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Effects of various feed forms on some gut bacteria and subsequent effects on productivity, egg quality, and intestinal morphology in Indigenous laying hens

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ABSTRACT

Background: Local hen layers play a crucial role in egg production and the poultry industry. Optimizing their performance, egg quality, and overall health is of paramount importance.
Aim: This research aims to examine the effects of different feed forms on gut bacteria and subsequent effects on productivity, egg quality, and intestinal morphology in indigenous laying hens.
Methods: Forty-five hens aged 73 weeks were randomly distributed into three treatment groups, each with three replicates of 5 chickens in ground cages. The dietary treatments included a 100% mash diet (T1), 50% mash + 50% pellet diet (T2), and 100% pellet diet (T3).
Results: Compared to the mash-fed group (T1), birds in the pellet and mixed-diet groups (T2 and T3) exhibited significant improvements ($p \leq 0.05$) in productive performance and egg quality. Furthermore, there were significant reductions ($p < 0.05$) in intestinal weight, intestinal length, and gizzard weight, accompanied by significant increases ($p < 0.05$) in lactic acid bacteria and Spore-forming bacteria, along with a decrease in Aerobic bacteria and Colibacilli bacteria. Increases seen in beneficial bacteria were accompanied by increases in egg quality, especially when pellet form was utilized by the layer hens.
Conclusion: These findings support the advantages of using pellet diets to enhance productive performance and intestinal health and morphology in local hen layers.
Keywords: Local laying hen, Feed form, Productive performance, Intestinal morphology, Microbial content.

Introduction

Nutritionists and the egg industry are researching ways to improve feed utilization and production efficiency for laying hens (Wan *et al.*, 2021). The feed form is crucial in determining poultry growth, nutrient absorption, intestinal health, and egg production (Massuquetto *et al.*, 2020). Poultry breeders in the United States were among the first to adopt the granulation method, a technique that involves forming feed into pellets. This method aims to reduce feed wastage during consumption by birds and improve the ease of transportation (Edith *et al.*, 2024).

In commercial egg production, two primary types of bird feed are commonly utilized: mash and pellets. Mash feed, characterized by its smaller particle size, is produced through the meticulous blending and fine grinding of ingredients to ensure a homogeneous mixture and prevent ingredient separation (Guzman *et al.*, 2015). In contrast, the pelletizing process involves agglomerating ingredients into larger pellets using mechanical action, moisture, pressure, and temperature (Edith *et al.*, 2024). Reports suggest several advantages of feeding chicken pellets, including enhanced growth, improved feed consumption, a better feed conversion ratio (FCR), manageability, smoother flow of feed in machinery, and reduced feed composition costs due to the use of cost-effective ingredients while minimizing ingredient segregation. However, it is worth noting that in certain circumstances, pellet diets may lead to reduced starch digestibility and nutrient utilization in broilers (Ruhnke *et al.*, 2015).

In contrast, mash nourishments have been found to be more effective than pellet diets in increasing the feed conversion rate, starch metabolism, and intestinal glucose usage in broilers (Herrera *et al.*, 2017). The composition of chicken feed significantly influences physiological processes and the development of the digestive tract, leading to improved feed utilization and gut motility (Gunjević *et al.*, 2023). The gizzard's remarkable grinding ability to feed into uniformly sized particles is truly impressive (Olson *et al.*, 2022). Birds fed purified diets with finely ground particles had larger proventriculi and modest gizzards than birds fed purified diets with coarsely ground particles. Additionally, the proventriculi of birds fed fine diets were filled with fluid instead of food, leading to faster

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passage through the gastrointestinal tract (GIT) (135 *vs.* 165 minutes). These birds were observed drinking more water, likely aiding in swallowing, and their crops tended to be pendulous (Boumans *et al.*, 2022).

In terms of dietary choices for the digestive system, mash diets resulted in duodenum hypertrophy than pellet feeds. In a follow-up study, Kiarie and Mills (2019) found that changing the size of maize particles from 0.6 to 2.17 mm had no impact on the weight of intestinal segments. However, (Ball *et al.*, 2015) reported the proportional length of all GIT constituents decreased as the size of the wheat particle increased. Reduced maintenance costs could enhance feed efficiency with decreased intestinal weight or length. The impact of food material size and intestinal structure has garnered inconsistent research attention and few reports. Sampath and Kim (2023) found that the duodenal villus length was positively correlated with a particle of the diet, while (Ademola *et al.*, 2013) found no impact of maize particle size on duodenal villus length, crypt deepness, or epithelial width. The study by Ademola *et al.* (2013) confirmed the above conclusions. However, (Bao *et al.*, 2016) found that the inclusion of coarse maize lowered the mast cells in the small intestine, intestinal jejunum, and intestinal ileum, compared to finely ground maize.

Notably, there is limited information on how feed size affects the composition of the gut microbiota. In an environment with high acidity, the entry of pathogen bacteria into the GIT through feed can be prevented. According to Kim *et al*. (2015), the increase in a number of *Lactobacilli* spp. is typically beneficial to the chicken, as *Lactobacilli* inhibit the colonization of virulent bacteria like *Escherichia coli*. Researchers found that birds fed a coarse mash diet had higher levels of *Lactobacilli* spp. populations in their caeca, compared to birds fed a finely ground mash diet or a pelleted feed. Conversely, feeding birds a finely ground pelleted diet resulted in lower levels of lactic acid bacteria.

The influence of feed size on the gut microbiome can be explained by two processes. First, the development of the gizzard and enhanced secretion of hydrochloric acid lower the pH in the digestive tract, which can kill harmful bacteria that enter the gut (Nguyen *et al.*, 2017). Second, feeding coarse feed can promote the growth of beneficial bacteria, which can outcompete harmful bacteria for space and nutrients (Ege *et al.*, 2019).

Materials and Methods

This experiment was conducted on a private poultry farm from 5/9/2022 to 30/10/2022, spanning 10 days, which included an adaptive period with no recorded data. It involved 45 local laying hens aged 73 weeks, randomly distributed into three treatments, each with three replicates containing 5 chickens in ground cages. Water and fodder were provided through waterers and feeders distributed throughout the cages. The hall was equipped with 16 hours of lighting, using energy-efficient 15-watt lamps.

Table 1. Basal diet composition and chemical analysis.

Ingredients	Percentage
Corn	34
Soybean meal	16
Barley	10
Wheat bran	18
Limestone	6
Concentrated protein	4
Premix 1	2
NaCl	2
Dicalcium phosphate	8
Total	100
Chemical analysis	
Crude protein	26.16
Ca	6.4
Available P	3
Lysine	8
Methionine	3
Metabolizable energy (Kcal/kg)	2,800

The nutritional treatments were as follows: (T1) Treatment 1: 100% mash diet; (T2) Treatment 2: 50% mash + 50% pellet diet; (T3) Treatment 3: 100% pellet diet.

A diet with different physical forms (mash and pellet) was employed, and the birds were fed the experimental diet detailed in Table 1, with chemical analysis and feed composition calculated according to (Lázaro *et al.*, 2003).

Various parameters, including feed utilization rate, feed conversion efficiency, protein conversion efficiency, daily egg production, egg weight, mass rate, and qualitative traits such as eggshell, egg yolk, and egg white weight, were calculated.

After the experiment, nine intestinal content samples (in sterile containers) were collected from nine birds in each group to observe intestinal morphology, including intestine weight, intestine length, and gizzard weight. Additionally, the intestinal content bacterial count of aerobic bacteria, colibacilli, lactic acid bacteria, and spore-forming bacteria was recorded for each group.

Statistical analysis

Data analysis and presentation in this study utilized GraphPrism v7 and SPSS software (USA). The data were represented in graphs and tables using the average (mean) and standard error of the mean. Significant differences were defined with p values under 0.05. The study employed the two-way ANOVA test.

Table 2. The effects of different feed forms on production performance of local hen layers.

Group Parameter	T1	T2	T3
Egg production %	84.2 ± 0.14[c]	87.4 ± 0.63[b]	87.64 ± 0.23[a]
Egg weight (g)	68.34 ± 0.24[c]	69.83 ± 0.12[b]	70.3 ± 0.125[a]
Egg mass (g)	65.45 ± 1.24[c]	66.30 ± 0.62[b]	67.57 ± 0.63[a]
Feed conversion ratio (FCR)	1.247 ± 0.74[c]	1.219 ± 0.329[b]	1.168 ± 0.27[a]
PER	0.368 ± 0.07[b]	0.352 ± 0.06[a]	0.355 ± 0.04[a]
Feed intake (g)	124.3 ± 1.02[b]	126.2 ± 1.04[a]	125 ± 1.07[a]

Different letters (a, b, c) indicate significant differences between the groups.

Ethical approval

Not needed for this study.

Results

In all Tables, the groups (T1, T2, T3) are listed along with various parameters related to egg production and feed performance. The corresponding values are provided in a clear and organized manner, and for each parameter, different letters (a, b, c) indicate significant differences between the groups.

Table 2 and Figures 1 and 2 present a summary of how feed form impacts production performance. Birds fed pellet diets in both T1 and T3 had higher laying rates, average daily feed intake, egg weight, and egg mass than those fed mash nourishments. Furthermore, they also exhibited significantly lower feed conversion rates and protein efficiency ratios (PER) ($p < 0.05$), indicating improved feed efficiency. In contrast, hens fed mash diets had higher FCR and PER values than those on pellet diets.

In essence, pellet diets resulted in improved laying rates, larger eggs, and higher egg mass, while also enhancing feed efficiency, as indicated by lower FCR and PER values compared to mash diets.

The impact of feed form on egg quality parameters is depicted in Table 3 and Figure 3. When comparing pellet diets to mash diets, hens in T2 and T3, which were fed a mixed diet, showed a significant increase in egg white and yolk weight ($p < 0.05$). On the other hand, eggshell weight slightly decreased ($p < 0.05$).

Table 4 and Figure 4 display the impact of different feed forms on intestinal morphological parameters. Hens in T2 and T3, which were fed mixed diets (combining mash and pellet), exhibited a significant reduction ($p < 0.05$) in intestinal weight, intestinal length, and gizzard weight when compared to hens in T1, who were fed mash diets exclusively.

Table 5 and Figure 5 provide a visual representation of how different feed forms affect the microbial content parameters in the small intestine of the studied hens. When comparing two specific diets to the conventional local mash diets (T1), thus: -T2 Pellet Diets: Hens fed pellet diets showed a significant increase ($p < 0.05$) in lactic acid bacteria and Spore-forming bacteria in their small intestines compared to hens that received mash diets. - Mixed Diets in T3: Similarly, when hens were fed mixed diets in T3 (a combination of mash and pellets), there was also a significant increase ($p < 0.05$) in lactic acid bacteria and Spore-forming bacteria in their small intestines compared to hens on mash diets. Aerobic Bacteria and Colibacilli Bacteria: In both T2 and T3 treatments, there was a decrease in the levels of Aerobic bacteria and Colibacilli bacteria

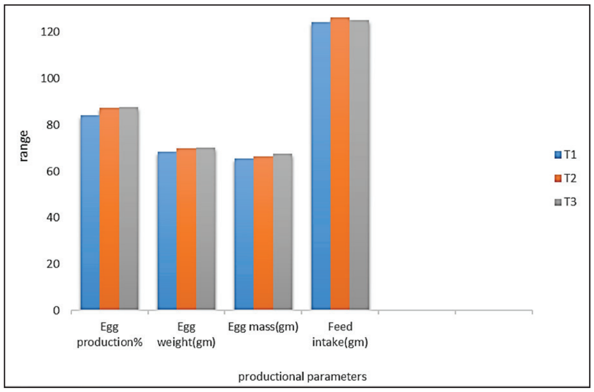

Fig. 1. The effects of different feed form on the productive performance of local hen layers.

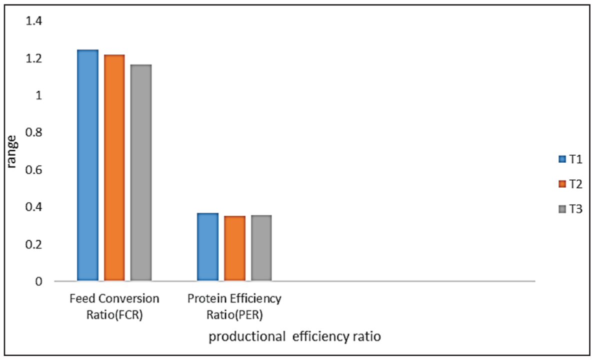

Fig. 2. The effects of different feed form on the productive efficiency ratio of local hen layers.

in the small intestine compared to hens fed with mash diets in T1. The results show that hens fed pellet diets (T2) and mixed diets (T3) had higher levels of lactic acid bacteria and Spore-forming bacteria in their small intestines but lower levels of Aerobic bacteria and Colibacilli bacteria when compared to hens on mash diets (T1). This suggests that the choice of feed form has a significant influence on the composition of microbes in the small intestine of these hens.

The correlation matrix summarises the relationships between the variables in the dataset, measured by the correlation coefficient r with values between −1 and 1. A positive correlation is an indication that as a variable increases so does the other, while a negative correlation is an indication that as one variable increases the other decreases. A positive correlation between egg weight and egg production would mean that heavier eggs are more likely to be produced at a higher rate, whereas a negative correlation between the FCR and

Table 3. The effects of different feed forms on egg quality parameters of local hen layers.

Group Parameter	T1	T2	T3
Egg shell weight (g)	8.93 ± 0.21^{c}	8.76 ± 0.07^{b}	8.45 ± 0.09^{a}
Egg white weight (g)	40.87 ± 0.1^{c}	41.35 ± 0.14^{b}	41.68 ± 0. 25^{a}
Egg yolk weight (g)	18. 54 ± 0.02^{b}	19.72 ± 0.13^{a}	19.9 ± 0. 32^{a}

Different letters (a, b, c) indicate significant differences between the groups.

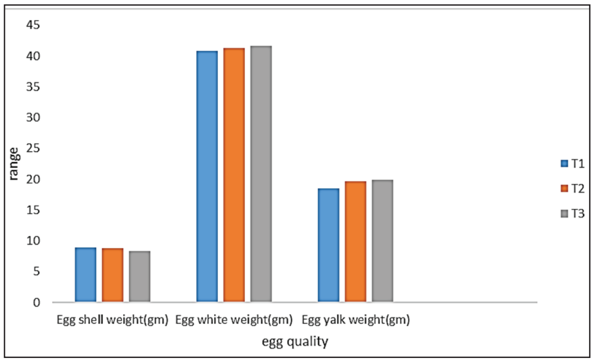

Fig. 3. The effects of different feed form on egg quality parameters of local hen layers.

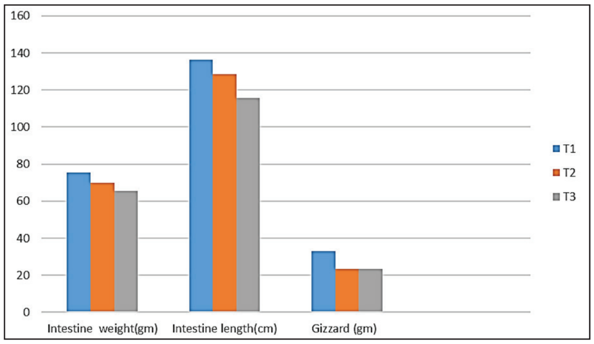

Fig. 4. The effects of feed form on the egg of local hen layers.

Table 4. The effects of feed form on the intestinal structure of local hen layers.

Group Parameter	T1	T2	T3
Intestine weight (g)	75.4 ± 0.05^{a}	70 ± 0.07^{b}	65.34 ± 0.21^{c}
Intestine length (cm)	136.4 ± 3.24^{a}	128.5 ± 2.14^{b}	115.7 ± 2. 93^{c}
Gizzard weight (g)	32.6 ± 0.03^{a}	23.57 ± 0.05^{b}	23.42 ± 0. 06^{b}

Different letters (a, b, c) indicate significant differences between the groups.

Table 5. The effects of different feed forms on intestinal microbiota composition of local hen layers.

Group Parameter	T1	T2	T3
LacticA bacteria (log cfu/g)	12.9 ± 0.01^{b}	12.74 ± 0.03^{a}	12.73 ± 0.05^{a}
Aerobic bacteria (Log cfu/g)	10.2 ± 0.12^{a}	9.5 ± 0.08^{b}	9.3 ± 0.06^{b}
Collibacilli (Log cfu/g)	8.76 ± 0.04^{a}	8.65 ± 0.13^{a}	8.3 ± 0. 21^{b}
Spore former bacteria (Log cfu/g)	3.45 ± 0.16^{b}	3.72 ± 0.25^{a}	3.68 ± 0.15^{a}

Different letters (a, b) indicate significant differences between the groups.

egg production would mean that more efficient feed conversion rates are likely to make more eggs. The matrix also clearly demonstrates which improvements in productivity are more likely to be complementary which variables are positively correlated and which are more likely to clash: two variables that mutually lead to improvement in prediction. There is evidence that beneficial gut flora could have a positive effect on egg production. This could be a creative trait manipulation to select for both beneficial gut flora and egg production. Of special importance is the multicollinearity between the independent variables (Fig. 6).

The given vertical scatter plots portray the relations between selected two bacteria listed in the given dataset such as lactic acid bacteria and Aerobic Bacteria and, Colibacilli and Spore-Forming Bacteria. On close perusal of the graph, the correlation co-efficient values help in determining the strength and direction of the relation between these bacteria. The central scatter plot depicts how the relationship potentially changes between the population of lactic acid bacteria and Aerobic Bacteria. There is a positive correlation, an increase in the population of lactic acid bacteria would lead to an increase in the population of Aerobic Bacteria. Similarly, Correlation Colibacilli and Spore-Forming Bacteria analyses the relationship between these two types of bacteria. Understanding these correlations is important because even small changes in gut microbiota composition could point to important interactions among bacterial groups that influence and affect gut health and overall productivity (Fig. 7).

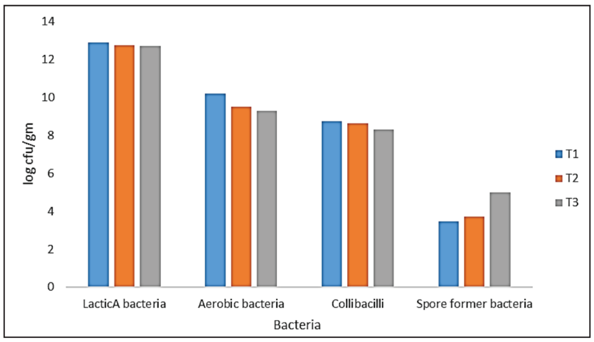

Fig. 5. Effect of different feed form on intestinal microbiota composition of local hen layers.

Figure 8 depicts the different levels of impact of gut bacterial balance on various factors concerning egg quality. With an extreme value of 9, it is revealed that nutrient absorption influences egg quality the most. This is because gut bacteria promote healthy digestion, allowing hens to absorb important nutrients. To produce it is essential that all required nutrients are efficiently absorbed from the food. Similarly, eggshell quality has also a high impact at level 8 as gut bacteria is significantly involved in calcium metabolism. Thus, the nutrients obtained from the diet are channeled to strengthen the eggshell, making it more durable. On the other hand, immune function is the next important factor influenced by bacterial balance with an impact of 6 which suggests that a healthy gut microbiome

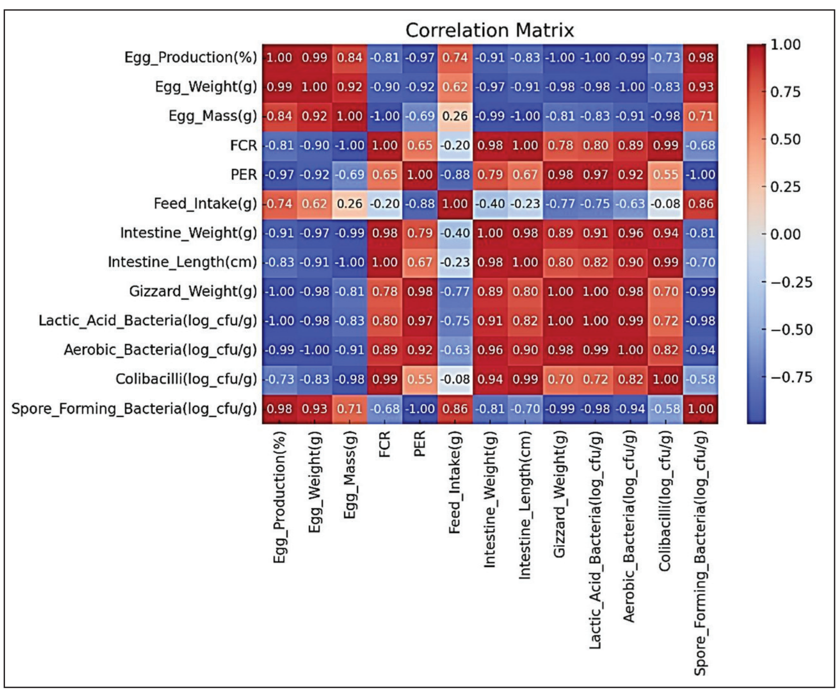

Fig. 6. Corelation matrix between different parameters

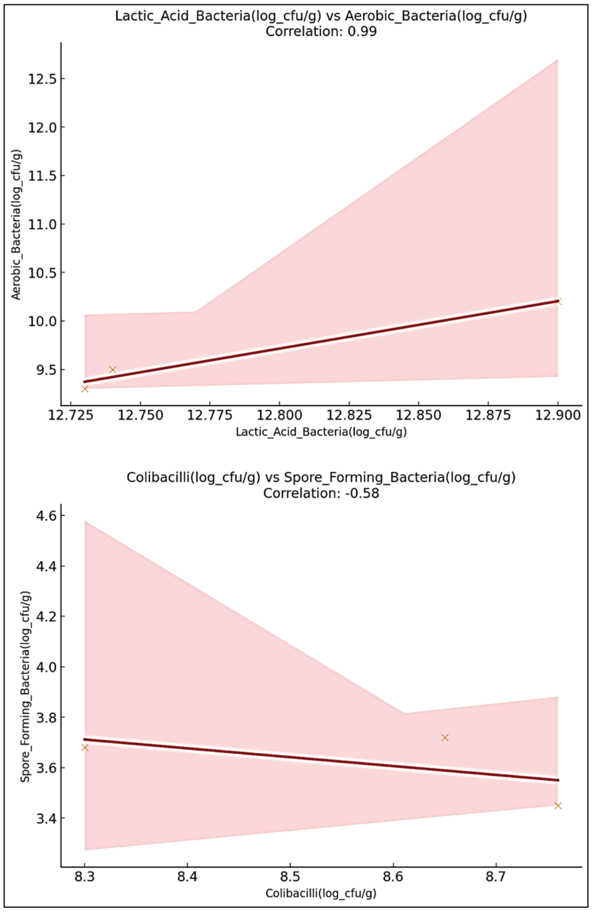

Fig. 7. Scatter plots of bacterial correlation between bacterial species.

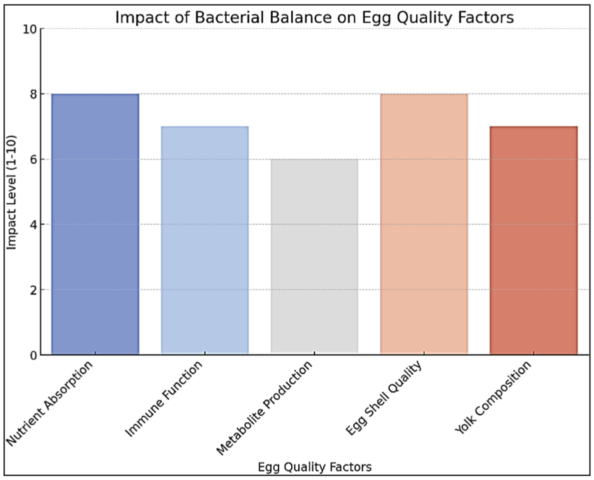

Fig. 8. Impact of gut bacteria on the egg quality.

enhances the hen's immune system by reducing stress and boosting egg production. Metabolite production is moderately influenced by the balance of bacterial population as only certain bacteria can produce beneficial metabolites which contribute to the efficient energy metabolism and production of high-quality eggs. Finally, bacterial balance impacts yolk composition mainly in terms of fatty acid profile and quality.

Figure 9 illustrates the impacts of feeding forms (Mash, Mash + Pellet, and Pellet) on intestinal bacteria counts and egg quality in laying hens. It is clearly detectable that an increment in the parts of pellets in the feed diets causes a considerable decrement in the counts of aerobic bacteria and colibacilli, while spore-forming bacteria enjoy a slight increment, suggesting a transformation of a beneficial intestine bacteria environment in the gut due to the utilization of pellet diets. Regarding the aspect of egg quality, the two measurements of egg white and yolk weights demonstrate a trend of an increase in the form of pellet diets, indicating a trend of egg quality enhancing, while the measurement of eggshell weights suffers a little reduction. To sum up, it is concluded that pellet diets are beneficial for the gut health and egg production enhancement of layers, which is thought of as a positive choice for promoting the productivity of layers.

Discussion

In this study, the primary evaluation criteria for layer performance are laying rate and FCR. It is broadly identified that feed pelleting increases production economics by improving egg-laying rates and feed productivity (NRC, 1994). Both hens fed pellets and those on a mixed diet of pellets and mash exhibited higher daily feed intake, consistent with previous research (Bao *et al.*, 2016). The pelleting process, involving steam and mechanical pressure to agglomerate feed particles and improve texture, may contribute to this increased feed intake in pellet-treated hens (Nguyen *et al.*, 2017).

Pelleted diets increase bulk density, making feed intake more convenient. While pelleting incurs higher costs compared to mash, these expenses can be offset by improved production performance, especially in layers. In every treatment, the results consistently endorse pellet feed, despite the interaction effects between feed form and laying performance (Melo-Durán *et al.*, 2021).

The choice of feed form significantly impacts most egg quality traits, including eggshell quality and increased egg white and yolk content. This aligns with findings reported in previous research (Herrera *et al.*, 2018) that emphasized the significant effect on egg quality traits.

Moreover, in the current study, both hens fed pellet diets and those on a mixed diet of mash and pellets exhibited higher egg weight and egg mass compared to hens on mash diets, indicating a significant albumen quality. This increase in egg weight in the pellet treatment can be credited to greater feed intake and enhanced protein digestion in birds (Herrera *et al.*, 2018). Similar to the findings by Kljak *et al.* (2019), which demonstrate that larger feed particle size stimulates gizzard activity

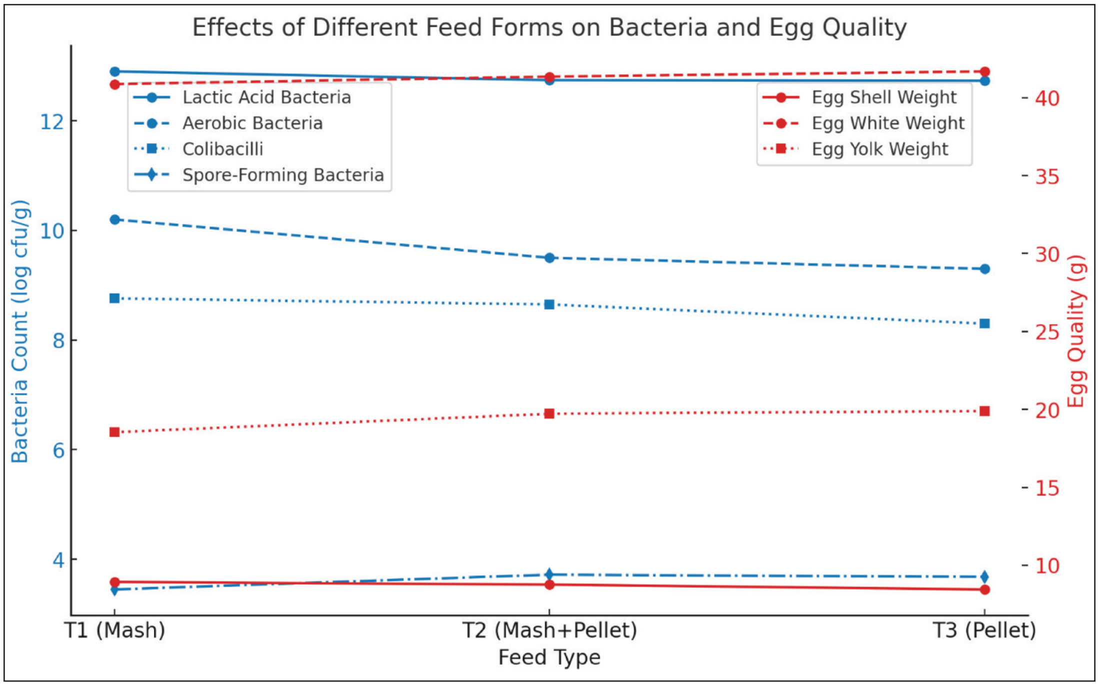

Fig. 9. Impact of feed forms on gut bacteria and egg quality.

and intestinal peristalsis, leading to increased nutrient digestion rates in birds fed smaller feed particles.

The findings regarding intestinal length align with those of (Odekunle *et al.*, 2021), who observed significantly shorter intestinal lengths in hens fed pellet diets ($p < 0.05$). Abadi *et al.* (2019) also noted a decrease in the proportional length of all GIT constituents when pellets were fed. However, due to lower maintenance costs, reduced intestinal mass or length may improve feed efficiency (Kljak *et al.*, 2019). The findings on intestinal and gizzard weight are in line with (21), who confirmed that pelleted diets reduced the weights of intestinal components. (Melo-Durán *et al.*, 2021) affirmed that when using highly processed pelleted feeds, birds' upper GITs do not fully develop.

According to this theory, Odekunle *et al.* (2021), a developed gizzard prevents virulent bacteria from getting into the distal GIT. Nevertheless, there is a scarcity of research examining the impact of feed particle size on the composition of intestinal and gut microbiota. In a highly acidic environment, pathogenic bacteria getting into the GIT through feed are likely suppressed. NRC (1994) suggests that an increase in the population of *Lactobacilli* spp. is useful as they inhibit colonization of pathogens. These researchers compared *Lactobacilli* spp. populations in the caeca of birds fed diets consisting of coarse grain or finely ground mash or pelleted feed, with the lowest number of lactic acid bacteria documented in birds given finely ground pellet nourishments (Kljak *et al.*, 2019).

Either or both of the following mechanisms may account for the impact of feed particle size on gut microbiome profiles: First, the stimulation of gizzard development and increased hydrochloric acid secretion initially lowers pH levels, then exerting an antimicrobial effect on pathogenic bacteria entering the distal portion of the GIT (Hosseini and Afshar, 2017). Second, fostering the growth of commensal bacteria while inhibiting the colonization of harmful bacteria may promote competitive exclusion (Abadi *et al.*, 2019; Sonu *et al.*, 2022).

Conclusion

In summary, the beneficial impacts of pellet, mash, and their combination on production performance and egg quality are counterbalanced by their effects on the development of intestinal components like the gizzard, intestinal weight, and length. This results in reductions in these parameters and decreased microbial content in local laying hen breeds. Pellet diets enhance egg-laying rates, daily feed intake, egg weight, and overall egg quality. These findings strongly endorse the advantages of using pellets during the egg-laying period for local hen breeds.

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Conflict of interest

No conflict of interest is found in the present study.

Funding
No specific fun is available for the current study, but it depends on the authors' assets.

Author contributions
All authors have their own parts in this study.

Data availability
The data of the current study can be provided by the corresponding author upon request.

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