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Effect of feed form on performance, gut morphology and cecal microflora on late-phase laying hens under cyclic high ambient temperature

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Abstract Feed form and heat stress (HS) are two major critical issues that affect feed intake (FI) in poultry. Limited research has been conducted on the impact of feed form on the intestinal morphology of laying hens. Additionally, the influence of feed form on the intestinal microbiota of laying hens under HS conditions remains unexplored. One hundred and forty-four laying hens (90 weeks of age) were used in a completely randomized design with two treatments (pellet and mash). Each treatment contained six replicates of 12 hens each. Laying hens were subjected to a cyclic heat stress regimen of 32 ± 3 °C for 6 hours per day at a humidity of 35-40% during the late laying period (weeks 90-97). Feed form had no effect on egg production and feed conversion ratio (P>0.05), but egg weight and mass, FI, and body weight gain increased in the birds fed the pelleted diet (P<0.05). Feeding the pelleted diet decreased the shell weight and thickness, as well as the yolk color score (P<0.05), but did not affect other egg quality parameters (P>0.05). Villus width and villus surface area were higher in hens fed the pelleted diet (P<0.05) than those fed the mash diet, but villus height, crypt depth, and the villus height-to-crypt depth ratio were not affected by the treatments (P>0.05). There were no significant differences between treatments regarding cecal *bifidobacteria* spp. *lactobacillus* spp. and coliforms populations (P>0.05). However, feeding laying hens with pelleted diet resulted in greater numbers of cecal *Enterococcus* spp. compared to those fed the mash diet (P<0.05). This study indicated that feeding the pelleted diet improved the egg mass production and intestinal morphology, reduced the shell weight and thickness, and increased the population of *Enterococcus* spp. in the ceca of late-laying hens exposed to HS. The economic benefit from performance improvement with the pelleted diet may have offset the higher feeding cost.

Keywords: feed form, gut morphology, laying hens, microbial population, pellet

Introduction

During the end of the laying cycle, there is a decline in egg production and quality, including eggshell breaking strength and albumen height. This necessitates the development of strategies to enhance both egg production performance and quality parameters in late-laying hens. These advancements could potentially extend the laying cycle and thus improve the overall breeding efficiency (Chen et al., 2021). More than 30% of the world's land surface and 60-70% of Iran's land surface are arid or semi-arid, facing high ambient temperatures for at least several months of the year. Heat stress (HS) is one of the major stressors in laying hen production, adversely affecting the reproductive capability, growth, feed intake (FI), egg quality, gut health, physiological status, and immune function of laying hens. Additionally, high ambient temperature increases the mortality rate of laying hens (Luo et al., 2018; Xing et al., 2019). Some studies have showed

that HS alters the intestinal microbiota in poultry, which could negatively affect performance (Wang et al., 2018; Shi et al., 2019). Furthermore, it has been shown that HS reduces the integrity of the intestinal barrier, leading to decreased nutrient absorption in laying hens (Zhang et al., 2017).

As well as, feed form (Abdollahi et al., 2014; Saldaňa et al., 2015) is obviously one of the major critical issues in determining feed intake in poultry which can also affect the health and development of the gastrointestinal tract, nutrient digestion, and absorption. Feed form (mash vs. crumble or pellet) remains an obscure area in laying hen nutrition, as mash is commonly fed in practice. Over the past two decades, a growing body of research has investigated the influence of feed form on laying hens, aiming to optimize production efficiency under various rearing conditions (Hamilton and Proudfoot, 1995; Wahlström et al., 1998, 1999a, 1999b; Scott et al., 1999; Frikha et al., 2009; Ruhnke et al., 2015; Mousavi et al., 2016; Wan et al., 2021). However, the results are quite divergent (Ruhnke et al., 2015; Mousavi et al., 2016; Wan et al., 2021). For example, feeding crumble diet to laying hens increased FI, feed conversion ratio (FCR), pancreatic activities of amylase, villus height (VH), villus height-to-crypt depth ratio (VCR), villus surface area (VSA) than in hens fed mash diets. However, egg production, egg weight, and egg mass were not affected by feed form (Ege et al., 2019). Compared to hens fed mash diet, those consuming pelleted feed exhibited increased egg weight, improved FCR, and reduced FI. However, no significant difference in egg production was observed between the two dietary forms (Mousavi et al., 2016). Moreover, laying rate, egg mass, egg weight, FI, shell strength, yolk proportion, Haugh unit, VH, crypt depth (CD), VCR, the apparent digestibility of dry matter, crude protein, phosphorus and Ca were all greater in hens receiving pelleted feed compared to those fed mash diet (Wan et al., 2021). In a previous study by Röhe et al. (2014), histological analysis revealed that the villi in the duodenum of hens fed the expanded diet were elongated, whereas those in the ileum were conversely shortened. Furthermore, these hens exhibited an elevated VCR in the duodenum, alongside significantly lower intestinal glucose transport rates. Zheng et al. (2020) reported that laying hens fed pelleted diet had higher egg mass and FI and numerically higher egg production and egg weight in comparison to those fed mash diet. In their study, feed form had no significant effect on counts of *Colibacillosis* spp. *Salmonella* spp. and total colonies on eggshell. Scott et al. (1999) found that replacing a mash diet with expanded pellets caused a decline in FI, egg production, egg mass, FCR, and eggshell percentage. The results are probably confounded by the bird's genotype, stage of production, dietary nutrient density, and feed processing method.

Our literature review revealed a scarcity of data on the impact of dietary physical form on the intestinal morphology of laying hens. Additionally, there is no existing research on the potential relationship between feed form and the intestinal microbial community in laying hens under HS conditions. This study investigated the hypothesis that pelleted feed could enhance the performance, egg quality, and intestinal morphology and microbiota in late-laying hens exposed to HS conditions. To test this hypothesis, the study evaluated the effects of dietary feed form (mash vs. pellet) on performance, egg quality, jejunal morphology, and cecal microbiota in laying hens experiencing HS from 90 to 97 weeks of age.

Materials and methods

The experimental procedures were approved by the Ethical Animal Care and Use Committee of the Department of Animal Science of Razi University, Iran (IR.RAZI.REC.1400.029).

Experimental design, animals and diet

One hundred and forty four 90 weeks old laying hens (LSL-Lite) were randomly divided into 2 groups (mash vs. pellet) with 6 replicates of 12 birds each. The birds were exposed to 32±3°C for 6 hours per day and 35-40% humidity. All birds were placed in cages (3 birds/cage, 44×40×40 cm) and kept under 16 hours light and 8 hours dark for eight weeks. A corn-soybean meal basal diet was formulated to meet LSL-Lite strain nutrient requirements (Lohmann Tierzucht GmbH, 2016). The calculated and determined nutrient contents of the basal diet are given in Table 1. A 4-mm screen (GHM Hammer Mill, Zarin Persia Sanat Pars, Iran) was used to grind the cereal of the diet and mixed in a horizontal mixer (Paddle Mixer, Zarin Persia Sanat Pars, Iran). To prepare pelleted feed, mash feed was processed at 75°C for 35 s in a conditioner, and then pelleted with a die ring of 4-mm screen and 35-mm thickness (Pellet Mill Progress 580, PTN, The Netherlands). Within 10 min after pelleting, the pellets were cooled to a temperature of around 20°C. The moisture content of the pelleted feed was adjusted to the level of the mash diet. The hens were housed in a caged layer house. Feed and water were provided *ad libitum*. The bird's weight, egg production, egg weight and egg quality were monitored before the experiment for two weeks (88 and 89 weeks of age) to ensure that replicates are distributed correctly. After that, there was a one-week preliminary feeding as the acclimation period. The crude protein, dry matter and ash contents in corn, soybean meal, wheat bran and mixed diet and dry matter content of the pellet were determined (AOAC, 2005). Physical properties of the pellet are presented in Table 2. The pellet durability index and hardness of 15 pelleted samples were measured using a Holmen pellet durability tester (60 seconds, Model: NHP100, TEKPRO, Norfolk, United Kingdom) and pellet hardness tester (Model: K3157, Amandus Kahl, Reinbek, Germany). To determine the fine powder content of the pellet, six samples (one kg) from different parts of the feed were weighed, sieved through a 4-mm sieve, and the amount of powder-like c-

ontent was weighed to calculate the percentage of fine powder content.

Table 1. Ingredients and chemical composition of nutrients in the basal diet (90-97 weeks of age)

Item	(%)
Corn	614.4
Soybean meal	241.0
Soybean oil	9.00
Wheat bran	7.00
DL-methionine	2.00
Di-calcium phosphate	18.0
Limestone	95.0
Salt	2.10
$NaHCO_3$	2.50
Vitamin and mineral premix[1]	4.00
Bentonite	5.00
Calculated composition	
Metabolizable energy (Kcal/Kg)	2660
Crude protein (%)	15.50
Digestible lysine (%)	0.73
Digestible methionine (%)	0.42
Digestible methionine + cysteine (%)	0.64
Digestible threonine (%)	0.50
Calcium (%)	4.00
Available phosphorus (%)	0.37
Analyzed composition (%)	
Crude protein	15.31
Ash	15.20
Dry matter	91.57

[1]Vitamin and mineral premix provided (units per kg/feed): retinol (vitamin A), 3.9 mg; cholecalciferol (vitamin D_3), 0.09 mg; α-tocopherol acetate (vitamin E), 21 mg; menadione (vitamin K_3), 4.2mg; thiamine (vitamin B_1), 3.0 mg; riboflavin (vitamin B_2), 10.2 mg; pantothenic acid (vitamin B_5), 16mg; pyridoxine (vitamin B_6), 8.0 mg; biotin (vitamin H), 0.15 mg; cobalamin (vitamin B_{12}), 0.024mg; choline, 500 mg; folic acid, 0.9 mg; iron, 66 mg; iodine, 0.45 mg; manganese, 80 mg; zinc, 83 mg; selenium, 0.3 mg.

Table 2. The physical properties of the pellet

Index	Value
Dry matter %	90.62 ± 0.43
Post pelleting temperature (℃)	68.20 ± 3.8
Post Cooling temperature (℃)	20.80 ± 1.3
PDI (%)	84.22 ± 2.64
Hardness (kg)	3.90 ± 0.27
Fine powder content (%)	2.10 ± 0.51
Diameter (mm)	4.00 ± 0.26
Length (mm)	7.00 ± 1.29

The values are mean ± SD

Production performance and egg quality

The birds were weighed individually at the beginning and end of the study. Egg production, egg weight, and abnormal eggs were recorded daily by replicate. Egg mass was calculated daily by multiplying egg production by egg weight. Feed was supplied to the birds two times per day, and FI was calculated by weighing the remaining feed at the end of each week. The FI and FCR (kg feed consumed per kg egg produced) were determined at one-week interval and for the entire experimental period. Any mortality was recorded and weighed as it occurred. Egg production and FI were adjusted for hen mortality. The abnormal egg proportion was calculated by dividing the total number of abnormal eggs (shell less, soft shell, cracked eggs) by the total number of eggs in each replicate.

At 97th week of age, 18 eggs per treatment were randomly collected for egg quality analysis on the day of collection. The egg shape index (ratio of length to width) was calculated by measuring the length and width of the egg with a caliper with an accuracy of 0.01. Egg weight, eggshell weight, albumen weight and yolk weight were measured using an electronic balance to the nearest 0.01 g and expressed as a percentage of the egg weight. Eggshell thickness was measured in three points using a micrometer gauge (VIJAY SURGICAL Micrometer Delhi, India) as described by Bozkurt et al. (2012). The egg yolk color was measured using yolk color fan Roche. The egg specific gravity was measured by the flotation of the egg in five salt solutions with specific gravities of 1.070, 1.075, 1.080, 1.085, and 1.090 as described by Butcher and Miles (2017). The Haugh unit (HU) was calculated using the following formula proposed by Haugh (1937).

$HU (\%) = 100 \times \log (H + 7.57 - 1.7W^{0.37})$

where H is the height of the albumen in mm and W is the weight of the egg in g,

Intestinal morphology

At the end of study, six birds per treatment (closer to the mean body weight of every replicate) were selected, killed via cervical dislocation. Immediately after slaughter, two-cm section of the jejunum (5 cm anterior to the Meckel's diverticulum) was excised, fixed in 10% neutral buffered formalin, processed in graded alcohol series and then cleared in xylene. The samples were embedded in paraffin and cut using a CUT4055 manually microtome (microTec Laborgeräte GmbH, Germany). Histological sections were stained with hematoxylin-eosin and digitalized with a computer-aided light microscope (Olympus BX51TF, Japan). The histological parameters recorded included VH, villus width (VW), at the half height point of villus, CD and VCR. Villus surface area was calculated using a formula described by Sakamoto et al. (2000).

$VSA = 2\pi \times (VW/2) \times VH$,

where. π= 3.14, VW=villus width in µm and VH=villus height in µm.

Cecal microbial population

At the same time as the intestinal sampling, cecal samples were also collected. One gram of cecal contents was aseptically mixed with 9 mL of sterile phosphate buffered saline solution (PBS) and serially diluted to 10^{-1} to 10^{-10}. For incubation, one mL of each dilution was used in triplicate. *Coliforms* were enumerated on MacConkey agar, *Enterococcus* spp. on Slanetz-Bartley agar, *Lactobacillus* spp. on de Man–

Rogosa–Sharpe agar and *Bifidobacterium* spp. on Eosin Methylene Blue agar. The plates were then incubated at 37°C, for 24-48 h (Slanetz-Bartley agar, Eosin Methylene Blue agar and MacConkey agars), or 48-72 h at 37°C (Man–Rogosa–Sharpe agar), and the colonies were counted. Microbial population was expressed as base-10 logarithm colony-forming units per gram (log CFU/ g) of cecal sample.

Economic consideration

Economic performance was assessed by calculating the income over feed cost, which was derived from the revenue generated from egg sales and the cost of feed consumed by the hens. The net profit was determined by subtracting the feed cost from the selling price of all eggs. It is important to note that fixed costs related to the experiment, such as the initial purchase price of hens, veterinary care, housing, and labor, were not included in the analysis as they were consistent across all treatment groups (Elsherbeni et al., 2024).

Statistical analysis

Statistical analysis of the data was conducted using SAS software version 9.2 (SAS Institute, Cary NC, 2017). Each treatment consisted of 6 replicates. The independent sample t-test was used because each measure had only two groups. A difference was deemed significant only when the *P*- value was less than 0.05; *P*-values between 0.05 and 0.10 were considered as trends.

Results

Productive performance and egg quality

The effects of experimental diets on body weight gain (BWG) and productive performance of laying hens are shown in Table 3. Mortality, 1.3% and 1.8% for mash and pellet treatments, respectively, was not related to treatments (data not shown). The BWG was greater in birds fed the pelleted diet (P<0.05). Physical form of the diet had no significant effect on egg production ad FCR, but feeding pelleted feed increased the egg weight, egg mass and FI compared with the mash diet (P<0.05).

The influence of physical form of the diet on the egg quality parameters is summarized in Table 4. A Significant decrease in the shell weight and shell thickness (P<0.05) was observed in birds receiving the pelleted feed compared with those fed the mash diets. Abnormal eggs, egg-shape index, Haugh unit, yolk weight, albumen weight and specific gravity were not affected by treatments (P>0.05). Birds fed the mash diet had higher yolk color score than the birds fed the pelleted diet (P<0.05).

Table 3. Effect of the diet physical form on performance and body weight gain in laying hens (90-97 weeks of age)

Form of the diet	Body weight gain (g- 90-97 weeks)	Egg production (H.D. %)	Egg weight (g)	Egg mass (g)	Feed intake (g/hen/day)	FCR
Mash	17.5 *	73.5	63.1 *	46.5*	102.5*	2.28
Pellet	122.2	76.8	66.3[a]	50.9	113.5	2.26
SEM	26.1	14.9	0.91	1.5	3.2	0.066
P-value	0.0004	0.13	0.009	0.03	0.007	0.854

*: significantly different from the pelleted form (P>0.05; t-test)

Table 4. Effect of the diet physical form on egg quality parameters in laying hens (at the end of experiment)

Form of the diet	Abnormal eggs (%)	Shape index (%)	Haugh unit	Yolk weight (%)	Albumen weight (%)	Shell weight (%)	Shell thickness (mm)	Specific gravity (g/cm^3)	Yolk color score
Mash	2.67	0.753	88.6	28.7	60.9	9.42 *	0.380 *	1.088	6.73*
Pellet	2.87	0.748	88.4	28.4	61.8	8.68	0.363	1.087	6.14
SEM	0.346	0.0054	1.27	0.6670	0.7241	0.1117	0.0063	0.001	0.13
P-value	0.71	0.602	0.918	0.713	0.326	<0.0001	0.0352	0.655	0.025

*: significantly different from the pelleted form (P>0.05; t-test)

Intestinal morphology

The birds fed the pelleted diet had higher VW and VSA in comparison with the fed mash diet (P<0.001, Table 5). Furthermore, laying hens fed the pelleted diet showed a tendency towards shallower crypts and a higher VCR (P < 0.1). The VH was not affected by the physical form of the diet (P>0.05).

Table 5 Effect of the diet physical form on the intestinal morphology in laying hens (at the end of experiment)

Form of the diet	Villus height (µm)	Villus width (µm)	Villus surface area (mm^2)	Crypt depth (µm)	VH/CD
Mash	1514.0	130.3 *	0.620 *	98.5	15.3
Pellet	1520.7	143.7	0.686	94.3	16.1
SEM	16.3	3.31	0.0156	1.74	0.3168
P-value	0.79	0.0004	<0.0001	0.089	0.0877

*: significantly different from the pelleted form (P>0.05; t-test)

Cecal microbial population

There was no effect of feed form on the cecal microbial population including *Bifidobacteria* spp. and *Lactobacillus* spp. (P>0.05, Table 6). Feeding pelleted diet promoted greater numbers of *Enterococcus* spp. in cecal samples than in hens fed the mash diet (P<0.05). There was also a tendency toward higher *Coliforms* population in the birds fed the pelleted diet compared with the mash diet (P=0.055).

Table 6. Effect of the diet physical form on the fecal microbial population (log CFU/ g) in laying hens (at the end of experiment)

Form of the diet	*Enterococcus*	*Coliforms*	*Bifidobacteria*	*Lactobacillus*
Mash	5.11 *	4.76	6.00	7.47
Pellet	5.27	4.82	5.99	7.52
SEM	0.046	0.023	0.020	0.031
P-value	0.004	0.055	0.656	0.360

*: significantly different from the pelleted form (P>0.05; t-test)

Economics

Feed intake of the hens fed the pelleted diet was approximately 10.7% higher compared to those consuming the mash diet. Additionally, pelleting the feed (at a cost of 3000 Iranian Rials per kg) led to a 9.32% increase in feeding costs compared to the mash diet. Despite the higher feeding cost, hens fed the pelleted diet showed greater gross revenue, with a 17 Rials/day/hen increase compared to those fed the mash diet (Table 4). While the observed profit margin may appear modest, its cumulative impact across large-scale operations could be substantial.

Table 7. Effect of the diet physical form on economic efficiency of experimental groups at the end of the experiment

Form of the diet	Egg production (H.D. %)	Abnormal eggs (%)	Total income/hen/day (Rial)	Feed intake (g/hen/day)	feed cost/hen/day (Rial)	Net Gross revenue/hen/day[1] (Rial)
Mash	73.5	2.67	7613.03 *	102.5 *	3297.19 *	4315.84
Pellet	76.8	2.87	8324.01	113.5	3991.81	4332.20
SEM	14.9	0.346	223.561	3.2	77.27124	185.562
P-value	0.13	0.71	0.05	0.007	0.0001	0.95

*: significantly different from the pelleted form (P>0.05; t-test)

1. The percentage of abnormal eggs was considered in the calculation of the net revenue.

Discussion

Heat stress can negatively impact egg production and the overall health of laying hens. In the summer months in Iran, indoor farm temperatures can reach up to 38 °C for several hours daily. This study revealed that hens fed with the pelleted feed gained more weight compared to those fed the mash feed. Previous research on the impact of feed form (crumbled or pelleted vs. mash) on BWG in poultry has not been consistent. Studies on pullets (Frikha et al., 2009; Saldaňa et al., 2015a; Bozkurt et al., 2019) and laying hens (Wahlström et al., 1999a) reported higher BWG with the pelleted diet, possibly due to increased FI associated with the higher bulk density of pellets (Frikha et al., 2009). Frikha et al. (2009) suggested that the increased FI with pellet might lead to better BWG due to the consumption of more nutrients, especially energy. Moreover, improved nutrient digestibility linked to pelleted diet (Wahlström et al., 1999b; Wan et al., 2021), could further contribute to increased BWG. Conversely, no significant effect of feed form on BWG was observed in laying hens by Hamilton and Proudfoot (1995), Koçer et al. (2016), Ruhnke et al. (2015), and Ege et al. (2019).

The present study found that hens fed the pelleted diet had increased FI compared to those fed the mash diets. However, the FCR was not significantly affected by the treatment. The pelleting process may increase FI due to the improved texture and density of feed, and potentially its faster passage rate through the digestive system (Mateos et al., 2012; Zheng et al., 2020). These findings are consistent with previous research by Zheng et al. (2020) and Wan et al. (2021), who reported increased FI in pullets and laying hens fed pelleted diet compared to mash. However, Wahlström et al. (1999a) observed no difference in FI or egg production between crumbled and mash diets, but hens fed crumbled diets had higher egg weight and mass. They attributed this discrepancy to the potential effects of the housing system or crumbled feed compared to intact pellets. Mousavi et al. (2016) reported higher egg weight and better FCR but lower FI with pelleted feed, although egg production was not significantly affected. Inconsistencies in research findings likely stem from a complex interplay of factors. These include inherent differences in the birds themselves (breed, age, laying rate), variations in experimental design and duration, the specific qualities of the pellets (pressure, humidity, temperature used during production, pellet size, and hardness), the overall composition of the diet and ingredient types used, and finally, the environmental temperature, which is known to affect laying hens and may further interact with the chosen feed form.

Our study found higher egg weight with pelleted feed compared to previous research under normal temperatures (Hamilton et al., 1995; Ruhnke et al.,

2015). The key difference might be the temperature. Our hens experienced high temperatures (32 °C for 6 hours), which can reduce FI in mash diets, potentially explaining the difference in results. Previous investigations have established a link between HS and suppressed FI in laying hens. Wan et al. (2021) demonstrated that pelleting improved the dry matter and crude protein digestibility compared to mash form for in laying hens. The combined effects of increased FI and improved nutrient digestibility may explain the observed improvements in egg weight. An alternative explanation for the positive impact of the pelleted feed on egg weight could be related to increased availability of linoleic acid in the intestine. Wahlström et al. (1998, 1999b) reported higher crude fat digestibility in a crumbled diet during a metabolic trial. This suggests that increased availability of linoleic acid, a key fatty acid, might contribute to higher egg weight.

The present study indicated that laying hens fed the pelleted diet produced more egg mass compared to those on the mash diet. This improvement in performance may be attributed to several potential mechanisms associated with pelleting: (a) Fine grinding and pelleting processes disrupt the seed coat and fracture the endosperm of grains. This facilitates access of digestive enzymes to intracellular oils (e.g., those contained in corn spherosomes) and other nutrients, potentially leading to increased nutrient utilization (Mateos et al., 2002); (b) The suppression of FI in HS conditions is hypothesized to be a key factor influencing alterations in the cecal microbiota composition and the subsequent decline in egg production (Xing et al., 2019). Several studies reported elevated FI in hens fed the pelleted diet (Saldaňa et al., 2015; Mahdavi et al., 2018; Ege et al., 2019; Zheng et al., 2020). This translates to a higher intake of nutrients, which can positively impact the egg production; (c) Pelleting may reduce the energy required for feed consumption and decrease the feed waste (Serrano et al., 2012). Furthermore, research suggested that pelleting can enhance intestinal absorption capacity (Mahdavi et al., 2018) and increase the pancreatic enzyme activity (Abdollahi et al., 2013; Mahdavi et al., 2018; Ege et al., 2019). Additionally, in line with previous research by Hamilton and Proudfoot (1995) and Wan et al. (2021), mortality rates were not significantly affected by the feed form.

Our findings indicated a minimal influence of feed form on most egg quality parameters. However, hens fed the mash diets exhibited greater shell weight, shell thickness, and yolk color scores compared to those on the pelleted diet. Consistent with our observations, prior research suggested a minimal impact of feed form on most egg quality parameters. These studies, including Wahlström et al. (1999a), Hafeez et al. (2015), Koçer et al. (2016), Ege et al. (2019), Wan et al. (2021), and Zheng et al. (2020), observed no significant differences in the prevalence of abnormal eggs, yolk weight, albumen weight, Haugh unit scores (a measure of albumen quality), or other internal quality parameters. However, unlike the current study, these studies did not report any changes in shell weight or thickness. Our study identified a significant reduction in both shell weight and thickness within the treatment group consuming the pelleted diet. The decline in sell thickness may be attributable, in part, to the concurrently observed increase in egg size (Roland, 1980). Further research is required to elucidate the contributions of other potential factors to these observed declines. The impact of feed form on yolk color score in laying hens is inconclusive. Our study showed a reduction in yolk color score with the pelleted vs. mash diets, which is consistent with the findings by Koçer et al. (2016), Ege et al. (2019), and Zheng et al. (2020), who also observed decreased yolk color intensity with crumbled feed. In contrast, Wahlström et al. (1999a) reported increased scores with crumbled or pelleted feed, while Wan et al. (2021) found no significant effect. The decrease in yolk color score for the hens fed the pellet could be due to the potential degradation of dietary xanthophylls (pigments responsible for yolk color) during pelleting as a result of high processing temperatures (Ege et al., 2019).

Our study found that feeding the pelleted diet increased the jejunal VW and VSA compared to the mash diet. These findings align with those of Ege et al. (2019), who reported increased VH, VW, and VSA in the ileum of laying hens fed crumbled diet compared to mash. However, our results partially differ from Röhe et al. (2014), who observed shorter ileal villi, longer duodenal villi, and increased duodenal VCR in mash-fed hens. The improved VW and VSA in our pellet-fed group corresponded to enhanced production performance and potentially indicated an increase in intestinal digestive and absorptive capacity, as suggested by Cera et al. (1988). The observed increase in VSA in the hens fed the pelleted diet could be an adaptive response to the higher FI and nutrient flow associated with this feed form (Amerah et al., 2007).

Previous research established that HS altered the composition and abundance of the intestinal microbiota in laying hens and broiler chicks (Wang et al., 2018; Shi et al., 2019). Reduced FI is a known negative consequence of HS (Xing et al., 2019), which may partially explain the changes in the gut microbiota. Our study observed an increase in the cecal population of potentially harmful bacteria (*Enterococcus* spp.) in birds fed with the pelleted diet. Svihus (2014) suggested that a higher gizzard pH may be associated with increased pathogenic bacteria in birds fed with the pellets. The mash feed, containing coarser particles, typically remains longer in the gizzard, stimulating its activity and leading to a lower gizzard pH (0.2–1.2-unit reduction) due to increased hydrochloric acid secretion (Thomas et al., 1998; Svihus, 2014). The lower gizzard pH in mash-fed hens might suppress the growth of harmful bacteria entering the digestive tract. The economic benefit of the pelleted diet, driven by performance improvement, may have compensated for the increased feeding cost.

Conclusion

This study demonstrates the significant influence of feed form on the production performance, egg quality, intestinal morphometry, and cecal microbiota composition in laying hens under HS conditions. Hens fed the pelleted diet exhibited increased FI, BWG, egg weight, and egg mass production compared to those on the mash diet. However, these benefits were accompanied by a negative impact on the shell quality and yolk color. Interestingly, the pelleted diet promoted positive changes in the intestinal morphology, characterized by increased VW and VSA. However, this feed form also led to alterations in cecal microbial populations, with a notable increase in *Enterococcus* spp. These findings highlight the importance of carefully considering the feed form to achieve a balance between optimal laying hen productivity and gut health. The enhanced performance linked to the pelleted diet may have surpassed the economic burden of its higher feeding cost. To elucidate the potential benefits of feed form for laying hens exposed to HS, further investigations are warranted to explore its effects and delineate the underlying physiological mechanisms.

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