In addition, the high prevalence of *fimA* supports its consideration as a promising marker for molecular diagnostics and for monitoring the risk of product contamination.

The high detection rates of *csgD* and *bcsA* highlight the active involvement of biofilm formation mechanisms, which is consistent with the findings of Yuan et al. (2023) on the importance of these genes for bacterial survival in harsh environments. The strong expression of these genes indicates the ability of bacteria to actively form amyloid fibrils and a cellulose matrix, thereby creating robust three-dimensional structures that protect cells from external stresses. This is particularly significant in poultry production, where bacteria are exposed to various disinfectants and mechanical damage. However, Dai et al. (2021) reported low detection of *csgD* in poultry farms, which may be explained by differences in the research objects selected and the analytical methods employed. Such discrepancies may result from temporary fluctuations in *csgD* expression or the use of less sensitive molecular diagnostic techniques, underscoring the need to standardize approaches for assessing the biofilm potential of pathogens.

The *rpoS* gene exhibited the greatest variability, reflecting the heterogeneity of stress responses among isolates. Dallal et al. (2023) similarly observed that *rpoS* levels are strongly influenced by external conditions, a finding confirmed in this study. Unlike the conclusions of Kim et al. (2022), who described *rpoS* as having a secondary role in biofilm formation, the present data emphasize its importance for bacterial survival in production environments. The high variability in *rpoS* expression suggests significant differences among isolates in their ability to activate universal protective mechanisms in response to stressors such as disinfectants, temperature fluctuations, and mechanical forces along production lines. This supports the role of *rpoS* as a crucial regulator that enables pathogen adaptation to adverse conditions and enhances their persistence within the technological environment of poultry enterprises.

Expression analysis revealed higher transcriptional activity of the main biofilm formation genes in *S. typhimurium*. These findings are consistent with Musa et al. (2024), who also reported increased activation of biofilm pathways in this serotype. Elevated expression of key regulatory and structural genes, including *csgD*, *bcsA*, and *adrA*, in *S. typhimurium* confirms its capacity to intensively produce extracellular matrix and form stable biofilms. This provides the serotype with a significant advantage in industrial environments, where resistance to sanitation and survival under adverse conditions are critical. By contrast, Ramatla et al. (2024) reported predominant gene expression in *S. enteritidis*, a finding not corroborated in the present study, where *S. typhimurium* demonstrated greater molecular activity. These discrepancies may be attributed to geographical factors, differences in poultry management conditions, isolate characteristics, or analytical methods. Overall, the results highlight the importance of considering local factors when assessing the biofilm potential of different *Salmonella* serotypes.

Of particular interest is the high expression of *bcsA* and *adrA* in *S. typhimurium*, which is fully consistent with the conclusions of Abou Elez et al. (2021) and Hull et al. (2022) regarding the formation of dense and stable biofilm structures. These mechanisms provide this serotype with a clear advantage in poultry processing plants, where resistance to external influences is essential. The strong activity of *bcsA*, which encodes cellulose synthase, indicates the ability of bacteria to actively synthesize cellulose, thereby enhancing the strength and stability of the extracellular matrix. Increased expression of *adrA*, which regulates cGMP production, further reinforces this process by activating the relevant signaling pathways. This molecular organization grants *S. typhimurium* the ability to survive long-term on technological equipment and reduces the effectiveness of standard sanitary measures aimed at biofilm removal.

A phenotypic assessment of biofilm formation ability also revealed an advantage for *S. typhimurium*, confirming its high persistence potential. These results are consistent with the findings of Metaane et al. (2022), who emphasized the leading role of this serotype in the formation of persistent biofilms. In contrast, Chandra et al. (2023) reported the predominance of *S. enteritidis* under similar conditions. However, the larger sample size and broader coverage of technological stages in the present study make its results more robust and objective. The high proportion of strong biofilm formers among *S. typhimurium* isolates underscores the importance of monitoring and controlling this serotype in processing plants. Similar conclusions were drawn by Middlemiss et al. (2023), who highlighted the threat posed by stable biofilm formation. In contrast, Albicoro et al. (2024) argued that biofilms play only a minor role in the survival of *Salmonella* spp.; however, the present results refute this claim by demonstrating a direct link between biofilm activity and the likelihood of long-term bacterial persistence.

Cumulative data analysis confirms that biofilms play a vital role in the survival and dissemination of *Salmonella* spp. in poultry farms. Biofilm formation provides pathogens with significant protection against antiseptic agents, promotes their long-term persistence on abiotic surfaces, and increases the likelihood of horizontal transfer of antibiotic resistance genes (Zhusanbayeva et al., 2024; Boiko et al., 2025). Both high gene activity and strong phenotypic biofilm formation ability are particularly pronounced in *S. typhimurium*, necessitating strengthened control of this serotype at all stages of the production cycle. These biological features of *S. typhimurium* reflect its high epidemiological potential and risk of long-term persistence on equipment and within the environment of poultry processing plants. The findings parallel the conclusions of Brenner and Wang (2022), who stressed the need for targeted control of biofilms to minimize product contamination risks and ensure sanitary safety. They also emphasize the urgency of developing effective biofilm eradication methods and implementing innovative sanitation strategies.

Ultimately, this study confirms the critical role of slaughter lines in the spread of *Salmonella* spp. and reveals significant differences in biofilm potential between the serotypes. The high activity of *S. typhimurium* highlights the need for enhanced sanitary measures and the development of strategies to disrupt biofilms at all stages of the production cycle. The established patterns can serve as a foundation for optimizing monitoring systems and preventing bacterial contamination in the poultry industry.

## CONCLUSIONS

In the course of the study conducted to identify serotype-specific molecular mechanisms regulating biofilm formation in *Salmonella* spp., substantial differences in the prevalence of serotypes were observed in poultry farms in Kazakhstan, Latvia, and Turkey at various stages of the technological process. A total of 238 bacterial strains were isolated from 340 samples, of which 52.1% belonged to *S. enteritidis* and 47.9% to *S. typhimurium*. The highest level of contamination was recorded on slaughter lines (43.3% of positive samples), confirming their critical role in the spread of pathogens. Country-

specific differences revealed the dominance of *S. enteritidis* in Kazakhstan (45.9% of isolates), which may be associated with poultry-keeping practices and the level of biosafety measures. In Latvia, *S. typhimurium* was predominant (64.7%), particularly on slaughter lines, indicating its adaptation and persistence in the production environment. In Turkey, the ratio of serotypes was approximately equal. Molecular analysis confirmed the high prevalence of genes responsible for biofilm formation. The highest detection rates were observed for *fimA* (94.1%) and *csgD* (88.7%), indicating a strong adhesive potential of the isolates. Expression analysis revealed significantly higher activity of key biofilm formation genes in *S. typhimurium*, particularly *csgD*, *bcsA*, and *adrA* ($P < 0.01$), which correlated with its phenotypic ability to form denser and more stable biofilms. Phenotypic evaluation using crystal violet staining further confirmed the higher biofilm activity of *S. typhimurium*: the proportion of strong and very strong biofilm formers reached 65%, compared to 45% for *S. enteritidis*. This finding underscores the greater persistence and resistance potential of *S. typhimurium* in production environments.

The practical significance of this study for poultry production lies in the need to strengthen sanitary control at slaughter lines and to develop targeted strategies for biofilm eradication. Such measures are essential to reduce the persistence of *Salmonella* species in production environments and to improve overall food safety. By identifying the genetic factors contributing to biofilm formation, the study supports the development of more effective disinfection protocols and highlights the importance of prioritizing disinfectants capable of targeting these resilient biofilms. The inclusion of molecular monitoring of biofilm-associated genes in food safety control systems is recommended. A key limitation of the study was the absence of an assessment of seasonal factors and technological differences between enterprises, which warrants further in-depth analysis for a more comprehensive understanding of the epidemiological significance of the observed patterns. A promising direction for future research is the investigation of the effects of different disinfectants on the eradication of *Salmonella* spp. biofilms.

## DECLARATIONS

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**Data availability**

The authors confirm that the data supporting the findings of this study are available in the article.

**Authors' contribution**

A. Zhusanbayeva, B. Biyashev, and Zh. Kirkimbaeva: conceptualization, methodology, data curation, writing-original draft preparation. B. Biyashev: visualization, investigation, and supervision. A. Zhylkaydar and G. Nurgozhaeva: software, validation, writing-reviewing, and editing. All authors read and approved the final manuscript.

**Ethical considerations**

All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with EU Directive 2010/63/EU for animal experiments.

**Consent to publish**

All authors agree to the publication of this manuscript.

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**Competing interests**

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

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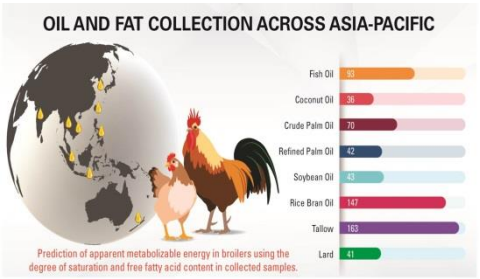

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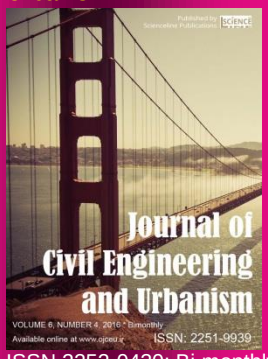


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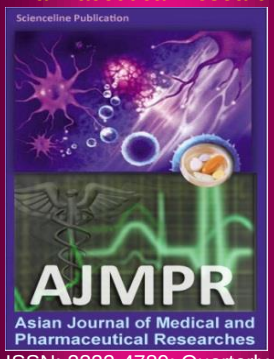


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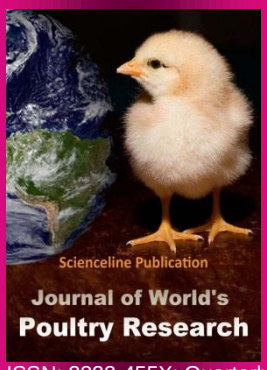


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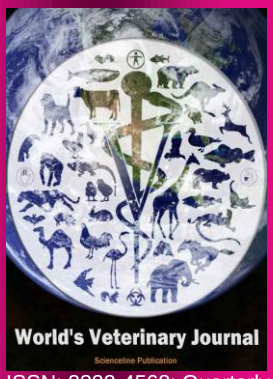


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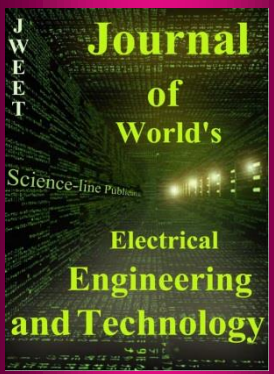


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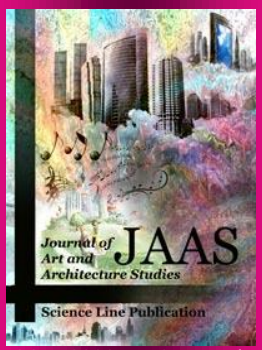


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