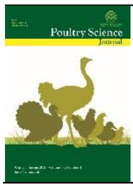

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Performance, Body Weight Uniformity, Feather Pecking Behavior, Bone Quality and Intestinal Histomorphology in Pullets Fed Mash or Pelleted Diets at Different Rearing Stages

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Abstract

Feed form affects the physiology of the gastrointestinal tract of pullets, but the optimum time of pelleted diet feeding during the rearing period should be determined to achieve optimum performance. This experiment evaluated the effects of feed form (mash or pelleted) on pullets' productive performance when fed during different rearing periods. Five treatments included: feeding a mash diet from hatching to 14 wk (MA14), feeding a pelleted diet from hatching and shifting to the mash diet at the end of the 4th (PE4), 8th (PE8), and 12th (PE12) wk, and feeding a pelleted diet from hatching to the 14th wk (PE14). Results showed that the average daily feed intake (ADFI) and average daily gain (ADG) increased linearly with increasing pelleted feeding time from 0 to 14 weeks ($P< 0.01$). Pullets shifted from a pelleted to a mash diet at any age had a poorer overall FCR than those fed either diet continuously ($P<$ 0.05). The gizzard weight was linearly decreased by increasing the pelleted diet feeding time from 0 to 14 wk ($P<0.01$). The jejunal absorptive surface area was lower ($P<0.01$) in pullets fed PE4, PE8, and PE12 diets than in those fed the MA14 diet. In examining the relative weights of internal organs, tibia bone quality, and feather score, no significant differences were observed among the experimental treatments. The results showed that feeding pullets a pelleted diet throughout the rearing period increased ADFI and ADG but led to poor gizzard development. However, pullets that shifted from a pelleted to a mash diet at 4, 8, and 12 wk of age had a well-developed gizzard compared to those fed a pelleted diet continuously.

Introduction

Genetic selection has led to a significant increase in productive traits and a reduction in the feed intake (FI) and body weight (BW) of the layer hens. Additionally, at the beginning of the laying period, it is beneficial to have heavier birds with a more uniform body weight distribution, as at their peak, laying hens may use body tissues due to lower FI and a negative nutrient balance (Leeson and Summers, 2005). Furthermore, the low FI of some laying hen strains during the early laying period (less than 85 g per bird/d) necessitates denser feeds with higher costs to meet their nutrient requirements (Hy-Line 2016). Therefore, nutritional strategies should focus on achieving the desired BW and on gastrointestinal tract (GIT) development to enhance FI during peaking (Leeson and Summers, 2005). Therefore, we hypothesized that feeding pullets with pelleted diets during defined periods of the rearing phase would enhance body weight gain, uniformity, gastrointestinal development, and skeletal quality compared with mash feeding. Additionally, we expected that the timing of pelleting (early, mid, or late rearing) would differentially influence feather pecking behavior and feed intake capacity at the onset of lay.

Feed form has a substantial influence on pullet performance. Compared with mash diets, pelleted

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feeds offer several advantages, such as improved growth responses, reduced ingredient segregation, lower feed wastage, shorter feeding time, and decreased energy required for feed prehension (Attia *et al.*, 2012; Attia *et al.*, 2014; Frikha *et al*., 2009). In addition, pelleting diets may destroy pathogens, alter starch and protein structures, and improve feed palatability (Axe, 1995; Amerah *et al*., 2007). Body weight uniformity during rearing is another key factor affecting future laying performance, and previous studies have shown that pelleted diets can improve BW uniformity relative to mash diets (Guzmán *et al*., 2015).

On the other hand, it has been reported that finer particles produced by the pelleting process had shorter retention times in the GIT, leading to lower nutrient digestibility (Zaefarian *et al*., 2016). Also, feeding pellets or crumbles to pullets may reduce the development and relative weight of different organs of the GIT, and alter the pH of the gizzard. Besides that, Appleby and Hughes (1991) suggested that the risk of feather pecking might be higher in hens fed pellets than in those fed mash, as time spent on feeding is reduced with the pelleted feed.

It has also been reported that pullets' responses to feed forms may depend on feed particle size (Bozkurt *et al.*, 2019) or diet energy concentration (Guzmán *et al*., 2015; Saldaña *et al.*, 2015a). Furthermore, as discussed previously, GIT development may be compromised when pullets are fed pelleted or crumbled diets; however, the adverse effects of crumble feeding on the relative weight of the gizzard and proventriculus increased over time (Saldaña *et al*., 2015a). In fact, these results suggest that the optimal time to feed pelleted diets throughout the rearing period should be determined to achieve optimal growth performance and future egg production.

As the two major indices of pullet quality are BW uniformity and BW at a specific age, we hypothesized that feeding a pelleted diet during the early rearing period may enhance performance and BW uniformity. Providing a mash diet during the late rearing period may enhance gut development, helping to achieve optimal FI, performance, and gut development outcomes. Therefore, the purpose of this study was to investigate the possible impacts of the two feed forms (mash and pelleted) at different times during the rearing period (1-14 wk) on the productive performance, BW uniformity, the relative weight of various segments of the GIT, and the intestinal histomorphology of the commercial laying pullets.

Materials and methods

Husbandry and experimental design

All animal care and experimental procedures were approved by the Animal Policy and Welfare Committee of Isfahan University of Technology (Isfahan, Iran), in accordance with the comprehensive guidelines on animal welfare adopted by FASS (2010). A total of 340-day-old commercial Hy-Line W-36 chicks with a hatching BW of 35.3±0.25 g were obtained from a commercial hatchery and housed in an environmentally controlled barn from 1 to 14 wk. Pullets were reared based on a 23 h/d light program for their first two weeks of life. Then, a 2-hour/week light-reduction program was followed until 12 hours of light/24 hours at 49 days of age. The rearing house temperature was maintained at 32 °C for the first three days, then gradually reduced to approximately 21 °C by day 35. Pullets were housed in cages (0.60 × 1.20 × 0.45 m) equipped with an open-through feeder and three nipple drinkers, which were adjusted in height as the birds grew. Pullets were vaccinated against the primary local diseases, including Newcastle disease on days 9, 25, 32, 40, and 45 of age; infectious bronchitis on days 13 and 45 of age; and infectious bursal disease on days 21 and 27 of age. All vaccines were administered in drinking water according to the commercial practices and local recommendations. Water and feed were available for *ad libitum* consumption during the experimental period (0-14 wk). Pullets were fed according to a completely randomized design with five treatments, involving 6 or 8 replicates (as follows) of 10 birds per cage. Experimental treatments included: feeding a mash diet continuously from hatching time to the 14^{th} wk of age (MA14), feeding a pelleted basal diet from hatching time, and shifting to the mash diet at the end of the 4^{th} (PE4), 8^{th} (PE8) and 12^{th} (PE12) wk of age, and feeding a pelleted basal diet continuously during 0-14 wk (PE14), as shown in Table 1.

A corn-soybean meal-based diet was formulated to meet or exceed the nutrient requirements of the pullets in three phases, including starter (0-6 wk of age), grower (7-12 wk of age), and developer (13-14 wk of age), as recommended by Hy–Line W-36 (Hy-Line 2016). Two batches of corn and soybean meal were ground to pass through a 3- and 4-mm screen, respectively, using a hammer mill to produce the pelleted and mash diets. After grinding, all components were mixed with a horizontal paddle mixer. For manufacturing pelleted diets, ingredients were conditioned at 75 °C and pelleted using a mill (Model 7700, CPM Co., Crawfordsville, IN, USA) with a 2 mm die for the starter and grower periods, and a 3 mm die for the developer period. Ingredient composition and calculated analysis of the basal diet at various rearing periods are presented in Table 2.

Table 1: The schedule of experimental treatments

Treatment	Pullet age (wk)			
	Hatch (0)-4	**5-8**	**9-12**	**13-14**
MA14	Mash	Mash	Mash	Mash
PE4	Pelleted	Mash	Mash	Mash
PE8	Pelleted	Pelleted	Mash	Mash
PE12	Pelleted	Pelleted	Pelleted	Mash
PE14	Pelleted	Pelleted	Pelleted	Pelleted

For analyzing performance data, the number of replications varied across periods. From hatching to wk 4, MA14 and PE14 included 8 and 26 replicates, respectively. From wk 5 to 8, MA14, PE4, and PE14 had 8, 6, and 20 replicates, respectively. From wk 9 to 12, MA14, PE4, PE8, and PE14 included 8, 6, 6, and 14 replicates, respectively. From wk 13 to 14, MA14, PE4, PE8, PE12, and PE14 included 8, 6, 6, 6, and 8 replicates, respectively. For the other measured parameters, MA14 and PA14 had eight replicates, and the others had six.

Table 2: Ingredient composition and calculated analysis of the experimental diets at various rearing periods

Period	**Starter (0-6 wk of age)**	**Grower (7-12 wk of age)**	**Developer (13-14 wk of age)**
Ingredient (%)			
Yellow corn	64.74	70.15	75.73
Soy bean meal, 45.22% CP	29.53	23.98	18.95
Wheat Gluten	1.00	1.00	1.00
Soybean oil	0.81	0.50	0.52
Bentonite	0.00	0.57	0.00
DL-Met, 99%	0.20	0.17	0.13
L-lysin hydocholoride, 98%	0.19	0.16	0.13
L- Thr, 98%	0.07	0.05	0.09
Mono calcium phosphate	1.35	1.24	1.17
Calcium carbonate	1.33	1.43	1.51
Vitamin and mineral mix	0.20	0.20	0.20
Salt	0.19	0.22	0.20
$NaHCO_3$	0.33	0.28	0.31
Choline chloride (60%)	0.055	0.045	0.055
Phytase	0.005	0.005	0.005
Calculated analysis			
AME (Kcal/Kg)	2977	2977	2977
Crude protein (%)	20.0	17.5	16.0
Digestible Lys (%)	1.05	0.88	0.76
Digestible Met (%)	0.47	0.40	0.36
Digestible Met +Cys (%)	0.74	0.67	0.59
Digestible Thr (%)	0.69	0.66	0.52
Calcium (%)	1.00	1.00	1.00
Available phosphorus (%)	0.50	0.47	0.45
Sodium (%)	0.18	0.17	0.18

[1]The vitamin premix supplied the following per kilogram of feed: vitamin A, 10000 IU; cholecalciferol, 3300 IU; niacin, 40 mg; α-tocopherol, 25 mg; pantothenic acid, 10 mg; riboflavin, 6.6 mg; pyridoxine, 4.5 mg; menadione, 3.5 mg; thiamine, 2.2 mg; folic acid, 1 mg; biotin, 40 µg; and vitamin B12, 23 µg and the mineral premix supplied the following per kilogram of feed: Zn, 85 mg; Mn, 90 mg; Fe, 30 mg; Cu, 15 mg; I, 1.5 µg; Co, 1.0 µg.

Before feed formulation, the feedstuffs were evaluated for specific nutrients and chemical composition using near-infrared reflectance (NIR) analysis by Evonik Co., Germany, and laboratory analysis, as shown in Table 3.

Nitrogen content was analyzed using the Kjeldahl method (Kjeltec 1030 Auto Analyzer, Tecator, Höganäs, Sweden) according to AOAC official method 976.05 (2002). Crude protein was calculated as 6.25 × N. Additionally, the ether extract (920.29) and ash (942.05) contents were analyzed according to the AOAC (2002) method. Feed particle sizes were determined by dry sieving using a sieve shaker equipped with sieves (W. S. Tyler, Inc., Mentor, OH) arranged in descending order of sieve size (3350, 1700, 850, 420, 212, 106, and 53 µm, and a pan). Feed particle sizes were determined after grinding and mixing and before conditioning, according to ASABE (2007), as shown in Table 4. The pellet durability index (PDI) of the pelleted feeds (Table 4) was measured by the Holmen method (Holmen NHP200, UK). In brief, a 100 g sample of pellets was introduced into a perforated mesh hopper. Compressed air was applied from the bottom of the hopper at 70 mBar for 120 seconds. The forced airflow caused the pellets to collide with each other and with the inner walls of the test chamber, subjecting them to both impact and shear forces. The resulting fines were collected and weighed internally, and the Pellet Durability Index (PDI) was automatically calculated and displayed by the Holmen NHP 200 device.

Table 3: Proximate analysis (mean±SD) and estimated by NIRS of yellow corn, soybean meal, and wheat gluten (as % of dry matter, n=3)

Item (%)	Yellow corn	Soybean meal	Wheat gluten
Apparent metabolizable energy, kcal/kg[2]	3299	2248	NA[3]
Dry matter [1]	87.20	88.41	93.11
Crude protein[1]	7.44	46.52	68.53
Crude ash,[1]	1.33	6.62	0.84
Crude fiber[1]	2.42	6.77	0.56
Ether extract[1]	4.11	1.42	6.52
Starch,[2]	63.31	0.71	NA
Acid detergent fiber[2]	3.38	8.24	NA
Neutral detergent fiber[2]	10.81	14.51	NA
Digestible methionine[2]	0.151	0.605	NA
Digestible cysteine[2]	0.154	0.669	NA
Digestible methionine + cysteine[2]	0.309	1.075	NA
Digestible lysine[2]	0.217	2.508	NA
Digestible threonine[2]	0.242	1.473	NA
Digestible tryptophan[2]	0.050	0.549	NA
Digestible arginine[2]	0.330	3.172	NA
Digestible isoleucine[2]	0.247	1.864	NA
Digestible leucine[2]	0.791	3.119	NA
Digestible valine[2]	0.343	1.925	NA
Digestible histidine[2]	0.216	1.110	NA
Digestible phenylalanine[2]	0.330	2.120	NA

[1]All analyses were performed in triplicate (N=3).
[2]Estimated by NIR analysis by Evonik-Degussa Co., Essen, Germany.
[3]Not analyzed.

Table 4: The particle size distribution of the final feed as determined by the dry sieving technique

	Starter and Grower diets		Developer diets	
	Mash	Pellet	Mash	Pellet
Amount retained on each sieve after sieving, %				
Sieve size, µm				
3350	0.80	0.30	5.50	1.10
1700	13.8	7.10	25.6	13.1
850	33.7	24.5	32.2	26.9
420	44.0	48.6	30.2	44.7
212	7.00	15.6	6.00	12.0
106	0.70	2.50	0.50	1.80
53	0.10	1.40	0.10	0.30
X_{gm},[1] µm	880	660	1150	790
SD_{gm},[2] µm	510	440	950	570
PDI[3] (%)		90		86

[1] Geometric mean particle size was determined by ASAE (2007; method S319.3).
[2] Geometric standard deviation was determined by ASAE (2007; method S319.3).
[3] Pellet durability index was measured in 3 replicates by the Holmen method (Holmen NHP200, UK).

Data collection

Growth performance of the pullets, including ADFI and individual BW, was measured every other week from hatching to the 14th week of age, after correction for mortality. FCR and BW uniformity were calculated every other week. Mortality was recorded daily. The BW uniformity was expressed as the coefficient of variation (CV) of BW, and the CV was calculated as follows:

$$CV (\%)=(\text{Standard deviation (g)})/(\text{Average body weight (g)}) \times 100$$

All pullets were scored individually for feathers coverage at the end of the study when they were 14 age, according to the 5-point feather score scale (Webster and Hurntk 1990), where 1 = smooth and complete plumage, 2 = ruffled, no naked spots, 3 = naked spots up to 5 cm at the widest part, 4 = naked spots greater than 5 cm wide, and 5 = naked spots with skin injury.

To assess the behavioral sensitivity of the birds, all pullets were evaluated at the 14th week of rearing based on the method described by Efranji et al. (2022). Behavioral analysis was conducted in a box measuring 80 × 40 cm and 80 cm high. Each bird was released from a height of 80 cm into the box, and its behavioral response upon landing was assessed using a 5-point scale: 1) The bird sat quietly after landing, showing no effort or reaction; 2) The bird moved slowly and stood while briefly observing its surroundings with minimal effort; 3) The bird rapidly wagged its head and moderately pushed its feet against the box floor, showing a mild scrabbling response; 4) The bird vigorously shook its head, forcefully pushed with its feet, and exhibited strong, intentional escape attempts accompanied by intense scrabbling; 5) The bird displayed highly agitated behavior, continuously attempting to escape with intense movements. Higher scores indicated a greater fear response to unfamiliar situations and a higher susceptibility to behavioral stress and sensitivity (He *et al*., 2016).

For carcass traits assessment, at the end of the 14th week, one pullet per replicate (at least six birds per treatment) was randomly selected, weighed, and humanely euthanized by CO2 after four hours of feed deprivation. The emptied proventriculus, gizzard, duodenum, jejunum, ileum, cecum, and liver were weighed, and all values were expressed as a percentage of the live BW. For pH value measurement, the gizzard contents of the pullets were collected after euthanasia. One gram of the gizzard content was mixed with 9 mL of distilled deionized water in a 25 mL centrifuge tube using a vortex mixer for 5 min. The pH values of the diluted mixture were measured using a pH meter (HI 2211 pH/ORP). The pH probe was inserted directly into the center of the diluted mixture (At a temperature of 25 ℃) and the pH value was recorded after the reading became stable (Pang and Applegate, 2007). A section of about one cm in length was cut from the middle segment of the jejunum and placed into a 10% buffered neutral formaldehyde solution. After dehydration, the samples were processed by routine histology methods (Lee *et al*., 2010). Briefly, the tissue samples were cleared and embedded in paraffin wax blocks, then sectioned at a thickness of 5 µm using a microtome. The sections were mounted on glass slides and stained with hematoxylin and eosin (H&E) for microscopic evaluation. Twelve intact villi from each sample were examined, and morphometric indices including villus length (VL; measured from the crypt mouth to the apex of the villus), crypt depth (CD; defined as the depth of the invagination between adjacent villi), and epithelial thickness were measured at 40×magnification using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The apparent villus surface area was calculated using the formula described by Iji et al. (2001): Apparent villus surface area = (villus width at one-third + villus width at two-thirds of the height of the villus)/2× villus height.

To assess bone quality, specifically the tibiotarsal index and robusticity index, the left tibia of each euthanized bird (one sample per replicate) was collected and stored at −20 °C until further analysis. The thicknesses of the medial and lateral walls of the tibia at the midpoint were measured using a digital caliper. The diameter of the medullary canal was then calculated by subtracting the combined thickness of these walls from the overall diameter at the diaphysis. The tibiotarsal and robusticity indices were calculated using the following formulas (Riesenfeld, 1972):

$$\text{Tibiotarsal index} = \frac{\text{diaphysis diameter} - \text{medullary canal diameter}}{\text{diaphysis diameter}} \times 100$$

$$\text{Robusticity index} = \frac{\text{bone length}}{\sqrt[3]{\text{bone weight}}}$$

Tibia samples were dried at 105°C for 16 h for DM determination, and subsequently the samples were ashed at 550°C for 8 h (AOAC 2005; methods 930.15 and 942.05, respectively).

Statistical Analysis

Data normality was first assessed using the Shapiro–Wilk test, and the homogeneity of variances was checked by Levene's test. All data were subjected to statistical analysis using a completely randomized unbalanced design in SAS (SAS Inst. Inc., Cary, NC), with each cage of birds as the experimental unit. Each experimental unit consisted of 10 birds. The model included the effect of dietary treatment. Contrast statements were used to test the linear and quadratic effects of increasing the pelleted diet feeding time during the whole experimental period. Differences between treatment means were considered significant at $p < 0.05$, using Tukey's honestly significant difference post-hoc test.

Due to the unequal number of replicates among treatments across different periods, data were analyzed using the GLM procedure with Type III sums of squares in SAS, which appropriately accommodates unbalanced designs. No weighting was required, and each cage was considered an independent experimental unit.

Results

The performance traits and BW uniformity of the pullets during various times of the rearing period are presented in Table 5. Pullets fed the PE14 diet had greater ADFI (*P*<0.05) than those fed the MA14 diet during the first two weeks of age. Similarly, pullets fed the PE14 diet or pullets shifted from a pelleted diet to mash diets at any age (e.g., PE4, PE8, and PE12 diets) had greater ADFI (*P*<0.05) compared to those fed the MA14 diet from wk 5 to 12 of age (*P*<0.01). In addition, ADWG was greater in pullets fed PE14 diet than those fed MA14 diet from 1 to 4 wk of age (*P*<0.01; Table 5). Pullets fed PE4, PE12, and PE14 diets had greater ADWG during wk 13 to 14 of age than those fed MA14 and PE8 diets ($P<0.05$). The body weight of pullets fed the PE14 diet, or those shifted from the pelleted diet to the mash diet at any age (e.g., PE4, PE8, and PE12 diets), was greater than that of those fed the MA14 diet at wk 2, 4, 6, and 8 of age (Table 5). Also, BW was greater in pullets fed PE4 and PE14 diets compared to those provided MA14 and PE8 diets ($P<0.01$) at week 10 of age, but this difference disappeared at wk 12 and 14 of age. The daily growth of the pullets exhibited a decreasing trend from wk 9 to 14 of age (13.65 to 9.39 g/d; Table 5).

From wk 3 to 4 of age, feeding the pelleted diet decreased FCR ($P = 0.01$) compared to the mash diet (Table 5). On the other hand, pullets fed the MA14 diet showed a lower FCR compared to those fed the PE14 diet ($P<0.05$), while pullets fed the other treatments had intermediate FCR from 11 to 12 wk of age.

Table 5: Effect of feeding different forms of diets (mash and pellet) at different bird ages on performance during different periods of rearing in commercial laying pullets

		Treatments[2]						
Period	**Items[1]**	**MA14**	**PE4**	**PE8**	**PE12**	**PE14**	**SEM[3]**	***P*-value**
		n = 8				n = 26		
0-2weeks	ADFI (g)	12.31[b]	- [4]	-	-	14.53[a]	0.329	0.04
	ADG (g)	6.75[b]	-	-	-	7.26[a]	0.265	< 0.01
	FCR (g/g)	1.83	-	-	-	2.00	0.062	0.18
	CV (%)	5.50	-	-	-	5.58	0.241	0.85
	BW (g)	129.96[b]				137.16[a]	1.813	< 0.01
3-4weeks	ADFI (g)	25.64	-	-	-	25.12	0.313	0.39
	ADG (g)	10.79[b]	-	-	-	11.48[a]	0.379	< 0.01
	FCR (g/g)	2.38[a]	-	-	-	2.19[b]	0.039	0.01
	CV (%)	4.87	-	-	-	5.25	0.287	0.49
	BW (g)	281.15[b]				298.00[a]	3.536	< 0.01
		n = 8	n = 6			n = 20		
5-6weeks	ADFI (g)	37.45[b]	40.30[a]	-	-	39.52 [a]	0.635	0.01
	ADG (g)	13.36	14.04	-	-	13.96	0.307	0.16
	FCR (g/g)	2.80	2.86	-	-	2.84	0.033	0.70
	CV (%)	5.50	4.85	-	-	4.86	0.417	0.30
	BW (g)	468.33[b]	491.09[a]			493.82[a]	3.927	< 0.01
7-8weeks	ADFI(g)	42.69[b]	46.57[a]	-	-	45.06[a]	0.453	< 0.01
	ADG (g)	12.81	13.39	-	-	12.66	0.549	0.48
	FCR (g/g)	3.36	3.48	-	-	3.62	0.247	0.53
	CV (%)	5.75	5.19	-	-	5.56	0.569	0.75
	BW (g)	647.69[b]	678.99[a]			671.12[a]	7.196	0.01
		n = 8	n = 6	n = 6		n = 14		
9-10weeks	ADFI (g)	43.02[b]	49.73[a]	47.50[a]	-	50.39[a]	1.488	<0.01
	ADG (g)	13.57	13.81	14.70	-	14.45	0.756	0.56
	FCR (g/g)	3.17	3.64	3.31	-	3.55	0.180	0.14
	CV (%)	4.62	3.86	4.86	-	4.65	0.445	0.36
	BW (g)	837.70[b]	871.00[a]	854.26[ab]		882.03[a]	10.245	< 0.01
11-12weeks	ADFI (g)	50.62[b]	54.07[a]	54.32[a]	-	53.87[a]	1.140	0.04
	ADG (g)	11.73	10.20	11.71	-	10.09	0.681	0.11
	FCR (g/g)	4.42[b]	5.27[ab]	4.61[ab]	-	5.37[a]	0.353	0.04
	CV (%)	4.50	4.88	4.72	-	4.66	0.476	0.93
	BW (g)	1002.03	1004.41	1009.01		1009.35	8.940	0.75
		n = 8	n = 6	n = 6	n = 6	n = 8		
13-14weeks	ADFI (g)	54.42	57.66	54.05	58.89	56.83	1.664	0.15
	ADG (g)	8.59[b]	9.48[a]	8.66[b]	10.59[a]	10.08[a]	0.580	0.04
	FCR (g/g)	6.40	6.19	6.59	5.59	5.69	0.443	0.33
	CV (%)	4.00	3.66	4.47	4.81	4.12	0.531	0.53
	BW (g)	1122.30	1136.17	1128.67	1147.80	1157.10	13.254	0.20

[a–b] Means within a row with different superscripts differ significantly ($p \leq 0.05$)

[1] ADFI: average daily feed intake, ADG: average daily weight gain, FCR: feed conversion ratio, CV: coefficient of variation and BW: body weight (at the end of each period).

[2] MA14- pullets fed a basal mash diet from hatching to 14[th] wk of age, PE4- pullets fed a pelleted basal diet from hatching time to the end of 4[th] wk of age and after that fed a basal mash diet from 5 to 14[th] wk of age, PE8- pullets fed a pelleted basal diet from hatching time to 8[th] wk of age and after that fed a basal mash diet from 9[th] to 14[th] wk of age, PE12- pullets fed a pelleted basal diet from hatching time to 12[th] wk of age and after that fed a basal mash diet from 13[th] to 14[th] wk of age and PE14- pullets fed a pelleted basal diet throughout the experimental period (Hatch-14 wk).

[3] Standard error of the means.

[4] treatment is not active during this period.

During the whole experimental period (e.g., 0-14 wk of age), pullets fed the PE4, PE8, PE12, and PE14 diets had greater ADFI (*P*<0.01) compared to those fed the MA14 diet (*P*< 0.01; Table 6). Moreover, FCR was quadratically affected by increasing the time of pelleted diet feeding (*P*<0.01; Table 6), indicating that shifting from pelleted to a mash diet at 4, 8, and 12 wk of age resulted in poorer FCR than the other treatments. Moreover, our results showed that BW uniformity (expressed as CV) was similar across different dietary treatments from 0 to 14 wk of age (Table 6; *P*>0.05).

Table 6: Effect of feeding different forms of diets (mash and pellet) at different times on performance during the whole rearing period (0-14 wk of age) in commercial laying pullets

Treatments[1]	**ADFI (g)**	**ADG (g)**	**FCR (g/g)**	**CV (%)**
MA14	38.02[c]	11.09[b]	3.48[b]	4.96
PE4	41.23[ab]	11.24[ab]	3.74[a]	5.02
PE8	39.81[b]	11.16[b]	3.70[a]	5.07
PE12	41.48[a]	11.36[ab]	3.71[a]	5.11
PE14	40.36[ab]	11.56[a]	3.51[b]	5.01
SEM[3]	0.431	0.148	0.05	0.193
P-value				
Treatment effect	< 0.01	0.05	0.01	0.98
Linear treatment effect	< 0.01	0.03	0.89	0.69
Quadratic treatment effect	< 0.01	0.80	< 0.01	0.67

[a–b] Means within a column with different superscripts differ significantly ($p \leq 0.05$)

[1] ADFI: average daily feed intake, ADWG: average daily weight gain, FCR: feed conversion ratio, and CV: coefficient of variance.

[2] MA14- pullets fed a basal mash diet from hatching to 14th wk of age (n = 8), PE4- pullets fed a pelleted basal diet from hatching time to end of 4th wk of age and after that fed a basal mash diet from 5 to 14th wk of age (n = 6), PE8- pullets fed a pelleted basal diet from hatching time to 8th wk of age and after that fed a basal mash diet from 9th to 14th wk of age (n = 6), PE12- pullets fed a pelleted basal diet from hatching time to 12th wk of age and after that fed a basal mash diet from 13th to 14th wk of age (n = 6) and PE14- pullets fed a pelleted basal diet throughout the experimental period (Hatch-14 wk; n = 8).

[3] Standard error of the means.

As shown in Table 7, at the end of the experiment, the relative gizzard weight was linearly decreased by increasing the pelleted diet feeding time from 0 to 14 wk of the rearing period (*p*<0.01). Treatments did not affect the relative weights of proventriculus, liver, abdominal and gizzard fat, duodenum, jejunum, and ceca (*p*>0.05; Table 7).

There was no treatment effect regarding the length of different segments of the GIT, including the duodenum, jejunum, and ileum (*p*>0.05; Table 7). However, results showed that pullets fed PE8 or PE12 diets had a heavier relative weight of the ileum than those fed the MA14 diet (*p*<0.05). As shown in Table 7, the digesta pH of the gizzard was not influenced by the form of feeds (pelleted or mash). The effect of the feed form on the morphological indices of the jejunum is shown in Table 8. Results showed that villus height, muscle layer thickness, crypt depth, and absorptive surface area of the jejunum were quadratically affected by increasing the time of pelleted diet feeding (*P*<0.01).

The observed quadratic effect indicated that shifting from the pelleted diet to the mash diet at any age resulted in lower values compared to those continuously fed a mash or pelleted diet (*P*< 0.05).

The feed form did not affect the weight (mg), length (mm), weight: length ratio, medullary canal, tibiotarsal index, bone robusticity index, and ash (% of dry matter) content of the left tibia of the pullets (*p*>0.05, Table 9).

The effects of the form of diets (pelleted or mash) on the feather score and stress-related behavioral score are shown in Table 10. The results showed that dietary treatments did not affect the feather and stress-related behavioral scores (*p*>0.05).

Table 7: Effect of feeding different forms of feeds (mash and pellet) at different times on relative weight (% BW) of internal organs (as %BW), and length (L) (cm) of duodenum, jejunum, ileum, and digesta pH of gizzard of commercial laying pullets at 14 wk of age

Treatments[1]	Proventriculus (% BW)	Gizzard (% BW)	Liver (%BW)	Gizzard fat (% BW)	Abdominal fat (% BW)	Ceca (% BW)	Duodenum[2]		Jejunum[2]		Ileum[2]		gizzard pH
							(% BW)	L	(% BW)	L	(% BW)	L	
MA14	0.250	2.01[a]	1.67	0.623	2.66	0.44	0.37	20.66	0.55	44.00	0.47[b]	41.85	4.18
PE4	0.238	1.91[ab]	1.70	0.93	2.86	0.44	0.40	22.20	0.66	49.50	0.62[ab]	43.25	4.10
PE8	0.254	1.71[b]	1.88	1.05	2.45	0.39	0.41	22.20	0.69	47.20	0.69[a]	45.40	4.26
PE12	0.260	1.80[ab]	1.92	0.86	2.80	0.38	0.40	22.50	0.62	44.25	0.72[a]	43.75	4.49
PE14	0.251	1.44[c]	1.79	0.90	2.82	0.38	0.42	21.16	0.60	45.50	0.59[ab]	45.83	4.35
SEM[3]	0.006	0.030	0.051	0.23	0.803	0.017	0.032	2.35	0.022	4.15	0.036	3.71	0.262
P-value													
Treatment effects	0.91	< 0.01	0.48	0.07	0.92	0.64	0.80	0.67	0.29	0.26	< 0.01	0.35	0.28
Linear treatment effects	0.60	< 0.01	0.19	0.55	0.40	0.13	0.35	0.82	0.65	0.73	0.13	0.39	0.32
Quadratic treatment effects	0.92	0.37	0.26	0.21	0.45	0.86	0.89	0.37	0.05	0.68	0.01	0.46	0.37

[a–c] Means within a column with different superscripts differ significantly ($P \leq 0.05$)

[1] MA14- pullets fed a basal mash diet from hatching to 14th wk of age (n = 8), PE4- pullets fed a pelleted basal diet from hatching time to end of 4th wk of age and after that fed a basal mash diet from 5 to 14th wk of age (n = 6), PE8- pullets fed a pelleted basal diet from hatching time to 8th wk of age and after that fed a basal mash diet from 9th to 14th wk of age (n = 6), PE12- pullets fed a pelleted basal diet from hatching time to 12th wk of age and after that fed a basal mash diet from 13th to 14th wk of age (n = 6) and PE14- pullets fed a pelleted basal diet throughout the experimental period (Hatch-14 wk; n = 8).

[2] W: the relative weight of duodenum, jejunum, and ileum (% of BW). L: the length of the duodenum, jejunum, and ileum (cm).

[3] Standard error of the means.

Table 8: Effect of feeding different forms of feeds (mash and pellet) at different times on jejunum histomorphology traits of commercial layer pullets at 14 wk of age

Treatments[1]	**Villus height (µm)**	**Villus width (µm)**	**Crypt depth (µm)**	**VH/CD**[2]	**Muscle thickness (µm)**	**Surface area (µm²)**
MA14	800.49 [a]	117.80	73.98	10.77	271.96 [a]	286195 [a]
PE4	620.58 [b]	77.90	68.06	8.90	141.59 [c]	153103 [b]
PE8	545.43 [b]	85.17	63.54	8.37	139.95 [c]	143115 [b]
PE12	552.65 [b]	105.51	57.92	8.98	213.50 [b]	154786 [b]
PE14	644.47 [ab]	120.37	76.62	8.51	237.87 [ab]	243893 [a]
SEM[3]	91.94	13.39	4.88	0.79	19.15	16121.8
P-value						
Treatment effects	0.01	0.15	0.13	0.19	< 0.01	< 0.01
Linear treatment effects	0.01	0.46	0.76	0.08	0.94	0.09
Quadratic treatment effects	< 0.01	0.02	0.02	0.18	< 0.01	< 0.01

[a–b] Means within a column with different superscripts differ significantly ($P \leq 0.05$)
[1] MA14- pullets fed a basal mash diet from hatching to 14th wk of age (n = 8), PE4- pullets fed a pelleted basal diet from hatching time to end of 4th wk of age and after that fed a basal mash diet from 5 to 14th wk of age (n = 6), PE8- pullets fed a pelleted basal diet from hatching time to 8th wk of age and after that fed a basal mash diet from 9th to 14th wk of age (n = 6), PE12- pullets fed a pelleted basal diet from hatching time to 12th wk of age and after that fed a basal mash diet from 13th to 14th wk of age (n = 6) and PE14- pullets fed a pelleted basal diet throughout the experimental period (Hatch-14 wk; n = 8).
[2] VH/CD=Villus height to Crypt depth ratio.
[3] Standard error of the means.

Table 9: Effect of feeding different forms of feeds (mash and pellet) at different times on the bone quality of the left tibia of commercial layer pullets at 14 wk of age

Treatments[1]	**Weight (mg)**	**length (mm)**	**Weight: length ratio**	**medullary canal (mm)**	**Tibiotarsal index**[2]	**Tibia robusticity index**[2]	**Ash (% of dry matter)**
MA14	4731.0	115.35	40.94	4.28	29.73	6.88	46.65
PE4	4720.8	115.86	40.72	4.16	30.96	6.91	49.00
PE8	4718.0	114.02	41.36	4.08	31.07	6.80	54.40
PE12	4631.3	113.46	40.74	4.15	30.46	6.81	47.17
PE14	4700.2	114.90	40.92	4.43	29.44	6.86	45.21
SEM[3]	171.36	1.12	1.22	0.14	1.40	0.05	3.06
P-value							
Treatment effects	0.99	0.71	0.99	0.51	0.90	0.74	0.34
Linear treatment effects	0.78	0.37	0.99	0.54	0.81	0.49	0.64
Quadratic treatment effects	0.91	0.48	0.92	0.10	0.35	0.47	0.09

[a–b] Means within a column with different superscripts differ significantly ($P \leq 0.05$)
[1] MA14- pullets fed a basal mash diet from hatching to 14th wk of age (n = 8), PE4- pullets fed a pelleted basal diet from hatching time to end of 4th wk of age and after that fed a basal mash diet from 5 to 14th wk of age (n = 6), PE8- pullets fed a pelleted basal diet from hatching time to 8th wk of age and after that fed a basal mash diet from 9th to 14th wk of age (n = 6), PE12- pullets fed a pelleted basal diet from hatching time to 12th wk of age and after that fed a basal mash diet from 13th to 14th wk of age (n = 6) and PE14- pullets fed a pelleted basal diet throughout the experimental period (Hatch-14 wk; n = 8).
[2] The tibiotarsal index, representing the relative cortical thickness of the bone, was calculated based on the ratio of diaphysis and medullary canal diameters. The robusticity index, reflecting bone strength, was determined from the bone length relative to its weight (Riesenfeld, 1972).
[3] Standard error of the means.

Table 10. Effect of feeding different forms of feeds (mash and pellet) at different times on the feather score and the stress-related behavioral score of pullets at 14 wk of age

Treatments[1]	Feather score[2]	Stress-related behavioral score[2]
MA14	2.925	1.350
PE4	2.933	1.933
PE8	2.866	1.933
PE12	2.933	1.800
PE14	2.850	1.875
SEM[3]	0.063	0.182
P-value		
Treatment effects	0.84	0.17
Linear treatment effects	0.475	0.13
Quadratic treatment effects	0.74	0.12

[1] MA14- pullets fed a basal mash diet from hatching to 14th wk of age (n = 8), PE4- pullets fed a pelleted basal diet from hatching time to end of 4th wk of age and after that fed a basal mash diet from 5 to 14th wk of age (n = 6), PE8- pullets fed a pelleted basal diet from hatching time to 8th wk of age and after that fed a basal mash diet from 9th to 14th wk of age (n = 6), PE12- pullets fed a pelleted basal diet from hatching time to 12th wk of age and after that fed a basal mash diet from 13th to 14th wk of age (n = 6) and PE14- pullets fed a pelleted basal diet throughout the experimental period (Hatch-14 wk; n = 8).
[2] Pullets were scored for feather coverage according to the 5-point feather score scale (Webster and Hurntk 1990) and for behavioral response according to a 5-point scale (He *et al*. 2016).
[3] Standard error of the means.

Discussion

In the present study, we demonstrated that transitioning from a pelleted diet to a mash diet at 4, 8, 12, and 14 weeks of age resulted in greater feed intake compared to those fed a continuous mash diet throughout the entire experimental period. In addition, the ADWG during the whole rearing period was greater in pullets fed a continuous pelleted diet than those fed a mash diet continuously or shifted from a pelleted to a mash diet at 8 wk of age. In agreement with our results, it has been reported that ADFI and ADWG from 5 to 17 wk of age were greater in the pullets fed the crumbled diet continuously, as compared to those using the mash diet continuously or shifted from the pelleted to mash diet at 5 or 10 wk of age (Saldaña *et al*., 2015b). The overall means of the daily growth of the pullets exhibited a decreasing trend from week 8 to 14 of age (13.65 to 9.39 g/d; Table 5), which may be related to the adverse effects of unknown stresses, such as disease or overstocking challenges. Therefore, our results suggest that feeding pelleted diets during weeks 12 to 14 of age could have beneficial effects on improving performance by increasing feed intake or other alleviating mechanisms, such as enhanced nutrient digestion, homogeneous nutrient intake, or reduced pathogen load.

Our findings showed that shifting pullets from a pelleted diet to mash at 4, 8, or 12 weeks of age resulted in poorer FCR compared with the continuously fed treatments. This reduction in feed efficiency can be attributed to several physiological and behavioral adjustments associated with the abrupt change in feed form. Pelleted diets facilitate rapid feed intake, lower energy expenditure during prehension, and provide higher nutrient accessibility due to partial starch gelatinization. When birds adapted to pellets are switched to mash, they must re-adjust their feeding behavior and gizzard activity, as mash requires greater mechanical grinding and longer eating time. This transition often increases feed sorting and feed wastage, reducing the proportion of nutrients actually consumed. Furthermore, the sudden loss of the higher digestibility and uniform particle structure associated with pellets may temporarily impair nutrient utilization, thereby negatively affecting FCR. Similar trends have been reported by Saldaña et al. (2015b), who found improved FCR in pullets continuously fed crumbles compared with those switched to mash.

Body weight uniformity is a key indicator of flock development and future laying performance. Consistent with our observations, previous studies have shown that pelleted diets do not necessarily improve BW uniformity in pullets (Frikha *et al.*, 2009; Bozkurt *et al.*, 2019), suggesting that factors beyond feed form—such as management, feeder access, and social dynamics—may play a larger role in determining uniformity during rearing.

At the end of the experiment, the relative gizzard weight was linearly decreased by increasing the pelleted diet feeding time from 0 to 14 weeks of the rearing period, while the relative weights of proventriculus, liver, abdominal and gizzard fat, duodenum, jejunum, and ceca were similar among treatments. As presented in Table 4, the geometric mean particle size of the ingredients in the mash feeds was coarser than that of the pelleted diets during the starter and grower stages (880 vs. 660 µm) and the developer stage (1150 vs. 790 µm). Additionally, secondary grinding of feed particles can occur during pelleting due to the narrow gap between the pellet rollers and die, as well as frictional forces within the die holes, leading to a finer particle size.

Therefore, in the current study, the particle size of the pelleted diet might be finer than determined by the dry sieving technique and reported in Table 4. Coarse particles in the mash diet might be selectively retained in the gizzard until they are ground to a certain critical size (Xu *et al*., 2015), resulting in more mechanical stimulation of the gizzard and an increase in organ size. These results were consistent with those reported by Frikha *et al*. (2009), who found that the relative weight of the gizzard was 35% heavier in pullets fed a mash diet from the age of 1 to 45 days compared to those receiving a pelleted diet. Moreover, the heavier gizzard relative weight of the pullets that shifted from the pelleted diet to the mash one at any age compared to PE14-fed pullets might indicate that they readapted quickly to the mash diet in terms of gastrointestinal tract development. These results agreed with those of Saldaña *et al*. (2015b). Furthermore, in agreement with our results, Ege *et al.* (2019) showed that the feed form (as mash and crumble) and particle size (as coarse and fine) could not affect the abdominal fat content of hens at their 52 wk of age.

The lengths of different segments of the gastrointestinal tract, including the duodenum, jejunum, and ileum, were not significantly affected by dietary treatments. However, pullets fed PE8 or PE12 diets exhibited a heavier relative ileum weight compared with those fed the MA14 diet. Interestingly, a quadratic effect was observed in jejunal histomorphology, with the intermediate transition groups (PE4–PE12) showing less favorable morphology than either of the continuous feeding treatments (MA14 or PE14). This pattern may be explained by the abrupt shifts in feed form, which likely required birds to adjust their gizzard activity, intestinal motility, and nutrient digestion. Such sudden changes can impose temporary stress on the intestinal epithelium, slow villus development, and increase variability in histomorphological measurements. In contrast, birds continuously fed a consistent feed form were able to develop their gastrointestinal tract steadily, resulting in better histological outcomes. These findings suggest that maintaining a consistent feed form supports optimal intestinal development, whereas mid-period transitions can transiently compromise intestinal morphology.

The pH of gizzard digesta was not influenced by feed form in the current study. This is in contrast to previous studies reporting that mash-fed birds may exhibit lower gizzard pH than pellet-fed birds (Engberg *et al.*, 2002; Serrano *et al.*, 2013), indicating that factors such as particle size distribution and feed intake behavior may interact with diet form to influence gastric acidity. Moreover, Ege et al. (2019) reported that feed form could affect jejunal length, with crumble-fed hens having longer jejunum than mash-fed birds, highlighting that the effect of feed form on intestinal morphology can vary depending on diet characteristics and management.

Results showed that some jejunum histomorphology traits, including villus height, muscle layer thickness, crypt depth, and absorptive surface area, were affected by increasing the time of pelleted diet feeding, as shifting from the pelleted diet to the mash diet at any age led to lower values than those continuously fed a mash or pelleted diet. The capacity of the birds to absorb dietary nutrients could be related to intestinal villus development (Wang *et al*., 2008). Greater villus height can increase the absorptive surface area, thereby enhancing nutrient transport across the villus surface. In other words, jejunum histomorphology traits did not differ between pullets fed continuously mash or pelleted diets. Bozkurt *et al*. (2019) reported that despite similar villus height, crumbling the feed increased the villus width and total absorptive surface area in the ileum compared to the mash. Conflicting results between these studies might be attributed to the difference in the composition of basal diets, such as anti-nutritional factors or the presence of pathogens, which could be destroyed by pelleting (Abdollahi *et al*., 2013).

In our study, the feed form did not affect the bone quality of the left tibia of the pullets. In contrast to our results, Saldaña *et al*. (2015b) observed that the pullets fed the mash diet had a longer and wider tarsus than those fed crumbles. To the best of our knowledge, the effects of the feed form or pelleted diet feeding schedule during the grower period on the mineralization and quality of the pullets' bones have not yet been widely considered.

Moreover, dietary treatments did not affect the feather pecking and stress-related behavioral scores. It has been reported that laying hens given the pelleted diets show a more feather pecking behavior when compared to those using the mash diet (El-Lethey *et al*., 2000; Lindberg and Nicol, 1994); this is probably due to spending less time on the FI. However, in agreement with our results, Savory (1974) reported that the feed form (mash or pelleted) did not affect the feather pecking behavior of the laying hens. Feather pecking is a multifactorial issue influenced by the birds' genetic background, early life experiences, and environmental factors, including nutrition, appropriate lighting, housing systems, group size, and stocking density (Khumput *et al*., 2019; Kjaer and Sørensen, 2002). For example, a study of 64 Swiss flocks suggested that feather pecking in grower-laying hens might be reduced at a lower stocking density (Huber-Eicher and Audigé, 1999). Therefore, in our study, factors other than the feed form might have a masking effect on the pecking behavior of pullets. Moreover, some evidence suggests that feather pecking is more likely to be initiated by birds that are fearful (Vestergaard *et al*., 1993; Johnsen *et al*., 1998). In the current study, there was no difference in stress-related responses when pullets faced an unfamiliar condition, thus suggesting that there was no relationship between the feed form in early life and the stress response of the pullets.

Conclusion

Our results showed that feeding pullets a pelleted diet throughout the rearing period could enhance ADFI and ADWG, but this approach also resulted in poor gizzard development. However, pullets that shifted from a pelleted to a mash diet at 4, 8, and 12 wk of age had a heavier relative gizzard weight than those fed a pelleted diet continuously. Regardless of pelleting costs, feeding a pelleted diet could increase FI and possibly improve the body weight or energy balance of hens at the onset of the egg production phase to maximize egg weight. Furthermore, a period of pellet feeding to increase BWG, followed by mash to improve gut development, could be advisable.

Authors contributions

Mohammad Sedghi: Supervisor, article editor; Mohsen Moaiedi: Experimenter; Farzad Hashemzadeh: Statistical analyst, article editor; Majid Olyayee: article writer.

Ethics approval

Animal welfare statement

The authors confirm that they have adhered to the animal welfare statement in this manuscript and that all EU standards for the protection of animals and/or feed legislation have been met. We also confirm that we have followed the animal welfare guide, as adopted by FASS (2010).

Conflicts of interest

The authors declare that they have no conflicts of interest.

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