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## Volume 15 (1); January 30, 2025

#### **Research Paper**

#### Calcium and phosphorus status of goats grazing in northwest Moroccan forest

Al Rharad A, Bouassab A, Acherkouk M and Ayadi M. Online J. Anim. Feed Res., 15(1): 01-07, 2025; pii: S222877012500001-15 DOI: <u>https://dx.doi.org/10.51227/ojafr.2025.1</u>

#### Abstract

In the present study, leaves and twigs from 17 shrubs and trees consumed by the west-north Moroccan indigenous goats were collected and evaluated for their calcium and phosphorus (Ca and P) content. The potential mineral needs of adults and young goats of both sexes (male and female) from three localities were estimated to assess their mineral deficiency. This assessment was based on their weight and the diet composition determined through direct observation and the bites method. The browse species had a higher Ca content than P (1.79 vs 1.57 g/kg DM). The adult female goats had the highest P intake (2.04 g/day) with the highest deficit



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compared to the male adult (-29 vs -26) % of their daily requirements. Young kids (males and females) had the lowest Ca intake (0.81 and 0.75 g/day, respectively) and recorded the lowest deficit (-17 vs -19) %, respectively. Goats also showed a higher Ca deficit than P. In conclusion, the present results offer valuable information about the main mineral intake of the goats in the forest pasture of this region. Supplementing these two minerals is essential for enhancing goat performance in the traditional semi-extensive goat farming system that relies on forest pastures in the western-northern region of Morocco.

Keywords: Diet composition, Goat, Indigenous breeds, Mineral requirement, Pastoral plants.

[Full text-PDF]

## Growth performance and profitability of weanling pigs (*Sus scrofa domesticus* L.) fed pre-starter diet supplemented with nucleotide

Verdijo A and Mondejar H. Online J. Anim. Feed Res., 15(1): 08-14, 2025; pii: S222877012500002-15 DOI: <u>https://dx.doi.org/10.51227/ojafr.2025.2</u>

#### Abstract

Nucleotides can improve intestinal health by modulating the local immune response and intestinal mucosa development in weaned piglets. This study was conducted to evaluate the growth performance of post weaned piglets and evaluated the economic analysis of nucleotides supplementation for 30 days. A total of 120 mixed breed piglets were selected at the weaning stage and were used in the experiment as control group vs. treatment 2 supplemented with nucleotide. Each treatment consisted of 60 heads with three replications with 20 heads per replication arranged in Complete Randomized Design. Results were analyzed through Pairwise T and LSD tests. In terms of growth performance, results showed that supplementation of nucleotide had



significantly increased the average daily gain, feed conversion ratio and weight gain by 0.50, 1.20 and 15.02 kg, respectively. However, there was no significant difference in terms of the average daily feed intake. With regards to the economic analysis, total production input had no effect with or without nucleotide supplementation but surprisingly, it had a gross margin of Php 95,900 (Philippine peso money) which was 5% more as with those that have supplementation. As to the net income, supplementation of nucleotides increased about 40.7% in comparison to control. Furthermore, a peso of investment could have a return of about 18 cents (1050 Php) more returns with supplementation, which apparently had 0.11 cents leverage compared to control group (0.07 cents). In conclusion, nucleotide supplementation not only improved the growth performance of post-weaned piglets but also enhanced profitability, offering a significant return on investment for swine producers. This makes nucleotide supplementation a promising strategy for improving both animal health and economic outcomes in swine production.

Keywords: Benefit Cost Ratio, Daily weight gain, Feed conversion ratio, Net Income, Nucleotides.

#### **Research Paper**

## Sarcocystosis in alpacas and llamas: regional, market, and muscle-specific prevalence patterns

Garcia-Olarte E, Ninahuanca J, Suarez-Reynoso W, Mauricio-Ramos Y, Guillen MF, Payano IU, Tacza AA, and Condor WG.

*Online J. Anim. Feed Res.*, 15(1): 15-20, 2025; pii: S222877012500003-15 DOI: <u>https://dx.doi.org/10.51227/ojafr.2025.3</u>

#### Abstract

The objective of this study was to determine the effect of species (alpacas and llamas), markets in the city of Huancayo (Ferrocarril Commercial Center, Nueva Esperanza, and Nazareth), and muscle groups on the prevalence of Sarcocystis sp. Between January and October 2023, a total of 2,211 carcasses were inspected, comprising 1,716 alpacas and 495 llamas. The results indicated a prevalence of 21% (104/495 carcasses) in llamas and 8% (138/1,716 carcasses) in alpacas. By region of origin, the prevalence in alpacas was reported as follows: Huancavelica (7.7%) with 14/181 carcasses, Junín (6.7%) with 55/820 carcasses, and Lima (9.7%) with 69/715 carcasses. For llamas, the Lima region exhibited the highest prevalence of sarcocystosis (33.9%) with 72/212 carcasses, and



Junín (9.8%) with 18/181 carcasses. Regarding the markets, the Ferrocarril market presented the highest risk of contamination, serving as the reference group for comparison. In contrast, the Nazareth and Nueva Esperanza markets showed significantly lower odds of Sarcocystis sp. presence, with Odds Ratios (ORs) of 0.38 and 0.25, respectively. For muscle groups, the anatomical distribution of Sarcocystis sp. cysts revealed a preferential localization in the leg (OR = 1.65) and neck (OR = 1.20) compared to the shoulder. This investigation provides significant data on the prevalence of Sarcocystis sp. in alpacas and llamas, highlighting a higher prevalence in llamas despite their smaller sample size. These findings emphasize the need for targeted interventions to address this parasitic infection in camelid production systems.

**Keywords:** Animal products, Camelids, Carcass quality, Mantaro valley, Parasite.

[Full text-PDF]

#### **Research** Paper

## Influence of feather genotype, storage duration and temperature on the external and internal qualities of chicken table eggs

Kanasuah DN, Adomako K, Hagan BA and Olympio OS.

*Online J. Anim. Feed Res.*, 15(1): 21-32, 2025; pii: S222877012500004-15 DOI: <u>https://dx.doi.org/10.51227/ojafr.2025.4</u>

#### Abstract

A study was carried out to determine the influence of the feather genotype, storage duration, temperature and method on the internal and external qualities of chicken table eggs. A total of 864 table eggs collected from naked neck (Nanaff), frizzle (nanaFf) and normal feathered (nanaff) birds were used in the study. A Completely Randomized Design of four factors namely, feather genotypes, storage temperatures (5°C and 26°C), storage duration (0, 7, 14, 21 and 28 days) and storage methods (with or without vegetable oil application) was used. The GLM procedure of GenStat (17th Edition) was used to determine the effects of the four factors and their interactions on external qualities (egg weight, length, and width, shell weight and



nasuah DN, Adomako K, Hagan BA and Olympio OS (2025). Influence of feather genotype, storage duration and temperature on the externa d internal qualities of chicken table eggs. Online J. Anim. Feed Res., 15(1): 21-32. DDI: <u>https://dx.doi.org/10.51227/ojsft.2025.4</u>

thickness) and internal qualities (albumen height and weight, yolk height, weight, diameter and colour and Haugh unit) of table eggs. The effect of chicken genotype on proximate composition and nutritional values of table eggs were also determined. Feather genotype had significant (P<0.05) effect on yolk colour and weight whilst storage duration, temperature and method had significant (P<0.05) effects on all the internal qualities of eggs studied except effect of storage duration on yolk colour. The 2-way and 3-way interactions of the factors studied were important sources of variation for many of the internal qualities of eggs studied. With the exception of storage temperature, the other factors studied had significant (P<0.05) effects on many of the external qualities of eggs. The interactions of the factors were not significant (P<0.05) sources of variation for most of the external qualities of eggs. Mutant feather genes (Na and F) positively influence egg qualities which could be utilised to segment the commercial chicken egg market.

Keywords: Feather, Frizzle, Naked neck, Nutritional value, Yolk colour,.

#### **Research Paper**

## Primal cuts of carcass and meat characteristics of Kacang goat fed total mixed ration containing different sources of ruminally undegraded protein

Adiwinarti R, Kustantinah, Rusman, Rianto E, Purnomoadi A, Arifin M, Sutaryo, and Restitrisnani V.

*Online J. Anim. Feed Res.*, 15(1): 33-40, 2025; pii: S222877012500005-15 DOI: <u>https://dx.doi.org/10.51227/ojafr.2025.5</u>

#### Abstract

This study was designed to evaluate the effect of feed quality improvement using gliricidia and different sources of protein in total mixed ration (TMR) on the primal cuts, loin eye area, and fatty acids profile of goat meat. This study used twenty yearling Kacang goats weighing 17.42±1.63 kg. The goats were randomly allocated into 4 different treatments in a completely randomized design. The treatments involved the use of natural grass from rangeland (NGFR; control) as well as improving the quality of feed through TMR containing various ruminally undegraded protein sources, i.e. TMR contains fish meal (TMR-FM), TMR contains soybean meal (TMR-SBM) and TMR contains formaldehyde treated soybean meal (TMR-TSBM). The parameters



observed were primal cuts yield, loin eye area, meat, fat, bone of primal cuts, and fatty acids profile. Data were analyzed using a one-way analysis of variance. The results showed that the goats fed TMR-FM and TMR-TSBM produced significantly higher meat percentage than control goats. The meat yield of TMR-SBM and TMR-TSBM goats were significantly higher than those of control goats. Goats fed TMR-SBM produced the highest primal cuts yield and shoulder weight, while the weight of rib, loin, and leg of TMR-SBM goats were similar to those of TMR-TSBM goats. Loin eye area was similar between the treatments. Saturated fatty acids content in TMR groups was similar to those in control. It can be concluded that improved feed quality using TMR-SBM produced significantly higher primal cuts weight, while TMR-TSBM goats. Fatty acid profiles were similar between treatments.

**Keywords**: Fatty acids, Fish meal, Goat meat, Meat quality, Total mixed ration.

[Full text-PDF]

#### Short Communication

## Hematological and biochemical parameters of captive fallow deer (*Dama dama*) in a zoo environment

Hažimusić N, Škapur V, Hadžijunuzović-Alagić D, and Livnjak A.

*Online J. Anim. Feed Res.*, 15(1): 41-46, 2025; pii: S222877012500006-15 DOI: <u>https://dx.doi.org/10.51227/ojafr.2025.6</u>

#### Abstract

Accurate health assessment of wild, semi-captive, or domesticated animals is essential for their well-being. Despite this necessity, limited studies have been conducted on deer species, and there is a paucity of information on the hemato-biochemical parameters of different deer species globally. Present study aimed to fill this gap by determining the hematological and serum biochemical parameters of fallow deer (*Dama dama*) maintained in semi-captivity within zoo environments for the first time in Bosnia and Herzegovina. Present research involved six healthy male fallow deer, aged 2 to 5 years. The deer were immobilized using xylazine hydrochloride and ketamine hydrochloride, and blood samples



were collected from the external jugular vein. The hematological parameters measured included RBC, PCV, HGB, MCV, MCH, MCHC, RDW, RETIC, WBC, WBC differential, PLT, MPV, PDW, and PCT. Biochemical parameters included glucose, urea, creatinine, albumin, triglycerides, cholesterol, and enzymes (AST, ALT, ALKP, and GGT) activities. The results showed the higher glucose and urea concentrations and the same values for creatinine, triglycerides, and enzyme activities when compared to some previous reports. These findings highlighted the importance of considering handling methods and environmental conditions when interpreting biochemical parameters, contributing to improved health assessments and management practices for deer in captivity.

**Keywords**: Biochemical and hematological parameters, Captive wildlife, Domesticated animals, Fallow deer.

## Effect of graded levels of dietary tomato waste on performance and carcass characteristics of Japanese quail reared under intensive system

Bhawa S, Moreki JC and Manyeula F.

*Online J. Anim. Feed Res.*, 15(1): 47-59, 2025; pii: S222877012500007-15 DOI: <u>https://dx.doi.org/10.51227/ojafr.2025.7</u>

#### Abstract

This study was carried out to evaluate the effects of partial replacement of soybean meal (SM) with tomato waste (TW) in Japanese quail diets on the resulting yield, internal organs, and carcass characteristics. Eighty unsexed 1-day-old chicks were housed in battery cages with cardboard boxes used as solid floors and randomly assigned to 1 of 4 dietary groups, 46.2% SM, 44.2% SM + 2% TW, 42.2% SM + 4% TW, or 40.2% SM + 6% TW, over a 6 weeks growth period. Yields and carcass characteristics were then determined. Data were analysed using the General Linear Model (GLM) procedures followed by a response procedure for surface regression analysis (Proc RSREG; SAS 9.4) to describe the



parameters' responses to graded levels of dietary tomato waste. Repeated measures analyses showed significant week  $\times$  diet interaction effects on feed intake (FI, P = 0.03), body weight gain (WG, P = 0.0006), feed conversion ratio (FCR, P = 0.002), protein efficient ratio (P = 0.0001), and growth efficiency (P = 0.0001). By supplementing the diets of quails with a 2% inclusion level, a diet significantly affected quails' FI on weeks 1, 2, 3, and 6. A diet containing 2% TW significantly affected live weight (LW), hot carcass weight (HCW), and cold-dressed weight (CDW). It is concluded that the diet supplementation with 44.2% SM + 2% TW seemed ideal for optimum performance in Japanese quails based on the insignificant change in feed intake and growth efficiency results compared to 46.2% SM for weeks 1 and 2. Further research is needed on the application method that could be used to enhance the utilization of tomato waste in Japanese quails.

**Keywords**: Carcass characteristics, Dietary replacements, Growth performance, Japanese quails, Tomato waste.



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# CALCIUM AND PHOSPHORUS STATUS OF GOATS GRAZING IN NORTHWEST MOROCCAN FOREST

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Supporting Information

**ABSTRACT**: In the present study, leaves and twigs from 17 shrubs and trees consumed by the west-north Moroccan indigenous goats were collected and evaluated for their calcium and phosphorus (Ca and P) content. The potential mineral needs of adults and young goats of both sexes (male and female) from three localities were estimated to assess their mineral deficiency. This assessment was based on their weight and the diet composition determined through direct observation and the bites method. The browse species had a higher Ca content than P (1.79 vs 1.57 g/kg DM). The adult female goats had the highest P intake (2.04 g/day) with the highest deficit compared to the male adult (-29 vs -26) % of their daily requirements. Young kids (males and females) had the lowest Ca intake (0.81 and 0.75 g/day, respectively) and recorded the lowest deficit (-17 vs -19) %, respectively. Goats also showed a higher Ca deficit than P. In conclusion, the present results offer valuable information about the main mineral intake of the goats in the forest pasture of this region. Supplementing these two minerals is essential for enhancing goat performance in the traditional semi-extensive goat farming system that relies on forest pastures in the western-northern region of Morocco.



Keywords: Diet composition, Goat, Indigenous breeds, Mineral requirement, Pastoral plants.

#### INTRODUCTION

Calcium (Ca) and phosphorus (P) play pivotal roles in the productivity and health of small ruminants, particularly goats, as they are essential macrominerals for bone development, metabolic processes, and overall performance. Adequate levels of these minerals are critical for growth, reproduction, lactation, and maintaining physiological functions, including energy metabolism and enzymatic activity. Deficiencies in Ca and P can lead to reduced productivity, poor skeletal development, and metabolic disorders, highlighting the importance of understanding their availability and contribution to grazing systems (Drogoul et al., 2004).

In Morocco's northern region, recognized as the most forested area in the country with a woodland rate of 26% (MAPMDREF, 2018), forest pastures serve as a vital year-round feed source for grazing goats. Trees and shrubs, the primary feed sources in this region, provide energy, protein, and potentially essential minerals to support the nutritional needs of goats. These forest ecosystems underpin traditional smallholder livestock production systems, where farmers rely heavily on grazing goats (Chebli et al., 2021; Ayadi et al., 2022; Chebli et al., 2022a, 2022b).

While numerous studies have explored goat feeding behavior in Mediterranean regions (Glasser et al., 2012; Manousidis et al., 2016, and 2018), research on forage mineral content, mainly Ca and P, remains limited. Most evaluations focus on the chemical composition of forage, such as protein and energy, with insufficient attention to the quantitative assessment of mineral contributions to animal productivity (Chebli et al., 2022c; Jimenez et al., 2024). Understanding the mineral content of forage and its contribution to goat requirements is essential for improving feeding and grazing strategies and enhancing the sustainability of livestock production systems.

This study aims to estimate the Ca and P contents in pastoral shrubs and trees and evaluate their potential intake by four goat categories grazing in forest pastures in the western-northern region of Morocco. This information is crucial for addressing potential mineral deficiencies and optimizing the productivity and sustainability of goat farming in the region.

#### MATERIALS AND METHODS

#### Study area

This study was conducted in the Chefchaouen region, specifically in the Talassemtane high mountains. This region is characterized by local goat breeding. The herds are managed in an extensive system on natural forest pastures, from which the animals obtain over 90% of their nutritional needs, which the breeders consider "free" feed. The climate of the

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study area is Mediterranean, with dry summers. The mean annual temperature is 18.6°C, with an average rainfall of around 640 mm yearly (Acherkouk et al., 2022).

#### Animals and feeding behaviors

The study was conducted in the autumn and winter of 2022. It took place in three localities to represent the Chefchaouen region: Bouhalla  $(35^{\circ}6'0^{\circ} N \text{ and } 5^{\circ}6'36^{\circ} W)$ , Chrafat  $(35^{\circ}4'60^{\circ} N \text{ and } 5^{\circ}6'8^{\circ} W)$ , and Kalaâ  $(35^{\circ}3'50^{\circ} N \text{ and } 4^{\circ}31'46^{\circ} W)$ , with one breeder by locality. A group of 21 indigenous goats of each category (male adult, female adult, male kids, female kids) was chosen from each breeder. Each group within each category had approximately the same age and similar body weight (Table 1). The goats grazed on natural pasture for about seven hours daily when conditions permitted.

#### **Diet selection**

For each goat category, we were interested in two key parameters: (i) their daily dry matter intake (DDMI) on forest pasture and (ii) their weight. DDMI (Table 2) was deduced by the difference in the goat's weight before being released for grazing and upon their return in the evening, taking into account excretion-related losses (Delagarde et al., 2019). The importance of grazed pastoral species in the four categories of animal diets (Table 3) was assessed by direct observation of the animals during their grazing in the cold season during the study period and by the bite method described by Meuret et al. (1985).

Table 1 - The age and body weight of the chosen goats			
Animal categories	Heads/category	Age	Mean body weight
Mole adult	number	(months)	(kg)
Male adult	7	24-30	36±9.07
Female adult	7	24	33±9.81
Male kids	7	9	17±1.02
Female Kids	7	9- 12	<b>18±6.56</b>

Table 2 - The goat's daily dry matter intake in the three localities			
Daily dry matter i	ntake (g) Breeder 1	Brooder 2	Brooder 3
Animal category	Dicedel T	Diceuci 2	Diecuel 3
Male adult	1500	1500	1800
Female adult	1500	2000	2500
Male kids	1200	1000	1200
Female Kids	1000	1000	1200

Table 3 - Qualitativ	e and quantitative o	liet composition of th	ie goats		
Breeder 1 Breeder 2			Breeder 3		
Species	Percentage (%)	Species	Percentage (%)	Species	Percentage (%)
Quercus ilex	67	Quercus ilex	50	Quercus ilex	80
Quercus ilex fruit	22	Quercus ilex fruit	20	Pistacia lentiscus	10
Phillyrea media	5	Phillyrea media	15	Cistus albidus	5
Cistus albidus	4	Arbutus unedo	5	Grass plants	5
Grass plants	2	Grass plants	10		

#### The mineral content of the browse species grazed by the goat in forest pasture

17 collected samples of shrubs and tree species from forest pastures in the mountainous region of northwest Morocco were collected to determine their Ca and P content. The collected samples were dried in an oven until a constant weight at 40°C temperature. Then, the dried samples were milled with a sieve mesh size of 1 mm to evaluate their Ca and P content. The analyses were conducted on triplicate from each species, which were subjected to dry ashing at 600 °C for 4 hours and then prepared for mineral analysis using the wet ashing (HCI–HNO3) procedure. The Ca content analysis was performed using the manganometric method according to AOAC (1997), and for P determination, the nitrovanadomolybdate technique was employed (AOAC, 1997).

#### Data analysis

The Ca and P content were analyzed using a general linear model (GLM) with the SAS software 9.4 version. To determine the variability between forage species, the variance of the means was analyzed according to a one-factor variation model Yij= $\mu$ +Ti+eij with  $\mu$ : overall mean, Ti: forage species, and ei: residual error. Differences between mean values were tested using the LSD "Last Square Deviation" test.

#### Estimation of the macromineral deficit of the goats

#### A) The macromineral requirement for goats

The primary mineral (Ca and P) requirements for goats are expressed in absorbable elements obtained by the equations of Meschy (2007) (1 and 2) based on the dry matter intake (DMI) in kg/day and body weight (BW) in kg. They are expressed by g per day.

P abs= 0.905 DMI+ 0.3 + 0.002 BW (1)

Ca abs= 0.67 DMI + 0.01 BW (2)

#### B) The mineral deficit of the goat's diet

With the data collected previously (daily feed intake, diet composition, and Ca and P requirement), the potential mineral intake was calculated based on their daily dry matter intake and Ca and P absorbable of the feed using the real absorption coefficients (RAC) for Ca and P by Meschy (2007). Then, we define the deficit by subtracting the intakes from the calculated needs based on each animal's body weight. Finally, the deficit is estimated as a percentage of the requirements.

#### RESULTS

#### Mineral content of pastoral fodder

The mineral content of the pastoral fodder selected by indigenous grazing goats is presented in Table 4. The P concentration ranged from 0.99±0.07 to 2.30±0.01 g/kg DM, which varies significantly between the forage species (P<0.001). The pastoral species with the highest P content are *Olea europaea* L. (2.30 g/kg DM), *Rosmarinus officinalis* L. (2.25 g/kg DM), *Ceratonia siliqua* L., and *Erica arborea* L. (1.93 and 1.91 g/kg DM, respectively). The other species' content varies between 1.78 and 0.99 g/kg DM. Moreover, the average Ca content of the selected plants is 1.79 g/kg DM. *Erica arborea* L. and *Genista scorpius* L. showed the lowest content (0.86 and 0.75 g/kg DM, respectively). At the same time, the highest values were observed in *Olea europaea* L., *Arbutus unedo* L., and grass plants (2.63, 2.61, and 2.42 g/kg DM, respectively). The Ca and P ratio oscillates between 0.45 and 2.39 (Table 4). The higher ratios were observed in grass plants (2.39), *Cistus ladanifer* L. (1.88), and *Arbutus unedo* L. (1.71). In contrast, *Vaccinium myrtillus* L., *Genista scorpius* L. showed lower ratios (0.62, 0.58, and 0.45, respectively).

Table 1 - Average content of macrominerals in species browsed by goats in forest pasture of Northwest Morocco (g/kg DM)				
Pastoral species	Phosphorus (g/kg DM)	Calcium (g/kg DM)	Ca:P ratio	
Olea europaea L. (Olive tree)	<b>2.30</b> ª	2.63ª	<b>1.14</b> <sup>fg</sup>	
Cistus albidus L. (White-Leaf Rockrose)	<b>1.78</b> °	2.17 <sup>cd</sup>	<b>1.21</b> <sup>fg</sup>	
Cistus ladanifer L. (Gum Rockrose)	<b>1.02</b> <sup>j</sup>	<b>1.92</b> <sup>e</sup>	<b>1.88</b> <sup>b</sup>	
Quercus canariensis (Algerian Oak)	<b>1.15</b> <sup>i</sup>	1.35 <sup>fg</sup>	<b>1.17</b> <sup>fg</sup>	
Phillyrea media L. (Mock privet)	<b>1.41</b> <sup>g</sup>	2.31 <sup>bcd</sup>	1.64 <sup>cd</sup>	
Quercus Ilex (Holm Oak)	<b>1.44</b> <sup>fg</sup>	<b>1.91</b> <sup>e</sup>	1.32 <sup>ef</sup>	
Quercus Ilex (Fruit)	<b>1.64</b> <sup>de</sup>	<b>1.18</b> <sup>gh</sup>	0.72 <sup>jk</sup>	
Arbutus unedo L. (Strawberry tree)	1.53 <sup>ef</sup>	<b>2.61</b> ª	1.71 <sup>bc</sup>	
Pistacia lentiscus L. (Mastic tree)	<b>1.60</b> <sup>de</sup>	2.37 <sup>bc</sup>	1.48 <sup>de</sup>	
Ceratonia siliqua L. (Carob tree)	<b>1.93</b> <sup>b</sup>	2.19 <sup>bcd</sup>	<b>1.14</b> <sup>fg</sup>	
Erica arborea L. (White heather)	<b>1.91</b> <sup>b</sup>	0.86 <sup>ij</sup>	0.45 <sup>i</sup>	
Quercus suber L. (Cork oak)	<b>1.68</b> <sup>cd</sup>	<b>1.46</b> <sup>f</sup>	0.87 <sup>ij</sup>	
Genista scorpius L. (Mediterranean broom)	<b>1.30</b> <sup>h</sup>	0.75 <sup>j</sup>	0.58 <sup>ki</sup>	
Vaccinium myrtillus L. (European blueberry)	<b>1.78</b> °	<b>1.10</b> <sup>h</sup>	0.62 <sup>kl</sup>	
Lavandula stoechas L. (Butterfly lavender)	<b>0.99</b> <sup>j</sup>	<b>1.06</b> <sup>hi</sup>	<b>1.07</b> gh	
Rosmarinus officinalis L. (Rosemary)	2.25ª	2.09 <sup>de</sup>	0.93 <sup>hi</sup>	
Grass plants	<b>1.01</b> <sup>j</sup>	2.42 <sup>ab</sup>	2.39ª	
Mean	1.57	1.79	1.20	
SEM	0.06	0.09	0.07	
Probability (P) value	<0.0001	<0.0001	<0.0001	
a,b,c: Mean values in the same row with different lette	ers are significantly different. D	M: Dry Matter SEM: Standard en	ror of the means	

#### Macro mineral balance of goats

Based on the previous results, we derived the Ca and P intake (Table 5). The female goat's intake from forest pasture resulted in a moderately high average daily P intake of approximately 2.04 g/day, representing a deficit of about 6% of requirements. As for Ca, the forage intake was 1.07 g/day, representing a deficit of approximately 29% of requirements. On the other hand, the intake by male goats from forest pastures resulted in an average daily P intake of 1.63 g/day (i.e., a deficit of about 10% of requirements). Regarding Ca, the male goat's intake from forest pastures was about 1.06 g/day (i.e., a deficit of about 26% of requirements). The male kids' daily absorbable P intake was 1.15 g/day, corresponding to a 14% deficit of requirements. In contrast, female kids had an intake of 1.27 g/day of absorbable P (i.e., a deficit of 11% of requirements) and an intake of 0.81 g/day of absorbable Ca, reflecting a 19% deficit of requirements compared to an intake of 0.75 g/day in absorbable Ca for the male kids (i.e., a deficit of 17% of requirements).

Table 5 - Phosphorus and Calcium intake by goats in forest pastures (g/day).									
Minoral intoka and deficit	Male goats F		Female	Female goats		Male kids		Female kids	
	Р	Ca	Р	Ca	Р	Ca	Р	Ca	
Daily potential mineral intake per day <sup>a</sup> (g/day)	1.63	1.06	2.04	1.07	1.15	0.75	1.27	0.81	
Daily potential mineral requirements ${}^{b}(g/day)$	1.82	1.44	2.18	1.51	1.33	0.91	1.42	1	
Deficit (g/day)	-0.19	-0.37	-0.13	-0.44	-0.18	-0.16	-0.15	-0.19	
Deficit (% of daily requirements)	-10%	-26%	-6%	-29%	-14%	-17%	-11%	-19%	
a: assumed daily DM intake by the method cited in D (2007) equations (in g/day).	elagarde e	t al. (2019)	(in g/day).	b: Recomn	nended ave	erage requi	rements by	Meschy	

#### DISCUSSION

The phosphorus content in the studied plant species oscillates between 0.23 % and 0.10 % DM. In contrast, Dione et al. (2022) showed that the highest P content in forage plants in the agro-pastoral zone of Senegal varies between 0.82 % and 0.06 % DM. Additionally, Abdelkefi et al. (2004) reported a P concentration among some pastoral species in semiarid and arid North Africa that varied from 0.05 to 0.52 % DM. However, Abdullah et al. (2013) found a lower P concentration in some browse species used as feed for livestock (0.016%).

Phosphorus has been known as a "master mineral" given that it affects the majority of metabolic processes (Rasby et al., 1997). The National Research Council (1984) recommended a P range of 0.12 to 0.48% for all ruminant classes, which aligns with present findings regarding the P concentration in the pastoral species used as feed for the northern goats of Morocco. The soil's P status, the plant's maturation stage, and the climate all impact the P content of forages, which varies widely (Underwood and Suttle, 1999).

The average Ca concentration in the studied pastoral plants was 1.79 g/kg DM. However, present findings were lower than the results reported by Abdullah et al. (2013) for browse species fed to livestock (1.79 vs 3 g/kg DM). Mirzaei (2012) revealed Ca concentration ranging from 4.17 to 2.42 g/kg in grass plants grazed by ruminants, which the results of the present experiment for grass plants (24.2 g/kg) are consistent with the findings of these researchers. Moreover, Chhabra et al. (2015), reported higher Ca content, approximately 0.77% (equivalent to 7 g/kg), in winter fodder in India.

These pastoral plants have Ca concentrations lower than the levels recommended by the NRC literature cited in Ghazanfar et al. (2011) and by Kessler (1991) in Ramírez-Orduña et al. (2005) for goat requirement. An exceeding of 1% of Ca content can decrease DM intake and reduce the absorption of trace minerals, especially zinc. However, the Ca requirements in grazing animals are a widely debated subject, as they are influenced by factors such as the type of animal, age, and production level (Khan et al., 2007).

The variations in Ca levels between the results of this study and values published in the literature are attributed to differences in forage species, species composition, seasonal and maturational stages, and changes in soil properties (Mirzaei, 2012). However, information about minerals in pastoral species, particularly those browsed by ruminants, is limited. These findings will provide a comprehensive knowledge of the mineral composition of grazing forage, thereby improving and ensuring goats' welfare and growth. In cases of deficiency, coupled with previous research regarding energy and protein content, they will enable the optimization of goat diets through informed adjustments.

Ca and P were studied together due to their close metabolic association, as an excess of either in the diet restricts the availability of both nutrients. The Ca:P ratio ranged from 0.45 to 1.88, which is in line with the recommended ratio by Abdulrazak et al. (2000), except for the grass plants with the highest Ca:P ratio (2.39). A higher ratio might interfere with the animal's ability to use Ca effectively (Fadel Elseed et al., 2002), and can also decrease livestock P absorbance.

The mineral elements are not produced in the body; the feed usually provides them. The concentrations of these elements in bodily fluids will vary depending on the availability of minerals, the quantity of dietary sources consumed, and the mineral content of feed (Suttle, 2010). The mineral concentrations of fodder plants are influenced by a wide range of environmental and plant parameters, such as the type of soil, species or strain/variety, seasonal circumstances during plant growth, plant maturity stage, and other management techniques (Underwood and Suttle, 1999).

P is a crucial mineral for animals, essential for their nutritional requirements. Approximately 80% of P is found in their skeleton, a critical bone and teeth component. Moreover, it plays a vital function in the transfer and utilization of energy. A P deficiency can decrease ruminant appetite, reduce fiber digestibility, and lower growth rates, weight gain, and reproduction (Drogoul et al., 2004). In cattle and small ruminants, a severe P deficiency (less than 1g/kg DM) can cause locomotor abnormalities, followed by paralysis of the rear end and spontaneous fractures (Meschy, 2010). P deficiency can also lead to losing appetite and consuming abnormal materials, such as bones, soil, wood, and flesh (Underwood and Suttle, 1999). Ramírez-Orduña et al. (2005) reported that the potential P intake of goats consuming shrubs in Mexico didn't fulfill their requirement, especially during years of low rainfall, which can harm goat performances.

Calcium is the most abundant mineral in the body, comprising 99% of the skeleton. A Ca deficiency can cause soft, weak, or deformed bones, leading to lameness, a condition known as osteomalacia or rickets. Ca is also required for blood clotting, nerve conduction, and muscular contraction (Hart, 2009). It was advised that the Ca requirements for maintaining, growing, and lactating sheep should be 1.2 to 2.6 g/kg (Mirzaei, 2012), which is higher than the potential mineral intake of the studied goats.

An excess or deficiency of these macrominerals can cause disruptions, slow growth, and limit the digestion of nutrients (NRC, 2007). To optimize animal well-being, it is preferable to provide them with the precise amounts they need based on their species and body weight. Mineral deficiency can also be caused by the feeding behavior of goats and the quality of their forage. As mentioned by Chebli et al. (2022a), there was a notable decline in forage production in the forested rangeland of Beni Arouss, located in the northern region of Morocco, with a 31% reduction in summer and a 47% decrease in autumn compared to the spring season. Furthermore, the intake rate was lower in the summer and autumn compared to the spring (4.94, 4.52 vs 5.57 g DM/min). The intake rate is influenced by season, as the goats tend to extend the duration of their grazing during summer days in comparison to the rainy season, as they strive to meet their intake requirements (Safari et al., 2011).

In this study, it is imperative to acknowledge several limitations that may have influenced the results to ensure the transparency and integrity of this research's findings. Methodological constraints, particularly concerning the estimation of dry matter intake on pasture and the estimation of mineral requirements, may have contributed to uncertainties in the results. In summary, present findings contribute to identifying the deficiencies in P and Ca within the goats' diet, thus emphasizing the necessity of considering them in ration formulation.

#### CONCLUSION

Mineral concentrations in browse plants differ significantly. Most plants had higher Ca levels than P during the rainy season (0,15 vs 0,10 g/kg DM). The levels of these macrominerals found in the browse shrubs grazed by goats in the northern region of Morocco were insufficient, resulting in a deficiency in their estimated daily mineral requirement, especially during the cold season (-0,23% deficit in Ca vs – 0,10% deficit in P). To fulfill the mineral needs of goats, it is essential to formulate feeding strategies and implement grazing management. To ensure a balanced diet for goats, it is essential to include supplements rich in calcium (Ca) and phosphorus (P), such as a vitamin-mineral complex. The diet can also be enhanced by incorporating concentrated feed options like bitter vetch, barley, wheat bran, and sorghum.

This approach aims to meet their mineral requirement and improve the production and performance of goats. The findings could offer valuable and specific information for herders to design supplementary diet formulations, considering grazing activities and the quality of the consumed plant species.

#### DECLARATIONS

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#### **Ethical approval**

All procedures involving animals in this study have been conducted according to the ethical standards of the Regional Center of Agricultural Research of Tangier. All the authors complied with the ARRIVE guidelines

#### Authors' contribution

Conceptualization: A. Al Rharad, A. Bouassab, M. Acherkouk, M. Ayadi

Data curation: M. Ayadi, M. Acherkouk, A. Al Rharad, A. Bouassab Formal analysis: A. Al Rharad, A. Bouassab, M. Acherkouk, M. Ayadi Investigation: M. Ayadi, M. Acherkouk, A. Al Rharad, A. Bouassab Methodology: A. Al Rharad, A. Bouassab, M. Acherkouk, M. Ayadi Validation: A. Al Rharad, A. Bouassab, M. Acherkouk, M. Ayadi Writing – original draft: A. Al Rharad, M. Ayadi, A. Bouassab, M. Acherkouk, Writing – review & editing: A. AL RHARAD, M. Ayadi, A. BOUASSAB, M. Acherkouk

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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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## GROWTH PERFORMANCE AND PROFITABILITY OF WEANLING PIGS (Sus scrofa domesticus L.) FED PRE-STARTER DIET SUPPLEMENTED WITH NUCLEOTIDE

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Supporting Information

ABSTRACT: Nucleotides can improve intestinal health by modulating the local immune response and intestinal mucosa development in weaned piglets. This study was conducted to evaluate the growth performance of post weaned piglets and evaluated the economic analysis of nucleotides supplementation for 30 days. A total of 120 mixed breed piglets were selected at the weaning stage and were used in the experiment as control group vs. treatment 2 supplemented with nucleotide. Each treatment consisted of 60 heads with three replications with 20 heads per replication arranged in Complete Randomized Design. Results were analyzed through Pairwise T and LSD tests. In terms of growth performance, results showed that supplementation of nucleotide had significantly increased the average daily gain, feed conversion ratio and weight gain by 0.50, 1.20 and 15.02 kg, respectively. However, there was no significant difference in terms of the average daily feed intake. With regards to the economic analysis, total production input had no effect with or without nucleotide supplementation but surprisingly, it had a gross margin of Php 95,900 (Philippine peso money) which was 5% more as with those that have supplementation. As to the net income, supplementation of nucleotides increased about 40.7% in comparison to control. Furthermore, a peso of investment could have a return of about 18 cents (1050 Php) more returns with supplementation, which apparently had 0.11 cents leverage compared to control group (0.07 cents). In conclusion, nucleotide supplementation not only improved the growth performance of post-weaned piglets but also enhanced profitability, offering a significant return on investment for swine producers. This makes nucleotide supplementation a promising strategy for improving both animal health and economic outcomes in swine production.

Keywords: Benefit Cost Ratio, Daily weight gain, Feed conversion ratio, Net Income, Nucleotides.

#### INTRODUCTION

Major adjustments in technologies in swine management and nutritional programs have been significantly improved for efficiency and quality in commercial swine production. Providing the primary needs of the weanling pigs, like feeds, water, and air, is crucial to their growth performances (Gaillard et al., 2020). One of the stressful moments in pig's life is on weaning, which is often accompanied by physiological variations in the gastrointestinal tract (GIT) (Pluske, 2016). Weaning is also challenging because of not only, the new atmosphere but also the transition to dry feed from sow's milk causing nutritional stress (Van Kerschave et al., 2023). One of the most health challenging experiences for pigs after weaning, is post-weaning lag (Vasa et al., 2024). Post-weaning lag is the condition where changes in the weaned pig's intestinal biochemistry can contribute to diarrhea, weight loss, a decline in appetite, and reduced growth after a new environment and the initial separation from the sow and the littermates (Dunshea et al., 2003; Muro et al., 2023). Also, after weaning, nutrient digestibility is reduced by the collapse of the intestinal barriers due to intestinal inflammation and oxidative stress (Lallès et al., 2004; Moeser et al., 2017). Thus, reducing oxidative stress by dietary nucleotides can be associated with improved health status and growth performance in nursery pigs (Jang and Kim, 2019; Duarte et al., 2019).

Dietary nucleotide supplementation to pre-starter feeds (after weaning) may provide a readily available nucleotide needed by the animals that can be used as a precursor for the maturation of intestinal mucosa, which can help alleviate the effect of early nutritional stress, brought about commonly by weaning (Correa et al., 2021). Thus, improved intestinal maturation when nucleotides are included in the diet can reduce the onset of diarrhea in weaned piglets (Martinez-Puig et al., 2007).

Nucleotides are organic molecules made up of a nitrogenous base, a pentose sugar, and a phosphate. They serve as the building blocks of nucleic acids. Nucleic acids such as deoxyribonucleic acids (DNA) and ribonucleic acids (RNA) are essential in cellular growth (Minchin and Lodge, 2019). Nucleotides can be synthesized in the body via two pathways; the first type is the de novo pathway which starts with metabolic precursors such as amino acids, ribose, carbon dioxide, and

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ammonia (Watson and Crick, 1953). The synthesis using the de novo pathway is energy-requiring. It utilizes many metabolic pathways that require a large amount of energy to proceed. In addition, some tissues in the body, like intestinal mucosa, have a limited capacity to synthesize purine nucleotides via the de novo pathway (Boza et al., 2002). Therefore, there can be a need for an exogenous supply of bases that can be utilized via salvage pathway for optimal function (Uauy et al., 1990). If the nucleotide requirements of the intestinal mucosa are quickly met, this may result in rapid intestinal development and maturation. On the other hand, the second type is the salvage pathway, which retrieves free purine and pyrimidine bases, as well as nucleosides, from the degradation of nucleic acids or from the diet (Moffatt and Ashihara, 2002; Dinardo et al., 2022). The salvage pathway is much more efficient than the de novo pathway, and this is what the study aims to develop.

The nucleosides and nucleotides are identified as bioactive compounds with significant potential for application in food and nutrition (Bezerra et al., 2024). These compounds could be integrated into functional foods to improve metabolic processes, enhance immune function, and support cellular regeneration. For infant nutrition, nucleotide fortification in infant formulas offers a strategy to replicate the immune and gut health benefits of human breast milk. Additionally, immune-boosting foods, such as soups or fortified beverages, could benefit from nucleotide inclusion to strengthen immune responses (Prakash et al., 2020). Lastly, the development of nucleotide-based dietary supplements presents an opportunity for promoting cellular health and DNA repair, highlighting the broader potential of these compounds as bioactive ingredients in food products designed for health enhancement. Moreover, previous studies found that supplementation of dietary nucleotides in nursery diets enhanced intestinal morphology (Jang and Kim, 2019; Valini et al., 2021) and intestinal immunity (Waititu et al., 2017). A research report has shown that the inclusion of a nucleotide base at a concentration of 0.5% in diets resulted in enhanced weight gain and increased feed intake among weaned pigs (Zomborszky-Kovacs et al., 2000). Moreover, it is still speculative if the efficiency of absorption of free nucleotides dissolved in water is different from that of bound nucleotides in feed ingredients (Sauer et al., 2012). Meanwhile, separate from the pig's environment, feeding strategies, age at injecting, and pig genotype and age are mediated by psychological and behavioral stress. A study has indicated that the addition of nucleotide supplementation within the range of 50 to 250 mg/kg in diets can potentially benefit newly weaned pigs. These benefits include improved growth performance, potentially attributed to reduced intestinal inflammation and oxidative stress, as well as enhanced intestinal villi structure and energy digestibility (Jang and Kim, 2019). However, little is known about the information on the effects of nucleotide on the growth of newly weaned piglets. Thus, present study was conducted to evaluate the effects of nucleotide supplementation on the nutrient digestibility and growth performance of pigs when added to pre-starter feedings over a period of 30 days.

#### MATERIALS AND METHODS

#### Time and place of study

The experiment was conducted at the Nursery Section, Department of the Research and Development Farm of UBC Stock Farm, Incorporated at Sitio Dao, Brgy. Singsing, Balamban, Cebu, Philippines from October to November 2023.

#### Experimental design and layout

The 120 heads total of mixed-breed newly weaned pig (Landrace × Duroc × Large White) were chosen at the weaning stage. A Completely Randomized Design (CRD) was used in the experiments with two experimental treatments, the control group (T1) consisted of weaned pigs given pure pre starter diet (without nucleotide supplementation) and treatment T2 were pigs supplemented with nucleotides added in the formulated feeds. Each group contained 60 heads which was further divided into 3 subgroups representing the three replications. The selection of the newly weaned piglets was based on their size and uniformity, as well as their sex, to ensure a fair distribution of males and females in each treatment group.

#### Care and management

Before conducting the experiment, the pen underwent thorough cleaning, disinfection, and sanitation process for approximately 14 days. This was done prior to transferring the pigs from the farrowing section to the weaning area. Each pen was provided with rotary feeders and one linear feeder. Feeds and clean water were provided in an ad libitum scheme. Vitamins and mineral supplements were administered via drinking water upon loading via medicator tank and during stressful periods such as challenging weather (extreme cold and extreme heat) and disturbance stress. Curtains and lighting were also installed on a case-by-case basis to provide the most comfortable conditions for the newly weaned pigs. Vaccination of Hog Cholera at 35 days old and *Mycoplasma pneumonia* and *Haemophilus parasuis* 2 at 42 days old was administered to all the experimental pigs.

#### **Experimental diets**

Both groups were administered the same feed formulations of the pre-starter diet. Group 2 animals received the prestarter diet with 500 grams of nucleotide per ton of feeds. The nucleotides were included during the mixing of feeds in the feed mill where the feeds were produced. The source of nucleotide was provided from the yeasts extracts through hydrolysis (Bioiberica, Spain, Table 1).

Table 1 - Nucleotide concentration (pp	n) in some commonly used feed	d ingredients (as-is basis	s) (Mateo et al., 2004
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Ingredient	Nucleotide: 5' -AMF	<b>P</b> 5'- CMF	P 5'-GMP	5'-IMP	5'-UMP
Barley	1	2	1	1	0
Casein	0	1	0	0	0
Corn	2	3	3	1	0
Fishmeal	11	26	2	35	1
Naked oats	3	3	3	1	1
Non-fat dried milk	0	65	0	195	106
Plasma protein, spray dried	2	2	2	1	0
Red blood cells, spray dried	44	0	3	6	2
Soybean meal, 44%	8	16	3	2	9
Soy protein concentrate	1	0	2	1	0
Whey dried	19	270	0	4	1
Whey protein concentrate	0	34	0	159	89
Adenosine 5' monophosphate (5' AMP), cytidine 5' monop monophosphate (5' IMP), and uridine 5' monophosphate (5' UM	phosphate (5' CMP), ( P)	guanosine 5'	monophosphate	(5' GMP),	inosine 5'

#### **Data Collection**

#### Body weight and feed measurements

From the first day to the day 30 of feeding, both feed consumption and body weights of the experimental animals were monitored. The collected data was analyzed, summarized, and categorized according to metrics like the ADG or Average Daily Gain, the ADFI or Average Daily Feed Intake, and the FCR or Feed Conversion Ratio (FCR) during the entire feeding duration. Additionally, calculations were made: 1) feed price per pig; 2) feed price per kilo of gain; 3) income over feed and 4) pig price.

#### Body weight and weight gain

The body weight at weaning (start of feedings) and after 30 days of feedings (end of feedings) was determined and recorded. The average body weight for each replicate was determined by dividing the group weight by the number of pigs in each pen. To determine the body weight gain for each replicate, the final weight at the end of the experiment was subtracted from the initial body weight.

#### Feed consumption

The feed intake of the pigs was be calculated by subtracting the number of left-over feeds from the amount of feed offered divided by the corrected number of pigs (less mortality).

#### Feed conversion ratio

The feed conversion ratio was determined by dividing the feed consumption by the corresponding weight gain at the conclusion of the pre-starter feeding period for each replicate.

#### Economic analysis

#### Variable cost of the treatments

Cost of Treatment 1 = total cost of compounded feeds given + other cost, if any

Cost of Treatment 2 = total cost of compound feeds + nucleotide supplements, other cost, if any.

To calculate the financial return, the total expenses were subtracted from the total expected sales.

Net profit = Total sales – Total Expenses

#### Where:

Total sales = were calculated by multiplying the final weights of the pigs by the current selling price. Total expenses = were expenses incurred throughout the experiments.

#### **Benefit-cost ratio**

The discounted cash inflows and outflows ratios, which must be equal to or larger than one, are known as the Benefitcost ratio (BCR). The ratio must be at least 1:1, indicating that the expense incurred and the benefit received are equal. If the benefits outweigh the costs, the ratio should be greater than 1. This parameter shows the rate of return and is worked out by dividing the total gross return by the total cost return.

BCR = <u>Total Gross Return</u> Total Cost

#### Statistical analysis

The data were analyzed using STAR version 2.0.1 or Agricultural Statistics software. Pairwise t-test analysis was used to compare treatments, while Least Significant Difference (LSD) test was used to determine significant differences between responses variables among different treatments in the study to ensure the reliability of the results obtained.

#### **RESULTS AND DISCUSSION**

#### Growth performance of pigs

The effectivity of nucleotide supplementation on post-weaned pigs has been studied earlier, showing contradicting results including but not limited to its dietary effects (Perricone et al., 2020), physiological attributes (Reina et al., 2014), and immune system (Superchi et al., 2012). Nonetheless, the effectiveness of nucleotide supplementation on post-weaned piglets has been the focus of the study.

Different growth performance or test variables have been tested in the study, including the average daily gain (ADG), feed conversion ratio (FCR), and weight gain (WG). As presented in Table 2, the effect of supplementation of nucleotide to the pre-starter on post-weaned had significantly improved their ADG, FCR, and WG, respectively. Results showed that nucleotide supplementation had significantly increased the daily gain by approximately 0.07 kg compared to the treatment without nucleotide supplementation. Regarding this, the results may be attributed to the positive effects of nucleotide supplementation to enhance nutrient absorbability in the small intestine by promoting the development of enhanced villi. This, in turn, leads to improved performance in pigs. The findings of this study are consistent with previous literature that has reported varying outcomes concerning nucleotide supplementation in pigs. For instance, Perricone et al. (2020) found that nucleotide supplementation can positively affect dietary intake and growth metrics, albeit with differing results based on the specific composition and dosage of nucleotides used. Similarly, Reina et al. (2014) reported improvements in physiological attributes associated with nucleotide intake, while Superchi et al. (2012) highlighted enhancements in immune response. While some studies have reported contradictory outcomes regarding the effectiveness of nucleotides on growth performance, this research supports the notion that, when utilized correctly, nucleotides can significantly enhance growth metrics. The discrepancies observed in previous studies may be attributed to various factors, including differences in experimental design, genetic backgrounds of the pigs, and environmental conditions, which can all influence nutrient metabolism and growth performance (Rocadembosch et al., 2016; Pierozan et al., 2016).

Treatment	Average daily gain (kg)	Feed conversion ratio	Average daily feed intake (kg)	Weight gain (kg)
T1 (Without Nucleotide)	0.430 <sup>b</sup>	1.47 ª	0.64	13.04 <sup>b</sup>
T2 (With Nucleotide)	0.500 ª	1.20 b	0.63	15.02 ª
P-Value	0.03	0.04	0.56	0.03
F Value	9.50	8.74	0.40	9.52
CV %	5.41	8.49	2.04	5.60
Means with the same letter designations were	not significantly differ	rent at 5% level LSD test.		

**Table 2** - Growth performance of pigs during the pre-starter stage in the post-weaning period (PWP), comparing those feds with and without nucleotide supplementation.

Feed conversion ratio served as the feed used per pound of weight gain. The results of the study demonstrated that the supplementation of nucleotides effectively reduced the amount of feed required to produce a kilogram of live weight. It takes only 1.20 kg of feeds to have a kilogram of its equivalent live weights. A lower FCR indicates that pigs are efficiently converting feed into body weight. The Feed Conversion Ratio (FCR) in pigs can differ across countries (Rocadembosch et al., 2016), stages of growth (Pierozan et al., 2016), environmental conditions (Agostini et al., 2014), and genetic factors (Bereskin, 1986). However, as a general guideline, a pig's FCR should ideally fall within the range of 3:1. In terms of the weight gain, results showed that the higher the average daily gain, this also resulted to a higher weight gain at harvesting (Table 2). Statistical evidence demonstrated that nucleotide supplementation resulted in a significant increase in live weight by 15.02 kg, which corresponds to a 7% higher weight compared to the group without nucleotide supplementation.

Statistical analysis showed no significant difference between the two treatments, indicating no effects on the daily feed intake. Based on the findings, the addition of nucleotide supplementation increased the average daily gain, feed conversion, and ultimately the live weight gain of post-weaned pigs. This indicates that nucleotide supplementation positively impacted the growth and performance of the pigs. Other scientists have also confirmed the effectiveness of

nucleotides, not only in terms of improving growth performance but also in boosting the immune system and reducing environmental stresses. This indicated that nucleotides have multiple beneficial effects beyond just promoting growth in animals. Nucleotides have important effects on the growth and development of rapid turnover cells, such as those in the immune system and the gastrointestinal (GI) tract (Dancey et al., 2006) However, it has been observed that animals fed diets lacking in nucleotides exhibit lower immune responses, as noted by Chandra and Kumari (1994). This suggests that the absence of nucleotides in the diet can negatively impact the immune system of animals. Dietary nucleotides recognized a potential antibiotic alternative, have been found to exhibit beneficial effects on intestinal hyperemia, systemic immunity, small-intestinal growth, and hepatic composition in pigs (Jang and Kim, 2019). Moreover, Sauer et al. (2011) summarized that supplementation of nucleotides affects immune function, nutrient metabolism, hepatic morphology and function and accelerates T-cell-dependent antibody production (Grimble et al., 2001).

#### Economic analysis of piglets

Economic analysis of pigs differs due to several reasons like breed type, cost of feeds fed, operating expenses, vitamins and medicine for diseases of pigs, market niche, current buying price, location, and demand pool in the society. In this study, the computation of the total variable cost is based on the existing care and maintenance practices that are uniformly applied to all blocks (Table 3). This means that the cost calculations consider the standard care and maintenance procedures that are consistently implemented across all blocks. The variable cost includes the price of pigs (20 heads per block), feeds, electricity, water, managerial pay/labor cost, as well as vaccines and vitamins. According to the analysis, there is no significant difference observed in terms of variable cost. This means that the addition of nucleotides does not result in any significant variation in the production inputs required for raising pigs. In terms of its gross income, calculation includes the total live weight of 20 pigs at harvest times at a price of each pig at 210 per kilogram, which was applied and computed to all blocks.

**Table 3** - Economic analysis of swine production at the pre-starter stage during the post-weaning period (PWP), comparing the outcomes with and without the inclusion of nucleotides.

Trootmont	Total per Philippine Peso		Not incomo	Bonofit east ratio	
reatment	Variable cost	Gross income	Net income	Deneni Cost ratio	
T1 (Without nucleotide)	81,497.67	87,535 b	6,037.33 Þ	1.07 b	
T2 (With nucleotide)	81,574.33	95,900 ª	14,325.49 ª	1.18 ª	
P Value	0.82	0.05	0.04	0.04	
F Value	0.06	7.67	9.20	8.98	
CV %	0.49	4.03	32.86	3.75	
Means with the same letter designations wer	e not significantly differe	ent at 5% level LSD test.			

The study results indicate that nucleotide supplementation leads to higher live weight, resulting in a higher gross income during harvesting. The statistical results indicated that nucleotide supplementation has significantly increased income compared to the non-supplemented group. A gross income of Php 95,900 (1648.39 USD) was incurred with nucleotide supplementation, which is 3 percent (3.84%) higher compared to Php 87,535 (1504.61 USD) without nucleotide supplementation. It simply means that the higher the gross income compared to variable cost, the higher is the net income. It is true that this study has significant implications, as the supplementation of nucleotides in T2 resulted in a 40.70 percent leverage compared to T1 (without nucleotide supplementation). Moreover, in terms of its benefit-cost ratio, the use of nucleotide supplementation incurred 1050 Php (18 cents) return on a peso of investment. This demonstrated a significant difference, with a lower return on the treatment without supplementation, yielding only 0.07 cents of return on each peso of investment.

Generally, the study implies that the addition of nucleotide supplements does not affect the overall production cost. However, it positively impacts the gross income by increasing pig live weights, net income, and the return on peso investment by 18 cents per peso, respectively.

#### CONCLUSION

The addition of nucleotide supplementation resulted in developments in average daily gain, feed conversion ratio, and the weight gain, although it did not have a significant impact on the average daily feed intake. Also, in terms of its economic analysis, supplementation of nucleotide had shown no effect on the total production cost but had higher gross income with 5% leverage as compared to control group, 40.70% increase in net income, and a higher benefit of 18 cents for a peso spending compared to treatment without supplementation.

#### 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 considerations**

This study adhered to the Animal Welfare Act of the Philippines 1998 (The Philippine Animal Welfare Society, 1998) to ensure the humane treatment of the post-weaned piglets. Proper care, including adequate housing, nutrition, and veterinary support, was provided throughout the experiment. Stress and pain were minimized by handling the piglets gently and using trained personnel for all procedures. Clear criteria for humane endpoints were established. The number of animals used was kept to the minimum necessary to achieve valid results, following the principle of reduction. The study-design ensured that the use of animals was ethically justified by the potential benefits to swine production (FAO, 2015).

#### Authors' contribution

Both authors contribute on conceptualization of the study, data analysis and the write up of the manuscript

#### **Competing interests**

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

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# SARCOCYSTOSIS IN ALPACAS AND LLAMAS: REGIONAL, MARKET, AND MUSCLE-SPECIFIC PREVALENCE PATTERNS

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Supporting Information

ABSTRACT: The objective of this study was to determine the effect of species (alpacas and llamas), markets in the city of Huancayo (Ferrocarril Commercial Center, Nueva Esperanza, and Nazareth), and muscle groups on the prevalence of Sarcocystis sp. Between January and October 2023, a total of 2.211 carcasses were inspected, comprising 1,716 alpacas and 495 llamas. The results indicated a prevalence of 21% (104/495 carcasses) in Ilamas and 8% (138/1,716 carcasses) in alpacas. By region of origin, the prevalence in alpacas was reported as follows: Huancavelica (7.7%) with 14/181 carcasses, Junín (6.7%) with 55/820 carcasses, and Lima (9.7%) with 69/715 carcasses. For llamas, the Lima region exhibited the highest prevalence of sarcocystosis (33.9%) with 72/212 carcasses, followed by Huancavelica (14.7%) with 14/102 carcasses, and Junín (9.8%) with 18/181 carcasses. Regarding the markets, the Ferrocarril market presented the highest risk of contamination, serving as the reference group for comparison. In contrast, the Nazareth and Nueva Esperanza markets showed significantly lower odds of Sarcocystis sp. presence, with Odds Ratios (ORs) of 0.38 and 0.25, respectively. For muscle groups, the anatomical distribution of Sarcocystis sp. cysts revealed a preferential localization in the leg (OR = 1.65) and neck (OR = 1.20) compared to the shoulder. This investigation provides significant data on the prevalence of Sarcocystis sp. in alpacas and llamas, highlighting a higher prevalence in llamas despite their smaller sample size. These findings emphasize the need for targeted interventions to address this parasitic infection in camelid production systems.



Keywords: Animal products, Camelids, Carcass quality, Mantaro valley, Parasite.

#### INTRODUCTION

Peru boasts a diverse range of climatic conditions, coupled with ample availability of natural pastures (Estremadoyro et al., 2024), which serve as the primary food source for South American camelids. These animals exhibit remarkable efficiency in converting feed, requiring only 1.5 - 2% of dry matter (DM) relative to their body weight for survival (Coleman et al., 2010). With approximately 4.3 million alpacas and 1.2 million llamas, Peru sustains a substantial population of these valuable species (Catoa et al., 2016).

Sarcocystosis is a parasitic disease caused by protozoa of the genus Sarcocystis sp. (Fayer et al., 2015), this protozoan belongs to the domain Protozoa, phylum Apicomplexa, class Sporozoa, suborder Eimeriorina, and family Sarcocystidae (Yang et al., 2005), which affects a wide range of intermediate hosts, including domestic and wild animals (Amalfitano et al., 2017). In camelids, particularly alpacas (Vicugna pacos) and Ilamas (Lama glama), this parasitosis is of growing concern due to its significant impact on meat quality (Gareh et al., 2020), food safety, and public health (Fernandez-F et al., 2022; Rodríguez et al., 2023). The life cycle of Sarcocystis sp., involves both definitive and intermediate hosts, with the parasite undergoing asexual reproduction in the musculature of the intermediate host (Lucas et al., 2019; Wu et al., 2022), forming cysts that can compromise meat suitability for human consumption. Definitive hosts, such as domestic and wild canids, play a crucial role in the dissemination of the parasite, perpetuating its cycle (Lindsay and Dubey, 2020). Despite the cultural and economic importance of alpacas and llamas in the Andean regions, there is limited information on the prevalence and distribution of sarcocystosis in their carcasses (Shams et al., 2022), particularly in commercial markets. Previous studies have documented prevalence rates varying widely between regions and production systems, suggesting that environmental conditions, animal management practices, and market dynamics may significantly influence the epidemiology of this disease (Valentine and Martin, 2007; Condori-Quispe et al., 2019). However, these studies often lack granular insights into the role of specific factors, such as geographic location, market practices, and anatomical distribution of cysts, which are essential for designing targeted control strategies.

This study addresses a critical gap in knowledge by evaluating the prevalence of Sarcocystis macrocysts in alpaca (Vicugna pacos) and Ilama (Lama glama) carcasses marketed in three key regions of Peru: Lima, Junín, and Huancavelica

(Bartl et al., 2023). These regions are not only pivotal for livestock production but also showcase diverse environmental and commercial dynamics, which may influence the epidemiology of sarcocystosis (Ayala Vargas, 2018). Additionally, this research explores the association between the prevalence of *Sarcocystis* sp., and specific commercial markets, as well as the distribution of cysts across different muscle groups, providing a nuanced understanding of this parasitic disease. In recent years, the province of Huancayo has experienced a marked increase in the consumption of alpaca and llama meat (Caulfield et al., 2022). However, the scarcity of authorized slaughterhouses and suboptimal sanitary conditions in animal processing has created significant information gaps. These include limited data on the prevalence of *Sarcocystis* sp., as well as the economic losses associated with carcass seizures due to parasitic contamination.

In this context, the objective of this study was to determine the prevalence of *Sarcocystis* sp. in Ilama and alpaca carcasses marketed in Huancayo. Furthermore, it sought to evaluate the economic implications of carcass seizures caused by this parasitosis. The findings aim to provide valuable insights for the better management of camelid resources and to support the implementation of effective strategies for controlling this parasitic disease.

#### MATERIALS AND METHODS

#### Area study

The study was conducted in three markets located in the city of Huancayo, situated in the southern part of the Mantaro Valley at an altitude of 3,200 meters above sea level. The selected markets were Centro Comercial Ferrocarril (12°4'18.80"S, 75°12'17.47"W), Nueva Esperanza (12°4'12.28"S, 75°12'16.35"W), and Nazaret (12°4'12.94"S, 75°12'16.18"W) (Senamhi, 2023). These markets primarily offer Ilama and alpaca carcasses, which are sourced from various regions, including Lima, Huancavelica, and Junín. The climate of the study area is characterized by an average annual temperature of 11°C and a total annual rainfall of 625 mm. A map indicating the location of the markets is presented in Figure 1.



#### Samples

During the period from January to October 2023, a total of 2211 carcasses were collected, of which 1716 corresponded to alpacas and 495 to llamas. These were from 3 regions: Huancavelica (181/alpaca and 95/llama), Junín (820/alpaca and 108/llama), and Lima (715/alpaca and 292/llama). These carcasses from the 3 regions were received by 3 markets: Centro Comercial Ferrocarril (n = 572 alpaca, with 223, 126, and 223 for the neck, shoulder, and leg muscles, respectively; and 160 llama, with 52, 61, and 47 for the same muscles, respectively), Nazareth (n = 482 alpaca, with 154, 149, and 179 for the neck, shoulder, and leg muscles, respectively), and Nueva Esperanza (n= 662 alpaca, with 249, 168, and 245 for the neck, shoulder, and leg muscles, respectively; and 153 llama, with 53, 52, and 48 for the same muscles, respectively). This collection was carried out in collaboration with the sanitary control personnel from the Bromatology area of the Provincial Municipality of Huancayo.

#### Parasitological analysis

Macroscopic examinations were conducted on the neck, shoulder, and legs of each camelid (alpacas and llamas) to assess infection rates of macroscopic cysts (Regensburger et al., 2015). The detection of cysts involved meticulous and thorough visual inspections of the carcasses, ensuring accuracy and consistency in observations (Apaza Jimenez and Chipana Mendoza, 2021). These examinations were performed in designated markets and at the processing center managed by the National Agricultural Health Service (SENASA), adhering to standardized protocols to ensure the reliability of the results.

#### **Statistical analysis**

The prevalence of macrocysts in alpacas and llamas were calculated using the equation:

Prevalence (%) =  $\frac{\# \text{ positive cases}}{T \text{ otal number of individuals}} x100$ 

A generalized linear mixed model (GLMM) with binomial distribution was applied, with the response variable being the presence of the parasite (macrocysts). Random effect variables included Species (Alpaca and Llama), Region (Huancavelica, Junín and Lima) and arm (BRAZO), neck (CUELLO) and leg (PIERNA) muscles. Additionally, the logit function was used to calculate Odds Ratios (OR), providing a measure of association between the variables of interest. All statistical analyses and calculations were performed using Excel (Microsoft Office® v2013) and the open-source software R (Team et al., 2018), using the packages descTools, ROCR, agricolae and stats to ensure accurate and reproducible results.

#### **RESULTS AND DISCUSSION**

Table 1 presents the total number of carcasses examined per camelid. The results showed the presence of Sarcocystis sp. in both species, with a prevalence of 8.0% (138 carcasses) for alpacas and 21.0% (104 carcasses) for llamas. The results of this study provide valuable insights into the prevalence and distribution of Sarcocystis sp. in alpaca and llama carcasses, emphasizing species, regional, and market-specific differences, as well as muscle-specific predilections. These findings highlight critical epidemiological patterns and their implications for food safety and public health. Table 2 shows that the highest prevalence of sarcocystosis in alpacas was recorded in the Lima region (9.7%) with 69 out of 715 carcasses, followed by Huancavelica (7.7%) with 14 out of 181 carcasses, and Junín (6.7%) with 55 out of 820 carcasses. Similarly, concerning llamas, the Lima Region exhibited the highest prevalence of sarcocystosis (33.9%) with 72 out of 212 carcasses, followed by Huancavelica (14.7%) with 14 out of 102 carcasses, and Junín (9.8%) with 18 out of 181 carcasses. A significant difference (p < 0.05) in the prevalence of sarcocystosis was observed between Lima and Junín, as well as between Lima and Huancavelica in the case of alpacas. Similarly, significant differences (p < 0.05) in the prevalence of sarcocystosis were found between the Huancavelica region and the Lima and Junín regions in Ilama carcasses. It was observed that the regions of Lima are the most at-risk places for consumption. These results are similar to those reported by Castro and Leguía (1992) in Lima and by Santiago and Leguía (2018) in the Junín region. These findings highlight that, despite being recognized as prominent livestock areas, the measures implemented for the management and prevention of parasitosis have not yielded satisfactory results in terms of food safety. In this situation, it is imperative to prioritize control efforts, such as health education and implementing authorized slaughterhouses for more effective resource management, thus ensuring food safety and public health.

Table 1 - Prevalence of Sarcocystis sp. in alpace	a and llama carcasses.		
Carcasses Total examined Positive infected			
	Total examined	N	%
Alpaca	1716	138	8.0
Llama	495	104	21.0

Table 2 - Prevalence of Sarc	ocystis sp. in alpaca and llama ca	rcasses.		
Animal	Region	Total	Infected	%
	Huancavelica	181	14	7.7ª
Alpaca	Junin	820	55	6.7ª
	Lima	715	69	9.7ª
P-value				NS
	Huancavelica	102	14	<b>14.7</b> ª
Llama	Junín	181	18	<b>9.9</b> <sup>a</sup>
	Lima	212	72	33.9 <sup>b</sup>
P-value				**
Equal letters in the same column	do not differ significantly ( $p > 0.05$ ).			

Variable		N	Odds ratio	References	P values
SPECIES	ALPACA	1716	,	Ref.	
SFECIES	LLAMA	495	-	2.82 (2.06, 3.84)	<0.001
	HUANCAVELICA	276	•	Ref.	_
REGION	JUNIN	928	-	1.45 (0.85, 2.52)	0.179
	LIMA	1007	⊶∎→	3.79 (1.68, 8.69)	0.001
	FERRO	732		Ref.	_
MARKET	NAZA	664	⊢ <b>∎</b> ⊣	0.38 (0.22, 063)	<0.001
	NESPE	815	⊶∎→	0.25 (0.12, 0.51)	<0.001
	ARM	624		Ref.	_
MUSCLES	NECK	789	-	1.20 (0.81, 1.79)	0.364
	LEG	798	-	1.65 (1.17, 2.35)	0.005

In Table 3, the Odds Ratios (OR) are observed, with the Llama species obtaining a value of 2.82, indicating that llamas have a higher chance of the presence of Sarcocystis sp. macrocysts compared to Alpacas. Regarding the region, Lima shows the highest chance of reporting macrocysts, reaching an OR of 3.79. This suggests a significantly higher presence compared to other regions. The higher prevalence of Sarcocystis sp. in llamas (21.0%) compared to alpacas (8.0%) reflects significant differences in susceptibility between the two species. This discrepancy may be attributed to llamas' greater exposure to definitive hosts, such as domestic and wild canines, and their distinct grazing behaviors, which may increase the risk of infection (Rosenthal, 2021). Additionally, management practices, herd density, and environmental conditions likely play a role in the higher parasitic burden observed in llamas. These findings align with existing literature, which suggests that llamas are often more vulnerable to parasitic infections due to less intensive management systems compared to alpacas (Wu et al., 2022). A notable finding of this study is the significant regional disparity in the prevalence of Sarcocystis sp. Lima exhibited the highest prevalence among regions, with an OR of 3.79 compared to other locations, such as Junín and Huancavelica. This suggests that factors specific to Lima, including urbanization, higher population density, and suboptimal slaughterhouse conditions, may contribute to the elevated risk of contamination, an idea also shared by Rene et al. (2019). Moreover, the environmental conditions in Lima, characterized by more intensive camelid trade and market activity, likely exacerbate the exposure of animals to parasitic contamination (Raymond et al., 2020). The lower prevalence observed in Junín and Huancavelica may be explained by differences in climatic conditions, such as cooler temperatures and reduced rainfall, which may limit the survival of Sarcocystis sp. in the environment (Baitzel et al., 2022). The results also highlight significant differences in prevalence within regions for alpacas and llamas. For instance, Lima showed a prevalence of 9.7% in alpacas and 33.9% in llamas, compared to Junín, which had lower rates for both species. These regional variations underscore the need for tailored interventions that account for the specific epidemiological and environmental characteristics of each location (Jauregui et al., 2024).

The prevalence of *Sarcocystis* sp. also varied significantly among markets (Table 3). The Ferrocarril market showed the highest risk of contamination, serving as the reference group with which other markets were compared. Conversely, the Nazareth and Nueva Esperanza markets demonstrated significantly lower odds of *Sarcocystis* sp. presence, with ORs of 0.38 and 0.25, respectively. These differences may be attributed to variations in sourcing practices, transportation conditions, and sanitary measures implemented at each market (Fernandez-F et al., 2022; Rodríguez et al., 2023). Markets with better infrastructure and stricter quality control measures are likely to exhibit lower prevalence rates of parasitic infections (Baitzel et al., 2022). The findings suggest that market-specific factors, such as hygiene standards and meat handling protocols, play a critical role in determining the risk of contamination. The anatomical distribution of *Sarcocystis* sp. cysts revealed a preferential localization in the leg (OR = 1.65) and neck (OR = 1.20) compared to the shoulder. This finding is consistent with previous studies that report a higher prevalence of macrocysts in muscle groups with greater vascularization and proximity to infected tissues (Regensburger et al., 2015). The preferential accumulation of cysts in the leg and neck muscles may reflect physiological differences in blood flow or tissue composition, which facilitate parasite development and cyst formation (Lucas et al., 2019; Wu et al., 2022). From a practical standpoint, this information is essential for meat inspection processes, as it highlights the importance of focusing on high-risk anatomical sites during routine examinations.

#### CONCLUSION

The research reveals significant data on the prevalence of *Sarcocystis* sp. in alpacas and llamas, highlighting a higher presence in llamas despite their smaller number of examined samples. This finding suggests a potential risk to public

health, especially in areas like Lima, where the prevalence is notably high. The results also underscore the importance of addressing risk factors such as extreme weather conditions and commercial practices that may influence the spread of parasitosis. There is a clear need to implement effective control and resource management measures to ensure food safety and public health in these regions. These findings provide a solid foundation for future research and preventive actions in the region.

#### DECLARATIONS

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#### Authors' contribution

Edgar Garcia-Olarte: Execution of the research; Jordan Ninahuanca Carhuas: Statistical analysis and editing; Wilder Suarez-Reynoso and Wilhelm Guerra Condor: laboratory analysis; Yakelin Maurico-Ramos: data collection; María Flores Guillen: macroscopic analysis of cysts; Ide Unchupaico Payano: macroscopic analysis of cysts; Armando Aquino Tacza: carcasses non-monitoring.

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#### **Ethical approval**

The procedures and ethics of this research work were based on the international and national guidelines for the care and use of animals in the scientific research.

#### **Consent to publish**

All authors agree to the publication of this manuscript.

#### **Competing interests**

The authors have not declared any competing interest.

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## INFLUENCE OF FEATHER GENOTYPE, STORAGE DURATION AND TEMPERATURE ON THE EXTERNAL AND INTERNAL QUALITIES OF CHICKEN TABLE EGGS

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Supporting Information

ABSTRACT: A study was carried out to determine the influence of the feather genotype, storage duration, temperature and method on the internal and external qualities of chicken table eggs. A total of 864 table eggs collected from naked neck (Nanaff), frizzle (nanaFf) and normal feathered (nanaff) birds were used in the study. A Completely Randomized Design of four factors namely, feather genotypes, storage temperatures (5°C and 26°C), storage duration (0, 7, 14, 21 and 28 days) and storage methods (with or without vegetable oil application) was used. The GLM procedure of GenStat (17th Edition) was used to determine the effects of the four factors and their interactions on external qualities (egg weight, length, and width, shell weight and thickness) and internal qualities (albumen height and weight, yolk height, weight, diameter and colour and Haugh unit) of table eggs. The effect of chicken genotype on proximate composition and nutritional values of table eggs were also determined. Feather genotype had significant (P<0.05) effect on yolk colour and weight whilst storage duration, temperature and method had significant (P<0.05) effects on all the internal qualities of eggs studied except effect of storage duration on yolk colour. The 2-way and 3-way interactions of the factors studied were important sources of variation for many of the internal qualities of eggs studied. With the exception of storage temperature, the other factors studied had significant (P<0.05) effects on many of the external qualities of eggs. The interactions of the factors were not significant (P>0.05) sources of variation for most of the external qualities of eggs. Mutant feather genes (Na and F) positively influence egg qualities which could be utilised to segment the commercial chicken egg market.



Keywords: Feather, Frizzle, Naked neck, Nutritional value, Yolk colour,.

#### INTRODUCTION

Eggs contain nutrients which are essential for improving human health. Proper functioning of the body is impeded if essential amino acids, which are the main nutrients in eggs, are lacking. Chicken egg albumen and yolk are reported to contain essential amino acids (Ali et al., 2019; Attia et al., 2020). The United States Department of Agriculture (USDA) (2008) reported that eggs are rich foundations of minerals and vitamins.

A complete egg is composed of parts such as the shell (exterior and interior shell membranes), albumen, air cell, cuticle or bloom, chalazae, germinal disk, nucleus of pander, yolk and vitelline membrane (EDINFORMATICS, 2013). Egg quality is built around a number of traits including albumen height, albumen weight, yolk height, yolk diameter, yolk index, yolk weight, shell ratio, shell thickness, shell weight, egg length, egg weight, egg width and Haugh Unit (Murshed and Qaid, 2024). Several studies have reported significant and positive relationships among egg quality parameters of poultry (Zhang et al., 2005; Inca et al., 2020; Guni et al., 2021). Farooq et al. (2001a) reported positive and significant relationships among egg weight, egg width and egg length for eggs from Fayoumi birds. Similarly, largely positive correlations were reported among egg quality traits of two layer chicken breeds in South Africa (Tyasi et al., 2022). In Japanese qualis, Farooq et al. (2001b) reported that there were positive correlations among shell weight, egg weight and shell thickness of quail eggs. Several external factors such as cleanliness, freshness, egg weight and shell weight are important for consumers' acceptability of eggs (Hamilton, 1982; Sonaiya and Swan, 2004; Batkowska et al., 2023). Internal characteristics such as yolk index, Haugh Unit and chemical composition are also important in poultry breeding because of their influence on growth of chicks, breeding performance and egg quality for consumption (Yahaya et al., 2021). The external and internal quality traits of eggs of hens have influence on the hatchability of fertile eggs, and the weight and development of chicks (Sahan et al., 2014; Iqbal et al., 2016; Hegab and Hanafy, 2019).

The external and internal egg qualities are also influenced by storage duration and storage temperature. Eggs stored at low temperature maintain better egg quality (Samli et al., 2005). Egg weight, shell weight, albumen height, albumen viscosity, Haugh Unit and yolk colour decreased with increasing storage temperature of hens (Lee et al., 2016; Martínez et al., 2021). Eggs maintain qualities better when stored for a short period of time (Jin et al., 2011). Prolonged length of egg

storage deteriorates egg quality of chicken eggs (Nasri et al., 2019; Melo et al., 2020). Tebesi et al. (2012) reported that eggs were able to maintain higher yolk height when stored within 7 days.

The naked neck (*Na*) and frizzle (*F*) genes are two mutant thermoregulatory genes that aid chickens to adapt to high ambient temperatures in, especially, the tropics (Asumah et al., 2022). Layer chickens carrying the *Na* or *F* alleles have been reported to record higher percentage of fertile eggs (Asumah et al., 2022), increased egg production (Fathi et al., 2013; Adomako et al., 2014) and improved egg shell quality (Salahuddin and Howlider, 1991). However, El-Rahman and Makled (2006) reported reduction in shell quality in birds carrying *Na* alleles compared to birds with only *na* alleles.

Whilst there have been several studies on egg quality traits of frizzle, naked neck and normal feathered birds in Sub-Saharan Africa and other parts of the world (Salahuddin and Howlider, 1991; Abou-Emera et al., 2017; Fathi et al., 2022), these studies have barely focused on the nutrient contents of the eggs produced by the birds. In addition, information is scanty on the interactions between feather genotype and egg storage methods on the egg quality traits of chicken eggs. The objective of this study therefore was to determine the influence of feather genotype, storage duration, storage temperature and storage method on external and internal egg quality characteristics, amino acid profile and proximate composition of chicken table eggs.

#### MATERIALS AND METHODS

#### Location and duration

The research was conducted at Akate Farms and Trading Company Limited (AFTC) at Saaman, Kumasi, Ghana and the Department of Animal Science, Kwame Nkrumah University of Science and Technology within a period of six months.

#### Experimental birds and eggs

The experimental birds kept at the AFTC were offspring of crosses between naked neck and frizzle feathered cocks and hybrid commercial Lohmann hens. The naked neck and frizzle feathered, both heterozygotes, were bred with normal feathered Lohmann Brown classic layers in two separate matings to produce offspring which were heterozygous naked neck, heterozygous frizzle feathered and normal feathered chickens in the first filial (F1) generation. Eight hundred and sixty-four (864) table eggs were collected from the naked neck (*Nanaff*), frizzle (*nanaFf*) and normal feathered (*nanaff*) layer chickens (288 per genotype) kept as experimental birds by AFTC, Kumasi, Ghana. The layer birds were 28 weeks old at the start of the experiment. The external and internal egg qualities were determined after collection, using the procedures described by Fayeye et al. (2005).

#### **Experimental design**

A Completely Randomized Design in a 3x2x5x2 factorial was applied. Eggs were obtained from three genotypes being *Nanaff*, *nanaFf* and *nanaff*, stored at two storage temperatures (26°C and 5°C) for four storage durations with a control of 0 days (0, 7, 14, 21 and 28 days) using two storage methods (with or without the application of vegetable oil to the egg shells). For eggs which received oil treatment, Sunny vegetable oil manufactured in Ghana was applied by immersion.

The experiment was conducted in three phases and eggs were collected from the chicken genotypes which were housed in deep litter pens. The three chicken genotypes were placed into nine different pens, with each bird genotype put into three different pens labelled as treatments (T1, T2 and T3) with about 20 birds in each pen. A total of 864 table eggs from the three genotypes were further used in the study with 288 table eggs obtained from each genotype.

#### Parameters studied and their measurement

a. The egg width and length was measured using a pair of vernier calipers in centimetres.

b. Egg weight was measured with a digital electric balance in grams.

c. Egg shell thickness was measured with a micrometer screw gauge in mm. Shell thickness was calculated from the average of three measurements taken at the middle, broad end and the small end of the eggs.

e. Yolk diameter was measured with a vernier caliper in centimeters.

f. Yolk colour was determined with the DSM yolk colour fan (formerly Roche Yolk Color Fan). Higher figures indicate deeper yolk colour while lower figures indicate lighter yolk colour.

g. Yolk weight was determined with a digital weighing scale in grams.

h. Yolk height was determined by the use of a tripod spherometer.

i. Albumen weight was also determined by the use of a digital weighing scale.

j. Albumen height was determined with a tripod spherometer in mm.

k. Egg weight loss was determined by subtracting the final weight from the initial weight and expressed as a percentage.

I. Haugh unit was determined using the formula,  $HU = 100 \times \log (H + 7.57 - 1.7W^{0.37})$  introduced by Haugh (1937).

where HU = Haugh Units; H = Observed albumin height (mm); W = Observed weight of egg (g) (Roush, 1981).

Twenty-four table eggs from each of the three genotypes were analysed on each storage period (0, 7, 14, 21 or 28), storage method (with or without vegetable oil application).

Proximate composition of the eggs from the three genotypes was determined by drying egg samples (albumen and yolk) in an oven at 65°C for 72 hours. The dried samples were transferred to the Crops and Soil Science Laboratory, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology for the proximate composition analyses. The nutritional values of egg albumen from the three genotypes were analyzed by Evonik Nutrition South Africa Limited. Similar to the proximate analysis, the albumen was also dried in an oven at 65°C for 72 hours and later transferred to South Africa for the amino acid profile analyses.

#### Data analysis

The data on external, internal quality characteristics of eggs, and proximate composition and amino acid profiles of the albumen using the general linear model procedure of GenStat (17th Edition). The model used for the analysis of the data collected is presented below.

 $Y_{ijklm} = \mu + G_i + T_j + D_k + M_l + GT_{ij} + GD_{ik} + GM_{il} + TD_{jk} + TM_{jl} + DM_{kl} + GTD_{ijk} + GTM_{ijl} + TDM_{jkl} + e_{ijklm}$ 

Where Y<sub>ijklm</sub> = measured or calculated variables;

 $\mu$  = overall mean;

G<sub>i</sub> = fixed effect of the i<sup>th</sup> chicken genotype (naked neck, frizzle or normal feathered);

 $T_j$  = fixed effect of the j<sup>th</sup> storage temperature (26°C and 5°C);

 $D_k$  = fixed effect of the k<sup>th</sup> egg storage duration (0, 7, 14, 21 or 28 days);

M<sub>i</sub> = fixed effect of the I<sup>th</sup> egg storage method (with or without cooking oil treatment);

GT<sub>ij</sub> = fixed interaction of the i<sup>th</sup> genotype and the j<sup>th</sup> storage temperature;

GD<sub>ik</sub> = fixed interaction of the i<sup>th</sup> genotype and the k<sup>th</sup> storage duration;

GM<sub>il</sub> = fixed interaction of the ith genotype and the I<sup>th</sup> storage method;

TD<sub>jk</sub> = fixed interaction of the j<sup>th</sup> storage temperature and the k<sup>th</sup> storage duration;

TM<sub>jl</sub> = fixed interaction of the j<sup>th</sup> storage temperature and the l<sup>th</sup> storage method;

 $DM_{kl}$  = fixed interaction of the k<sup>th</sup> storage temperature and the l<sup>th</sup> storage method;

GTD<sub>ijk</sub> = fixed interaction of the ith genotype, jth storage temperature and the kth storage duration;

GTM<sub>ijl</sub> = fixed interaction of the i<sup>th</sup> genotype, j<sup>th</sup> storage temperature and the I<sup>th</sup> storage method;

TDM<sub>jkl</sub> = fixed interaction of the j<sup>th</sup> storage temperature, k<sup>th</sup> storage duration and l<sup>th</sup> storage method;

 $e_{ijklm}$  = random error term associated with each observation ~  $N(0, \sigma^2_e)$  where  $\sigma^2_e$  is residual variance.

Differences between means were separated using Tukey's Test at 5% probability level.

#### **RESULTS AND DISCUSSION**

#### Internal qualities of table eggs as influenced by genotype

The effect of chicken genotype on internal egg qualities of table eggs are presented in Table 1. There were no significant differences (P>0.05) among various genotypes in relation to albumen height, albumen weight, yolk diameter, Haugh unit and yolk height. The absence of significant differences for these parameters agrees with the findings of Rajkumar et al. (2009) who observed no significant differences in albumen height, albumen weight, yolk height (at 28 weeks old) and Haugh unit for *NaNa, Nana* and *nana* chicken genotypes in India. Udoh et al. (2012) also reported no significant difference (P>0.05) among three local genotypes in terms of yolk weight, albumen height and yolk height in Nigeria. Frizzle genotype recorded significantly (P<0.05) heavier yolk weight than normal feathered genotype, with the normal feathered showing the lowest value in this trait. The higher yolk weight for the frizzle eggs could probably be due to their efficient feed conversion ratio in converting protein for feather production into their eggs. The heavier yolk weight of eggs from frizzle feathered genotype in this study is contrary to the report of Yakubu et al. (2008) who reported that naked neck chicken eggs had heavier yolk weight compared to eggs from normal and frizzled feathered birds. However, Rajkumar et al. (2009) recorded a significantly (P<0.05) heavier yolk weight for normal feathered birds than naked neck ones in India, and noted that lower yolk weight in naked neck birds indicated lower fat percentage in these birds than their normal feathered counterparts. Non-significant (P>0.05) effect of feather genotype on yolk weight has also been reported by Udoh et al. (2012) and Ogundero et al. (2019) in Nigerian local indigenous chickens.

The yolk colour for naked neck and the normal feathered bird eggs were not significantly (P>0.05) different but both were significantly (P<0.05) different from the frizzle hens which recorded a lower yolk colour value (Table 1). Islam et al. (2011) recorded higher yolk colour values from Bangladesh naked neck chicken which is in agreement with the results of the current study. However, Rajkumar et al. (2009) reported higher yolk colour in normal feathered (8.00) and naked neck (7.49) than observed in the present findings. Yolk colour is probably controlled mainly by nutrition than genetics (Grashorn, 2016), hence the varying results for yolk colour as influenced by feather genotype in literature and this study.

Table 1 - Internal quality charactenstics of table eggs as influenced by chicken reather genotype, egg storage duration, egg storage temperature and storage method							
Items	Albumen height (mm)	Albumen weight (g)	Haugh unit (%)	Yolk colour	Yolk diameter, (cm)	Yolk height, (mm)	Yolk weight, (g)
Genotype							
Nanaff	4.52	33.74	60.36	<b>4.94</b> <sup>a</sup>	4.21	13.38	<b>17.37</b> ª
nanaFf	4.52	33.69	60.47	4.52 <sup>b</sup>	4.23	12.88	<b>17.48</b> ª
nanaff	4.43	32.30	59.92	<b>4.93</b> ª	4.22	13.18	<b>17.15</b> <sup>b</sup>
SEM	0.092	0.295	0.737	0.098	0.019	0.013	0.098
P-value	0.544	0.512	0.855	0.003	0.822	0.116	0.042
Storage duration (days)							
0	<b>4.82</b> ª	34.57ª	62.25ª	4.99	4.05°	<b>14.62</b> ª	17.36
7	4.49 <sup>b</sup>	34.28 <sup>ab</sup>	60.94 <sup>b</sup>	4.80	<b>4.19</b> °	<b>13.15</b> <sup>b</sup>	17.33
14	<b>4.44</b> <sup>b</sup>	33.75 <sup>b</sup>	59.95°	4.83	4.23 <sup>b</sup>	<b>12.91</b> °	17.25
21	<b>4.40</b> <sup>b</sup>	<b>32.58</b> ⁰	59.80°	4.60	4.25 <sup>b</sup>	<b>12.93</b> °	17.31
28	<b>4.31</b> ℃	<b>32.70</b> ℃	58.30 <sup>d</sup>	4.76	<b>4.41</b> <sup>a</sup>	<b>12.13</b> <sup>d</sup>	17.38
SEM	0.084	0.354	0.873	0.116	0.022	0.015	0.112
P-value	<0.001	<0.001	0.005	0.216	<0.001	<0.001	0.938
Storage temperature							
Refrigeration (0°C)	<b>5.21</b> <sup>a</sup>	<b>34.22</b> ª	68.27ª	<b>5.07</b> ª	4.00 <sup>b</sup>	15.13ª	<b>17.16</b> <sup>b</sup>
Room temperature (26°C)	3.77 <sup>b</sup>	32.93 <sup>b</sup>	52.32 <sup>b</sup>	<b>4.52</b> <sup>b</sup>	<b>4.46</b> ª	<b>11.15</b> <sup>b</sup>	<b>17.51</b> ª
SEM	0.247	0.059	0.617	0.082	0.015	0.144	0.079
P-value	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.008
Storage method							
Vegetable oil	<b>4.79</b> <sup>a</sup>	<b>33.60</b> ª	63.77ª	<b>4.89</b> ª	4.08 <sup>b</sup>	<b>14.08</b> ª	<b>17.44</b> ª
No oiling	3.85 <sup>b</sup>	32.86 <sup>b</sup>	54.00 <sup>b</sup>	4.55 <sup>b</sup>	4.39ª	<b>11.75</b> <sup>b</sup>	17.15 <sup>b</sup>
SEM	0.059	0.247	0.617	0.116	0.015	0.144	0.079
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

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#### Internal qualities of table eggs as influenced by storage duration

The effect of storage duration on the internal qualities of table eggs is presented in Table 1. Storage duration of eggs did not have any significant (P>0.05) effect on yolk colour and yolk weight. However, Jin et al. (2011) reported significant effect of storage time on yolk colour of laying hens at peak production. Duration of egg storage significantly (P<0.05) influenced albumen height, albumen weight, Haugh unit, yolk diameter and yolk height. Albumen height, albumen weight, Haugh unit and yolk height largely decreased with increase in length of egg storage. The importance of storage duration on albumen height in this study corroborates the findings of Raji et al. (2009) and Santos et al. (2019) who observed decline in albumen height with increase in storage length. Akinola and Ibe (2014) and Abioja et al. (2021) also reported similar findings to the present study. Tebesi et al. (2012), however, reported different findings with eggs stored for 14 days showing higher albumen height. Yolk diameter significantly (P<0.05) increased with increase in storage length. This could be due to the expansion of yolk as storage length increases. This finding agrees with results of Abioja et al. (2021) in FUNAAB-a chicken eggs. The reduction in yolk height with increase in storage length could be attributed to loss in moisture from the yolk resulting in shrinkage of the yolk. The current result agrees with Raji et al. (2009) and Tebesi et al. (2012) who reported higher yolk height for day 7. Similarly, the decline in Haugh unit with increase in storage duration is an indication of deterioration of egg quality. USDA (2000) reported that higher Haugh unit determines the protein content and freshness of eggs. Several authors (Raikumar et al., 2009; Raii et al., 2009; Akinola and Ibe, 2014; Abioia et al., 2021) have presented results of higher Haugh unit with reduced storage duration of eggs which corroborate the findings from this work.

#### Internal qualities of table eggs as influenced by storage temperature

Results of the influence of storage temperature on internal egg quality is presented in Table 1. Albumen height, albumen weight, Haugh unit, yolk height, yolk colour, yolk diameter and yolk weight were significantly (P<0.05) affected by storage temperatures.

Albumen height was higher for eggs stored in a refrigerator than those stored under room temperature. This finding corroborates the results of Scott and Silversides (2000) and Samli et al. (2005) who observed increased albumen height for refrigerated eggs compared to eggs stored at room temperature. This could be attributed to the fact that eggs stored in a refrigerator maintain better albumen quality than those stored at room temperature. Refrigeration of eggs enhance the ability to retard carbon dioxide loss and breakdown of carbonic acid to carbon dioxide leading to the maintenance of egg quality (Qin et al., 2024). The heavier weight of the albumen for refrigerated eggs could be due to the prevention of evaporation of moisture from eggs stored in a refrigerator as a result of low temperature. The retention of Mucin fiber in the albumen of eggs stored in a refrigerator could have prevented the albumen from becoming watery and losing weight (Mountney, 1976). Khan et al. (2013) noted that albumen quality deterioration could be due to the effect of evaporation of moisture and carbon dioxide from the egg when stored under room temperature.

The mean yolk weight of eggs stored in a refrigerator was significantly heavier (P<0.05) than those stored at room temperature. This could be due to the retention of moisture in the yolk of eggs stored in a refrigerator. Samli et al. (2005) observed that there was a decrease in yolk weight with increase in storage temperature. Eggs stored at room temperature showed significantly lower yolk colour value (4.52) than eggs stored in a refrigerator (5.07) and this agrees with Jin et al. (2011) who reported significant effect of storage temperature on yolk colour. Yolk diameter also exhibited a significant difference (P<0.05) with eggs stored at room temperature showing higher yolk diameter than the ones stored in a refrigerator. Yolk height was significantly higher (P<0.05) for eggs stored in a refrigerator than eggs stored at room temperature. This result was similar to the finding of Raji et al. (2009) who recorded higher yolk height for eggs stored in a refrigerator compared to those stored at room temperature. Haugh unit showed a significant difference (P<0.05) with eggs stored at room temperature. Haugh unit showed a significant difference (P<0.05) with eggs stored at room temperature. Haugh unit showed a significant difference (P<0.05) with eggs stored in a refrigerator recording higher Haugh unit (68.27) than eggs stored at room temperature (52.32). The higher Haugh unit indicates the freshness of eggs stored in a refrigerator as Haugh unit value determines the changes of the interior qualities of eggs. Park et al. (2003) and Grashorn et al. (2016) also recorded a decrease in Haugh unit for eggs stored under high temperature. The present result is similar to that of Dudusola (2009) and Raji et al. (2009) who recorded higher Haugh unit values for eggs stored in a refrigerator compared to eggs stored in a refrigerator compared to eggs stored at eremines the changes of the interior qualities of eggs. Park et al. (2003) and Grashorn et al. (2016) also recorded a decrease in Haugh unit for eggs stored under high t

#### Internal qualities of table eggs as influenced by storage method

The effect of storage method on internal egg quality traits is presented in Table 1. There were significant (P<0.05) differences in internal egg qualities between eggs coated with vegetable oil and those that were not coated with vegetable oil. This difference could be attributed to the fact that oil has the ability to seal egg pores, preventing evaporation of moisture and carbon dioxide from the eggs during storage. Eggs coated with vegetable oil had heavier albumen weight and higher albumen height values than those that were not coated with vegetable oil. This might be due to the retention of moisture within the albumen of oiled eggs in the absence of osmotic pressure as observed by Orji et al. (1981). Eggs coated with vegetable oil were significantly higher (P<0.05) in yolk weight and yolk height than those eggs stored without vegetable oil and these values could be due to increase of fat within the yolk through absorption or reduction in moisture evaporation from the yolk. Raji et al. (2009) also observed higher yolk height in eggs of laying hens in a dry climate stored with vegetable oil.

The yolk colour was significantly (P<0.05) higher for oiled eggs (4.89) compared to eggs stored without oil application (4.55). The significantly higher yolk colour value observed for oiled eggs indicates that eggs stored after oil application maintained better yolk colour than eggs stored without oil application. Yolk colour has effect on the nutritional value of eggs. Eggs stored without oil application showed significantly higher (P<0.05) yolk diameter than those stored after vegetable oil application. The higher yolk diameter indicates the spread of yolk as a result of moisture loss from the yolk. This could be attributed to the evaporation of moisture from the eggs during storage as a result of high temperature. Oil helps to seal the various pores on the eggs preventing evaporation of moisture during storage.

Haugh unit showed a significant difference (P<0.05) with eggs coated with vegetable oil showing higher value (63.77) than eggs without vegetable oil (54.00). Güçlü et al. (2008) observed Haugh unit similar to the current results in Table 1, with eggs stored with fish oil showing higher (P<0.05) Haugh unit than other storage methods. Dudusola (2009) reported results that were similar to the current study. The current result also agrees with the finding of Grobas et al. (2001) and Eke et al. (2013) who reported significantly (P<0.05) higher Haugh unit for eggs stored with oil.

## Internal qualities of table eggs as influenced by the two-way and three-way interactions of feather genotype, storage duration, storage temperature and storage method

The P values of the influence of the two and three-way interactions among genotype, storage duration, storage temperature and storage method on internal egg quality traits are presented in Table 2. No significant interaction of genotype by storage duration on internal egg qualities was observed except for yolk weight (0.008). This suggests that variation in yolk weight of chicken genotypes is dependent on the storage durations of the eggs. Significant interaction (0.034) of genotype and storage method on yolk colour of eggs was also observed.

Storage duration x storage temperature had significant effect on all the internal egg qualities studied except yolk colour. This is in agreement with other studies who have reported important storage duration x temperature influence on Haugh unit (Chung and Lee, 2014), albumen height (Samli et al., 2005), albumen weight and yolk weight (Jin et al., 2011). However, Jin et al. (2011) reported significant storage duration x storage time interaction on yolk colour which was contrary to the finding in this study. The variation between the two studies could be attributed to the differences in the breeds of chicken used.

Yolk height was significantly higher (P<0.05) for eggs coated with vegetable oil during storage; although the yolk height values slowly decreased with increase in storage time. Eggs stored without oil application rapidly deteriorated in yolk height as storage time increased. This result agrees with Tebesi et al. (2015) and Raji et al. (2009) who recorded higher yolk height values for eggs stored with oil application for shorter periods of time.

Storage temperature x storage method had significant (P<0.05) effect on albumen height, Haugh unit, yolk diameter, yolk height and yolk weight. There were significantly higher (P<0.05) albumen height values for oiled eggs stored in refrigerator as compared to eggs stored without oil application under room temperature. The eggs coated with oil stored at room temperature or refrigerator also recorded higher albumen height values than eggs stored at room temperature or refrigerator without oil application. This indicates that eggs coated with vegetable oil and stored in a refrigerator maintained better albumen quality possibly due to the prevention of moisture loss by evaporation thus retention of moisture in the albumen as the oil seals the egg pores. Dudusola (2009) reported that eggs coated with oil and refrigerated eggs did not lose much solvent as compared with those in polythene bag and uncoated. The significant variations in yolk weight and diameter due to storage temperature x storage method corroborate the findings of Dudusola (2009) and Orji et al. (1981) respectively who observed increased in yolk weight and diameter as a result of increase in storage temperature and storage time. The increased yolk weight during storage at room temperature could be due to movement of water from albumen to the yolk due to some high pressures. In addition, the significant variation in yolk height due to storage temperature x storage method agrees with Raji et al. (2009) who recorded higher yolk height values for oiled eggs stored at low temperature. Similarly, the important variation in Haugh unit due to storage temperature x storage method is an indication that changes in egg quality due to storage temperature is dependent on the presence or absence of oil application on the egg.

The three-way interactions of the factors studied were significant sources of variations for some of the internal egg parameters except yolk colour (Table 2). The explanation of some of these complex interactions could be quite complicated.

#### External qualities of table eggs as influenced by chicken genotype

The effect of genotype on external egg qualities are presented in Table 3. Chicken genotype had significant (P<0.05) effect on all the external qualities of eggs studied except egg weight. The differences in the external qualities could be attributed to the differences in the alleles controlling the genotypes. These results corroborate the findings of Egahi et al. (2013) who also found significant (P<0.05) effect of genotype on all the external egg qualities studied including egg weight. Rajkumar et al. (2009) however observed no significant differences in shell weight and egg weight for *NaNa*, *Nana*, and *nana* genotypes in India. Udoh et al. (2012) also reported no significant difference (P>0.05) among three local genotypes in terms of shell thickness in Nigeria.

Naked neck showing higher shell thickness value could be attributed to the result of their feed intake and feed conversion ratio. As the naked neck take in more feed, calcium from the feed is being converted into the egg shell thereby making their shell thicker than the other birds. However, the frizzle and the normal feathered birds showed no significant difference (P>0.05) in terms of shell thickness. The current result for naked neck was similar to that of Nwachukwu et al. (2006), who also recorded shell thickness between 0.30 mm to 0.34 mm in naked neck, frizzle and normal feathered birds. Yakubu et al. (2008) observed 0.38 mm of shell thickness in naked neck chickens from Nigeria which was higher than the values realized in the present study (0.31 mm). Egahi et al. (2013) also reported shell thickness of 0.33 mm in naked neck, 0.36 mm in frizzle, and 0.32 mm in normal feathered birds.

Table 2 - P-values of the 2 and 3-way interactions among the effects of feather genotype, storage duration, storage
temperature and storage method on internal egg quality characteristics

Source of variation	Albumen helght (mm)	Albumen weight (g)	Haugh unit (%)	Yolk colour	Yolk diameter, (cm)	Yolk height, (mm)	Yolk welght, (g)
Genotype*SD	0.994	0.384	0.505	0.986	0.099	0.112	0.008
Genotype*ST	0.937	0.408	0.945	0.936	0.958	0.715	0.856
Genotype*SM	0.467	0.467	0.580	0.034	0.397	0.583	0.568
SD*ST	0.052	<0.001	0.035	0.623	<0.001	<0.001	0.003
SD*SM	0.830	0.493	0.830	0.643	1.000	<0.001	0.126
ST*SM	0.017	0.696	<0.001	0.144	<0.001	<0.001	<0.004
Genotype*SD*SM	0.183	0.018	0.308	0.657	0.375	0.010	0.488
Genotype*SD*ST	0.077	0.140	0.111	0.423	0.506	0.020	0.173
Genotype*ST*SM	0.300	0.850	0.350	0.670	0.040	0.561	0.402
SD*ST*SM	0.043	0.824	0.008	0.655	<0.001	<0.001	0.008
<sup>1</sup> SD: Storage duration; ST: Storage time; SM: Storage method							

 Table 3 - External qualities of table eggs as influenced by genotype, storage duration, storage temperature and storage method

Factors		Shell thickness	Shell weight	Egg weight	Egg length	Egg width
		(mm)	(g)	(g)	(cm)	(cm)
Geno	otype					
I	Nanaff	0.30a	6.01ª	61.46	5.90 <sup>a</sup>	<b>4.32</b> <sup>a</sup>
I	nanaFf	0.25 <sup>b</sup>	6.09 <sup>a</sup>	61.21	5.81 <sup>b</sup>	<b>4.33</b> ª
I	nanaff	0.22 <sup>b</sup>	5.95 <sup>b</sup>	60.95	5.80 <sup>b</sup>	<b>4.28</b> <sup>b</sup>
:	SEM	0.004	0.042	0.333	0.016	0.010
I	p-value	<0.001	0.043	0.569	0.035	0.003
Stora	age duration					
(	0	<b>0.27</b> ª	6.16ª	<b>63.21</b> ª	5.95ª	<b>4.37</b> ª
-	7	<b>0.27</b> ª	6.02 <sup>b</sup>	<b>61.45</b> ⁵	<b>5.88</b> <sup>a</sup>	<b>4.31</b> <sup>a</sup>
:	14	<b>0.27</b> ª	5.96 <sup>b</sup>	61.34 <sup>b</sup>	<b>5.87</b> ª	<b>4.31</b> <sup>a</sup>
:	21	0.26ª	5.96 <sup>b</sup>	60.04°	5.83 <sup>b</sup>	<b>4.28</b> <sup>b</sup>
1	28	0.23 <sup>b</sup>	5.99 <sup>b</sup>	59.98°	5.85 <sup>b</sup>	<b>4.28</b> <sup>b</sup>
	SEM	0.005	0.049	0.395	0.019	0.012
1	p-value	<0.001	0.043	<0.001	<0.001	<0.001
Storage Temperature						
I	Refrigeration	0.26	6.02	61.45	5.86	4.30
1	Room Temperature	0.25	6.01	60.96	5.86	4.32
	SEM	0.004	0.034	0.279	0.013	0.009
1	p-value	0.901	0.943	0.467	0.326	0.643
Stora	age method					
١	Vegetable oil	<b>0.27</b> ª	6.05	<b>61.50</b> ª	5.87	4.30
1	No oiling	0.24 <sup>b</sup>	5.98	60.40 <sup>b</sup>	5.86	4.31
9	SEM	0.003	0.035	0.300	0.013	0.009
	p-value	<0.001	0.176	<0.001	0.709	0.752
<sup>abc</sup> Me	ans within the same sub-colu	mn with different subscript	ts are significant at I	P<0.05.		
#### External qualities of table eggs as influenced by storage duration

Storage duration significantly (P<0.05) influenced shell thickness and weight, egg weight, length and width (Table 3). Shell thickness was lower (P<0.05) for eggs stored for 28 days compared to all the other storage durations. The significant variation in shell thickness due to storage duration is in agreement with the report of Grashorn et al. (2016) but contrary to the finding of Lee et al. (2016) who reported non-significant effect of storage duration on shell thickness. Shell weight and egg weight significantly (P<0.05) decreased with increase in length of storage duration and this corroborates the findings of Samli et al. (2005), Jin et al. (2011), Akinola and Ibe (2014) and Lee et al. (2016). The loss in weight is attributed to water loss through evaporation from the pores in the egg shell and escape of carbon dioxide from the egg albumen (Samli et al., 2005). Dudusola (2009) also indicated that the loss of egg weight due to prolonged storage might be due to loss of carbon dioxide, ammonia, nitrogen, hydrogen sulphide gas and water from the eggs.

#### External qualities of table eggs as influenced by storage temperature and storage method during storage

The external egg qualities were not significantly (P>0.05) different between the two storage temperatures. This finding does not agree with report of Raji et al. (2009) who observed higher egg weight for eggs stored in the refrigerator than those stored under room temperature. Oil application during egg storage had important (P<0.05) effect on shell thickness and egg weight (Table 3). Eggs with oil application had thicker shells and heavier egg weight than those without oil application. The higher shell thickness of oil coated eggs is in agreement with Raji et al. (2009) who also reported higher shell thickness values for eggs coated with oil during storage. The high shell thickness of oiled eggs is due to the layer of oil applied on the shells. In addition, the heavier egg weights of oil applied eggs compared to non-oil applied eggs is probably due to the reduction in moisture loss through the pores on the shells.

# External qualities of table eggs as influenced by the two-way and three-way interactions of genotype, storage duration, storage temperature and storage method

All the two-way and three-way interactions of genotype, storage duration, storage temperature and storage method on the external qualities of eggs were not significant (P>0.05) except the interaction of storage duration x storage method on shell thickness, storage temperature x storage method on egg weight and genotype x storage duration x storage temperature on egg weight (Table 4). The significant (P<0.05) interaction of storage duration x storage method observed in this study corroborates the findings of Tebesi et al. (2012) and Akinola and Ibe (2014) but contrary to the report of Raji et al. (2009).

temperature and storage method on external egg qualities								
Factors	Shell thickness (mm)	Shell weight (g)	Egg welght (g)	Egg length (cm)	Egg width (cm)			
Genotype*SD	0.989	0.702	0.477	0.156	0.550			
Genotype*ST	0.870	0.876	0.939	0.577	0.045			
Genotype*SM	0.910	0.736	0.986	0.069	0.580			
SD*ST	0.513	0.841	0.401	0.256	0.034			
SD*SM	0.007	0.386	0.237	0.632	0.590			
ST*SM	0.100	0.279	0.031	0.765	0.502			
Genotype*SD*SM	0.390	0.500	0.420	0.464	0.886			
Genotype*SD*ST	0.756	0.763	0.009	0.164	0.142			
Genotype*ST*SM	0.820	0.930	0.460	0.402	0.511			
SD*ST*SM	0.453	0.142	0.166	0.685	0.642			
<sup>1</sup> SD: Storage duration; ST: Storage time; SM: Storage method								

 Table 4 - P-values of the 2 and 3-way interactions among the effects of genotype, storage duration, storage temperature and storage method on external egg qualities

#### Effect of genotype on the proximate composition of egg albumen and egg yolk (as-fed basis)

Table 5 shows the effect of chicken genotype on proximate composition of chicken egg albumen. There were no significant (P>0.05) difference among chicken genotypes for all the proximate compositions of egg albumen except ether extract (EE). The EE content of egg albumen from frizzle feathered hens were significantly lower (0.08%) than those of normal feathered and naked neck birds. There was no significant (P>0.05) difference among chicken genotypes with respect to the proximate composition of egg yolk except for ash content. Eggs from frizzle feathered hens recorded higher levels of ash compared to those from the naked neck and normal feathered birds.

#### Effect of chicken genotype on the amino acid profile of table egg albumen and egg yolk

There were no significant differences (P>0.05) among the chicken genotypes with respect to amino acid profile of the egg albumen (Table 6) and egg yolk (Table 7). The absence of significant differences among frizzle, naked neck and normal feathered birds with regard to amino acid profiles in the albumen and yolk might be due to the similarity of diet fed to the birds and the same environmental conditions under which they were raised.

Egg part	Moisture (%)	NFF (%)	<b>Ash</b> (%)	FF (%)	CF (%)	CP (%)
Genotype			71011 (70)	(//)	••• (,,•)	••• (,••)
Egg albumen						
Nanaff	89.04	5.14	0.27	0.18ª	0.02	5.35
nanaFf	89.39	4.93	0.19	0.08 <sup>b</sup>	0.03	5.38
nanaff	88.66	5.49	0.17	0.20 <sup>a</sup>	0.02	5.45
SEM	0.27	0.23	0.04	0.01	0.04	0.13
P-value	0.18	0.24	0.12	<0.01	0.21	0.83
Egg yolk						
Nanaff	57.06	6.78	<b>1.24</b> <sup>b</sup>	27.08	0.06	7.78
nanaFf	57.00	6.95	<b>1.58</b> ª	26.86	0.08	7.53
nanaff	57.17	6.74	<b>1.16</b> <sup>b</sup>	27.05	0.07	7.81
SEM	0.09	0.18	0.09	0.18	0.03	0.05
P-value	0.40	0.70	0.01	0.67	0.86	0.20

Table 6 - Effect of feather genotype on amino acid profile as a percentage of egg albumen

	Genotype		nonoff	CEM	Duckus
Amino acid profile		nt nanart	папап	SEM	P-value
ALA (%)	0.5	9 0.59	0.59	0.01	0.95
ARG (%)	0.43	3 0.44	0.45	0.01	0.41
ASP (%)	1.02	2 1.03	1.03	0.01	0.78
CYS (%)	0.1	5 0.14	0.15	0.04	0.58
GLU (%)	1.29	9 1.31	1.32	0.02	0.50
GLY (%)	0.3	5 0.35	0.35	0.03	0.90
HIS (%)	0.24	4 0.24	0.25	0.03	0.76
ILE (%)	0.5	0.50	0.50	0.01	0.86
LEU (%)	0.83	2 0.84	0.84	0.01	0.92
LYS (%)	0.54	4 0.53	0.56	0.01	0.38
MET (%)	0.3	1 0.31	0.31	0.06	0.94
MET + CYT (%)	0.4	5 0.45	0.46	0.06	0.57
PHE (%)	0.5	7 0.59	0.59	0.08	0.82
PRO (%)	0.34	4 0.34	0.35	0.04	0.32
SER (%)	0.64	4 0.64	0.65	0.08	0.62
THR (%)	0.43	3 0.42	0.43	0.03	0.75
VAL (%)	0.6	7 0.67	0.67	0.01	0.90

<sup>1</sup>SEM – Standard Error of Means; P-value: Probability Value; ALA: Alanine, ARG: Arginine; ASP: Aspartic acid; CYS: Cystine; GLU: Glutamic acid; GLY: Glycine; HIS: Histidine; ILE: Isoleucine; LEU: Leucine; LYS: Lysine; MET: Methionine; MET+CYS: Methionine+Cystine; PHE: Phenylalanine; PRO: Proline; SER: Serine; THR: Threonine; VAL: Valine.

	Table 7 - Effect	of genotype or	i amino acid	profile of e	egg volk
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	Genotype	Nonoff	n e n e 156	nonoff	CEM	Duralua
Amino acid profile		Nanaπ	nanart	nanaπ	SEM	P-value
ALA (%)		0.77	0.75	0.75	0.01	0.23
ARG (%)		0.56	0.56	0.57	0.01	0.72
ASP (%)		1.33	1.31	1.31	0.01	0.08
CYS (%)		0.19	0.18	0.18	0.01	0.66
GLU (%)		1.68	1.67	1.68	0.01	0.48
GLY (%)		0.46	0.46	0.45	0.01	0.45
HIS (%)		0.32	0.31	0.31	0.03	0.40
ILE (%)		0.65	0.64	0.63	0.01	0.63
LEU (%)		1.07	1.07	1.06	0.01	0.24
LYS (%)		0.70	0.68	0.71	0.01	0.39
MET (%)		0.40	0.39	0.39	0.01	0.48
MET + CYT (%)		0.59	0.57	0.58	0.01	0.31
PHE (%)		0.76	0.75	0.75	0.01	0.39
PRO (%)		0.45	0.43	0.45	0.03	0.19
SER (%)		0.84	0.82	0.83	0.01	0.39
THR (%)		0.55	0.55	0.55	0.03	0.08
VAL (%)		0.87	0.86	0.85	0.01	0.49
<sup>1</sup> SEM: Standard Error of Means; P-	value: Probability	Value; ALA: Alan	ine, ARG: Arginine;	ASP: Aspartic acid	; CYS: Cystine; GL	U: Glutamic acid;

PRO: Proline; SER: Serine; THR: Threonine; VAL: Valine.

#### CONCLUSION

Naked neck and frizzle genes had positive influence on egg quality traits. Shorter storage duration had positive influence on egg qualities during storage. Eggs stored at low temperature showed positive results in terms of internal egg qualities. Eggs coated with vegetable oil also showed better egg quality during storage. Naked neck recorded heavier egg weight than frizzle and normal feathered in their interactions with storage duration and temperature. Refrigerator and vegetable oil showed better yolk quality in their interactions with storage duration. Information from this study could be used in the preservation of the internal and external qualities of table eggs from chicken.

#### DECLARATIONS

This study is part of the thesis of the first author. The complete thesis is available in the library of of the Kwame Nkrumah University of Science and Technology (KNUST). No part of this thesis is however published in any journal or conference proceedings.

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#### Data availability

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

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None.

#### **Ethics Approval**

All experimental procedures which involved the use of birds were conducted in compliance with the ARRIVE guidelines.

#### Authors' contribution

Kanasuah DN performed the field work, analysed the data and drafted the manuscript, Adomako K designed the study, edited that manuscript and approved the final manuscript, Hagan BA designed the study, analysed the data and wrote the manuscript. Olympio OS edited the manuscript.

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#### **Competing interests**

None of the authors of this article has any competing interests in the publication of this article.

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# PRIMAL CUTS OF CARCASS AND MEAT CHARACTERISTICS OF KACANG GOAT FED TOTAL MIXED RATION CONTAINING DIFFERENT SOURCES OF RUMINALLY UNDEGRADED PROTEIN

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Supporting Information

ABSTRACT: This study was designed to evaluate the effect of feed quality improvement using gliricidia and different sources of protein in total mixed ration (TMR) on the primal cuts, loin eye area, and fatty acids profile of goat meat. This study used twenty yearling Kacang goats weighing 17.42±1.63 kg. The goats were randomly allocated into 4 different treatments in a completely randomized design. The treatments involved the use of natural grass from rangeland (NGFR; control) as well as improving the quality of feed through TMR containing various ruminally undegraded protein sources, i.e. TMR contains fish meal (TMR-FM), TMR contains soybean meal (TMR-SBM) and TMR contains formaldehyde treated soybean meal (TMR-TSBM). The parameters observed were primal cuts yield, loin eye area, meat, fat, bone of primal cuts, and fatty acids profile. Data were analyzed using a one-way analysis of variance. The results showed that the goats fed TMR-FM and TMR-TSBM produced significantly higher meat percentage than control goats. The meat yield of TMR-SBM and TMR-TSBM goats were significantly higher than those of control goats. Goats fed TMR-SBM produced the highest primal cuts yield and shoulder weight, while the weight of rib, loin, and leg of TMR-SBM goats were similar to those of TMR-TSBM goats. Loin eve area was similar between the treatments. Saturated fatty acids content in TMR groups was similar to those in control. It can be concluded that improved feed quality using TMR-SBM produced significantly higher primal cuts weight, while TMR-TSBM had better meat-tobone ratio than control. TMR-TSBM goats produced significantly leaner meat than TMR-SBM goats. Fatty acid profiles were similar between treatments.

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#### INTRODUCTION

Goats are widely raised traditionally by farmers in Indonesia. They are usulally kept in the yard and fed natural forages from the rangeland, so that they have low growth rate. Nevertheless, they produce high quality of meat (Slimeni et al., 2022) due to their high content of unsaturated fatty acids (Makmur et al., 2020). Goat meat is considered a healthier meat compared to other red meat products for its leaness (Slimeni et al., 2022; Gawat et al., 2023), and has a higher ratio of polyunsaturated fatty acids to saturated fatty acid (PUFA/SFA) when fed with forage-based diet (Lee et al., 2023). Lee et al. (2023) reported that dietary nutrient contents influence fatty acids composition of Korean native black goat meat, and forage-based diet increased PUFA to SFA ratio. To increase the goat production, Slimeni et al. (2022) added concentrate and hay for pasture grazing goats. Low production of goats kept extensively was caused by the scarcity of good quality forages, limited of herbaceous plants and the low quality of shrubs (Slimeni et al., 2022). Goats are selective eaters, preferring wild grass over soft grass (Lee et al., 2019) and showing a preference for woody plants (Chebli et al., 2022). Fish meal (FM) and soybean meal (SBM) are common protein sources in animal diet. Fish meal tends to be less degraded in the rumen than SBM (Falahatizow et al., 2015), but it is more expensive than SBM. Additionally, goats do not favor diets containing FM (Adiwinarti et al., 2016). Therefore, a better option is to use total mixed ration (TMR) for blending feed ingredients, ensuring that animals cannot selectively choose the feed based on their preference (Fluharty et al., 2017; Santana et al., 2017). Total mixed ration can be formulated based on the animals need. Santana et al. (2017) stated that TMR could maintain nutrient content in the ration. Protein in SBM is easily degraded in the rumen (Wang et al., 2021; Phesatcha et al., 2022) and provide about 65% of rumen-degradable protein (Phesatcha et al., 2022). In order to improve carcass weight, primal cuts and meat yield, protein in feed must be efficiently utilized by reducing rumen protein digestibility, so that post-rumen utilization of protein is more optimal. There have been many studies have been done to reduce the degradability of SBM in the rumen. Rooke et al. (1983) reported that degradability of formaldehydetreated SBM was lower than that of untreated SBM. Widyobroto et al. (2010) stated that formaldehyde protected SBM increased rumen undegraded protein about 50-80%, but the formaldehyde protected SBM did not decrease the degradability in the small intestine. This study was set up to improve carcass weight, primal cuts yield, meat yield, and goat meat quality by enhanching the feed quality using TMR. Natural grass from rangeland (forage-based diet) was used as control to represent the common practice of goat rearing in the rural area, while improved feed quality involved TMR consisting of Napier grass, gliricidia, and concentrate with different protein sources (fish meal, SBM, and formaldehydetreated SBM). The objectives of this study were to evaluate the carcass weight, primal cuts yield, meat yield, and meat

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quality of goats fed with forage-based diet compared to those fed with higher-quality feed (TMR containing different protein sources: fish meal, SBM, and formaldehyde-treated SBM).

#### MATERIALS AND METHODS

#### Animals, feeds, and experimental design

Twenty yearling Kacang goats with the initial body weight of 17.42±1.63 kg were reared 95 days before being slaughtered. The goats were randomly allocated into 4 different treatments using completely randomized design. The treatments were: 100% natural grass from rangeland (NGFR) as the control and 3 improved feed quality used total mixed ration consisted of 30% Napier grass, 30% gliricidia leaves, and 40% concentrate with different sources of protein, i.e. TMR containing fish meal (TMR-FM), TRM containing soybean (TMR-SBM) and TMR containing formaldehyde treated soybean meal (TMR-TSBM) (Table 1). A 1% of formaldehyde treated soybean meal was made by using formalin and it was formulated to contain 1% of formaldehyde that was used to treat SBM in dry matter bases calculation.

#### Animals care and management

In order to protect from internal and external parasites, all goats were treated orally with 1.5 mL/head of Valbendazol and injected by 0.5 mL/head of Intermectin. Goats were housed individually, provided feed and water *ad libitum* given at 8:00 AM, 12:00 PM, and 4:00 PM.

Table 1 - The composition of feed ingredients, chemical composition and Fatty acids composition (%) of the rations							
Parameters	NGFR	TMR-FM	TMR-SBM	TMR-TSBM			
Feed ingredients composition							
Natural grass from rangeland	100	-	-	-			
Napier grass	-	30	30	30			
Gliricidia leaves	-	30	30	30			
Concentrate feed consisted of:	-	40	40	40			
Cassava waste product		50.2	48.0	48.0			
Wheat bran		34.4	34.5	34.5			
Fish meal		15.4	0	0			
Soybean meal		0	17.5	0			
Formaldehyde treated soybean meal		0	0	17.5			
Chemical composition							
Dry matter	18.6	91.3	91.5	91.4			
Crude protein	10.9	15.3	15.6	14.3			
Total digestible nutrients	63.2	56.2	58.0	60.1			
Fatty acids composition							
Myristic acid (C14:0)	14.76	1.70	0.00	3.95			
Palmitic acid (C16:0)	53.62	29.02	28.56	28.93			
Stearic acid (C18:0)	12.54	11.97	9.34	10.62			
Palmitoleic acid (C16:1)	0.00	0.64	0.00	0.57			
Oleic acid (C18:1)	3.80	18.76	19.85	14.27			
Linoleic acid (C18:2)	15.27	24.37	29.50	28.48			
Linolenic acid (C18:3)	0.00	13.54	12.75	13.19			
SFA (saturated fatty acids)	80.93	42.69	37.91	43.50			
MUFA (mono unsaturated fatty acids)	3.80	19.40	19.85	14.84			
PUFA (poly unsaturated fatty acids)	15.27	37.91	42.25	41.66			
UFA (unsaturated fatty acids)	3.71	36.48	32.63	29.78			
NGFR: natural grass from rangeland, TMR-FM: total mixed ratio mixed ration containing formaldehyde treated soybean meal	on containing fish meal,	TMR-SBM: total mixed	ration containing soybea	n meal, TMR-TSBM: total			

During the experimental period, two goats from the TMR-FM and TMR-TSBM groups became ill and died, resulting in only 18 goats being available for data collection. After a treatment periods of 95 days, the goats were deprived of feed and given only clean water for 12 hours. Subsequently, the goats were weighed and slaughtered. Goats were slaughtered to obtain carcass, which was then cooled at a temperature of 4°C for 12 hours before further carcass observation were conducted. Carcass were cut and the primal cuts (shoulder, rib, loin, leg) were dissected for meat, fat, and bone separation to observe weight and percentage of carcass composition (meat, fat, and bone). Loin eye areas were measured following the method of Rezende et al. (2020).

#### Sampling and sample analysis

Samples from *Biceps femoris* muscle were frozen before fatty acids analysis. Fatty acids composition was analyzed using Gas Chromatography-mass Spectrometry (Stashenko and Martinez, 2014).

#### Statistical analysis

Data were analyzed by a one-way analysis of variance using significance level based on p<0.05 (Steel and Torrie, 1980). If there was a significantly different between the treatments, Duncan's Multiple Range test was used for further analysis (Steel and Torrie, 1980).

Parameters	NGFR	TMR-FM	TMR-SBM	TMR-TSBM	P-value
Performance					
Dry matter intake (g)	485.27 <sup>b</sup> ±58.31	620.71 <sup>ab</sup> ±47.54	740.24ª±132.16	648.00 <sup>a</sup> ±106.29	0.007
Protein intake (g)	53.00°±6.37	94.72 <sup>b</sup> ±7.25	115.43°±20.61	92.40 <sup>b</sup> ±15.16	0.0001
Total digestible nutrients intake (g)	309.28 <sup>b</sup> ±42.89	349.55 <sup>ab</sup> ±40.33	438.19ª±101.57	388.55 <sup>ab</sup> ±58.86	0.053
Average daily gain (g)	28.92°±7.05	57.56 <sup>b</sup> ±21.42	78.54ª±10.23	56.19 <sup>b</sup> ±4.94	0.0001
feed conversion ratio	17.60ª±4.67	12.52 <sup>ab</sup> ±6.35	9.45 <sup>b</sup> ±1.30	11.48 <sup>b</sup> ±1.11	0.035
Slaughter weight (kg)	20.04 <sup>c</sup> ±1.66	20.59 <sup>c</sup> ±1.72	25.09ª±1.33	22.97 <sup>bc</sup> ±0.55	0.0001
Carcass weight (g)	7, <b>1</b> 74.80°± <b>1</b> ,076.00	7,979.75 <sup>bc</sup> ±1,288.16	10,042.20ª±1,021.03	8,882.75 <sup>ab</sup> ±703.26	0.005
Primal cuts (% carcass)	74.25±2.02	73.68±1.32	75.48±1.36	72.47±3.13	0.218
Primal cuts yield (g)	5,322.29°±770.19	5,868.42 <sup>bc</sup> ±863.04	7,573.89ª±699.17	6,434.48 <sup>b</sup> ±541.31	0.002
Shoulder (g)	1,983.02 <sup>b</sup> ±323.04	2,271.63 <sup>b</sup> ±547.55	2,842.38ª±252.23	2,314.69 <sup>b</sup> ±280.65	0.015
Rib (g)	553.98 <sup>b</sup> ±125.82	595.60 <sup>b</sup> ±57.94	850.42ª±214.31	723.95 <sup>ab</sup> ±41.91	0.019
Loin (g)	636.04 <sup>b</sup> ±168.13	670.56 <sup>b</sup> ±89.49	925.54ª±85.19	826.59 <sup>ab</sup> ±169.77	0.016
Leg (g)	2,149.26 <sup>b</sup> ±289.18	2,330.63 <sup>b</sup> ±227.17	2,955.55ª±491.55	2,569.25 <sup>ab</sup> ±261.80	0.014
Primal cuts composition					
Meat (g)	3,758.10°±560.60	4,371.55 <sup>bc</sup> ±789.55	5,458.95°±445.56	4,815.94 <sup>ab</sup> ±384.64	0.002
Meat (%)	70.58 <sup>b</sup> ±1.24	74.21ª±2.53	72.17 <sup>ab</sup> ±3.16	74.87ª±0.51	0.039
Fat (g)	381.17 <sup>b</sup> ±133.70	322.29 <sup>b</sup> ±56.58	661.89ª±186.73	420.71 <sup>b</sup> ±182.34	0.019
Fat (%)	7.06±2.00	5.59±1.29	8.72±2.33	6.47±2.38	0.182
Bone (g)	1,183.02±163.40	1,174.58±89.05	1,453.04±243.81	1,197.83±120.25	0.069
Bone (%)	22.36ª±2.40	20.19 <sup>ab</sup> ±1.79	19.11 <sup>b</sup> ±1.70	18.67 <sup>b</sup> ±1.88	0.053
Edible portion (%)	77.64 <sup>b</sup> ±2.40	79.81 <sup>ab</sup> ±1.79	80.89 <sup>a</sup> ±1.70	81.33ª±1.88	0.053
Meat-bone ratio	3.19 <sup>b</sup> ±0.36	3.70 <sup>ab</sup> ±0.44	3.81 <sup>ab</sup> ±0.46	4.04 <sup>a</sup> ±0.39	0.043
Meat+fat-bone ratio	3.51±0.47	3.98±0.44	4.27±0.46	4.40±0.55	0.058
Loin eye area (cm <sup>2</sup> )	4.75±1.32	5.47±1.82	7.72±2.96	7.22±1.80	0.140

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# Table 3 - Fatty acids concentration of goat meat

Parameters	NGFR	TMR-FM	TMR-SBM	TMR-TSBM	P-value	Average
SFA (%)	52.77±1.63	43.45±8.92	42.25±6.72	42.93±10.27	0.127	45.59
Myristic acid (%)	1.24±0.89	1.92±1.69	1.38±1.04	1.29±0.64	0.798	1.44
Palmitic acid (%)	21.43±1.72	19.12±5.45	17.59±3.21	21.87±3.36	0.259	19.95
Stearic acid (%)	30.10±1.29	22.40±2.25	23.28±5.19	19.77±13.34	0.176	24.20
MUFA (%)	46.07±2.09	49.40±11.89	57.06±6.76	56.34±10.80	0.169	52.15
Palmitoleic acid (%)	1.58±0.97	1.66±0.76	1.61±0.84	<b>1.82±1.10</b>	0.981	1.66
Oleic acid (%)	44.50±2.32	47.74±11.14	55.45±7.47	54.52±9.83	0.153	50.49
PUFA (%)	<b>1.16±0.60</b>	0.77±0.91	0.69±0.34	0.73±0.55	0.602	0.85
Linolenic acid (%)	0.16±0.35	0.44±0.75	0.19±0.21	0	0.521	0.17
Arachidonic acid (%)	1.00±0.52	0.33±0.29	0.51±0.16	0.73±0.55	0.167	1.44
UFA (MUFA+PUFA) (%)	47.23±1.63	56.55±8.92	57.75±6.72	57.07±10.27	0.127	54.41
MUFA/SFA	0.88±0.07	1.19±0.45	1.41±0.45	1.43±0.68	0.235	1.22
PUFA/SFA	0.02±0.01	0.02±0.03	0.02±0.01	0.02±0.01	0.856	0.02

NGFR: natural grass from rangeland; TMR-FM: total mixed ration containing fish meal; TMR-SBM: total mixed ration containing soybean meal; TMR-TSBM: total mixed ration containing formaldehyde treated soybean meal; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids.

#### RESULTS

After being raised for 95 days, the performance of the goats including feed intake, growth rate, and feed conversion ratio are presented in the Table 2. Those parameters were significantly different between the treatments (P<0.01). Carcass weights and primal cuts yield also showed significant differences between the treatments (P<0.01). Carcass weights and primal cut percentages were similar across all treatments. Additionally, there were significant differences in the meat-to-bone ratio of primal cuts between the treatments (P<0.05). The fatty acids composition (%) of the rations is presented in the Table 1. The main fatty acids identified in goat meat in this study included myristic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid, linolenic acid, and arachidonic acid. Myristic acid, palmitic acid, stearic acid are grouped into saturated fatty acids (SFA), while palmitoleic acid and oleic acid belong to the category of mono-unsaturated fatty acids (MUFA), and linolenic acid are poly-unsaturated fatty acids (PUFA). There were no significant differences observed in the fatty acids content between the treatments (Table 3).

#### DISCUSSION

#### Productivity of goats fed natural grass and total mixed ration

Carcass weight of goats fed TMR containing SBM and TSBM were higher than those of control goats (NGFR) (Table 2). The higher carcass weight because of their average daily gain were higher than those of control goats. In addition, the faster growth of TMR goats was caused by the more dry matter and protein intake (Table 2).

Primal cuts were used as a parameter of goat production in this study because they represent about 74.07±2.16% of the total carcass. Primal cuts consisted of shoulder, rib, loin, and leg (Rezende et al., 2020). Total primal cuts yield (g) and shoulder (g) of TMR-SBM were higher than other treatments, while the weight of rib, loin, and leg (g) of TMR-SBM were similar to those of TMR-TSBM. This indicated that improved feed quality using TMR-SBM and TMR-TSBM produced higher product than control.

Goats in TMR-SBM and TMR-TSBM produced more primal cuts meat compared to control. In fact, the meat percentage of TMR-TSBM and TMR-FM was higher than those of control (Table 2). Goats from TMR-SBM and TMR-TSBM had bone percentage that was lower than control. In addition, the meat-to-bone ratio and the percentage of edible portion in TMR-TSBM were higher compared to the control group. However, the fat content in TMR-SBM was the highest (p<0.05) compared to the other treatments. These findings suggest that goats fed TMR produced better product than those in the control group, particularly when TMR containing SBM and TSBM. The meat from TMR-TSBM goats also appeared leaner compared to the meat from the TMR-SBM goats. Bambou et al. (2021) stated that diet is one of the factors influencing meat production. Goats fed with TMR-TSBM might increase the bypass protein level, thereby enhancing meat production. Rooke et al. (1983) reported that the degradation rate of formaldehyde-treated SBM decreased from 0.90 (untreated SBM) to 0.40 (formaldehyde-treated SBM). Widyobroto et al. (2010) stated that formaldehyde protected SBM can increase rumen undegraded protein by about 50-80%. However, it is essential to note that formaldehyde treatment did not reduce the protein degradation ability in the small intestine. Therefore, undegraded rumen protein can be efficiently utilized to enhance meat production. Additionally, tannin in gliricidia might reduce the digestibility of dry matter, organic matter, and crude protein as reported by Aguerre et al. (2016). Adiwinarti et al. (2019) reported that retained protein to meat conversion ratio of goat fed ration containing 50% untreated SBM+50% formaldehyde treated SBM is better than those of goats fed a diet containing untreated SBM or those of goats fed a diet containing treated SBM.

The meat content (%) in this study was relatively similar to the findings of Bambou et al. (2021), who reported values ranging from approximately 69.1% to 75.1%. The meat-to-bone ratio of primal cuts in TMR goats was higher compared to the meat-to-bone ratio reported by Cruz et al. (2023), which ranged between 3.08 to 3.28. However, Bambou et al. (2021) reported that the muscle-to-bone ratio in the left shoulder is between 2.8 and 4.2.

Loin eye area of goats was relatively similar between the treatments. Loin eye area of NGFR (4.75 cm<sup>2</sup>) and TMR-FM (5.47 cm<sup>2</sup>) were smaller than those reported by Cruz et al. (2023), but those of TMR-SBM (7.72 cm<sup>2</sup>) and TMR-TSBM (7.22 cm<sup>2</sup>) were relatively similar. Some researchers reported that loin eye areas are 7.35-8.00 cm<sup>2</sup> in goat having 21.8 to 23.4 kg of body weight (Cruz et al., 2023), 8.51-9.36 cm<sup>2</sup> in growing period and 12.81-13.01 cm<sup>2</sup> in fattening period of Nubian goats (Chen et al., 2022), 13.91-15.12 cm<sup>2</sup> in goat with body weight of 44.9 to 48.4 kg (Kafle et al., 2021).

#### Fatty acids of goats fed natural grass and total mixed ration

The main SFA of goat meat in this study consisted of stearic and palmitic acid, while the main UFA was oleic acid. Previous studies also reported that the dominant fatty acids in goat meat are palmitic acids, stearic acids, and oleic acids (Kafle et al., 2021; Akbas et al., 2022), along with linoleic acid and arachidonic acids (Kim et al., 2019). According to Kafle et al. (2021), palmitic acids, stearic acids, and oleic acids contribute approximately 80-85% of the total fatty acids.

In this study, goat meat had a higher content of SFA and MUFA compared to PUFA, aligning with the findings reported by Dinh et al. (2021). The high presence of SFA in ruminants was attributed to the biohydrogenation process in their

rumens. Slimeni et al. (2022) also reported that extensively raised goats had a higher proportion of SFA, while semiintensively raised goats produced more MUFA in goat meat.

The SFA content in goat meat from the goats in control group was relatively similar to that in the TMR group (Table 3), although the SFA content in natural grass from rangeland (control) was higher than those in TMR diets (Table 1). The average SFA content in TMR goat meat was 42.83% that was lower than the findings in Bambou et al. (2021) or Slimeni et al. (2022), but comparable to Lee et al. (2023) and higher than in Akbas et al. (2022). Slimeni et al. (2022) reported that extensively raised goat had 50.3% SFA, while semi intensively raised goat had 44.6% SFA. Bambou et al. (2021) reported SFA content in Creole goat meat ranging from about 42.8% to 52.6%. Lee et al. (2023) reported an SFA concentration of 42.48%, while Akbas et al. (2022) reported around 38.9%. Akbas et al. (2022) stated that low concentration of lauric acids, miristic acids, palmitic acids, and stearic acids indicated better meat quality. The concentration of UFA in the TMR group was relatively similar to those reported by García et al. (2019) and Lee et al (2023). The MUFA concentration in this study was higher compared to the findings reported by García et al. (2019): 42.9%, Akbas et al. (2022): 43.53%, and Lee et al. (2023): 36.27%. Meanwhile, the concentration of PUFA was lower compared to the results of García et al. (2019), Akbas et al. (2022), and Lee et al. (2023). Akbas et al. (2022) reported a PUFA content of 14.85%, Lee et al. (2023) reported 21.25%, García et al. (2019) reported 6.57%.

In this study PUFA/SFA ratio of forage diet (NGFR) was similar to other treatments. However, Lee et al. (2023) mentioned that different diets can influence the fatty acid composition of native Korean black goats meat, and a foragebased diet can enhance the PUFA/SFA ratio. The PUFA/SFA ratio in this study was lower compared to other researchers (0.08 reported by Guzmàn et al. (2020) and between 0.1 to 0.15 reported by García et al. (2019).

The ratio of MUFA to SFA between treatments did not show significant differences. The MUFA/SFA ratio in goats fed with TMR (1.19 to 1.43) was higher compared to the findings of García et al. (2019), Guzmàn et al. (2020) and Akbas et al. (2022) who reported MUFA/SFA ratios ranging from 0.32 to 0.83 (Guzmàn et al., 2020), 0.81 to 0.92 (García et al., 2019), and 1.07 to 1.15 (Akbas et al., 2022).

#### CONCLUSION

In conclusion, improving feed quality by using TMR-SBM (total mixed ration containing soybean meal) resulted in higher primal cuts weight, while TMR-TSBM (total mixed ration containing formaldehyde treated soybean meal) displayed a better meat-to-bone ratio compared to the control. Goats fed with TMR-TSBM produced leaner meat compared to those fed with TMR-SBM. The fatty acid profiles were similar between the treatments.

#### DECLARATIONS

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#### **Ethical considerations**

This experiment procedures related to the use of animals have been approved by the committee of animal ethics in the Animal and Agricultural Science, Universitas Diponegoro (59-04/A-08/KEP-FPP) and the authors have complied with the ARRIVE guidelines.

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#### Authors' contribution

R.Adiwinarti designed the research, supervised the fieldwork, analyzed the data, prepared and wrote the manuscript. Kustantinah and Rusman participated in designing the research, reviewed and edited the manuscript.

E.Rianto and A.Purnomoadi participated in supervising the fieldwork, reviewed and edited the manuscript.

M.Arifin and Sutaryo contributed to review and edit the manuscript.

V.Restitrisnani supervised the fieldwork and lab work.

All authors have read and approved the final manuscript.

#### **Competing interests**

There are no competing interests regarding the publication of this article.

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# HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF CAPTIVE FALLOW DEER (*Dama dama*) IN A ZOO ENVIRONMENT

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Supporting Information

**ABSTRACT**: Accurate health assessment of wild, semi-captive, or domesticated animals is essential for their well-being. Despite this necessity, limited studies have been conducted on deer species, and there is a paucity of information on the hemato-biochemical parameters of different deer species globally. Present study aimed to fill this gap by determining the hematological and serum biochemical parameters of fallow deer (*Dama dama*) maintained in semi-captivity within zoo environments for the first time in Bosnia and Herzegovina. Present research involved six healthy male fallow deer, aged 2 to 5 years. The deer were immobilized using xylazine hydrochloride and ketamine hydrochloride, and blood samples were collected from the external jugular vein. The hematological parameters measured included RBC, PCV, HGB, MCV, MCH, MCHC, RDW, RETIC, WBC, WBC differential, PLT, MPV, PDW, and PCT. Biochemical parameters included glucose, urea, creatinine, albumin, triglycerides, cholesterol, and enzymes (AST, ALT, ALKP, and GGT) activities. The results showed the higher glucose and urea concentrations and the same values for creatinine, triglycerides, and enzyme activities when compared to some previous reports. These findings highlighted the importance of considering handling methods and environmental conditions when interpreting biochemical parameters, contributing to improved health assessments and management practices for deer in captivity.

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Keywords: Biochemical and hematological parameters, Captive wildlife, Domesticated animals, Fallow deer.

Abbreviations: ALB: Albumin; ALKP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BASO: Basophils; CHOL: Cholesterol; CREA: Creatinine; EDTA K: Ethylenediaminetetraacetic Acid Potassium Salt; EOS: Eosinophils; GGT: Gamma-Glutamyl Transferase; GLU: Glucose; HGB: Hemoglobin; LYM: Lymphocytes; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean Corpuscular Volume; MONO: Monocytes; MPV: Mean Platelet Volume; NEU: Neutrophils; PCT: Plateletcrit; PCV: Packed Cell Volume; PDW: Platelet Distribution Width; PLT: Platelets; RBC: Red Blood Cells; RDW: Red Cell Distribution Width; RETIC: Reticulocytes; TG: Triglycerides; WBC differential: White Blood Cells Differential; WBC: White Blood Cells.

#### INTRODUCTION

Accurately assessing the health of wild, semi-captive, or domesticated animals is crucial for their wellbeing. However, there have been very few studies conducted on deer species, and limited reports exist on the haemato-biochemical parameters of different deer species worldwide (Gupta et al., 2007; Sinanović et al., 2013; Vukšić et al., 2016).

While normal hematological and serum biochemical values for several deer species are limited in the literature, some studies have established reference ranges for certain wild species. For instance, Rosef et al. (2004) provided hematological and serum biochemical reference values for free-ranging red deer (Cervus elaphus atlanticus) in Norway. Similarly, Miller et al. (2013) reported biochemical and hematologic reference values for free-ranging, chemically immobilized wild Norwegian reindeer (Rangifer tarandus tarandus) during early winter. Additionally, Karpiński et al. (2023) presented hematology, and serum chemistry values for free-ranging roe deer (Capreolus capreolus) in Poland. These studies provide valuable baseline data for health assessments and disease diagnosis in these species. However, for other deer species lacking specific reference values, comparisons are often made using baseline data from domestic small ruminants such as sheep and goats (Gupta et al., 2007).

The fallow deer (*Dama dama*) is a native Eurasian wild species of cervid (Pastrana et al., 2022) and among the most common cervid species in Europe and the most widely distributed cervid globally. Although the fallow deer has been introduced to most parts of Europe, it is native only to southern Anatolia, Sicily, southern Italy, and the southern Balkan peninsula. However, distribution data for the fallow deer in Bosnia and Herzegovina is not available, as they are only found in reserves (Bijl and Csányi, 2022). Fallow deer are the most common deer species found in both wild and captive environments in Bosnia and Herzegovina.

Several studies have identified differences in blood values among deer, which can be attributed to various factors including farming conditions, management practices, and sampling techniques. Methods such as collection from pasture, yarding, drafting, indoor confinement, isolation, and catheterisation have been shown to induce stress in deer (Vengušt et al., 2006). These variations in blood values can also result from genetic, environmental, nutritional, and physiological factors, as well as the stress of capture and the influence of different blood sampling techniques (Vengušt et al., 2006).

Assessing the health of wild, semi-captive, or domesticated animals is vital, yet studies on deer species and their haemato-biochemical parameters are limited (Gupta et al., 2007). Differences in blood values among deer have been linked to farming conditions, management, and sampling techniques (Vengušt et al., 2006). In the absence of specific data, comparisons are often made with baseline values from domestic small ruminants like sheep and goats (Gupta et al., 2007).

Fallow deer are the most common deer species found in both wild and semi-captive environments in Bosnia and Herzegovina. They are residents of forested areas and are also kept in semi-captivity in zoos. The data reported here detail the typical hematological and serum biochemical parameters of clinical importance for a deer species raised in captivity. Specifically, these parameters pertain to fallow deer maintained in captivity within zoo environments.

#### MATERIALS AND METHODS

The research was conducted on six male fallow deer (*Dama dama*), aged between 2 and 5 years, housed in the zoo in Sarajevo – Pionirska dolina, Sarajevo, Bosnia and Herzegovina; 43°52'41.8"N 18°24'44.1"E; elevation 518 m). The animals were healthy, well-fed, and clinically healthy. The deer were immobilized using a combination of 100 mg xylazine hydrochloride and 300 mg ketamine hydrochloride. Following immobilization, blood was collected from the external jugular vein. For hematological analysis, blood samples were collected into tubes containing the EDTA K as an anticoagulant. Hematological analysis was conducted within 2 to 3 hours after sampling using an automated veterinary hematology analyzer – The ProCyte IDEXX, PRC 1025236.

The following parameters were determined: red blood cells (RBC), packed cell volume (PCV), hemoglobin (HGB), the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), the mean corpuscular hemoglobin concentration (MCHC), the red blood cell distribution width (RDW), reticulocyte count (RETIC), white blood cells (WBC), white blood cells differential(WBC-D), the platelets (PLT), the mean platelet volume (MPV), the platelet distribution width (PDW), and the plateletcrit (PCT). Plain tubes were used to collect serum for the analysis of biochemical parameters, including glucose, urea, creatinine, albumin, triglycerides, cholesterol, and the activity of enzymes AST, ALT, ALKP, and GGT. Serum biochemical parameters were determined by the IDEXX Catalyst One veterinary chemistry analyzer, REF 89-92525-00.

#### **Ethical Regulations and animal welfare**

In present study, all procedures involving animals were conducted in accordance with recognized standards for animal welfare and ethical research. The fallow deer (*Dama dama*) were housed in managed care within a zoo environment, specifically in the Pionirska Dolina Zoo in Sarajevo, Bosnia and Herzegovina. Their health and well-being were closely monitored by experienced veterinarians, ensuring that all animals were kept in optimal conditions and handled according to established welfare protocols. Blood sampling was performed as part of routine veterinary care, and all immobilization and handling techniques were aimed at minimizing stress and discomfort to the animals. As the blood sampling occurred during routine health monitoring, it did not require specific approval from an ethical committee. However, all efforts were made to follow best practices for animal care and welfare, and the animals were never subjected to unnecessary pain or distress.

#### **Statistical analysis**

The results were analysed statistically. The values are presented throughout as a mean value and standard deviation (SD). Statistical analysis was performed by means of the SPSS package (SPSS Inc., Chicago, Illinois, USA).

#### **RESULTS AND DISCUSSION**

The hematological and biochemical analysis of six male fallow deer (*Dama dama*) maintained in a zoo environment revealed several notable findings. The glucose and urea concentrations were higher compared to some previous studies, while creatinine, triglyceride, and enzyme activity levels remained consistent with prior reports. The hematological parameters, including RBC, PCV, HGB, MCV, MCH, and MCHC, aligned closely with previously published values.

Table 1 - Mean values of haematology parameters in male fallow deer

Parameters	Mean ± SD
RBC (×1012/L)	$10.81 \pm 2.06$
PCV (L/L)	44.3 ± 37.1
HGB (g/L)	143.3 ± 23
MCV (fL)	41.2 ± 1.48
MCH	<b>14.27 ± 0.6</b>
MCHC	34.67 ± 0.59
RDW	31.97 ± 2.8
%RETIC	0
RETIC	1.13 ± 1.27
WBC (×109/L)	$1.95 \pm 0.94$
%NEU	56.27 ± 6.26
%LYM	23.03 ± 2.47
%MONO	5.6 ± 2.1
%EOS	13.23 ± 2.43
%BASO	<b>1.87 ± 0.25</b>
NEU	$1.07 \pm 0.44$
LYM	$0.46 \pm 0.26$
MONO	$0.11 \pm 0.06$
EOS	$0.27 \pm 0.17$
BASO	$0.04 \pm 0.02$
PLT	265.33 ± 92.45
MPV	7.77 ± 0.51
PDW	$6.55 \pm 0.35$
PCT	0.21 ± 0.07

Table 2 - Mean values of biochemistry parameters in male fallow deer						
Parameters	Mean ± SD					
GLU (mmol/L)	9.71 ± 2.35					
UREA (mmol/L)	$6.31 \pm 2.04$					
CREA (µmol/L)	<b>127 ± 20.58</b>					
ALB (g/L)	48 ± 2.67					
TG (mmol/L)	0.52 ± 0.46					
CHOL (mmol/L)	$1.99 \pm 0.12$					
AST (U/L)	99 ± 36.78					
ALT (U/L)	36 ± 5.87					
ALKP (U/L)	<b>212 ± 19.87</b>					
GGT (U/L)	38 ± 6					

Existing literature provides data on the blood parameters of deer which vary depending on the sampling technique used, including chemical immobilization (Peinado et al., 1999; Poljičak-Milas et al., 2004), physical restraint (Rehbein et al., 1999), or post-culling (Vengušt et al., 2002). The RBC values determined by present research are slightly lower than those determined by Tajchman et al. (2023) and the values determined by Vukšić et al. (2016), but correspond to the values determined by Venguš et al. (2006) for deer after sedation and culling.

Present study measured PCV, HGB, MCV, MCH, and MCH levels in deer, aligning closely with the findings with the values reported earlier (Vengušt et al., 2006) following sedation and culling. Comparable values were also reported by Kováč et al. (1997), as well as by Barić Rafaj et al. (2011) in red deer. Vukšić et al. (2016) noted that the hemoglobin concentration ranged from 157.00 to 164.00 g/L, averaging 160.50 g/L, which was similar to the concentration observed in present study but higher than that reported by Vengušt (2002). Present research identified significantly lower WBC values compared to other studies (Vengušt et al., 2006; Vukšić et al., 2016). Vukšić et al. (2016) determined the average leukocyte count in adults (7.07 x 10^9/L), which was higher compared to young deer, indicating that age influences the value of this parameter.

The PLT value was determined in fallow deer to be  $265.33 \pm 92.45$ , while Vukšić et al. (2016) reported a mean PLT value of 161.78. Additionally, Barić-Rafaj et al. (2011) found a PLT value of  $262 \pm 118$  in adult red deer. The significant difference between present findings and those of Vukšić et al. (2016), might be attributed to variations in sampling methods, environmental conditions, the health and physiological status of the animals, or differences in the populations studied. Interestingly, present PLT values are closer to those reported by Barić-Rafaj et al. (2011) for adult red deer, suggesting that species differences or age-related factors might play a role. Further investigation is needed to understand these discrepancies and to establish more comprehensive reference ranges for fallow deer and other cervids in different environments.

Red cell distribution width (RDW) measures the variation in erythrocyte size using their MCV (Mean Corpuscular Volume). Baric-Rafaj et al. (2011) in their study on farmed red deer, determined that the RDW was significantly higher in fawns. Although RDW determination is widely accepted in human medicine, there is limited information about this parameter in veterinary medicine, particularly concerning wild animals. The values for WBC and the differential blood count are presented in Table 1. Vukšić et al. (2016) determined a WBC value of 7.07 in adult fallow deer. Barić-Rafaj et al. (2011) found a WBC value of 15.41  $\pm$  4.87 in 11 adult red deer. Vengušt et al. (2006) reported WBC values in fallow deer as follows: 9.1  $\pm$  1.2 for restrained deer, 3.6  $\pm$  0.9 for tranquilized deer, and 2.9  $\pm$  1.3 for shot deer. In contrast, present research determined a WBC value of 1.95  $\pm$  0.94.

These differences highlight the significant variability in WBC counts depending on factors such as species, handling methods, and the physiological state of the animals. Present findings, which show lower WBC values compared to other studies (Vengušt et al., 2006) may be influenced by the specific conditions under which our samples were taken. This underscores the importance of considering these variables when interpreting hematological data and establishing reference ranges for wildlife.

Present research also determined values of some biochemical parameters (Table 2). Vengušt et al. (2006) determined the glucose values in deer as follows:  $2.9 \pm 0.4 \text{ mmol/L}$  for restrained deer,  $8.5 \pm 2.1 \text{ mmol/L}$  for tranquilized deer, and  $7.5 \pm 3.2 \text{ mmol/L}$  for deer that were shot. These values indicate that glucose levels vary significantly depending on the method of restraint or sedation. The glucose values determined in present research correspond to the values found for tranquilized deer. However, in reserch conducted by Vengušt and Bidovec (2002) authors described lower glucose values compared to those determined in present research. This discrepancy highlights potential differences in environmental conditions, handling, or physiological states of the deer between the studies.

The serum glucose concentration in fallow deer may exhibited significant individual variation (Rehbein et al., 1999; Slavica et al., 2000). Wilson and Pauli (1983) reported similar results in red deer. Compared to domestic ruminants, deer have higher serum glucose levels, which may be due to their more nervous temperament or higher metabolic rate (Wilson and Pauli, 1983).

Present research determined urea concentration of  $6.31 \pm 2.04 \text{ mmol/L}$  in deer, which presents an interesting comparison to the values obtained by other studies (Vengušt et al., 2006). While present methodology also involved the use of sedation, obtained values are somewhat different from those reported by Vengušt et al. (2006). In previous studies, the urea values were reported as follows:  $9.8 \pm 3.2 \text{ mmol/L}$  for restrained deer,  $8.1 \pm 0.7 \text{ mmol/L}$  for tranquilized deer, and  $6.5 \pm 1.6 \text{ mmol/L}$  for deer that were shot. These values indicate that the method of handling and sedation significantly impacts the biochemical parameters measured in deer. For instance, the highest urea concentration was observed in restrained deer, likely due to the stress response elicited by physical restraint. Tranquilized deer showed slightly lower urea levels, which can be attributed to the calming effects of sedation that reduce stress-induced metabolic changes. Deer that were shot had the lowest urea concentrations, potentially due to the rapid physiological changes occurring at the time of death, affecting metabolic waste levels. Although present research employed sedation similar to the aforementioned studies, the urea concentration we observed ( $6.31 \pm 2.04 \text{ mmol/L}$ ) is somewhat different from the  $8.1 \pm 0.7 \text{ mmol/L}$  reported for tranquilized deer in research conducted by Vengušt et al. (2006). This discrepancy could be due to differences in sedation protocols, environmental conditions, or the physiological status of the animals at the time of sampling. These variations underscore the importance of considering the method of animal handling and specific research conditions when interpreting biochemical parameters.

This research determined that the concentrations of creatinine and triglycerides were similar to those reported by Vengušt et al. (2006). This consistency suggests that, despite some variations in urea levels, the biochemical responses related to creatinine and triglycerides in present study align with existing literature (Vengušt et al., 2006). This finding reinforces the reliability of present methods and the comparability of present results with previous research. This research determined that the enzyme activities were similar to those reported in other studies (Vengušt et al., 2006; Sinanović et al., 2013). This similarity indicates that the enzymatic responses observed in present study align well with existing literature (Vengušt et al., 2006; Sinanović et al., 2013), further validating our methods and ensuring the comparability of present results with prior studies in this field.

Although Sinanović published a preliminary report in 2013 (Sinanović et al., 2013) on certain biochemical parameters in deer, present research is the first study in Bosnia and Herzegovina to determine both hematological and biochemical parameters in deer blood. The obtained values of hematological and biochemical parameters relate to deer

that were sedated for sampling purposes. Numerous authors mention significant differences in the frequency of the tested parameters depending on whether the animals were sedated, restrained, or shot and then sampled (Vengušt et al., 2006). Some researchers have proposed that two separate ranges of reference blood values should be established for wild animals, based on the capture method used (Vengušt et al., 2006).

#### CONCLUSION

This study provides essential baseline data on the hematological and biochemical parameters of captive fallow deer in Bosnia and Herzegovina. The findings highlight significant variations in these parameters depending on the method of restraint and sedation used during sampling. The glucose and urea concentrations determined in present research were higher than those reported in some previous studies, indicating potential influences from environmental conditions, handling techniques, and physiological states of the deer. Despite these variations, the consistency observed in creatinine, triglyceride concentrations, and enzyme activities with existing literature suggests that present methods are reliable and comparable to another research in the field. The hematological parameters such as RBC, PCV, HGB, MCV, MCH, and MCHC levels aligned closely with earlier findings, further validating our approach. Also, this study is the first comprehensive report in Bosnia and Herzegovina to document both hematological and biochemical parameters in deer blood, specifically under sedation. Present results emphasize the importance of standardized protocols in wildlife health assessments to ensure accurate and comparable data across different studies and regions. Establishing separate reference ranges for wild animals based on capture methods, as proposed by some researchers, could enhance the precision of health evaluations and contribute to better wildlife management and conservation practices. The results emphasize the importance of standardized protocols in wildlife health assessments to ensure accurate and comparable data across different studies and regions. Establishing separate reference ranges for wild animals based on capture methods, as proposed by some researchers, could enhance the precision of health evaluations and contribute to better wildlife management and conservation practices. In summary, present study underscores the need for continuous monitoring and evaluation of deer health in both wild and captive environments. The data generated from this study will serve as a valuable reference for veterinarians and wildlife biologists in assessing the health and well-being of fallow deer and other cervid species.

#### DECLARATIONS

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#### Authors' contributions

N.Hadžimusić contributed to the design of the study, data analysis, and the writing of the manuscript. N.Hadžimusić, A.Livnjak, DŽ.Hadžijunuzović-Alagić and V.Škapur were responsible for sample collection, laboratory analysis, and manuscript review. All authors have contributed to the interpretation of the data and approved the final manuscript for submission.

#### Ethics committee approval

As the blood sampling was performed during routine veterinary health checks and involved no invasive procedures beyond standard care. The study strictly adhered to internationally recognized animal care guidelines, ensuring minimal stress and maximum welfare for the animals throughout the study.

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#### Consent to publish

All authors have read and approved the final version of the manuscript and give their consent for publication.

#### **Competing Interests**

The authors declare that there are no competing interests regarding the publication of this paper.

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# EFFECT OF GRADED LEVELS OF DIETARY TOMATO WASTE ON PERFORMANCE AND CARCASS CHARACTERISTICS OF JAPANESE QUAIL REARED UNDER INTENSIVE SYSTEM

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Supporting Information

ABSTRACT: This study was carried out to evaluate the effects of partial replacement of soybean meal (SM) with tomato waste (TW) in Japanese quail diets on the resulting yield, internal organs, and carcass characteristics. Eighty unsexed 1-day-old chicks were housed in battery cages with cardboard boxes used as solid floors and randomly assigned to 1 of 4 dietary groups, 46.2% SM, 44.2% SM + 2% TW, 42.2% SM + 4% TW, or 40.2% SM + 6% TW, over a 6 weeks growth period. Yields and carcass characteristics were then determined. Data were analysed using the General Linear Model (GLM) procedures followed by a response procedure for surface regression analysis (Proc RSREG; SAS 9.4) to describe the parameters' responses to graded levels of dietary tomato waste. Repeated measures analysis showed significant week × diet interaction effects on feed intake (FI, P = 0.03), body weight gain (WG, P = 0.0006), feed conversion ratio (FCR, P = 0.002), protein efficient ratio (P = 0.0001), and growth efficiency (P = 0.0001). By supplementing the diets of quails with a 2% inclusion level, a diet significantly affected quails' FI on weeks 1, 2, 3, and 6. A diet containing 2% TW significantly affected live weight (LW), hot carcass weight (HCW), and cold-dressed weight (CDW). It is concluded that the dietary supplementation with 44.2% SM + 2% TW seemed ideal for optimum performance in Japanese quails based on the insignificant change in feed intake and growth efficiency results compared to 46.2% SM for weeks 1 and 2. Further research is needed on the application method that could be used to enhance the utilization of tomato waste in Japanese quails.



Keywords: Carcass characteristics, Dietary replacements, Growth performance, Japanese quails, Tomato waste.

#### INTRODUCTION

Due to domestic birds' short gestation and generation intervals, high productivity, rapid turnover rate, higher feed efficiency, and cheap land and labour needs, poultry products including meat and eggs have been encouraged for regular intake to fulfill the protein deficit (Olawumi, 2015). In many countries, including Botswana, the Japanese quail (*Coturnix coturnix japonica*) is quickly gaining recognition as a source of meat and eggs. In contrast to other poultry species, the Japanese quail has a sexually dimorphic body structure, with females being larger than males (Sezer et al., 2006). Bonos et al. (2010) reported that female quail have heavier carcasses and bodies than their male counterparts.

As a result of the lack of animal protein caused by the declining supply of meat and eggs in developing nations, rearing Japanese quail as food is seen as another aspect of poultry farming (Illgner and Nel, 2000; Agiang et al., 2011; Dalle Zotte and Cullere, 2024). The Japanese quail produces meat and eggs that are highly prized for their distinctive flavor (Ayaşan, 2013). Furthermore, the Japanese quail serves as a laboratory animal (Ophir et al., 2005), and has unique traits notably quick growth rates which allow the bird to be sold for human consumption at 5-6 weeks of age (Hemid et al., 2010). Compared quails to chickens and found that quails reach sexual maturity earlier leading to a small generation gap, have higher laying rates, and significantly reduced space requirements (Hemid et al., 2010).

The escalating costs of cereals and imported feedstuffs for chicken diets have driven a search for substitute ingredients that will be available as by-products from domestic agricultural producers (Adeniji and Oyeleke, 2008; Okello et al., 2023; Dou et al., 2024). The increased production of soybeans is causing worry because of greenhouse gas emissions and the devastation of wildlife habitats (Siamabele, 2018). Rahman et al. (2016) posited that utilizing alternate feed materials in chicken rations is a crucial aspect of effective poultry production in countries experiencing a food shortage. In many parts of the world, using alternative feed ingredients such as tomato pomace with a high protein value of 17-24% (Lu et al., 2022) for chicken diets is crucial to the poultry industry's success (Yitbarek, 2013). Therefore, the current experiment was conducted to determine the effect of feeding graded levels of tomato waste on the growth, slaughter performance, and carcass characteristics of Japanese quail reared under an intensive system. It was

hypothesized that replacing soybean meal (SM) with tomato waste in quail diets would not affect growth, slaughter performances, and carcass traits.

#### MATERIALS AND METHODS

#### Description of the study area

The study was conducted at BUAN farm in South-East Botswana. The farm is located at coordinates 24°34'54.41" S 25°58'14.64" E (Google Earth Pro, 2022). The farm is about 15 km north of Gaborone and is at an elevation of 978 m (Google Earth Pro, 2022). The vegetation type is Savannah, with tall grasses, bushes, and trees. Precipitation in Gaborone is about 457 mm per year (Climate-Data.Org, 2022). In Gaborone, the average temperature of the coldest month (July) is 13.5 °C, and that of the warmest month (January of 2022) is 26°C.

#### Source and sample preparation

Tomato waste was procured from the NAFTEC Investments plant in Selebi-Phikwe, located 404 km northeast of Gaborone, the capital of Botswana. Tomato waste was dried for 4 days in February 2023 (Aragaw et al., 2021) by spreading it in the sun. After reducing the particle size of the dried tomato waste by hand, it was run through a grinder (Polymix PX-MFC 90 D model, Kinematica<sup>™</sup>, Switzerland) and passed through a 1 mm sieve. Thereafter, samples were weighed and stored at room temperature in the Nutrition Laboratory storage room at BUAN before being subjected to chemical analysis and later used in this experiment.

#### **Proximate analyses**

Tomato waste was subjected to preliminary analysis using the Association of Official Analytical Chemists (AOAC) International methods before diet formulations. After formulations were performed, subsamples of the experimental diets (TW0, TW2, TW4, and TW6) and tomato waste were analysed using methods from AOAC (2005). For dry matter (DM), method number, 930.15 was used. Ash content was determined by ashing at 550 °C for about 6 hours (AOAC, 2005; method number 924.05), whereas nitrogen was determined using the Kjeldahl method (AOAC, 2005; method number 984.13). The percentage of nitrogen was multiplied by 6.25 to determine crude protein (AOAC, 2005; method number, 920.39). Energy content was determined using a bomb calorimeter and measured in joules. Fat was determined using AOAC, (2005); method number, 920.39. Crude fiber was determined following AOAC, (2005); method number, 978.10. Neutral detergent fiber (NDF), and acid detergent fiber (ADF) were determined by refluxing 0.45 g of samples with neutral detergent and acid detergent solutions respectively, for 1 hour using the ANKOM<sup>2000</sup> fiber analyser (ANKOM Technology, NY, USA). Acid detergent lignin (ADL) was determined by using the ADF residue that was solubilized by 72 % sulphuric acid, leaving the lignin (ADL), which was determined gravimetrically (AOAC, 2005; method number, 973.18). Table 1 shows results from a proximate analysis of tomato waste.

Table 1 - Proximate analysis (%, unless otherwise stated) of tomato waste used in this experi-	mental trial
Parameter	Value
Dry Matter	95.84
Ash	1.58
Crude protein	20.1
Energy (J)	22
Fat	13
Crude fiber	55
Neutral detergent fiber	56
Acid detergent fiber	60
Acid detergent lignin	25
J= Joule	

#### Diet formulations and composition of experimental diets

The following four isocaloric dietary treatments were formulated using Excel spreadsheet 2010 by partially substituting SM with tomato waste in the diets of Japanese quail: TWO = Diet with no tomato waste (control); TW2 = Diet with 2% SM replaced with tomato waste; TW4 = Diet with 4% SM replaced with tomato waste; TW6 = Diet with 6% SM replaced with tomato waste. The ration was prepared following the National Research Council (1994). Table 2 presents the ingredients and the calculated composition of the regular diet that was in mash form.

Diets <sup>2</sup>		Starte	er (%)		Grower (%)			
Ingredient <sup>1</sup>	TW0	TW2	TW4	TW6	TW0	TW2	TW4	TW6
Maize	42.31	42.31	42.31	42.31	55.79	55.79	55.79	55.79
Sorghum	9.84	9.84	9.84	9.84	9.93	9.93	9.93	9.93
Soybean meal (SM)	46.20	44.20	42.20	40.20	32.70	30.70	28.70	26.70
Tomato waste (TW)	0	2	4	6	0	2	4	6
DCP	0.17	0.17	0.17	0.17	0.02	0.02	0.02	0.02
Premix	0.50	0.50	0.50	0.50	0.47	0.47	0.47	0.47
Salt	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Limestone	0.10	0.10	0.10	0.10	0.20	0.20	0.20	0.20
Lysine	0.12	0.12	0.12	0.12	0.18	0.18	0.18	0.18
Methionine	0.40	0.40	0.40	0.40	0.35	0.35	0.35	0.35
Calculated nutrient content								
Metabolisable energy (MJ/kg)	12.13	12.12	12.30	13.38	13.38	13.38	13.40	13.40
Crude protein	25.90	25.40	24.90	24.40	21	20.50	20	19.60
Calcium	9.10	9	9	8.90	8.80	8.70	8.70	8.60
Phosphorous	5.40	5.30	5.20	5.10	2.20	2.10	2	1.90
Lysine	13.80	13.80	13.90	13.90	19.60	19.60	19.70	19.70
Methionine	5.20	5.20	5.20	5.20	4.90	4.90	4.90	4.90
Ingredient: TW = Tomato waste: DCP	= Dicalcium n	hosphate Pr	emix sunnlvii	ng ner kg feed	• 12 000 III vi	tamin A 50	00 III vitamir	D3 80 mg

Table 2 - Composition of experimental diets (%, unless otherwise stated) used in the experimental trial

<sup>1</sup>Ingredient: TW = Tomato waste; DCP = Dicalcium phosphate; Premix supplying per kg feed: 12,000 IU vitamin A, 5,000 IU vitamin D3, 80 mg vitamin E, 7 mg vitamin K, 5 mg thiamine, 6 mg riboflavin, 6 mg pyridoxine, 0.02 mg vitamin B12, 60 mg niacin, 15 mg pantothenic acid, 1.5 mg folic acid, 0.25 biotin, 10 mg vitamin C, 500 mg choline chloride, 100 mg Zn, 120 mg Mn, 20 mg Fe, 15 mg Cu, 0.2 mg, Co, 1 mg I, 0.3 mg Se. <sup>2</sup>Diets: TW0 = Diet with no tomato waste; TW2 = Diet with 2% of SM replaced with tomato waste; TW4 = Diet with 4% of SM replaced with tomato waste; TW6 = Diet with 6% of SM replaced with tomato waste.

#### **Experimental design**

A total of 80-day-old unsexed quail chicks with an initial weight of  $7.6 \pm 0.152$  g were acquired from Makhaya Quail farm in Gaborone. Japanese quails were randomly assigned to 4 dietary treatment groups at 20 chicks per treatment in a completely randomized design. The treatments were replicated 4 times with 5 chicks in each pen (experimental unit). The one-day-old chicks were housed in battery cages with cardboard boxes used as solid floors and were randomly assigned to 1 of 4 dietary groups, 46.2% SM (TW0), 44.2% SM + 2% TW (TW2), 42.2% SM + 4% TW (TW4), or 40.2% SM + 6% TW (TW6), over a 6 weeks growth period. Yields and carcass characteristics were then determined.

#### **Bird management**

The poultry house and equipment were cleaned two weeks before the arrival of quail chicks by using clean water and disinfected with Kenosan detergent from Shield Vet Gaborone. Virocid was used to disinfect the ceiling, walls, and floors. To eradicate and sterilize bugs from the base post crevices, formalin and a salt mixture were applied to the floor, wall junctions, and the surrounding region. Throughout the experimental period, the 750 ml water fonts were used and cleaned daily, and 1 kg feed trays were used. Feed was weighed and replaced every day. Quails were kept in battery cages with firm floors made of cardboard boxes. Sunflower husks were utilised as litter to make the dwelling more comfortable as recommended by Amedu et al. (2018). Feed and water were provided *ad libitum*. The illumination schedule was 23 hours per day for the first week, and thereafter 16 hours per day until the end of the experiment (Manyeula et al., 2019). The temperature of 32°C was maintained using electric heaters for the first two days and then decreased by 1°C every other two days until the third week, and thereafter a temperature of 20°C was maintained for the remainder of the feeding period. Mortality was recorded as it occurred throughout the experimental period.

#### Data collection procedure

#### Growth performance parameters

Feed was weighed using Adam's electronic scale sensitive to 0.01 g (Adam scale Pty Ltd, Gaborone, Botswana) in the morning (feed offered) and the following day the refusals were re-weighed and FI was then calculated by subtracting feed offered from the refusal (Tamburawa et al., 2018) as shown in formula 1.

AWFI(g/week) = Feed offered(g) - feed refusal(g)/7 days.....1

Quail chicks were individually weighed at the beginning of the experimental trial using Adam's electronic scale and subsequently weekly. Weekly weight gain was calculated using formula 2.

AWWG (g/week) = Finish weight (g) - start weight (g)/7 days.....2

The feed conversion ratio was calculated by dividing feed consumption by body weight gain (BWG) as shown in formula 3.

 $FCR = Feed intake (g) / weight (g) \dots 3$ 

Weekly protein consumed (PC, g/bird) was calculated by multiplying the concentration of crude protein (CPd) in the diet (g/kg DM consumed) by weekly feed intake (FI) g/bird) as illustrated in formula 4.

PC(g/bird) = Feed intake(g) X crude protein of the diet.....4

The protein efficiency ratio (PER, g/g) was calculated by dividing mean body weight gain (AWG) by the mean protein consumed (PC) as shown in formula 5.

PER(g/g) = Body weight gain (g)/protein consumed......**5** 

Growth efficiency was calculated by dividing BWG (g) by initial weight (g) as shown in formula 6.

GE = Body weight gain (g)/initial weight (g).....6

#### **Slaughter procedure**

All the Japanese quails were humanely sacrificed to evaluate the size of internal organs and carcass characteristics following a 6-week feeding trial. The night before slaughter, feed was withdrawn to clear the digestive system. The following morning quails were weighed (pre-slaughter weight) to determine the slaughter weight (SLW). Japanese quails were then transported to the BUAN slaughterhouse where they were electrically stunned and bled for 5 minutes by cutting the jugular vein following a procedure by Mnisi et al. (2021). Quails were then soaked for 2 minutes in the heated water (60 °C) for easy de-feathering as described by Narinc et al. (2014). Quail carcasses were then weighed, and all the internal organs were removed using a sharp knife. The carcasses were then individually weighed as hot carcass weight (HCW) and stored in the chiller at -4 °C for 24 hours. The entire internal organs (liver, gizzards, proventriculus, spleen, abdominal fat, heart, small and large intestines) were then separated and weighed. After 24 hours, the carcasses were weighed again to determine cold carcass weight (CCW), and the external parts (drumstick, thighs, breast muscle, wings, and back) were dissected (Mnisi et al., 2021) and weighed individually to determine their weight. Wings were detached by cutting at the humeroscapular joint, cutting it through the rib head to the shoulder girdle, and pulling the vertebrae out intact (Alikwe et al., 2010).

## Determination of carcass yield, cuts, and internal organ weights

Internal and external organs were obtained and expressed as the fraction of HCW as shown in equation 7.

Hot carcass yield was calculated by dividing HCW by pre-slaughter weight following equation 8.

Hot carcass yield percentage =  $\frac{Hot \ carcass \ weight \ (g)}{Live \ weight \ (g)} X \ 100.....8$ 

The dressing out percentage was calculated by dividing HCW by pre-slaughter weight as shown in equation 9.

Dressing out (%) =  $\frac{Carcass weight(g)}{Body weight(g)} X 100.....9$ 

The breast muscle percentage was calculated by dividing breast muscle weight by HCW as shown in equation 10.

Breast muscle (%) = 
$$\frac{Breast muscle(g)}{Hot carcass weight(g)} X 100.....10$$

The drumstick percentage was calculated by dividing drumstick weight by HCW as shown in equation 11.

 $Dumstick (\%) = \frac{Drumstick (g)}{Hot \ carcass \ weight(g)} X \ 100.....11$ 

#### **Data analyses**

The growth performance data were checked for interaction with time; where the interaction existed, data were analysed using repeated measures shown in model 1 and if no interaction existed, data were analysed using the general linear model procedure (GLM) of SAS (2013) version 9.4 for a completely randomized experimental design with pen as the experimental unit shown in model 2.

 $Y_{ijk} = \mu + \tau_i + w_j + w\tau_{ij} + e_{ijk}$ .....model 1

Where,  $Y_{ijk} = ijk^{th}$  response variable;  $\mu =$  General mean;  $\tau_i = i^{th}$  treatment effect (tomato waste);  $w_j = j^{th}$  week effect;  $(w\tau)_{ij} = ij^{th}$  interaction between week and diet effect;  $e_{ij} =$  random variation  $\sim N(0, \sigma_e^2)$ .

 $Y_{ij} = \mu + \tau_i + e_{ij}$ .....model 2.

Where,  $Y_{ij} = ij^{\text{th}}$  response variable;  $\mu =$  General mean;  $\tau_i = i^{\text{th}}$  treatment effect (tomato waste);  $e_{ij} =$  random variation  $\sim N(0, \sigma_e^2)$ .

The Tukey's Studentized (HSD) Range Test in the SAS (2013) was used to separate the means when the Analysis of Variance indicated the existence of a significant difference between the treatment means. Furthermore, all data were evaluated for linear and quadratic effects using polynomial contrasts. Response procedures for surface regression analysis (Proc RSREG; SAS 9.4, 2013) were applied to describe responses of parameters to graded levels of tomato waste in the diets fed to Japanese quails following the quadratic model:  $y = ax^2 + bx + c$ , where y = response variables, a and b are the coefficients of the quadratic equation; c is the intercept; x is dietary tomato waste level (%). The significance level was declared at P < 0.05.

#### RESULTS

#### Growth performance

Repeated measures analyses showed overall significant week × diet interaction effects on FI (P = 0.03), weight gain (P = 0.0006), FCR (P = 0.002), protein efficiency ratio (PER) (P = 0.0001), and growth efficiency (GE) (P = 0.0001) but not on protein consumed (P = 0.10). Diet significantly affected quails' FI at weeks 1, 2, 3, and 6 only (Table 3). In weeks 1, 3, and 6 quails fed on TWO and TW2 diets had significantly higher FI than those fed diet TW6. Quails fed on TW4 diets had significantly similar FI to those fed on TW0, TW2, and TW6 diets. In week 2, quails fed on TW0 and TW2 diets had significantly higher FI than those fed on TW4 and TW6 diets which were statistically similar. In weeks 4 (P = 0.20) and 5 (P = 0.08), diets did not affect FI. The regression analysis in Table 4 revealed that FI decreased linearly in weeks 1, 4, and 6 as tomato waste inclusion levels increased. In week 2, FI increased linearly with tomato waste. However, decreased quadratic trends in FI were observed in weeks 3 and 5 as tomato waste levels increased in the diets.

The results in Table 3 showed significant dietary effects in weeks 2, 3, and 4 but diets did not significantly affect PER in weeks 1, 5, and 6. In week 2, quails fed on TWO and TW2 diets had significantly higher PER compared to those fed TW4 and TW6 diets, which were statistically similar. In weeks 3 and 4, quails fed on TW0 diet had significantly higher PER than those fed TW2. TW4, and TW6 diets, which did not differ significantly. Regression analysis revealed that PER linearly decreased in weeks 1 and 6 (Table 4). However, there was a linear increase in week 2 in PER as tomato waste levels increased. Increasing quadratic effects were observed in weeks 3 and 4 with increased dietary tomato waste levels. In week 5, neither linear nor quadratic trends were observed in PER as dietary tomato waste increased.

The results revealed no significant dietary effects in weeks 5 and 6 in growth efficiency (GE) (Table 3). However, significant differences were recorded in weeks 1, 2, 3, and 4. At weeks 1 and 2, quails fed on TWO and TW2 diets had significantly higher GE followed by those fed TW4 diet and lastly, those fed on TW6 diet. In weeks 3 and 4, quails fed on TWO diet had higher GE followed by those on TW2 and TW4 diets which were statistically similar and lastly, those fed on TW6 diet. Regression analysis results in Table 4 revealed that PER linearly decreased in week 1 and linearly increased in week 5 with increased dietary levels of tomato waste. However, a negative trend was observed on GE in response to increases in dietary levels of tomato waste at 2, 3, 4, and 6 weeks of age.

		Signi	ficance <sup>3</sup>					
Parameter	TW0	TW2	TW4	TW6	SEM <sup>2</sup>	P value	Linear	Quadratic
Feed intake (g/quail)								
Week 1	6.56ª	6.54ª	5.96 <sup>ab</sup>	<b>5.87</b> ⁵	0.149	0.0091	*	NS
Week 2	8.78ª	<b>8.67</b> ª	7.29 <sup>b</sup>	7.19 <sup>b</sup>	0.140	< 0.0001	*	NS
Week 3	<b>12.80</b> ª	<b>12.76</b> <sup>a</sup>	<b>12.27</b> <sup>ab</sup>	<b>12.25</b> <sup>b</sup>	0.090	< 0.0001	*	*
Week 4	17.69	17.51	17.16	16.95	0.250	0.1978	*	NS
Week 5	28.08	27.96	27.56	27.03	0.280	0.0847	*	*
Week 6	33.51ª	33.13ª	32.78 <sup>ab</sup>	<b>31.95</b> <sup>b</sup>	0.220	0.0018	*	NS
Protein efficiency ratio								
Week 1	0.99	0.92	0.90	0.91	0.020	0.0831	*	NS
Week 2	2.61ª	2.58ª	2.26 <sup>b</sup>	2.24 <sup>b</sup>	0.050	0.0001	*	NS
Week 3	2.76ª	<b>2.45</b> ⁵	2.43 <sup>b</sup>	2.38 <sup>b</sup>	0.030	< 0.0001	*	*
Week 4	<b>1.51</b> ª	1.28 <sup>b</sup>	<b>1.2</b> 9 <sup>b</sup>	<b>1.28</b> <sup>b</sup>	0.020	< 0.0001	*	*
Week 5	0.81	0.79	0.78	0.80	0.010	0.4425	NS	NS
Week 6	0.67	0.65	0.63	0.63	0.010	0.0579	*	NS
Growth efficiency								
Week 1	<b>1.33</b> ª	<b>1.32</b> <sup>a</sup>	<b>1.14</b> <sup>b</sup>	<b>1.10</b> °	0.005	< 0.0001	*	NS
Week 2	<b>1.87</b> ª	<b>1.86</b> ª	<b>1.80</b> <sup>b</sup>	<b>1.74</b> °	0.010	< 0.0001	*	*
Week 3	<b>1.00</b> ª	0.81 <sup>b</sup>	0.80 <sup>b</sup>	0.78°	0.005	< 0.0001	NS	*
Week 4	0.77ª	0.47 <sup>b</sup>	0.46 <sup>b</sup>	0.44 <sup>c</sup>	0.010	< 0.0001	NS	*
Week 5	0.32	0.32	0.32	0.27	0.020	0.1034	*	NS
Week 6	0.24	0.24	0.24	0.23	0.010	0.0008	*	*
a,b,cIn row, means with commo	on superscripts	do not differ	r significantly.	<sup>1</sup> Diets: TW0 =	Diet with n	o tomato wast	e; TW2 = D	iet with 2% of

Table 3 - Weekly feed intake, protein efficiency ratio, and growth efficiency of Japanese quails fed graded levels of tomato waste as partial replacement of sovbean meal

meal replaced with tomato waste. 2SEM =standard error of mean; 3Significance: \* = Significant difference (P < 0.05); NS = Not significant.

**Table 4** - The linear and quadratic trends on weekly feed intake (g/bird), weekly protein efficiency ratio, and weekly growth efficiency of Japanese quails fed graded levels of tomato waste as partial replacement of soybean meal

Parameter	Equation	P value	R <sup>2</sup>
Feed intake (g/quail)			
Week 1	Y = 6.5 (± 0.7) – 0.8 (± 0.6) X	0.0001	0.69
Week 2	Y = 8.8 (± 1.2) + 0.6 (± 1.0) X	0.0002	0.70
Week 3	Y = 12.5 (± 0.7) + 0.5 (± 0.571.1) X- 0.2 (±0.09) X <sup>2</sup>	0.0300	0.70
Week 4	$Y = 17.7 (\pm 0.5) - 1.0 (\pm 0.4) X$	< 0.0001	0.82
Week 5	$Y = 28.0 (\pm 0.4) - 0.1 (\pm 0.3) X - 0.2 (\pm 0.1) X^2$	0.0020	0.92
Week 6	Y = 33.5 (± 1.2) – 1.4 (± 1.0) X	0.0030	0.50
Protein efficiency ratio			
Week 1	Y = 0.99 (± 0.02) – 0.05 (± 0.02) X	0.0300	0.41
Week 2	Y = 2.2 (± 0.1) + 0.05 (± 0.05) X	0.0020	0.67
Week 3	$Y = 2.7 (\pm 0.04) - 0.2 (\pm 0.03) X + 0.02 (\pm 0.005) X^2$	< 0.0001	0.80
Week 4	$Y = 1.5 \ (\pm \ 0.02) - 0.1 \ (\pm \ 0.02) \ X + \ 0.01 \ (\pm 0.003) \ X^2$	0.0010	0.81
Week 6	$Y = 0.7 (\pm 0.01) - 0.02 (\pm 0.01) X$	0.0100	0.45
Growth efficiency			
Week 1	Y = 1.3 (± 0.03) – 0.06 (± 0.02) X	< 0.0001	0.76
Week 2	$Y = 1.7 (\pm 0.01) + 0.05 (\pm 0.01) X- 0.05 (\pm 0.002) X^2$	0.0100	0.85
Week 3	$Y = 0.81 \ (\pm \ 0.04) + 0.08 \ (\pm \ 0.03) \ X - 0.02 \ (\pm 0.005) \ X^2$	0.0100	0.50
Week 4	$Y = 0.5 (\pm 0.1) + 0.1 (\pm 0.04) X - 0.02 (\pm 0.01) X^2$	0.0100	0.50
Week 5	Y = 0.3 (± 0.02) + 0.03 (±0.01) X	0.0500	0.36
Week 6	$\texttt{Y} = 0.2 ~(\pm 0.01) + 0.03 ~(\pm 0.01) ~\texttt{X} - 0.01 ~(\pm 0.001) ~\texttt{X}^2$	0.0002	0.73

The results showed that diet affected average weight gain at weeks 1, 2, 4, and 6 only but did not affect the weight gain of quails at weeks 3 and 5 (Figure 1). At week 1, quails fed on TWO diet had significantly higher WG than those fed on TW2, TW4, and TW6 diets. In week 2, quails fed on TW0 and TW2 diets had significantly higher WG than those fed on TW4 and TW6 diets. Significantly higher WG was recorded in week 4 in quails fed on TW0 and TW2 diets than those fed on TW6 diet. However, quails on TW4 diet had statistically similar WG to those fed on TW0, TW2, and TW6 diets. In week 6, quails fed on TW0 diet had significantly higher WG compared to those fed on TW2 diet followed by TW4 and lastly TW6. At 3 and 5 weeks of age, diet did not significantly affect WG of quails.

The results showed that there were significant dietary effects in week 2 only (Figure 2). However, there were no dietary effects in FCR at weeks 1, 3, 4, 5, and 6. In week 2, quails fed on TW0 and TW2 diets had significantly lower FCR compared to those fed on TW4 and TW6 diets which were statistically similar. The regression analysis results revealed that FCR decreased linearly from week 1 to 2 and then increased linearly from week 2 to 6.



Figure 1 - Weekly weight gain of Japanese quails fed graded levels of tomato waste as a partial replacement for soybean meal.



Figure 2 - Weekly FCR of Japanese quails fed graded levels of tomato waste as partial replacement of SM.

#### **Carcass characteristics**

Diets affected (SLW), (HCW), and (CDW) but did not affect (CL) and dressed weight percentage (Table 5). Quails fed on the TWO diet had significantly higher SLW followed by those fed on the TW2 diet, and lastly, those fed on the TW4 and TW6 diets, which were statistically similar. Similarly, quails fed on the TW0 diet had significantly higher HCW and CDW than those fed on the TW2, TW4, and TW6 diets. However, quails fed on the TW4 diet had significantly similar HCW and CDW compared to those fed on the TW2 and TW6 diets. Regression analysis showed positive trends in SLW of Japanese quails with tomato waste inclusion levels (Table 6). Linear decreases were recorded in SLW, HCW, and CDW with increased tomato waste inclusion levels. No significant linear and quadratic trends for dressed percentage were detected.

 Table 5 - Effects of feeding graded levels of tomato waste (g, unless otherwise stated) as partial replacement of soybean

 meal on carcass characteristics of six weeks old Japanese quails

			Significance <sup>4</sup>					
Parameter 1	TW0	TW2	TW4	TW6	SEM <sup>3</sup>	P value	Linear	Quadratic
SLW	<b>220.89</b> <sup>a</sup>	207.13 <sup>b</sup>	200.36°	<b>195.07</b> °	1.49	< 0.0001	*	*
HCW	<b>165.60</b> ª	156.44 <sup>b</sup>	151.55 <sup>bc</sup>	147.06°	1.25	< 0.0001	*	NS
CDW	<b>165.45</b> ª	<b>156.31</b> <sup>b</sup>	151.43 <sup>bc</sup>	<b>146.95</b> °	1.24	< 0.0001	*	NS
CL	0.15	0.13	0.12	0.11	0.02	0.50	NS	NS
Dressed (%)	75.00	75.5	75.67	75.17	0.31	0.43	NS	NS

<sup>1</sup>Parameter: SW= Slaughter weight; HCW = Hot carcass weight; CDW = Cold dressed weight; CL = Chilling loss. <sup>2</sup>Diets: TWO = Diet with no tomato waste; TW2 = Diet with 2% of soybean meal replaced with tomato waste; TW4 = Diet with 4% of soybean meal replaced with tomato waste; TW6 = Diet with 6% of soybean meal replaced with tomato waste. <sup>3</sup>SEM= Standard error of the mean. <sup>4</sup>Significance: NS = Not significant; \* = Significant; <sup>abc</sup>Within a row, different superscripts denote significant differences (P < 0.05) between treatments.

 Table 6 - Linear and quadratic trends of carcass characteristics of Japanese quails fed graded levels of tomato waste as

 partial replacement of soybean meal

Parameter	Equation	P value	R <sup>2</sup>					
SLW	$Y = 220.5 (\pm 1.6) - 7.3(\pm 1.3) X + 0.5 (\pm 0.2) X^2$	0.02	0.87					
HCW	$Y = 165.2 (\pm 1.4) - 4.7(\pm 1.1) X$	< 0.0001	0.83					
CDW	$Y = 164.9 (\pm 1.4) - 4.7 (\pm 1.1) X$	< 0.0001	0.83					
<sup>1</sup> Parameter: LW= Live weight (g); HCW = Hot carcass weight (g); CDW = Cold dressed weight (g); CL = Chilling loss (g).								

Diet significantly affected the quail back only but did not affect the drumstick, wings, thighs, and breast (Table 7). Quails fed on the TW0 diet had significantly heavier backs than those fed on the TW4 and TW6 diets. Quails fed on diet TW2 had significantly similar back weights to those fed on TW0 and TW4 diets. Quails fed on the TW4 diet had a lighter back but were statistically similar to quails fed on diets TW2 and TW6. The regression analysis revealed linear decreases for the drumstick [ $y = 12.9 (\pm 0.4) - 0.1(\pm 0.3) X$ ;  $R^2 = 0.29$ ; P = 0.01] as dietary levels of tomato waste increased. Neither linear nor quadratic effects were observed on wings, thighs, back, and breast.

Diet significantly affected the liver and heart weights only but did not affect the weights of gizzards, proventriculus, spleen, and fat (Table 8). Quails fed on TW0 diet had significantly heavier liver followed by those fed on TW2 diet and lastly Quails fed on TW4 and TW6 diets had statistically similar liver and hear weights. Quails fed on TW0 diet had heavier hearts than those fed TW4 and TW6 diets. However, quails fed on TW2 diet had significantly similar heart weights with those fed on TW0, TW4, and TW6 diets. The regression results showed that there were linear decreases for liver with incremental levels of tomato waste [ $y = 1.6 (\pm 0.1) - 0.05 (\pm 0.3) X$ ;  $R^2 = 0.31$ ; P = 0.03]. However, proventriculus increased linearly [ $y = 0.3 (\pm 0.02) + 0.02 (\pm 0.02) X$ ;  $R^2 = 0.22$ ; P = 0.04] with tomato waste incremental levels. No significant linear and quadratic trends were observed on gizzards, spleen, fat and heart.

Diet significantly affected the weight of the small intestines but did not affect the length of the small intestines, and length and weight of the large intestines (Table 9). Quails fed on the TW0 (4.55 %) diet had heavier small intestines than those fed on the TW4 and TW6 diets. However, the quails fed on TW2 diet had significantly similar small intestines weights with those on TW0, TW4, and TW6 diets. The regression analysis results showed a quadratic decrease on small intestines weight with tomato waste levels [ $y = 3.2 (\pm 0.2) - 0.3 (\pm 0.1) X + 0.1 (\pm 0.02) X^2$ ; R<sup>2</sup> = 0.28; P = 0.01]. No significant linear and quadratic trends were observed on small and, large intestines length, and large intestines weights.

 Table 7 - Effects of feeding graded levels of tomato waste (%HCW), as partial replacement of soybean meal on external organs of six weeks old Japanese quails

			Significance <sup>4</sup>					
Parameter 1	TW0	TW2	TW4	TW6	SEM <sup>3</sup>	P value	Linear	Quadratic
Drumstick	11.06	11.65	11.19	10.78	0.29	0.23	NS	NS
Wings	8.07	8.06	8.06	8.07	0.01	0.72	NS	NS
Thighs	21.00	20.52	20.28	20.15	0.53	0.68	NS	NS
Back	<b>18.65</b> ª	18.12 <sup>ab</sup>	17.15 <sup>bc</sup>	<b>17.25</b> °	0.31	0.01	*	NS
Breast	37.97	37.30	36.97	36.48	0.67	0.29	NS	NS

<sup>1</sup>Diets: TW0 = Diet with no tomato waste; TW2 = Diet with 2% of soybean meal replaced with tomato waste; TW4 = Diet with 4% of soybean meal replaced with tomato waste; TW6 = Diet with 6% of soybean meal replaced with tomato waste. <sup>2</sup>SEM= Standard error of the mean. <sup>3</sup>Significance: NS = Not significant; \* = Significant; <sup>abc</sup> Within a row, different superscripts denote significant differences (P < 0.05) between treatments.

 Table 8 - Effects of feeding graded levels of tomato waste (%HCW), as partial replacement of soybean meal on internal organs of six weeks old Japanese quails

		Significance <sup>4</sup>						
Parameter 1	TWO	TW2	TW4	TW6	SEM <sup>3</sup>	P value	Linear	Quadratic
Liver	3.62ª	3.39 <sup>b</sup>	3.19°	3.12°	0.04	< 0.0001	*	NS
Gizzards	2.03	2.08	2.05	2.04	0.02	0.13	NS	NS
Proventriculus	0.40	0.40	0.43	0.39	0.02	0.34	NS	NS
Spleen	0.18	0.08	0.08	0.08	0.05	0.47	NS	NS
Abdominal fat	0.07	0.19	0.08	0.08	0.06	0.42	NS	NS
Heart	0.83ª	0.80 <sup>ab</sup>	0.75 <sup>b</sup>	0.76 <sup>b</sup>	0.01	0.01	*	NS

<sup>1</sup>Diets: TW0 = Diet with no tomato waste; TW2 = Diet with 2% of soybean meal replaced with tomato waste; TW4 = Diet with 4% of soybean meal replaced with tomato waste; TW6 = Diet with 6% of soybean meal replaced with tomato waste. <sup>2</sup>SEM= Standard error of the mean. <sup>3</sup>Significance: NS = Not significant; \* = Significant; <sup>abc</sup> Within a row, different superscripts denote significant differences (P < 0.05) between treatments.

 Table 9 - Effects of feeding graded levels of tomato waste (%, unless otherwise stated) as partial replacement of soybean

 meal on intestines of six weeks old Japanese quails

		Signi	ficance 4					
Parameter 1	TW0	TW2	TW4	TW6	SEM <sup>3</sup>	P value	Linear	Quadratic
Small intestines (cm)	60.00	60.00	60.00	59.17	0.92	0.89	NS	NS
Small intestines (%)	4.55ª	4.39 <sup>ab</sup>	3.88 <sup>b</sup>	<b>3.85</b> ⁵	0.18	0.03	NS	*
Large intestines (cm)	12.58	12.22	11.88	11.58	0.40	0.36	NS	NS
Large intestines (%)	0.77	0.77	0.75	0.75	0.05	0.94	NS	NS

<sup>1</sup>Diets: TW0 = Diet with no tomato waste; TW2 = Diet with 2% of soybean meal replaced with tomato waste; TW4 = Diet with 4% of soybean meal replaced with tomato waste; TW6 = Diet with 6% of soybean meal replaced with tomato waste. <sup>2</sup>SEM= Standard error of the mean. <sup>3</sup>Significance: NS = Not significant; \* = Significant; <sup>a,b,c</sup> Within a row, different superscripts denote significant differences (P < 0.05) between treatments.

#### DISCUSSION

#### **Growth performance**

Repeated measures analysis showed significant week x diet interaction effects on weekly FI, WG, FCR, PER, and GE, demonstrating that tomato waste inclusion influenced quail growth performance over time. The significantly lower FI in quails fed TW6 diets in weeks 1, 3, and 6 suggest that the higher inclusion levels reduced FI. Common organoleptic characteristics associated with tannin compounds are astringency and bitterness which reduce voluntary FI (Choi and Kim, 2020) which could be linked with FI reductions. Similarly, Tabeidian et al. (2011) reported that growth performance was lowered even at modest inclusion levels of tomato waste at 3% in the starter and 9% in the finisher phase of broiler chicks. In contrast, Shehata et al. (2018) and Muhammad et al. (2023) reported increased feed consumption when tomato pomace-containing diets were fed to laying Japanese quails at 2.5 and 5% inclusion levels. This discrepancy could be due to the different species (broilers vs. Japanese quails) used. It is noted that different poultry species act differently when exposed to the same diet (Mnisi and Mlambo, 2018). Quails fed on the TWO diet had similar feed intake to TW4, TW2, and TW6 suggesting that tomato waste at 6% inclusion did not affect the voluntary FI of quails, suggesting that the diet (tomato waste) can be used up to 6% inclusion level. This is in line with the findings of Mohammed et al. (2021) who reported that supplementing tomato pomace at 6% did not affect FI of broiler chicks. Contrary to our expectations, in week 2, quails fed on TWO and TW2 diets had significantly higher FI than those fed on other diets, suggesting that tomato waste can be used at 2% inclusion without affecting the palatability of the diets. Lower FI of quails fed TW4 and TW6 diets could be due to tannins that exert a bitter taste resulting in decreased feed consumption (Hassan et al., 2020). The physicochemical characteristics of the entire diet are greatly affected by a little alteration in an ingredient during diet formation.

In weeks 4 and 5, diet did not affect FI suggesting that tomato waste inclusion rates up to 6% can produce similar results as the control diet. This could be linked with the maturity of the GIT of Japanese quails which efficiently handled the fiber contents of the diets. These results agree with Gungor et al. (2024) who reported that the supplementation of dried tomato pomace to broiler diets up to 10% had no significant effect on FI. Similarly, Jouzi et al. (2015) found no significant difference between the diet groups supplemented with 5% dried tomato pomace and the control group of Japanese quails.

Quails fed on TWO and TW2 diets gained more weight in weeks 1, 2, 4, and 6, indicating that they were able to use the nutrients in the diets implying that the ANFS such as tannins, pectins, and insoluble fiber were negligible. These ANFs temper with feed utilization (Szabo et al., 2019). The bioactive components found in tomato waste include lycopene and ß-carotene, which have substantial antioxidant capacity (Szabo et al., 2019). Lycopene is a powerful antioxidant that can protect muscle cells from the damaging effects of reactive oxygen species and biomolecule destruction (Mezbani et al., 2019). Our results agree with Sahin et al. (2008) who reported that a 5% inclusion level of tomato waste in broiler diets improved the body weight of broiler chickens.

At weeks 3 and 5 diet did not significantly affect the weight gain of quails, suggesting that tomato waste could be incorporated into the diet without affecting Japanese quails' feed utilization. This was due to statistically similar FI, nutrient intake, and feed utilisation. In line with our results, Nikolakakis et al. (2004) reported no significant differences in body weight and BWG on growing quails fed on diets containing tomato pulp at 5 and 10% levels. Contrary to this finding, Alagawany et al. (2021) noted a significant improvement in body weight and BWG when quails were fed on 6% sundried tomato pomace at 3 to 5 weeks of age compared to those fed on the control diet. The current result implies that sun-dried tomato waste can replace SM by 6% in the quail diet without detrimentally affecting weight gain.

There were no significant dietary effects on FCR at weeks 1, 3, 4, 5, and 6 implying that tomato waste can be used in quail diets as partial replacement of SM. In agreement with our results, Jouzi et al. (2015) obtained no significant effect on FCR in growing Japanese quails fed on tomato powder at 2, 4, 6, and 8% inclusion levels. Similar to the weight gain, at week 2, quails fed on TWO and TW2 diets in this study had comparable FCR suggesting that the quails utilized the feed regardless of the fiber content in the diet. The quails fed on TW4 and TW6 had improved FCR probably due to the high fiber content that is responsible for the improvements in villus height and overall epithelial cell arrangement which increases nutrient absorption and hence better FCR. In addition, high fiber content increases the retention time of the digesta along the GIT, causing optimum digestion and absorption of nutrients for anabolic purposes.

Fiber is known to be involved in the modification of the intestinal length, villus height, crypt depth, as well as, the passage rate and size through different segments of the intestines (Rezaei et al., 2018; Tejeda and Kim, 2021). However, as fiber increased the FCR began to increase from weeks 2 to 6 due to the presence of tannin and pectins that cause a reduction in nutrient absorption due to the formation of insoluble complexes with proteins leading to poorer FCR observed. It is known that monogastric animals do not produce the cellulolytic enzymes required to break down the fiber but utilise the microbial enzymes in their caeca, though less efficient in degrading fiber (Jha and Mishra, 2021). Additionally, fiber has been considered a diluent of the diet and is known to increase the passage rate of the gastrointestinal tract owing to the reduction of nutritional utilization (Mateos et al., 2012). Hosseini-Vashan et al. (2016)

found that supplementing 5% tomato pomace increased broiler FCR during heat stress. In contrast with the present results, Lira et al. (2010) reported that using more than 20% tomato pomace in the diet of broiler chickens from 1 to 28 days may decrease FCR. The current results indicated that tomato waste inclusions possibly interfered with the use of nutrients in the rations at higher inclusion levels of tomato waste. The current results suggest that the inclusion of tomato waste at 4 and 6% compromised FCR.

The comparison between the average body weight and the amount of protein in the diet is known as the protein efficiency ratio (PER) which is influenced by the productivity of the animals (Ratriyanto et al., 2017). The rise in PER values in the present study indicates that the birds were able to use the protein they consume more effectively. In this study, quails fed on TWO and TW2 diets in week 2 had similar PER suggesting that tomato waste can be used at the inclusion of 2% without affecting the PER. There is very limited literature on the PER of poultry-fed tomato waste. There was a significant reduction in PER at weeks 3 and 4 in quails fed tomato-based diet, suggesting that the inclusion of tomato waste in diets above 2% hindered protein utilization. This implies that as the tomato waste increases, the tannins and pectins that bind protein increase resulting in protein utilisation being hindered. Also, the feed was slowly digested since the quails do not produce the cellulolytic enzymes required to quickly break down fiber. This implies that the inclusion of tomato waste as a partial replacement of SM compromised PER. No significant dietary effects were noted at 1, 5, and 6 weeks of age across diets, suggesting that protein utilization was similar to the control diet. This implies that tomato waste can be incorporated in the quail diets at 2% without compromising the PER.

Quails fed on TW0 and TW2 diets at 1 and 2 weeks of age had significantly higher GE compared to those fed TW4 and TW6 diets, indicating that tomato waste reduced the GE when included in the quail's diets at higher levels. This reduction in GE could be due to the presence of pectins, tannins and fiber which increase with increasing tomato waste inclusion levels. According to Brenes et al. (2016) and Mnisi et al. (2022), anti-nutrients inhibit protein utilisation and nutrient digestibility owing to depressed growth. Furthermore, it is speculated that the capacity of the gut is very limiting in young quails; hence their inability to produce enough fibrinolytic enzymes that help in the digestion of fiber. Our results are in line with Cavalcante et al. (2007) who reported the negative effect of tomato waste in the early stages of quail life due to great sensitivity of young chicks to ingestion of diets with high fiber content. Fiber is a naturally occurring plant component associated with physiological, structural, and functional changes in the GIT (Deehan et al., 2022). At weeks 3 and 4, quails fed on tomato-based diets had reduced GE suggesting that tomato waste in the diet led to an increase in tannin contents, which interfered with protein utilization, thus suppressing growth. Therefore, the inclusion of tomato waste as a partial replacement for SM compromised GE. In agreement with our results, Tabeidian et al. (2011) found that growth performance in broilers was lowered even at a modest inclusion rate of 3% in the starter phase and 9% in the finisher phase.

#### **Carcass characteristics**

Among the variables that are known to affect carcass features in birds include age, sex, diet, genetics, and conditions of slaughter (Young et al., 2001). In this study, the results showed that diet had an impact on HCW, CDW, and SLW, and TW6 diets showed notably greater HCW and CDW in Japanese quails. This was due to reduced FI in tomato-based diets. In this study, the inclusion of tomato waste in diets linearly decreased carcass yield, HCW, CDW, CL, and weight of drumsticks, thigh, wing, and breast. This observation may be related to the lower FI observed in quails fed on diet containing tomato waste, which led to inadequate nutrient uptake for muscle growth and ineffective weight gains. This suggests that feeding tomato waste to quail could have a negative impact on the viability and profitability of quail businesses at high inclusion levels. These results agree with Jouzi et al. (2015) and Lira et al. (2010) who reported a linear decrease in carcass yield, wings, breast, and drumstick when quails were fed on diets containing 8% of tomato waste. In contrast to our results, Yitbarek (2013) reported that broilers fed a diet containing 15% dried tomato pomace had a higher carcass yield than other treatments. However, broiler chicks fed a diet containing 15% dried tomato pomace showed a lack of significant difference on carcass characteristics suggesting that tomato pomace can be used at 15% without detrimental effect on carcass characteristics.

The gastrointestinal morphology of poultry is related to variations in dietary fiber content as an adaptation mechanism to make use of the high levels of fiber (Jha et al., 2019). The lower liver weights on tomato-based diets in the present study could have been triggered by a negligible concentration of secondary plant compounds such as pectins and tannins in tomato waste, which require detoxification by the liver when available in the diets. This lower liver weight is linked with less ANFs in the diet. A previous study of Leke et al. (2018) showed that the heart and gizzard weights were not significantly affected by dietary tomato waste at 0, 3, 6, 9, and 12% inclusion levels. In contrast with our results, Mateos et al. (2012) observed that birds fed fibrous diets had enlarged gastrointestinal organs. Nevertheless, despite the presence of crude fiber in the tomato waste-containing diets in this study, examination of the visceral organs (fat, spleen, proventriculus, and gizzards) revealed no significant effects. It is likely that dietary tomato waste levels as high as 6% were not enough to cause physio-anatomical changes in the fat, spleen, proventriculus, or gizzards.

Mateos et al. (2012) found that the gastrointestinal organs of birds fed fibrous diets were larger. Moreover, Nikolakakis et al. (2004) found that the amount of dietary tomato pulp supplementation had no significant impact on the weight and length of the quail intestines. Despite the higher crude fiber content of the diets, the control group's small intestines were heavier than those of the tomato waste-containing diet implying that tomato waste diets did not promote the growth of the intestines. The current findings suggest that the inclusion of tomato waste as a partial replacement of SM compromised the weights of the small intestines.

#### **CONCLUSION AND RECOMMENDATIONS**

The inclusion level of tomato waste up to 6% as a partial replacement for soybean meal compromised the growth performance of Japanese quails. Higher tomato waste inclusion levels negatively affected slaughter liveweight, cold dressed weight, hot carcass weight, back weight, small intestines weight, liver weight, and heart weight of Japanese quails. The present results showed that higher inclusion levels of tomato waste compromised the performance of Japanese quails. The 2% inclusion level was more efficient compared to other inclusion levels. Therefore, it is recommended that where higher dietary levels of tomato waste are desired, fiber-degrading strategies including enzymes such as pectinases be used in Japanese quail diets. Further investigations on the amino acid profile of tomato waste need to be carried out.

#### DECLARATIONS

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#### Authors' contribution

Conceptualisation: J.C. Moreki, and S. Bhawa Data curation and writing of original draft: S. Bhawa Data analysis, writing-review and editing: F. Manyeula, J.C. Moreki, and S. Bhawa

#### Ethical approval

This study was conducted in line with the Ethics Committee Guidelines provided by the Faculty of Animal and Veterinary Sciences at the Botswana University of Agriculture and Natural Resources (BUAN). BUAN Ethics Committee approval number: BUAN-ACUC-2023-03.

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#### **Consent to publish**

All authors agreed to the publication of this manuscript.

#### **Data availability**

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

#### **Competing interests**

The authors declared no competing interests.

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1

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