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Effects of Feed Physical Form and Insoluble Fiber during Different Rearing Periods on Productive Performance, Immune Response, Behavior, Tibia indices and Gastrointestinal Alterations of W-36 Laying Pullets

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Abstract

This experiment was conducted to evaluate the effects of changing feed form in commercial laying pullets at their 9-14 weeks of age. In the first eight weeks of rearing, the birds were fed four basal diets, including two feed forms (mash;M vs. pellet;P) and two levels of sunflower hulls (SH) inclusion (0 vs. 4%); then, they were assigned to 11 treatments with eight replicates of 9 birds each. The experimental groups consisted of the mash diet during 0-8 and 9-14 weeks (control); mash from 0-8 week and pellet +SH from 9-14 weeks (M-PSH); and so on MSH-MSH; MSH-PSH; P-P; P-M; P-MSH; P-PSH; PSH-PSH; PSH-MSH; and M-MSH. During the whole period, average daily feed intake (ADFI) in the birds fed with pellet or mash and then fed with the pellet containing SH (P-PSH and M-PSH) was higher than with the other treatments ($P < 0.05$). Average daily weight gain (ADG) was affected by dietary fiber and feed form, whereas it was found lower in the PSH–MSH treatment birds and greater for the birds in MSH-PSH treatment than others ($P < 0.05$). Further, FCR improved in the birds fed the mash diet with and without fiber from during 1-14 weeks ($P < 0.05$). Feeding pellet diet without fiber during 1-14 weeks resulted in lower gizzard weight and deeper crypt than the other groups ($P < 0.05$). In addition, the greater and lower villus surface area belonged to the birds fed MSH-MSHP-P diets during 1-14 weeks, respectively. The greatest tibiotarsal index was related to the group fed the pellet or mash without fiber during 0-14 weeks ($P < 0.05$). In conclusion, considering the costs for pellet production, it seems that feeding pellets just during the first eight weeks of age could lead to better performance in commercial situations. Moreover, dietary inclusion of SH improved the function of gizzard.

Introduction

The quality of pullets at the end of their growing phase is critical for the performance of laying hens. Thus, the growing period should follow the nutritional objectives to raise healthy birds with the ideal body weight; this could ensure that they would be prepared at the expected age for a specific commercial strain. Intensive genetic selection has significantly reduced the feed intake (FI) and bodyweight of the laying hens. Therefore, FI in some laying hen strains may be a limiting factor at the beginning of the laying period to achieve optimal egg production. Based on the Hy-Line W-36 recommendation, if hens consume 74 g feed, the diets should contain 5.6% calcium, 21.62% crude protein, and 2955 kcal/kg metabolizable energy. The greater levels of these nutrients can result in a more dense diet and consequently increase feed cost (Hy-Line W-36 Management Guide, 2016). The efficacy of the feed form in pullets and layers is a subject of debate among nutritionists. The diet selected for pullets and laying hens is usually in the mash, crumble and pellet forms (Jiménez-Moreno *et al*., 2019). Commercial laying pullets do not achieve the standard weights

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when fed the usual mash feed at 18 weeks of age. Therefore, the use of pellet may help to improve this growth decline according to practical results. The feed form and composition of the feed ingredients can significantly affect the growth performance in the laying pullets (Jiménez-Moreno *et al*., 2016). Pellet feed has many potential benefits, including the improvement of the feed absorption and feed conversion ratio (FCR), (Saldaña *et al*., 2015a), reducingfeed wastes (Serrano *et al*., 2013), increasing feed intake, reducingselective feeding (Lv *et al*., 2015), increasing flock uniformity and subsequently providing the better growth performance (Jiménez-Moreno *et al*., 2019). In contrast, the pellet feed consumption in pullets increases cannibalism and reduces gizzard development due to the decreased feed consumption time; so, the role of the gizzard changes from a grinding organ to a transit one (Van Krimpen *et al*., 2005). The developed gizzard can boost cholecystokinin secretion in duodenum (Svihus *et al*., 2004) by improving gut motility (Ferket, 2000), consequently increasing the secretion of pancreatic enzymes and improving nutrient utilization (Amerah *et al*., 2007a). Therefore, identifying the beneficial structure of the diet and its components is critical to increasing the health and production of pullets.

To perform this goal, some of the previous studies have suggested that the consumption of insoluble fiber could promote the development of the gastrointestinal tract (GIT); but these effects depend on the type and level of fiber (Hetland and Svihus, 2007). Adding the soluble fiber to the birds' diet causes negative changes in the digesta viscosity, decreases the rate of gastric emptying, reduces the feed intake, and decreases the feed digestibility, thus reducing the production and performance of birds (Mateos *et al*., 2012). Adding the insoluble fiber in the diet of brown-egg laying pullets from hatching to 5 weeks of age increases the weight and size of the gizzard (Guzmán *et al*., 2015a) and improves nutrient digestibility and GIT development of birds (Kalmendal *et al*., 2011).

Various types of insoluble fibers, such as sunflower hulls (SH), have been considered in different studies (Jiménez-Moreno *et al*., 2019; Mateos *et al*., 2012; Viveros *et al*., 2009). In this regard, Kimiaeitalab *et al*. (2017) reported that the addition of SH to the pullet diets increased the weight and reduced the pH of the gizzard. A similar experiment has been reported by Guzmán *et al*. (2015b), who noted a 6.4% enhancement in the average daily gain (ADG) of brown-egg laying pullets with the inclusion of 2% SH in their diet.

It should be noted that the price of ration at the peak of egg production increases, but laying persistency decreases during the peak production. To cope with these problems, we hypothesized that the beneficial effects of SH as an insoluble fiber on gastrointestinal tract development and nutrient digestibility could reduce the adverse effects of consuming the pellet feed in the laying pullets. Furthermore, feeding with different feed forms during the first (1 to 8 weeks) and second (9 to 14 weeks) rearing periods may help achieve the standard weight and increase the GIT development and feed intake during production periods. So, this study aimed to evaluate the effects of feeding the SH as insoluble fiber on the growth performance, carcass characteristics, intestinal morphology, and tibia indices of W-36 laying pullets fed with a low fiber diet either in the mash or pellet form.

Materials and Methods

Birds and experimental design

All animal work in the present study was conducted according to the comprehensive guidelines of animal welfare adopted by FASS (2010). The experiment was started at eight weeks of age. To prepare birds for the trial, day-old Hy-line W-36 were fed with four different diets from 0 to 8 weeks of age, consisting of 1) a mash diet without SH, 2) a mash diet with SH, 3) a pellet diet without SH and 4) a pellet diet with SH. At the end of eight weeks of age, the birds were divided into 11 treatments (8 replicates of 9 birds in each cage) based on a completely random design. These 11 treatments were obtained by changing the feed form and adding SH during two ages (0-8 and 9-14 weeks).

Table 1. Experimental treatments

	0-8 week	9-14week
Control	Mash diet with 0%sunflower hull	Mash diet with 0%sunflower hull
M-PSH	Mash diet with 0%sunflower hull	Pellet diet with 4%sunflower hull
MSH-MSH	Mash diet with 4%sunflower hull	Mash diet with 4%sunflower hull
MSH-PSH	Mash diet with 4%sunflower hull	Pellet diet with 4%sunflower hull
P-P	Pellet diet with 0%sunflower hull	Pellet diet with 0%sunflower hull
P-M	Pellet diet with 0%sunflower hull	Mash diet with 0%sunflower hull
P-MSH	Pellet diet with 0%sunflower hull	Mash diet with 4%sunflower hull
P-PSH	Pellet diet with 0%sunflower hull	Pellet diet with 4%sunflower hull
PSH-PSH	Pellet diet with 4%sunflower hull	Pellet diet with 4%sunflower hull
PSH-MSH	Pellet diet with 4%sunflower hull	Mash diet with 4%sunflower hull
M-MSH	Mash diet with 0%sunflower hull	Mash diet with 4%sunflower hull

The experimental groups are shown in Table 1. In general, we tested the effects of switching the feed form or adding SH at eight weeks of age on the performance of pullets during 9-14 weeks of age. The environmental conditions were controlled according to Hy-Line W-36 Commercial Layer Management Guide (2016). The temperature was 32 to 33 °C from the hatch to day three and gradually decreased to 21°C when the chicks reached 56 d of age. The light was available for 22 hours during 0-3 days, then dropped to 21 hours at 4-7 days. After the first week, we reduce the lighting duration according to the Hy-Line W-36 recommendation (Hy-Line W-36 Management Guide, 2016) to 12 h per day. Feed and water were offered *ad libitum* during the experimental periods. The basal diets (Table 2) were formulated to meet the Hy-Line W-36 strain recommendations in two phases of 9 to 12 weeks and 13 to 14 weeks; so they differed only in the feed form (pellet or mash), with or without SH. Also, diets were formulated based on digestible amino acids (Table 2).

Table 2. Ingredient and nutrient composition of the experimental diets (as-is basis)

Item	Grower (9-12 weeks)		Developer (13-14 weeks)	
	0%SH	4%SH	0%SH	4%SH
Ingredient, %				
Corn grain	66.9	63.1	72.6	69.7
Soybean meal	27.6	26.7	22.2	19.6
Wheat gluten	1	2.5	1	3
Bentonite	0.82	0	0.53	0
Sunflower hull	0	4	0	4
DL-Methionine 99%	0.18	0.17	0.14	0.13
L-Lysine-HCL 78%	0.12	0.15	0.12	0.17
Monocalcium phosphate	1.22	1.23	1.15	1.17
Calcium carbonate	1.4	1.38	1.46	1.45
Vitamin and mineral premix[1]	0.2	0.2	0.2	0.2
Sodium chloride	0.21	0.2	0.2	0.18
Sodium bicarbonate	0.25	0.27	0.3	0.3
Choline chloride 60%	0.05	0.05	0.05	0.05
Phytase enzyme	0.05	0.05	0.05	0.05
Chemical Analysis				
Metabolizable energy, kcal/kg	2977	2977	2977	2977
Crude protein,%	17.5	17.5	16.0	16.0
Crude fiber,%	3.43	5.21	3.21	4.93
ADF	4.49	6.51	4.22	6.21
NDF	11.36	13.89	11.18	13.71
Calcium, %	1.00	1.00	1.00	1.00
Available phosphorus, %	0.47	0.47	0.45	0.45
Sodium, %	0.17	0.17	0.18	0.18
Digestible lysine, %	0.88	0.88	0.76	0.76
Digestible methionine, %	0.40	0.40	0.36	0.36
Digestible methionine + cystine, %	0.67	0.67	0.59	0.59
Digestible threonine, %	0.60	0.60	0.52	0.52

[1]Provides in kg of diet: vitamin A (retinol), 10,000 IU; vitamin D_3 (cholecalciferol), 3,300 IU; vitamin E ($_{DL}$-α-tocopheryl acetate), 25.0 g; vitamin K_3 (menadione), 3.50 mg; vitamin B_1 (thiamin), 2.38 mg; vitamin B_2 (riboflavin), 7.70 mg; vitamin B_3 (niacin), 39.20 mg; vitamin B_5 (pantothenic acid), 9.24 mg; vitamin B_6 (pyridoxine), 4.62 mg; vitamin B_9 (folic acid), 0.84 mg; vitamin B_{12} (cyanocobalamin), 0.03 mg; vitamin H_2 (biotin), 0.08 mg; choline chloride, 154 mg; antioxidant, 1.4 mg; Mn (manganese oxide), 90.00 mg; Zn (zinc oxide), 85.00 mg; Fe (iron carbonate), 30.00 mg; Cu (copper sulfate), 15.00 mg; I (calcium iodate), 0.96 mg; selenium (sodium selenite); 0.25 mg.

Feedstuffs were analyzed for chemical composition, as shown in Table 3. Amino acid, metabolized energy, and crude protein were predicted by near-infrared reflectance (NIR) by the Evonik Co., Evonik, Germany. Crude fiber (CF) (method 978.10), ether extract (method 920.29) and ash (method 942.05) were also analyzed, according to AOAC (2005). Starch concentration was measured according to Zhu *et al*. (2016) with some modifications. In brief, 0.2 g of sample was boiled with 50 mL distilled water for 20 min and then cooled to ambient temperature. Next, 20 mL of acetate buffer solution (pH = 4.80) was added to each tube, followed by adding 3 mL of glucoamylase solution (1 mg/mL), and samples were incubated at 40°C for 70 min. Next, 5 mL of trichloroacetic acid was added to halt hydrolysis. After the sample was cooled to room temperature, distilled water was added to make a final volume of 100 mL. Free d-glucose was then was measured enzymatically through a glucose oxidase-peroxidase assay kit (Pars Azmoon Inc., Tehran, Iran).

Before starting this experiment, the size of the fiber particles used in this study was determined by a sieve, as shown in Table 4. Briefly, more than 86% of the fiber particles were larger than 1180 µm in terms of size.

Table 3. Analyzed chemical composition of corn grain, soybean meal, sunflower hull, and wheat gluten (% as fed basis)

Item	Corn grain	Soybean meal	Sunflower hull	Wheat gluten
Apparent metabolizable energy, kcal/kg[1]	3299	2248	NA[4]	NA
Dry matter, %[1]	87.2	88.4	90.7	93.1
Crude protein, %[1]	7.4	46.5	5.2	68.5
Crude ash, %[1]	1.3	6.6	3.0	0.8
Crude fiber, %[1]	2.4	6.7	50.0	0.5
Ether extract, %[1]	4.1	1.4	4.2	6.5
Starch, %[1]	63.3	0.7	0	0
Acid detergent fibre, %	3.3	8.2	NA	NA
Neutral detergent fibre, %	10.8	14.5	NA	NA
Digestible methionine, %[2]	0.151	0.605	NA	NA
Digestible cystine, %[2]	0.154	0.669	NA	NA
Digestible methionine + cystine, %[2,3]	0.309	1.075	NA	NA
Digestible lysine, %[2]	0.217	2.508	NA	NA
Digestible threonine, %[2]	0.242	1.473	NA	NA
Digestible tryptophan, %[2]	0.050	0.549	NA	NA
Digestible arginine, %[2]	0.330	3.172	NA	NA
Digestible isoleucine, %[2]	0.247	1.864	NA	NA
Digestible leucine, %[2]	0.791	3.119	NA	NA
Digestible valine, %[2]	0.343	1.925	NA	NA
Digestible histidine, %[2]	0.216	1.110	NA	NA
Digestible phenylalanine, %[2]	0.330	2.120	NA	NA

[1]All analyses were performed in triplicate.
[2]Estimated by NIR analysis byEvonik-Degussa Co.,Kennesaw, GA.
[3]Methionine + cystine estimated with separate calibration equation.
[4]Not analysed.

Table 4. Particle size distribution of the sunflower hull (SH)

Sieve size, µm	Amount retained on sieve, %
750	0.2
2360	3.8
1180	81.9
600	9.7
300	3.5
150	0.4
75	0.4
X_{gm},[1] µm	1510
SD_{gm},[2] µm	450

[1] Geometric mean particle size determined by ASABE (2006; method S319.3).
[2] Geometric standard deviation determined by ASABE (2006; method S319.3).

Growth performance

At weeks 10, 12, and 14 of the experiment, the pullets were weighed, and the average daily weight gain (ADG), average daily feed intake (ADFI), and FCR were calculated for each unit biweekly. Also, mortality was recorded daily. In addition, chicks were vaccinated according to the regional vaccination program routine.

Carcass characteristics and internal organs weight

At week 14 of the experiment, one bird close to the mean body weight from each replication (8 pullets for each treatment) was selected randomly and humanly euthanized by CO_2 to study the relative weights (based on the % of live body weight) of the proventriculus, bursa of Fabricius, thymus, spleen, liver, gizzard, gizzard fat (fat tissue surrounding the gizzard), abdominal fat, duodenum, jejunum, ileum, and ceca. Also, the length of different sections of the small intestine was determined.

Gizzard pH

To calculate the gizzard pH at the end of the experimental period, one gram ofgizzard content was

mixed with 9 mL of distilled water, and its pH was determined using a digital pH meter (2211 pH/ ORP meters HI), as described by Al-Natour and Alshawabkeh (2005).

Histological examinations

At the end of the study, to investigate the jejunal morphology of euthanized birds (one bird from each replication), about 1.0 cm from the middle part of the jejunal was cut and washed with saline to remove digesta. To prevent degradation and preserve the physical structure of the intestine, the samples were immediately placed in containers containing 10% formalin as the stabilizer. Samples were dehydrated, cleared, and placed in paraffin. The samples were prepared for tissue expansion using the method described by Iji *et al*. (2001). Then, four samples of each replication were cut to 5 µm thickness by a sled microtome (Sakura SRM 200, Tokyo, Japan), fixed on the slide (Kazemi *et al.*, 2019) and stained with a mixture of hematoxylin and eosin. Samples were prepared for evaluation by optical microscope (Olympus CX31, Tokyo, Japan) and photographed with a digital microscope camera. Villus height (VH), villus width (VW), crypt depth (CD), and muscle layer thickness were measured by ImageJ software (version 1.47r, National Institutes of Health), as explained by Sakamoto *et al.* (2000). The surface area of the intestinal villus was calculated according to Sakamoto *et al*. (2000). A total of 10 intact, well-oriented villus–crypt units were selected for each intestinal cross-section (three cross-sections/sample and 30 villus/replicate).

Tibia indices

At the end of the experimental period, the left tibia was separated from each euthanized bird and kept at -20 °C for the analysis of the morphological characteristics (length, weight, strength and diaphyseal diameter, diameter of the medullary canal, lateral maxillary sinus wall thickness, tibia weight to length ratio, tibiotarsal index and robusticity index), mechanical characteristics (elasticity coefficient) and assessment of biochemical characteristics (dry matter and ash). For this purpose, the meat and fats were separated from the bones, and bones were placed in boiling water for 10 minutes. Then the femoral head of the bone was separated and dried for 24 h at room temperature (Kocabagli *et al*., 2001). Finally, all of the left tibias were weighted. Length of the left tibia and diaphyseal diameter in the central bone in both vertical and parallel directions were measured using the force applied by a digital calliper. Also, bone weight to bone length index was calculated by dividing the tibia weight by its length. The shear stress, flexural stress, and modulus of elasticity were calculated, as described by Kocabagli (2001). After breaking the bone, the wall thickness in both vertical and parallel directions was measured using the digital calliper. The diameter of the tibia medullary canal was calculated by subtracting the thickness of the middle and lateral walls from the diaphyseal diameter. Bones were dried for 24 h at 105 °C. Then, they were placed in a furnace at 600 °C for six h, and the amount of ash was obtained. The dry matter and ash of the tibia were determined according to AOAC (2005). The tibiotarsal and robusticity indexes were measured using the following formulas (Riesenfeld, 1972), respectively:

$$Tibiotarsal\ index = \frac{diaphysis\ diameter - medullary\ canal\ diameter}{diaphysis\ diameter} \times 100 \quad \text{(Equation 1)}$$

$$Robusticity\ index = \frac{bone\ length}{\sqrt[3]{bone\ weight}} \quad \text{(Equation 2)}$$

Immune system response

At 12 weeks of age, to evaluate the humoral immunity, one pullet per replicate was selected randomly for the intravenous injection with 1.0 mL of the 5% suspension of sheep red blood cell (SRBC). The injection was reapplied a week later. Seven days after each injection, about 2 mL of blood from the injected pullets was taken through the wing vein. Blood samples were centrifuged at 1500 rpm for 20 min, and serum was isolated. The antibody response to SRBC was measured by the microagglutination method (Peterson *et al*., 1999). Briefly, the serum of each sample was inactivated at 56°C for 30 minutes. Total, mercaptoethanol-sensitive (IgM) and mercaptoethanol-resistant (IgG) anti SRBC antibodies were measured as previously described by Cheema *et al.* (2003).

Behavior evaluation

To assess the behavioral sensitivity of the birds, five pullets per replicate were selected randomly at the 14th week of rearing and examined. For the behavioral analysis of the birds, a box with the size of 80 × 40 cm and the height of 80 cm was used. The birds were released from the height of 80 cm into the box, and their behavioral response was recorded on a 5-point scale after reaching the bottom of the box. Higher scores meant that birds were afraid of unfamiliar situations and were prone to high-sensitivity behavioral stress (He *et al*., 2016). Each scale was introduced as follows: 1) Sitting down quietly after falling in the box without any effort; 2) Moving slowly after falling in the box and standing looking around with a little effort; 3) Wagging head rapidly and pressing the feet to the box with the medium scrabble after falling in the box; 4) shaking head rapidly and powerfully pushing feet out with severe scrabble and strongly escaping intentionally after falling in the box and; 5) Showing intense behaviour and intending to escape every moment.

Statistical analysis

Data were statistically analyzed using the completely randomized design (CRD) of SAS/STAT® 9.2 (SAS Inst. Inc., Cary, NC. 2008); also, the Tukey's test was used to determine the significant differences among treatment mean values. The probability value ($P < 0.05$) was considered statistically significant.

Results

Performance

The results of the study in three periods, including 9-10, 11-12, and 13-14 weeks of age and the whole period, are shown in Table 5. Treatments did not influence ADG during 9-11 weeks of age, but during 11-12 weeks of age, the birds fed with the pellet without fiber (P-P) had the lowest ADG ($P < 0.05$). Also, at 13-14 weeks of age, ADG was higher in birds of P-P, P-PSH, and M-MSH treatments than the M-PSH treatment ($P < 0.05$). Therefore, these results indicate that at an early age probably, the insoluble fiber could prevent the increase of ADG; in other words, the best period to include the insoluble fiber in the pullet's diet is after week 9 of age.

At weeks 9-10, the control birds, MSH-MSH, M-MSH, and P-MSH treatments, had lower feed intake ($P < 0.05$); also, the birds in M-PSH and P-PSH treatments had higher ADFI as compared with other groups ($P < 0.05$). At weeks 11-12 of age, feeding the birds with P-PSH had the highest ADFI, while the control (M-M) had the lowest. In addition, at the 13-14 week period, the birds in M-PSH treatment showed the highest ADFI, while pullets in control and M-MSH groups had the lowest feed intake ($P < 0.05$). Further, the results of the study in the whole period showed that the M-PSH and P-PSH treatments had highest ADFI, as compared to other treatments ($P < 0.05$).

No significant differences were observed in FCR during the 9-10 weeks period; however, during 11-12 weeks of age, the highest FCR was related to the P-P treatment, and the lowest FCR was associated with the MSH-MSH treatment ($P < 0.05$). Also, at 13-14 weeks of age, FCR in the M-PSH experimental group showed a significant increase compared to the other treatments ($P < 0.05$). On the other hand, in the whole period, FCR was higher in birds that fed with mash and the pellet with SH during the first and second periods, respectively (M-PSH), as compared to the control, MSH-MSH, MSH-PSH, P-MSH, and M-MSH experimental groups ($P < 0.05$).

The coefficient of variation (CV) indicates body weight uniformity. As shown in Table 6, at the end of weeks 10 and 12, no significant differences were observed between treatments in terms of CV; but, at the end of 14 weeks, CV was higher in the P-PSH and M-MSH groups as compared to the PSH-PSH group ($P < 0.05$). In this study, the lowest final body weight at week 14 of age was related to the control treatment. In other words, it seems that feeding with a pellet diet (either with or without the addition of insoluble fiber to the diet) at least in one of the rearing periods could improve the final body weight of pullets.

This study showed that using a mash or pellet diet without SH from 1 to 8 weeks and changing it to the pellet with 4% SH after week 9 (M-PSH) could increase the pullet's ADFI ($P < 0.05$). In contrast, the mash diet with or without SH significantly decreased ADFI in all periods ($P < 0.05$). Furthermore, feeding the pelleted feed (without SH) from 1 to 14 weeks (P-P) could not increase the ADFI of pullets.

The pullets respond positively to the inclusion of insoluble fiber in their diet from 9 to 14 weeks of age. Based on the current findings, the inclusion of the insoluble fiber in the diet decreased ADFI in the earlier period of rearing because increasing the insoluble fiber in the diet increases the retention time of feed in the gastrointestinal tract and therefore reduces the rate of feed passage.

Carcass characteristics and internal organs weight

The effects of adding different physical forms of the diet with or without insoluble fiber on the relative weight of carcass and different organs in 14-wk pullets are shown in Table 7. The relative weight of proventriculus, bursa of Fabricius, thymus, spleen, liver, gizzard fat, abdominal fat, duodenum, jejunum, ileum, and cecum were not affected by dietary treatments. However, the lowest gizzard weight belonged to the birds fed pelleted feed from 1 to 14 weeks (P-P treatment). Also, the M-MSH experimental treatment had a heavier gizzard relative weight than the other treatments ($P < 0.05$). On the other hand, the pullets that received the MSH diet for 9 to 14 weeks (regardless of the diet they received in the first period) had the highest gizzard relative weight.

Histological examinations

As reported in Table 8, The experimental treatments did not affect the length of different parts of the GIT and the gizzard pH. Also, the dietary treatments did not affect the villus height, crypt depth, villus width, and muscular layer thickness. However, the consumption of pellets without SH in the 9 to 14 weeks period (P-P) resulted in a deeper crypt and smaller villus surface area ($P < 0.05$); so it seems that adding SH to the pellet diets could ameliorate this adverse effect. Also, by comparing the data, it could be concluded that the use of SH in the mash diets reduced the crypt depth and increased the villus surface area.

Table 5. The effects of feed form and insoluble fiber inclusion in the diet on the growth performance in the laying pullets

Treatments	9-10 week			11-12 week			13-14 week			9-14 week		
	ADG (g/b/d)	ADFI (g/b/d)	FCR (g/g)	ADG (g/b/d)	ADFI (g/b/d)	FCR (g/g)	ADG (g/b/d)	ADFI (g/b/d)	FCR (g/g)	ADG (g/b/d)	ADFI (g/b/d)	FCR (g/g)
Control	12.5	48.5^{c}	3.863	11.1^{ab}	54.0^{e}	4.922^{bc}	8.3^{ab}	54.5^{c}	6.818^{b}	10.6^{b}	52.4^{c}	4.926^{b}
M-PSH	14.1	55.7^{a}	3.986	11.4^{a}	57.4^{abc}	5.013^{bc}	7.4^{b}	62.3^{a}	8.483^{a}	11.0^{ab}	60.8^{a}	5.531^{a}
MSH-MSH	13.5	48.8^{c}	3.612	11.2^{ab}	54.4^{de}	4.784^{c}	8.4^{ab}	56.0^{abc}	6.596^{b}	11.1^{ab}	53.3^{c}	4.789^{b}
MSH-PSH	14.2	52.9^{ab}	3.725	11.5^{a}	57.1^{bcd}	5.023^{bc}	8.1^{ab}	58.6^{abc}	7.210^{b}	11.3^{a}	55.6^{c}	4.896^{b}
P-P	13.4	52.2^{abc}	3.889	10.1^{b}	59.2^{ab}	5.855^{a}	9.3^{a}	57.8^{abc}	6.255^{b}	11.0^{ab}	55.9^{bc}	5.116^{ab}
P-M	13.1	49.8^{bc}	3.768	10.4^{ab}	54.7^{cde}	5.213^{abc}	8.1^{ab}	55.7^{bc}	6.941^{b}	10.5^{b}	53.3^{c}	5.084^{ab}
P-MSH	13.0	48.4^{c}	3.692	10.9^{ab}	54.5^{de}	5.026^{bc}	8.4^{ab}	55.7^{bc}	6.767^{b}	10.8^{ab}	53.6^{c}	4.945^{b}
P-PSH	13.7	54.5^{a}	3.976	11.1^{ab}	60.1^{a}	5.395^{ab}	8.6^{a}	61.3^{ab}	7.014^{b}	11.2^{a}	59.3^{ab}	5.284^{ab}
PSH-PSH	14.0	51.9^{abc}	3.693	11.0^{ab}	57.0^{bcd}	5.189^{abc}	8.1^{ab}	59.0^{abc}	7.190^{b}	11.0^{ab}	56.0^{bc}	5.079^{ab}
PSH-MSH	11.8	49.1^{bc}	3.893	11.0^{ab}	56.9^{bcde}	5.098^{bc}	8.1^{ab}	58.3^{abc}	7.280^{b}	10.3^{b}	54.2^{c}	5.183^{ab}
M-MSH	13.5	48.4^{c}	3.554	10.9^{ab}	54.5^{de}	5.025^{bc}	9.0^{a}	54.3^{c}	6.219^{b}	11.1^{ab}	52.8^{c}	4.754^{b}
SEM	0.269	0.847	0.101	0.243	0.545	0.128	0.243	1.296	0.240	0.182	0.730	0.108
P-value	0.222	0.001	0.614	0.016	0.001	0.001	0.003	0.001	0.001	0.028	0.001	0.002

[abc] Values within a column followed by different superscripts are significantly different. $P < 0.05$; Tukey's pairwise test.

SEM: standard error of the mean, ADG: Average daily weight gain, ADFI: Average daily feed intake, FCR: Feed conversion ratio

Mash diet during 1-8 and 9-14, (control); mash from 1-8 and pellet+SH from 9-14 (M-PSH); mash diet +SH during 1-8 and 9-14 (MSH-MSH); mash diet +SH during 1-8 and pellet+SH from 9-14 (MSH-PSH); pellet diet during 1-8 and 9-14 (P-P); pellet diet during 1-8 and mash from 9-14 (P-M); pellet diet during 1-8 and mash+SH from 9-14 (P-MSH); pellet diet during 1-8 and pellet+SH from 9-14 (P-PSH); pellet +SH diet during 1-8 and pellet+SH from 9-14 (PSH-PSH);pellet diet +SH during 1-8 and mash+SH from 9-14 (PSH-MSH); Mash diet during 1-8 and Mash+SH from 9-14 (M-MSH).

Table 6. The effects of feed form and insoluble fiber inclusion in the diet on 14 weeks body weight and coefficient of variation (CV) in the laying pullets

Treatments	Bodyweight of 14 weeks old birds (g)	CV (%)		
		10 week	12 week	14 week
Control	1099.38[b]	5.333	5.666	6.166[ab]
M-PSH	1123.63[ab]	6.000	5.666	6.285[ab]
MSH-MSH	1115.50[ab]	6.666	6.000	6/000[ab]
MSH-PSH	1129.13[ab]	5.666	6.833	5.857[ab]
P-P	1136.50[ab]	6.500	6.166	6.500[ab]
P-M	1121.38[ab]	6.500	7.333	6.666[ab]
P-MSH	1134.50[ab]	6.333	6.166	6.666[ab]
P-PSH	1156.88[a]	5.500	6.333	7/600[a]
PSH-PSH	1149.75[ab]	5.333	5.500	4.833[b]
PSH-MSH	1131.25[ab]	6.000	5.500	5.666[ab]
M-MSH	1109.50[ab]	7.000	5.333	7.000[a]
SEM	11.443	0.412	0.411	0.437
P-value	0.0302	0.187	0.095	0.014

[abc] Values within a column followed by different superscripts are significantly different. $P < 0.05$; Tukey's pairwise test.

SEM: standard error of the mean, CV: Coefficient of variation, Mash diet during 1-8 and 9-14, (control); mash from1-8 and pellet+SH from 9-14 (M-Psh); mash diet +SH during 1-8 and 9-14 (Msh-Msh); mash diet +SH during 1-8 and pellet+SH from 9-14 (Msh-Psh); pellet diet during 1-8 and 9-15 (P-P); pellet diet during 1-8 and mash from 9-14 (P-M); pellet diet during 0-8 and mash+SH from 9-14 (P-Msh); pellet diet during 1-8 and pellet+SH from 9-14 (P-Psh); pellet +SH diet during 0-8 and pellet+SH from 9-14 (Psh-Psh); pellet diet +SH during 1-8 and mash+SH from 9-14 (Psh-Msh); Mash diet during 1-8 and Mash+SH from 9-14 (M-Msh).

Table 7. The effects of feed form and insoluble fiber inclusion in the diet on the relative weight of the digestive and lymphatic organs in the laying pullets (% of live body weight)

	Spleen	Bursa of Fabricius	Thymus	Proventriculus	Liver	Gizzard	Gizzard fat	Abdominal fat	Duodenum	Jejunum	Ileum	Cecum
Control	0.172	0.277	0.419	0.250	1.690	2.02^{ab}	0.754	2.195	0.378	0.554	0.527	0.444
M-PSH	0.166	0.232	0.424	0.430	1.768	1.58^{cde}	1.021	3.054	0.383	0.550	0.708	0.323
MSH-MSH	0.167	0.172	0.484	0.234	1.667	1.92^{abc}	1.250	2.896	0.452	0.612	0.650	0.457
MSH-PSH	0.160	0.249	0.428	0.263	1.637	1.66^{bcde}	1.127	2.477	0.424	0.614	0.634	0.373
P-P	0.173	0.235	0.445	0.251	1.791	1.45^{e}	0.903	2.977	0.422	0.607	0.596	0.383
P-M	0.177	0.222	0.404	0.247	1.652	1.84^{abcd}	1.321	3.011	0.395	0.571	0.667	0.469
P-MSH	0.158	0.159	0.382	0.225	1.704	1.81^{abcde}	1.080	2.475	0.388	0.622	0.596	0.370
P-PSH	0.168	0.271	0.496	0.243	1.826	1.50^{de}	0.847	2.415	0.370	0.562	0.590	0.409
PSH-PSH	0.198	0.278	0.358	0.235	1.812	1.60^{cde}	0.964	3.094	0.388	0.564	0.738	0.412
PSH-MSH	0.166	0.216	0.397	0.226	1.671	1.95^{abc}	1.225	2.466	0.407	0.676	0.716	0.375
M-MSH	0.163	0.218	0.503	0.249	1.714	2.03^{a}	1.054	2.763	0.384	0.577	0.599	0.415
SEM	0.012	0.035	0.054	0.050	0.088	0.067	0.143	0.328	0.021	0.037	0.046	0.036
P-value	0.795	0.565	0.837	0.461	0.920	0.001	0.319	0.703	0.469	0.619	0.161	0.795

^{abc} Values within a column followed by different superscripts are significantly different. $P < 0.05$; Tukey's pairwise test.

SEM: standard error of the mean. Mash diet during 1-8 and 9-14, (control); mash from 1-8 and pellet+SH from 9-14 (M-PSH); mash diet +SH during 1-8 and 9-14 (MSH-MSH); mash diet +SH during 1-8 and pellet+SH from 9-14 (MSH-PSH); pellet diet during 1-8 and 9-14 (P-P); pellet diet during1-8 and mash from 9-14 (P-M); pellet diet during 1-8 and mash+SH from 9-14 (P-MSH); pellet diet during 1-8 and pellet+SH from 9-14 (P-PSH); pellet +SH diet during1-8 and pellet+SH from 9-14 (PSH-PSH); pellet diet +SH during 1-8 and mash+SH from 9-14 (PSH-MSH); Mash diet during 1-8 and Mash+SH from 9-14 (M-MSH).

Table 8. The effects of feed form and insoluble fiber inclusion in the diet on the length of the gastrointestinal tract, gizzard pH and small intestinal morphology in the laying pullets

	Duodenum, cm	Jejunum, cm	Ileum, cm	Cecum, cm	Gizzard pH	Villus height, µm	Crypt depth, µm	Villus height: crypt depth	Villus width µm	Muscular layer thickness, µm	Villus surface area, mm^2
Control	1.975	3.804	3.606	2.295	4.187	735.762	73.98^{ab}	10.773	115.023	244.3	0.230^{ab}
M-PSH	1.827	3.847	3.779	2.002	4.125	608.611	69.17^{ab}	9.337	105.818	289.5	0.276^{ab}
MSH-MSH	1.813	3.715	3.560	2.264	4.115	748.563	64.72^{b}	11.030	120.497	294.9	0.303^{a}
MSH-PSH	1.830	3.932	3.580	2.160	4.248	740.253	70.14^{ab}	10.030	119.494	271.0	0.277^{ab}
P-P	1.816	3.896	3.923	2.343	4.350	582.423	85.65^{a}	8.510	120.965	256.7	0.189^{b}
P-M	1.774	3.412	3.377	2.244	4.550	670.522	63.97^{b}	10.412	121.456	241.6	0.246^{ab}
P-MSH	1.743	3.666	3.566	2.155	4.138	644.502	61.32^{b}	8.819	120.371	202.3	0.244^{ab}
P-PSH	1.749	3.704	3.648	2.237	4.310	608.092	79.62^{ab}	9.378	105.818	229.1	0.208^{ab}
PSH-PSH	1.774	3.618	3.647	2.199	4.221	613.252	68.32^{ab}	8.646	109.446	252.7	0.269^{ab}
PSH-MSH	1.808	3.650	3.660	2.156	4.485	608.822	64.37^{b}	10.856	111.228	265.6	0.220^{ab}
M-MSH	1.740	3.720	3.797	2.331	4.216	691.531	64.74^{b}	9.845	114.483	314.2	0.259^{ab}
SEM	0.093	0.143	0.131	0.084	0.121	3.880	3.620	0.165	4.230	19.1802	25.920
P-value	0.932	0.645	0.526	0.443	0.403	0.220	0.012	0.594	0.215	0.086	0.013

[abc] Values within a column followed by different superscripts are significantly different. $P < 0.05$; Tukey's pairwise test.

SEM: standard error of the mean. Mash diet during 1-8 and 9-14, (control); mash from 1-8 and pellet+SH from 9-14(M-PSH); mash diet +SH during 1-8 and 9-14(MSH-MSH); mash diet +SH during 1-8 and pellet+SH from 9-14(MSH-PSH); pellet diet during 1-8 and 9-14(P-P); pellet diet during 1-8 and mash from 9-14(P-M); pellet diet during 1-8 and mash+SH from 9-14(P-MSH); pellet diet during 1-8 and pellet+SH from 9-14(P-PSH); pellet +SH diet during 1-8 and pellet+SH from 9-14(PSH-PSH); pellet diet +SH during 1-8 and mash+SH from 9-14(PSH-MSH); Mash diet during 1-8 and Mash+SH from 9-14(M-MSH).

Table 9. The effects of feed form and insoluble fiber inclusion in the diet on the tibia parameters in the laying pullets at 14 weeks of age

Treatments	Morphometric parameters								Mechanical measurements	Biochemical measurements
	Weight, mg	Length, mm	Weight: length	Diaphysis diameter, mm	Medullary canal diameter, mm	Lateral wall thickness, mm	Tibiotarsal index	Robusticity index	Modulus of elasticity, kg/cm²	Ash, %
Control	4665.2	115.7	41.85	6.133	4.212	0.927	32.71[a]	6.844	7.628	46.28
M-PSH	4736.8	113.5	41.71	6.317	4.517	0.961	30.09[abc]	6.760	6.797	45.66
MSH-MSH	4551.4	113.4	41.12	6.275	4.435	0.986	30.06[abc]	6.797	8.140	45.64
MSH-PSH	4778.8	113.8	42.04	6.200	4.283	0.958	30.91[abc]	6.756	8.449	45.43
P-P	4559.4	114.9	40.92	6.100	4.127	0.920	32.41[ab]	6.865	8.048	45.22
P-M	4758.6	113.2	43.10	6.167	4.313	0.907	32.75[a]	6.676	7.128	44.19
P-MSH	4675.5	114.2	42.65	6.183	4.162	1.010	29.17[bc]	6.740	7.303	45.93
P-PSH	5126.8	115.2	43.08	6.220	4.262	0.979	29.39[abc]	6.758	7.300	44.12
PSH-PSH	4740.0	114.2	41.52	6.258	4.373	0.942	28.76[c]	6.798	7.090	45.64
PSH-MSH	4884.5	115.1	43.20	6.283	4.375	0.954	28.98[bc]	6.790	7.604	45.46
M-MSH	4622.3	113.6	40.66	6.175	4.210	0.982	31.81[abc]	6.826	7.590	45.94
SEM	103.856	0.972	0.996	0.107	0.127	0.033	0.861	0.049	0.311	0.714
P-value	0.252	0.819	0.811	0.980	0.777	0.764	0.039	0.570	0.183	0.770

[abc] Values within a column followed by different superscripts are significantly different. $P < 0.05$; Tukey's pairwise test.

SEM: standard error of the mean. Mash diet during 1-8 and 9-14, (control); mash from 1-8 and pellet+SH from 9-14(M-PSH); mash diet +SH during 1-8 and 9-14(MSH-MSH); mash diet +SH during 1-8 and pellet+SH from 9-14(MSH-PSH); pellet diet during 1-8 and 9-14(P-P); pellet diet during 1-8 and mash from 9-14(P-M); pellet diet during 1-8 and mash+SH from 9-14(P-MSH); pellet diet during 1-8 and pellet+SH from 9-14(P-PSH); pellet +SH diet during 1-8 and pellet+SH from 9-14(PSH-PSH); pellet diet +SH during 1-8 and mash+SH from 9-14(PSH-MSH); Mash diet during 1-8 and Mash+SH from 9-14(M-MSH).

Tibia indices

Results of tibia quality parameters are shown in Table 9. The results indicated that there were no remarkable differences in terms of weight, length, weight: length, diaphysis diameter, medullary canal diameter, lateral wall thickness, robusticity index, modulus of elasticity, and ash; however, the tibiotarsal index was affected by the experimental treatments. The greatest tibiotarsal index was related to the birds fed control and P-M diets; but, the lowest tibiotarsal index was related to PSH-PSH treatment. ($P < 0.05$).

Immune system response

The antibody titer against SRBC and serum levels of IgG and IgM were not affected by the dietary treatments (Table 10). Probably, the lack of significant difference between all dietary treatments at the end of the experiment was due to the fact that the sources of soluble and insoluble fiber may have have different effects on immune system stimulation.

Behavior evaluation

As presented in Table 11, the birds' behavior was not affected by the physical feed form and SH, such that they went through all periods of the experiment without any stress.

Table 10. The effects of feed form and insoluble fiber inclusion in the diet on immune system in the laying pullets (Log2)

Treatments	Total anti-SRBC	IgG	IgM
Control	2.343	1.009	1.396
M-PSH	2.150	0.917	1.186
MSH-MSH	2.013	0.896	0.969
MSH-PSH	2.408	0.948	1.594
P-P	2.221	0.512	1.644
P-M	2.139	0.573	1.448
P-MSH	2.142	0.948	1.094
P-PSH	2.456	1.476	1.271
PSH-PSH	2.533	1.344	1.615
PSH-MSH	2.656	1.162	1.851
M-MSH	2.321	1.219	1.271
SEM	0.156	0.276	0.229
P-value	0.137	0.383	0.231

SEM: standard error of the mean. Mash diet during 1-8 and 9-14, (control); mash from 1-8 and pellet+SH from 9-14(M-PSH); mash diet +SH during 0-8 and 9-14(MSH-MSH); mash diet +SH during 1-8 and pellet+SH from 9-14(MSH-PSH); pellet diet during 1-8 and 9-14(P-P); pellet diet during 1-8 and mash from 9-14(P-M); pellet diet during 1-8 and mash+SH from 9-14(P-MSH); pellet diet during 1-8 and pellet+SH from 9-14(P-PSH); pellet +SH diet during 1-8 and pellet+SH from 9-14(PSH-PSH); pellet diet +SH during 1-8 and mash+SH from 9-14(PSH-MSH); Mash diet during 1-8 and Mash+SH from 9-14(M-MSH).

Table 11. The effects of feed form and insoluble fiber inclusion in the diet on behavior of laying pullets

Treatments	Behavior score (1-5)
Control	1.300
M-PSH	1.600
MSH-MSH	1.700
MSH-PSH	1.650
P-P	1.800
P-M	1.800
P-MSH	1.750
P-PSH	1.550
PSH-PSH	1.500
PSH-MSH	1.960
M-MSH	1.800
SEM	0.160
P-value	0.733

SEM: standard error of the mean. Mash diet during 1-8 and 9-14, (control); mash from 1-8 and pellet+SH from 9-14(M-PSH); mash diet +SH during 1-8 and 9-14(MSH-MSH); mash diet +SH during 1-8 and pellet+SH from 9-14(MSH-PSH); pellet diet during 1-8 and 9-14(P-P); pellet diet during 1-8 and mash from 9-14(P-M); pellet diet during 1-8 and mash+SH from 9-14(P-MSH); pellet diet during 0-8 and pellet+SH from 9-14(P-PSH); pellet +SH diet during 1-8 and pellet+SH from 9-14(PSH-PSH); pellet diet +SH during 1-8 and mash+SH from 9-14(PSH-MSH); Mash diet during 1-8 and Mash+SH from 9-14(M-MSH).

Discussion

Some authors have suggested that pelleting of the diets could improve FCR in broilers (Amerah *et al*., 2007a; Serrano *et al*., 2012) and brown-egg laying pullets (Frikha *et al*., 2009), in agreement with our results, Howlider and Rose (1992) showed that pelleting increased FCR in the broiler chickens.

Based on a previous study, pelleting enhances the number of small particles in the feed, and small particles transit quickly from the gizzard to the small intestine (Abdollahi *et al*., 2013). Small particles have minor effects on gizzard function (Amerah *et al*., 2007a; Svihus, 2011). However, particle size reduction through pelleting can enhance feed intake due to the rapid evacuation of the upper part of the GIT (Svihus *et al*., 2010). In agreement with our findings, Shirzadegan and Taheri (2017) also reported that the use of pellets with 6% alfalfa meal, rice bran, or wood shaving as fiber additives in broilers from 11 to 24 days of age resulted in a higher ADFI, as compared to the other dietary treatments. However, this finding was different from those of Leung *et al*. (2018), who reported that ADFI was decreased with the fiber inclusion (40% of soy hulls, 33% of oat hulls, or 48% of flax meal) in the 65-week-old broiler breeder hens diets. In this regard, Serrano *et al*. (2012) suggested that pelleting improved ADFI and ADG in the broilers. Some authors have suggested that the pelleted diet increased the feed intake, and the reduction of the feed waste was detected (Jahan *et al*., 2006 ; Jiménez-Moreno *et al*., 2016).

Based on the previous study, because of the weak bonding strength between the pellet structure during consumption, rather than the internal binding between microstructures in the mash diets, gizzard tries to quickly pass the particles remaining in pellet diets, as the pellet would be dissolved in the following consumption immediately. Additionally, when a mash diet is introduced to the gizzard, given its size, it tries to grind the microstructure in the mash diet further. These functions could raise muscular activity, finally leading to an increase in the gizzard weight (Svihus, 2011).

Consistent with our results, (Saldaña *et al*., 2015b) suggested that pelleting could reduce the gizzard weight of pullets. Pelleting could reduce the retention times of digestion in the gizzard; therefore, it decreases the gizzard's weight and holding capacity (Amerah *et al*., 2007b). On the other hand, Jiménez-Moreno *et al*. (2019) reported that the insoluble fiber in the diet of broilers at 8 and 21 d of age increased the gizzard weight and gizzard contents. In this regard, it has been suggested that the insoluble fiber tends to accumulate in the gizzard and the upper part of the GIT, thus increasing the transit time and mechanically stimulating the development of the muscular layers of the gizzard (Mateos *et al*., 2012). Therefore, as mentioned above, the mash diet, due to the increase in the related weight of the gizzard, contributed to the growth of the pullets. In general, it can be concluded that mash diet (with or without SH) leads to an increase in the relative weight of the gizzard, although the inclusion of insoluble fiber does not affect the relative weight of the other organs.

In agreement with our data, Moran (2006) reported that with the inclusion of the insoluble fiber in the chicken feed increasevillus height and surface area. However, Baurhoo *et al*. (2007) reported that the broiler chickens given insoluble fibers had a significantly lower villus height than other treatment groups. Some authors suggested that the insoluble fiber could affect the microbial population of the GIT, hence reducing the necrotic enteritis lesions, as well as increasing the villus height, villus surface area, and crypt depth; this could be considered the primary indicator of the intestinal health (Amerah *et al*., 2009; Branton *et al*., 1997).

The tibiotarsal index is a morphometric method to describe the degree of bone mineralization (Mutuş *et al*., 2006). In the present experiment, regardless of the feed form, the inclusion of SH in the diet tended to reduce the tibiotarsal index. Asensio *et al*. (2020) also showed that fiber decreased bone mineral deposition. In contrast, Oikeh *et al*. (2019) revealed that broilers fed the diluted diets with the insoluble fiber exhibited improved femur and tibia ash weights. Therefore, present study suggest that the inclusion of fiber in the diets may result in lower absorption of minerals. In this regard, it is reported that the inclusion of fiber in the broiler breeder hens diet could accelerate the passage time of the digesta and reduce calcium and phosphorus absorption, depending on the type of fiber and its level in the diet. (Enting *et al*., 2007). The enhancement of the intestinal pH decreased the soluble particle of minerals and the ratio of minerals in little collections; consequently, their accessibility for absorption could be decreased (Shafey *et al*., 1991). Some studies reported that the insoluble fiber reduce pH in the gizzard (Hetland *et al*. 2003; Incharoen and Maneechote, 2013). In general, the results of the current study demonstrated that bone parameters were not remarkably affected by the feed form and the inclusion of insoluble fiber in the diet.

Some authors have shown that the antibody titer against SRBC could not be influenced by the inclusion of the fibrous materials in the feed (Sadeghi *et al*., 2015). However, this finding contrasted with Saadatmand *et al*. (2019) study in which a significant increase was reported in the antibody titer against SRBC in the broiler chickens fed soluble or insoluble fiber. In this regard, Wils-Plotz and Dilger (2013) pointed out that the soluble dietary fibers containing β-glucans and pectins may have a positive impact on the gut immune response against coccidiosis challenge and immune system stimulation.

Contrary to our results, Wechsler *et al*. (1998) reported an increase in cannibalism in laying hens fed pellet diets. In this regard, Savory *et al*. (1996) reported that with the addition of insoluble fibre, chickens would spend more time feeding, increasing the likelihood that the behavioral need for feeding will be satisfied (Moradi *et al*., 2013; Savory *et al*., 1996).

Vtlariño *et al*. (1996) reported that the feeding behavior of laying hens is affected by the physical characteristics of feed. They also noted that feeding a high level of fiber did not have any detrimental effect on productivity and welfare. Hartini and Choct (2010) also reported that rice hulls reduce cannibalism mortality more than the other diets. The finding in the present study accentuated the previous suggestion that longer retention of insoluble fiber in the gizzard helps birds feeling more 'settled'. Also, Van Krimpen *et al*. (2009) reported that feeding diets containing high levels of NSP increased eating time (Van Krimpen *et al*., 2009) whichmay reduce feather pecking behavior. Therefore, increasing feeding time by inclusion of insoluble fiber in mash diet might be an effective strategy to reduce the incidence of cannibalism in laying hens. (Choct and Hartini, 2003)

Conclusion

Generally, changing the feed form during different rearing periods could improve the pullet's performance. Also, feeding the commercial pullets with the pellet feed tended to improve their performance, feed efficiency, and uniformity compared to the regular mash feed. Furthermore, the inclusion of SH as fiber in the diet may be considered an enhancer of growth performance and serves as a valuable additive to improve gizzard function.

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