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Original Article

Effect of Different Conditioning Temperatures and Times on the Pellet Quality, Performance, Intestinal Morphology, Ileal Microbial Population, and Apparent Metabolizable Energy in Broiler Chickens

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ABSTRACT

This experiment was conducted for 42 days, and aimed to investigate the effect of conditioning temperature and time on feed quality, performance, jejunum morphology, ileal microbial population, and apparent metabolizable energy in broilers. According to the completely randomized design (CRD) in a factorial arrangement 2*3 (conditioning temperatures: 65 and 75 °C; conditioning times: 30, 60, and 90 second), 540 one-day-old male Ross 308 broilers were randomly distributed among six treatments with six replications, each replicate including 15 birds. Treatments included: 1) 65-30, 2) 65-60, 3) 65-90, 4) 75-30, 5) 75-60, 6) 75-90. The results showed that 60 seconds of conditioning at 75 °C increased the pellet durability index (PDI) in the starter diets ($p<0.05$). In the grower and finisher diets, groups (65-60) and (65-90) showed the highest PDI ($p<0.05$). Broilers fed diets conditioned at 75 °C for 60 s showed more body weight gain ($p<0.05$). On days 25 and 42, the highest villus height (VH) was observed in treatment (75-60), and 60 s steam conditioning increased crypt depth (CD) ($p<0.05$). At 75 ºC, the number of goblet cells decreased, while their highest number was observed at 30 and 60 s on 25 d ($p<0.05$). Conditioning at 75 °C for 60 s enhanced the apparent metabolizable energy (AME) in broilers ($p<0.05$). In conclusion, 60 s conditioning at 75 °C improved the PDI of starter diets, performance, villus height, and AME, while the suitable temperature and pelleting time for grower and finisher diets were (65-60) and (65-90).

INTRODUCTION

Poultry feed processing technology includes various heat treatment steps, including extrusion, expansion, conditioning, and pelleting (Abdollahi *et al.*, 2010a). Compressing feed particles using heat, moisture, and pressure is the main purpose of feed pelleting (Falk, 1985). During the pelleting process, conditioning of mash feed is an essential precondition (Peisker, 2006). Pelleted feed can lead to greater feed consumption as compared to mash feed, since it is easier for birds to take in (Meinerz *et al.*, 2001) and digest different portions of the diet better (Zelenka, 2003), increasing energy availability for production, as less energy is used for feed consumption (Mckinney & Teeter 2004), and finally improving broilers' performance. It has been reported that various aspects affect the physical quality of pelleted feeds, such as feed formulation, feed particle size, conditioning, general characteristics of the pellet-mill die, and pellet cooling and drying (Reimer, 1992). Various studies have shown that high temperatures can reduce the digestibility of heat-sensitive nutrients (Lundblad *et al.*, 2011; Loar *et al.*, 2014). In laying hens, some trace minerals in egg contents were retained differently due to the interaction between temperature and particle size (Hafeez *et al.*,2015). The retention of a few trace elements in egg contents was increased when using mash feed (Khoshbin *et al.*, 2023; Niknia *et al.*, 2022; Vakili *et al.,* 2022). Bedford *et al.* (2003) reported that higher temperatures during conditioning in wheat-based diets had adverse effects once the pelleting temperature overstepped 65 °C. Amylose and amylopectin chains are decomposed by the conditioning temperature, moisture, pressure, and time, thus increasing carbohydrate digestibility (Svihus *et al.*, 2004). Moreover, some researchers stated that increasing the conditioning time improves the physical quality of the pellet (Briggs *et al.*, 1999; Gilpin *et al.*, 2002; Fahrenholz, 2012). Differential diameter conditioners (DDC) are a new generation of conditioners, and several of their functions on feed and broiler chickens have received less attention. One of the advantages of these conditioners is the optimal management of continuous thermal stresses through the longer retention time during gelatinization. First, the diets are homogenized; then, by injecting enough steam, the ideal and constant temperature is reached and the gelatinization process occurs optimally (Sorensen *et al.*, 2011). Today, for complete processing in conditioning systems, double and triple conditioners are used in some factories to produce animal, poultry, and aquatic feeds. In the absence of two full steel double-walled chambers, DDC provide the possibility of better mixing of feed ingredients with steam during a shorter time (1 to 5 minutes). According to the studies conducted concerning the conditioning temperature and time and the dispersion of the results, the objective of this study is to evaluate the main and interaction effects of different temperatures and times of conditioning on the pellet quality of the starter, grower, and finisher diets, as well as the performance of broiler chickens and the appropriate time and temperature to achieve optimal performance.

MATERIAL AND METHODS

Birds, diets, and housing

This study was carried out based on procedures and guidelines approved by the Animal Care Committee of the Kashmar Branch, Islamic Azad University, Kashmar, Iran.

Five hundred and forty one-day-old Ross 308 broiler male chicks were reared until 42 days of age. Fifteen chickens were allocated in each pen. Broilers were fed with six different diets (2*3 (conditioning temperature (65 and 75 °C) and conditioning time (30, 60, and 90 s)) during the starter (1-10 d), grower (11-24 d), and finisher (25-42 d) periods. Six replicates were considered for each treatment. Conditioning temperature was determined at the conditioner outlet before the mash diet entered the die. The composition of the diets and their nutrient amount are indicated in Table 1 (NRC, 1994). Ross 308 nutrition guidelines were used to formulate the diets (Aviagen, 2014). All materials were milled using a hammer mill (Asiab Company, Tehran, Iran). The diets were conditioned for 30, 60, and 90 s at 65 and 75 °C in a DDC conditioner (Feed tech, Turkey, pellet system, and yemmak). The feeds were blended in the mixer (Feed tech, Turkey). Then, each diet was divided into six batches, which were conditioned individually with 65 or 75 °C steam for 30, 60, or 90 s, and pelleted by a mill with a 2 mm die for the starter and grower diets, and 4 mm die for the finisher diets (Graf Gmbh, Germany). House temperature was reduced by 3°C every week until it

Table 1 – Ingredients and nutrient composition of the experimental diets (as-fed basis).

Ingredients (%)	Starter (1–10 d)	Grower (11–24 d)	Finisher (25–42 d)
Corn	53.23	55.44	59.44
Wheat	5.00	6.00	7.00
Soybean meal (44%)	32.89	29.15	23.76
Corn gluten (62%)	3.00	3.00	3.00
Soybean oil	1.10	2.06	2.73
Calcium carbonate	1.06	0.98	0.91
Dicalcium phosphate	1.89	1.68	1.52
Sodium chloride	0.30	0.30	0.30
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
L-Lysine HCl	0.40	0.34	0.35
DL-Methionine	0.33	0.28	0.26
L-Threonine	0.14	0.11	0.09
Sodium Bicarbonate	0.10	0.10	0.10
Choline chloride (60%)	0.06	0.06	0.06
Nutrient composition (%)			
Metabolizable energy (kcal/kg)	2900	3000	3100
Crude protein	22.24	20.80	18.89
Lysine	1.39	1.25	1.12
Methionine + Cystine	1.04	0.96	0.88
Threonine	0.94	0.85	0.76
Calcium	0.93	0.84	0.77
Available Phosphorus	0.46	0.42	0.38
Sodium	0.16	0.16	0.16
Potassium	0.85	0.79	0.70
Chlorine	0.31	0.30	0.30

[1]Provided the followings per kg of diet: vitamin A (trans-retinyl acetate), 12100 U; vitamin D3 (cholecalciferol), 5000 U; vitamin E (D L-α tocopherol acetate), 80 U; vitamin K (menadione), 3.20 mg; riboflavin, 8.60 mg; pantothenic acid (D-Ca pantothenate), 18.00 mg; pyridoxine (pyridoxine-HCl), 4.60 mg; thiamine, 3.20 mg; vitamin B12 (cyanocobalamin), 0.02 mg; biotin, 0.20 mg; folic acid, 2.2 mg; nicotinic acid, 60.00 mg; ethoxyquin (antioxidant), 2.5 mg. [2]Provided the following per kg of diet: Fe, 20 mg; Zn, 110 mg; Mn, 120 mg; Cu, 16 mg; I, 1.25 mg; Se, 0.30 mg.

reached 21°C from the 32 °C on the first day, and was then fixed until the end of the trial period. The relative humidity during the study was considered to be 50–60%. The light schedule was set to 18 h lightness and 6 h darkness throughout the study. Birds had free access to feed and water during the study period.

Pellet quality

Pellet durability was determined at different days after feed manufacturing (0, 15, 30, and 45 days) in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, and Norfolk, UK). One hundred grams of pellet samples were circulated pneumatically in a closed chamber for 30 s before passing through a sieve. The Pellet Durability Index (PDI) was then determined (Abdollahi *et al.*, 2012).

Birds' growth performance

The broilers were weighed at the end of each age period (11, 25, and 42d). Body weight gain (BWG) and feed intake (FI) were measured for each replicate. Feed consumption was measured by deducting the residuary feed from the provided feed in each replicate during the experiment. Feed conversion ratio (FCR) was calculated as the feed consumed (gram) by broilers divided by the body weight gain (gram). Mortality was recorded daily.

Carcass characteristics

At the end of the experimental period (day 42), two birds were selected from each pen, weighed, and decapitated to measure carcass yield. The thigh, breast, heart, and liver weights were recorded and calculated as a percentage of the live weight.

Jejunum morphology

Two broilers from each replicate were chosen and euthanized via cervical dislocation at 25 and 42 d of age. The digestive tract was removed, and parts of approximately 1 cm were obtained from the middle segment of the jejunum. The fragments were fixed in 10% neutral buffered formalin solution and subsequently infixed in paraffin wax. All morphometric examinations are accomplished on 5 µm segments and stained with hematoxylin and eosin. In order to investigate the morphology of tissue samples, a computer-connected optical microscope (Olympus model BX51 microscope; magnification 100) was applied and villus height (VH), crypt depth (CD), villus height to crypt depth ratio (VH:CD) and goblet cells number per villus were thus measured (Garcia *et al.*, 2007).

Ileal microbiology

To investigate the intestinal microbial flora at 25 and 42 d of age, two birds from each replication were randomly selected and slaughtered, and one gram of digesta was removed from the ileum of each bird. It was diluted and homogenized with 9 ml of physiological serum (85%). The samples were used for counting bacteria, including *Lactobacillus*, which were cultured using MRS Agar and incubated at 37 °C for 72 and 48h (McCartney *et al.*, 1996). The number of colonies was determined in terms of CFU/ml and expressed as log10.

Apparent metabolizable energy determination

To measure the apparent metabolizable energy (AME), four broilers per replicate were carried over to separate cages on day 19 to adapt to cage situations for two days. On d 21, chickens were exposed to 8 h of hunger (in order to collect excreta, trays were set under each cage). Excreta were gathered twice a day between 21 and 24 days of age. Samples were dried, blended, weighed, milled, and stored in plastic containers at −20 °C. The birds' feed intake in each cage was noted in terms of excreta accumulation. The gross energy of feed and excreta were determined by a bomb calorimeter (C5003 ika, GMBIT CO., Staufen, Germany) (Harjo & Teeter, 1994). AME values were measured using the blow formula:

$$\text{AME (Kcal/Kg)} = [(\text{Feed intake} \times \text{Gross energy}_{\text{diet}}) - (\text{Excreta output} \times \text{Gross energy}_{\text{excreta}})] / \text{Feed intake}$$

Statistical Analyses

This experiment was performed based on a completely randomized design (CRD) in a factorial arrangement of 2*3 (with two conditioning temperatures and three conditioning times) with six replicates through the GLM[1] procedure of the SAS 9.4 (2012) software. All data were normalized by the Shapiro–Wilk test. The difference between the treatments was analyzed with ANOVA. Duncan's test was used to compare the mean of the treatments. ($p<0$ .05).

RESULTS

The PDI results of the starter, grower, and finisher diets at regular intervals after feed manufacturing (day 0, day 15, day 30, and day 45) are indicated in Tables 2, 3, and 4, respectively. The main effect of temperature

[1] General Linear Model

on the PDI was significant at 0, 15, 30, and 45 days in the starter diets (Table 2), with the durability index of pellets produced at 75 °C being remarkably higher than pellets produced at 65 °C ($p<0.05$). In relation to the main effect of time, the durability index of pellets that had been for 90 and 60 seconds in the conditioner was significantly higher than those that were for 30 seconds as soon as the pellet was produced ($p<0.05$). The interaction effects of temperature and time on the PDI were significant for 0, 15, 30, and 45 days after pellet production; thereby, treatments receiving 75 °C temperature during 60 s of conditioning (75-60) had the highest PDI among the groups ($p<0.05$).

Table 2 – Effect of different conditioning temperatures and times on the pellet durability index (PDI) of starter diets at regular intervals after feed manufacturing.

Experimental treatments	PDI (%)			
Main effect*	Day 0	Day 15	Day 30	Day 45
Temperature (°C)				
65	82.88^{b}	84.88^{b}	79.95^{b}	79.86^{b}
75	85.38^{a}	87.90^{a}	81.64^{a}	82.05^{a}
p-value	0.0001	0.0001	0.0001	0.0001
SEM	0.25	0.24	0.11	0.32
Time (second)				
30	83.00^{c}	85.87	80.64	80.42
60	84.12^{b}	86.40	80.74	81.52
90	85.27^{a}	86.90	80.90	80.91
p-value	0.0002	0.07	0.83	0.17
SEM	0.31	0.29	0.13	0.39
Interaction effects				
65-30	82.55^{d}	85.30^{b}	82.55^{d}	79.62^{bc}
65-60	80.30^{e}	82.30^{c}	80.30^{e}	78.52^{c}
65-90	85.80^{b}	87.05^{b}	85.80^{b}	81.42^{b}
75-30	83.45^{cd}	86.45^{b}	83.45^{cd}	81.22^{b}
75-60	87.95^{a}	90.50^{a}	87.95^{a}	84.52^{a}
75-90	84.75^{cb}	86.75^{b}	84.75^{bc}	80.40^{bc}
p-value	0.0001	0.0001	0.0001	0.0001
SEM	0.35	0.34	0.43	0.45

$^{a-d}$ Means within a column without a common superscript significantly differ ($p<0.05$).
Abbreviations: SEM, Standard error of mean.

In the grower diets (Table 3), the durability index of pellets manufactured at 75 °C was remarkably greater than the 65 °C group at all times after pellet production ($p<0.05$). A significant difference was observed between the PDI for 90 and 60 seconds and that of 30 seconds, with conditioning for 90 and 60 seconds notably increasing PDI ($p<0.05$). This was despite the fact that no substantial discrepancy was observed between 90 and 60 seconds of conditioning ($p>0.05$). In relation to interaction effects, the highest PDI was observed in treatments (65-60) and (65-90) in all measurement periods, which were notably different from others ($p<0.05$).

Table 3 – Effect of different conditioning temperatures and times on the pellet durability index (PDI) of grower diets at regular intervals after feed manufacturing.

Experimental treatments	PDI (%)			
Main effect*	Day 0	Day 15	Day 30	Day 45
Temperature (°C)				
65	86.38^{b}	87.86^{b}	85.71^{b}	85.32^{b}
75	90.27^{a}	89.56^{a}	88.83^{a}	88.01^{a}
p-value	0.0001	0.0010	0.0001	0.0001
SEM	0.34	0.31	0.28	0.25
Time (second)				
30	85.29^{b}	88.15^{b}	84.55^{b}	83.84^{b}
60	89.80^{a}	88.25^{a}	88.37^{a}	87.87^{a}
90	89.90^{a}	89.74^{a}	88.89^{a}	88.27^{a}
p-value	0.0001	0.01	0.0001	0.0001
SEM	0.42	0.38	0.34	0.31
Interaction effects				
65-30	85.15^{b}	88.05^{b}	83.90^{c}	82.65^{c}
65-60	92.15^{a}	91.75^{a}	90.50^{a}	90.00^{a}
65-90	93.52^{a}	90.97^{a}	92.10^{a}	91.37^{a}
75-30	85.42^{b}	88.45^{b}	85.20^{bc}	85.02^{b}
75-60	87.45^{b}	84.55^{b}	86.25^{b}	85.75^{b}
75-90	86.27^{b}	88.50^{b}	85.67^{bc}	85.17^{b}
p-value	0.0001	0.0001	0.0001	0.0001
SEM	0.48	0.44	0.39	0.36

$^{a-c}$ Means within a column without a common superscript significantly differ ($p<0.05$).
Abbreviations: SEM, Standard error of mean.

In the finisher diets (Table 4), at time 0 after feed manufacturing, the pellet durability index at 75 oC was considerably higher than at 65 °C ($p<0.05$). Conditioning for 90 and 60 s significantly enhanced the durability index of the pellet as compared to 30 seconds ($p<0.05$). With respect to interaction effects, at time 0 after feed manufacturing, groups (65-60) and (65-90) had the highest PDI ($p<0.05$).

The outcomes of BWG, FI, and FCR for the starter, grower, finisher and whole phases are shown in Table 5. During the starter period, only BWG was affected by the factors, with the highest increase in BWG being in chickens consuming the pellets produced under the temperature of 75 °C and the time of 60 seconds ($p<0.05$). The highest BWG was observed in treatment (75-60), which was considerably different from other treatments ($p<0.05$). During the grower phase, the highest BWG and the best FCR were observed at 60 s conditioning ($p<0.05$). In addition, by checking the interaction effects, it was understood that the highest BWG was in treatment (75-60), which showed a significant difference from other groups ($p<0.05$). The highest BWG was achieved with 60 seconds of conditioning during the finisher period, which was significantly greater than that obtained with 90 and

Table 4 – Effect of different conditioning temperatures and times on the pellet durability index (PDI) of finisher diets at regular intervals after feed manufacturing.

Experimental treatments	PDI (%)			
Main effect*	Day 0	Day 15	Day 30	Day 45
Temperature (°C)				
65	87.00^{b}	69.01	46.43	46.84
75	90.21^{a}	76.53	66.08	66.03
p-value	0.0001	0.59	0.31	0.31
SEM	0.35	9.94	13.25	13.02
Time (second)				
30	86.01^{b}	78.25	55.31	54.98
60	89.94^{a}	70.38	56.68	57.78
90	89.86^{a}	69.69	57.78	56.55
p-value	0.0001	0.86	0.99	0.99
SEM	0.43	12.17	16.23	15.94
Interaction effects				
65-30	85.97^{b}	88.10	44.95	45.00
65-60	91.77^{a}	68.25	47.50	48.07
65-90	92.87^{a}	70.67	46.82	47.45
75-30	86.05^{b}	68.40	65.67	64.95
75-60	88.10^{b}	92.50	65.85	67.47
75-90	86.85^{b}	68.70	66.72	65.65
p-value	0.0010	0.19	0.99	0.98
SEM	0.50	14.06	18.74	18.41

$^{a\text{-}b}$ Means within a column without a common superscript significantly differ ($p<0.05$).

Abbreviations: SEM, Standard error of mean.

30 seconds ($p<0.05$). Similar to the grower period, the highest BWG was observed in group (75-60) ($p<0.05$). The only effective parameter during the whole period was FCR, which was considerably improved by 60 s as compared to 30 s.

Table 6 shows the data related to the percentage of carcass components. Diets had no considerable effects on carcass traits ($p>0.05$).

The main and interaction effects of conditioning temperature and time on the jejunum morphology at 25 and 42 d of age are given in Table 7. Regarding the main effects of temperature, the 75 °C groups had higher VH than the 65 °C groups on days 25 and 42 ($p<0.05$). In terms of the main effects of time, the highest VH was observed for 60 s on 42d of age ($p<0.05$). On days 25 and 42, the highest VH was in treatment (75-60), which showed a significant difference from other treatments ($p<0.05$). The highest crypt depth was observed in the time of 60 s at 25 and 42 d of age, which were significantly different from 30 and 90 s ($p<0.05$). In relation to the number of goblet cells in the jejunum, the main effect of temperature and time was significant at the age of 25 days. Therefore, at the temperature of 75 °C, the number of these cells in the jejunum was significantly lower than at the temperature of 65 °C. In contrast, the highest number

Table 5 – Effect of different conditioning temperatures and times on the performance of broilers during the starter, grower, and finisher phases.

Treatments	Starter (1-10 d of age)			Grower (11-24 d of age)			Finisher (25-42 d of age)			Whole phase (1-42 d of age)		
Main effects*	BWG (g)	FI (g)	FCR (g:g)	BWG (g)	FI (g)	FCR (g:g)	BWG (g)	FI (g)	FCR (g:g)	BWG (g)	FI (g)	FCR (g:g)
Temperature (°C)												
65	242.49^{b}	261.61	1.08	967.77	1118.57	1.54	967.77	2299.99	1.73	2292.80	3680.16	1.60
75	252.39^{a}	268.11	1.06	974.41	1097.08	1.53	974.41	2268.81	1.71	2303.05	3634.01	1.58
p-value	0.0001	0.25	0.41	0.42	0.35	0.79	0.42	0.63	0.63	0.62	0.39	0.33
SEM	1.39	3.89	0.01	5.73	15.81	0.02	14.35	45.47	0.03	14.35	37.80	0.02
Time (second)												
30	235.86^{c}	258.44	1.09	921.76^{c}	1093.93	1.60^{a}	2224.20^{b}	2266.29	1.74	2224.20^{b}	3618.66	1.63^{a}
60	257.47^{a}	268.20	1.04	1038.80^{a}	1120.94	1.44^{b}	2396.16^{a}	2312.03	1.71	2396.16^{a}	3701.15	1.55^{b}
90	248.99^{b}	267.94	1.07	952.70^{b}	1108.61	1.57^{a}	2273.41^{b}	2274.89	1.72	2273.41^{b}	3651.44	1.61^{a}
p-value	0.0001	0.28	0.10	0.0001	0.62	0.0008	0.0001	0.83	0.85	0.0001	0.46	0.03
SEM	1.71	4.77	0.02	7.02	19.36	0.03	17.58	55.69	0.04	17.58	46.29	0.02
Interaction effects												
65-30	239.07^{dc}	264.20	1.10	909.25^{d}	1086.92	1.62	2220.32^{c}	2267.45	1.73	2220.32	3618.57	1.63
65-60	247.87^{bc}	258.27	1.04	1015.40^{b}	1121.47	1.45	2238.77^{b}	2346.32	1.77	2338.77	3726.05	1.59
65-90	240.52^{dc}	262.35	1.09	978.65b^{c}	1147.30	1.55	2319.30^{bc}	2286.20	1.71	2319.30	3695.85	1.59
75-30	232.65^{d}	252.67	1.08	934.27^{cd}	1100.92	1.57	2228.07^{bc}	2265.12	1.75	2228.07	3695.84	1.63
75-60	267.07^{a}	278.12	1.04	1062.20^{a}	1120.40	1.43	2453.55^{a}	2277.72	1.64	2453.55	3676.25	1.50
75-90	257.45^{b}	273.52	1.06	926.75^{d}	1069.92	1.60	2227.52^{bc}	2263.57	1.74	2227.52	3607.02	1.61
p-value	0.0001	0.08	0.80	0.0002	0.23	0.40	0.002	0.91	0.32	0.20	0.79	1.63
SEM	1.98	5.51	0.02	8.10	22.36	0.03	20.29	64.31	0.05	20.29	53.46	1.59

$^{a\text{-}d}$ Means within a column without a common superscript significantly differ ($p<0.05$).

Abbreviations: BWG, body weight gain; FI, feed intake; FCR, Feed conversion ratio; SEM, Standard error of mean.

Table 6 – Effect of different conditioning temperatures and times on the carcass characteristics (%) of broilers at 42 d of age.

Treatments					
Main effects	Carcass	Thigh	Breast	Heart	Liver
Temperature (°C)					
65	70.86	26.12	25.79	0.67	2.17
75	72.88	25.69	25.49	0.65	2.07
p-value	0.07	0.57	0.79	0.84	0.45
SEM	0.45	0.52	0.77	0.04	0.09
Time (second)					
30	71.64	26.28	25.41	0.66	2.18
60	71.22	25.92	26.47	0.69	2.17
90	72.76	25.51	25.05	0.63	2.02
p-value	0.05	0.69	0.55	0.71	0.59
SEM	0.43	0.63	0.95	0.05	0.12
Interaction effects					
65-30	70.77	26.19	26.16	0.77	2.31
65-60	69.39	26.73	26.16	0.62	2.10
65-90	72.41	25.42	25.06	0.57	2.11
75-30	72.50	26.37	24.65	0.54	2.05
75-60	73.06	25.11	26.78	0.76	2.23
75-90	73.09	25.59	25.04	0.69	1.92
p-value	0.07	0.52	0.72	0.05	0.49
SEM	0.49	0.73	1.09	0.06	0.14

Abbreviations: SEM, Standard error of mean.

of goblet cells was observed at 30 and 60 seconds, which were significantly different from those observed for 90 seconds ($p<0.0$).

The results of counting Lactobacillus in the ileum of broilers are shown in Table 8. The bacterial population was not affected by the treatment ($p>0.05$).

The results of the effect of conditioning temperature and time on the AME are also given in Table 8. Regarding the main effects of conditioning time, 60 s remarkably improved AME as compared to other times (30 and 90 s) ($p<0.05$). The interaction effects data showed that the highest amount of AME was observed in treatment (75-60), which was significantly different from other groups ($p<0.05$).

DISCUSSION

To increase the temperature of the conditioner, it is necessary to add water to the machine via steam infusion. This additional vapor concretes particles and makes the pellets per se, improving the physical quality of pellets (Froetschner, 2006). In the present study, 60 s steam conditioning at 75 °C remarkably increased the PDI in the starter diets, while in grower and finisher diets groups (65-60) and (65-90) showed the highest PDI. It has been said that PDI values

Table 7 – Effect of different conditioning temperatures and times on the on jejunum morphology at 25 and 42 d of age.

Treatments	Jejunum morphology characteristics							
Main effect	Villus height (µm)		Crypt depth (µm)		VH: CD		Number of goblet cells*	
	25d	42d	25d	42d	25d	42d	25d	42d
Temperature (°C)								
65	704.25[b]	807.47[b]	132.42	145.62	5.07	5.61	22.70[a]	19.95
75	756.92[a]	867.85[a]	137.92	149.30	5.10	5.83	19.60[b]	20.93
p-value	0.02	0.04	0.34	0.62	0.86	0.38	0.009	0.50
SEM	20.19	6.04	3.95	5.16	0.14	0.18	0.75	1.01
Time (second)								
30	722.88	802.45[b]	131.87[b]	141.62[b]	5.19	5.73	21.47[a]	20.65
60	758.13	934.13[a]	146.62[a]	164.40[a]	5.09	5.72	23.65[a]	21.55
90	710.75	776.40[b]	127.00[b]	136.35[b]	4.97	5.71	18.32[b]	19.12
p-value	0.18	0.0006	0.03	0.01	0.70	0.99	0.002	0.39
SEM	24.74	7.39	4.83	6.31	0.18	0.22	0.92	1.24
Interaction effects								
65-30	708.00[b]	811.90[b]	123.25	133.20	5.34	6.13	20.55	18.90
65-60	672.25[b]	800.25[b]	142.00	159.95	4.93	5.06	19.75	21.65
65-90	732.50[b]	810.25[b]	132.00	143.70	4.93	5.63	18.50	19.30
75-30	737.75[b]	793.00[b]	140.50	150.05	5.04	5.34	22.40	22.40
75-60	844.00[a]	1068.00[a]	151.25	168.85	5.24	6.37	27.55	21.45
75-90	689.00[b]	742.55[b]	122.00	129.00	5.02	5.79	18.15	18.95
p-value	0.0017	0.0003	0.15	0.21	0.48	0.12	0.16	0.48
SEM	25.36	8.54	5.58	7.29	0.25	0.31	1.06	1.43

[a–b] Means within a column without a common superscript significantly differ ($p<0.05$).

Abbreviations: VH: CD, villus height to crypt depth ratio; SEM, Standard error of mean.

* Per villus

Table 8 - Effect of different conditioning temperatures and times on the ileum microflora and apparent metabolizable energy

Treatments			
	*Lactobacillus**		AME*
Main effects	25d	42d	18-20d of age
Temperature (°C)			
65	139.67	150.35	3413.46
75	144.52	168.27	3412.54
p-value	0.10	0.05	0.94
SEM	3.57	12.79	9.20
Time (second)			
30	139.87	154.60	3378.80[b]
60	142.45	159.29	3485.03[a]
90	143.00	154.03	3375.18[b]
p-value	0.27	0.31	<0.0001
SEM	4.26	16.42	11.27
Interaction effects			
65-30	139.13	149.30	3405.90[bc]
65-60	139.40	142.00	3463.10[bc]
65-90	141.50	159.75	3371.41[c]
75-30	140.56	159.90	3351.70[c]
75-60	142.00	158.71	3507.00[a]
75-90	143.12	152.47	3379.00[c]
p-value	0.17	0.09	0.02
SEM	4.43	18.46	15.93

[a-c] Means within a column without a common superscript significantly differ ($p<0.05$).
Abbreviations: AME, Apparent metabolizable energy; SEM, Standard error of mean.
[*] *Lactobacillus*, mean log10 cfu/ml; AME, kcal/kg.

mounted as conditioning temperature enhanced, with diets conditioned at 88 °C showing more PDI than those produced at 60°C (Perera *et al.*, 2021). These findings agree with other studies that attributed better pellet quality to the greater gelatinized starch amount in response to enhancing conditioning temperatures (Abdollahi *et al.*, 2010b; 2011). Beaman *et al.* (2012) also found that the PDI increased from 82 to 95% when the temperature of conditioning increased from 82 to 93 °C. It has been suggested that an increment in the viscosity of diets, partly due to the gelatinization of starch, may improve the binding volume of feed particles, resulting in better pellet quality (Svihus *et al.*, 2005). Santos *et al.* (2020) reported that with the increase in the conditioner temperature and time, PDI increased. They reported that the highest PDI for finisher diets (21-42 d of age) was obtained when feed was conditioned for 20 s at 85 °C. In the current study, by checking the finisher diets (25-42 d of age), it can be seen that the pellet durability index decreased. This may be due to the use of higher levels of soybean oil in these diets, as increased fat levels in the diets can have a negative effect on pellet elasticity and PDI (Gehring *et al.*, 2011; Abadi *et al.*, 2019). Due to its lubricating effects, fat can decrease the force of friction formed in the die holes and lead to lower pellet quality. Moreover, high amounts of fat in diets can mask the particles of feed, creating an obstacle to steam infiltration in the feed particles, inhibiting gelatinization of starch and binding adherence (Lowe, 2007).

Providing pelleted diets is recognized to improve broilers' performance, mostly because the pellets' physical shape augments feed consumption (Massuquetto *et al.*, 2019). In the present study, the positive effect of increasing the conditioning temperature and time on broilers' performance could be observed. According to the results, the highest body weight gain was obtained in treatment (75-60) during starter, grower, and finisher phases. On the other hand, the lowest FCR was observed for 60 s of time in grower and whole periods. Meanwhile, feed consumption was not affected by the treatments. Abdollahi *et al.* (2010b) reported that the feed conversion ratio decreased as the conditioner temperature increased from 60 to 95 °C. Thereby, they reported the best FCR at 60 °C. Creswell & Bedford (2006) also previously reported the adverse impacts of greater conditioner temperatures on the birds' body weight gain and feed conversion ratio when fed with corn-based diets. They recommend a pellet conditioning temperature of around 80 °C to improve broiler performance. It has been reported that the consumption of feed processed at higher temperatures causes an increase in body weight. Heat breaks disulfide bonds, thus improving protein digestion and deactivating protease inhibitors (Dahlke *et al.*, 2003). More durable pellets can ameliorate performance via enhancement of nutrient agglomeration, and reduction of the amounts of fines, feed losses, and energy spent on consumption and conversion into productive energy (Jensen, 2000). Latshaw & Moritz (2009) stated that feed shape affects the energy from each feed unit that is used to produce and increase heat. As a result, pellet-fed broilers had lower heat increment and used more feed energy for production aims. Kirkpinar & Basmacioglu (2006) reported the negative impacts of higher pelleting temperature on the body weight gain of broilers fed with corn-based diets. They said that pelleting a corn–soybean meal diet at 65 ºC resulted in greater weight gain.

In the present study, none of the parameters measured related to the carcass were affected by the treatments. Mingbin *et al.* (2015) did not observe a significant difference between the weights of the carcass, breast, thigh, and abdominal fat of the birds when using crumble-pellet and mash diets. In contrast, Dozier *et al.* (2010) observed that broilers fed pelleted diets showed higher carcass and breast weights compared to mash diets. The reason for these diverse results can be different conditioning times and temperatures, basal diet composition, and birds' age.

The function and natural structure of the intestine are the biological basis of growth, digestion, and absorption of nutrients in animals (El Aidy *et al.*, 2015). Longer villi indicate improved intestinal health in birds, which, in addition to a greater capacity to absorb nutrients, create uniformity and integrity in the intestinal mucosa (Borsatti *et al.*, 2020). Attar *et al.* (2018) reported that birds fed with diets conditioned for 2 min had higher VH and deeper CD in the jejunum as compared to broilers fed diets steam-conditioned for 0 or 4 min. In the current study, the highest VH was observed in treatment (75-60) at 25 and 42 d of age. Birds consuming diets conditioned for 60 s showed greater CD compared to 30 and 90 s (on days 25 and 42). The improvement in VH for the jejunum of birds fed the pellet diets was related to an enhancement in growth performance. The growth and development of the villi may expand the entire villus absorption area of the lumen, and thus lead to a sufficient digestive area and more nutrient transport on the surface of the villi (Cera *et al.*, 1988). In line with this study, Amerah *et al.* (2007) stated that conditioned diets enhanced the morphometric specifications of the villus. It has been reported that broilers fed with pelleted diets showed an enhancement in VH and VH: CD compared to those fed with mash diets (Zang *et al.*, 2009). An increase in the number of goblet cells indicates a thicker mucosal layer, which is related to a decrease in the nutrient availability. This can cause an increase in energy requirements for the maintenance of the digestive system, and ultimately reduce birds' performance (Wils-Plotz & Dilger, 2013). It is assumed that pelleting reduces the population of heat-sensitive bacteria. Consequently, in response to a lower demand for mucin production to maintain the health of the host against harmful bacteria, a decrease in goblet cells in the villi occurs (Abadi *et al.*, 2019). In the present study, according to the main effects of temperature and time, it can be understood that the number of goblet cells decreased with the increase in the temperature of the conditioner (from 65 to 75 °C). On the other hand, paying attention to the conditioning time suggests that the increase in the conditioning time (90 s) can reduce the number of goblet cells as compared to the times of 30 and 60 seconds.

In the present experiment, the effect of the conditioning temperature (65 and 75 °C) and time (30, 60, and 90 S) on the population of ileum *lactobacilli* at 25 and 42 days was not significant. In accordance with these results, Engberg *et al.* (2004) also stated that thermal processing did not have a significant effect on the population of lactobacillus in the digestive tract. In addition, another study showed that pelleting temperatures of 70 and 85 °C had no considerable effect on the population of *Lactobacillus* and *Bacillus subtilis* in the caeca of broilers (Ighani *et al.*, 2017). In general, little information is available regarding the effect of pelleting and different conditioning temperatures and times on the population of intestinal bacteria, and it needs more study and research.

In this study, the highest level of AME was observed in diets conditioned at 75 °C for 60 s. Netto *et al.* (2019) highlighted that pelleted diets at 80 and 90 °C had higher AME.

Jimenez-Moreno *et al.* (2009) noted that the aleurone layer of grains, especially corn, contains oil bodies that processing factors, such as machinery friction, temperature, moisture, conditioning temperature and time, may act on to release more nutrients to the gut digestion enzymes, and as a result, have a higher ability to digest nutrients. Feed ingredients and texture are well-acknowledged factors affecting feed efficiency, and performance of broilers. Increasing dietary antioxidants is necessary for growth and antioxidant protection system (Vakili and Rashidi, 2011). On the other hand, the conditioning by gelatinizing feed starch increases its surface area (Itani & Svihus, 2019), and by exposing starch to the intestinal digestive enzymes, starch digestibility improves and AME is increased. Concurrently, physiological characteristics of the gut may boost the absorption of nutrients (Abdollahi *et al.*, 2013). An increase in the height of the villi was observed in this study, and there was an improvement in the digestion and performance of the birds as a result.

In general, the improvement of the pellet durability index of starter diets, body weight gain, villus height, and apparent metabolizable energy was obtained in the diets conditioned at 75 °C for 60 seconds. The highest pellet durability index of grower and finisher diets was observed at 65 °C for 60 and 90 s. *Lactobacilli* population was not affected by the different temperatures and times of the conditioner. However, the data in this field is limited and more research is needed to achieve reliable results.

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DATA AVAILABILITY

The data that support this study will be shared upon reasonable request to the corresponding author.

CONFLICTS OF INTEREST

No potential conflict of interest was reported by the author(s).

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