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The Interactive Impacts of Corn Particle Size and Conditioning Temperature on Performance, Carcass Traits, and Intestinal Morphology of Broiler Chickens

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Abstract: This study aimed to investigate the interactions between corn particle size (PS) and conditioning temperature (CT) on the performance, carcass traits, intestinal morphology, and immune responses in broilers fed a corn-soybean meal-based diet. A total of 360, one-day-old male broiler chicks (Ross 308) were randomly allocated into six dietary treatments in a 2×3 factorial arrangement, consisting of two corn PS (finely ground with geometric mean diameter (GMD) of 357 µm (PS_F) vs. coarsely ground corn with GMD of 737 µm (PS_C), and three CT [unconditioned (CT_U), conditioned at 75°C (CT_{75}) and 90°C (CT_{90})]. Birds accommodated in 30 pens with five replicates and 12 chicks per each. There was no interaction between corn PS and CT on growth performance and immune response of broilers at any growth phases. However, during the starter (0-10d) period, the average daily weight gain (ADWG) and feed conversion ratio (FCR) of PS_F-fed birds were significantly improved compared to those fed PS_C ($P<0.05$). During the starter (0-10d) and grower (11-24d) periods, increasing the conditioning temperature of corn increased the ADWG, while in the starter phase only the CT_{75} caused a lower FCR ($P<0.05$). Broilers fed PS_F corn showed lowest FCR during the finisher (25-42d) period compared to those fed PS_C ($P<0.05$). Conditioning corn at 75ºC reduced FCR during the finisher (25-42d) period compared to the birds fed CT_U and CT_{90} corn ($P<0.05$). In whole experimental periods (1-42d), PS_F and CT_{75} treatment increased the ADWG compared to the PS_C and CT_U ($P<0.05$). The CT_{75} treatment improved primary total anti-sheep red blood cell (SRBCs) titer (IgT) and IgM and secondary IgT and IgG responses compared to the other experimental groups (CT_U and CT_{90}) ($P<0.05$). No significant PS × CT interaction was found on Newcastle disease (ND) antibody titer of broiler chickens ($P>0.05$). Feeding CT_{75} corn reduced duodenum and jejunum relative length compared to the birds fed diets containing CT_U corn. Significant PS×CT interactions ($P<0.05$) were observed for villus height, villus height to crypt depth, crypt depth, muscle thickness, and absorption surface area of jejunum. The highest carcass yield observed in PS_F-CT_{75}-group ($P<0.05$). In conclusion, the use of finely ground corn (PS_F) conditioned at 75℃ (CT_{75}) was beneficial to growth performance, development of the digestive tract, jejunum histomorphometry and the immune responses of broilers.

Keywords: Broiler; Conditioning temperature; Corn; Particle size; Performance

1. Introduction

Corn (*Zea mays L.*), the primary dietary energy source in monogastric animals, especially poultry, is the dominant cereal grain produced worldwide, and its non-processed usage in the manufacture of bio-based products is increasing. Due to its better nutritional value, especially the excellent source of carbohydrates with low fiber and soluble non-starch polysaccharides content, corn grain has gained many attractions (compared to wheat and barley) in poultry nutrition. (Cowieson, 2005).

Due to several advantages such as improving nutrient digestibility, energy utilization and increasing growth performance and production economic, feed processing has become an essential

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step in the poultry feeding system (Putra et al., 2018). The degree of grain grinding affects not only the processing quality but also the nutritional value of the feed ingredients, broiler performance, and gastrointestinal tract (GIT) function (Abdollahi et al., 2019a). Studies have shown that coarsely ground corn? effectively improved FCR and the utilization efficiency of nutrients in broiler chickens (Xu et al., 2017). Finely ground cereals have been shown to increase nutrient digestibility because of the high relative surface area, which interacts better with digestive enzymes in the GIT (Hetland et al., 2002; Amerah et al., 2007). This improvement in the digestibility may attribute to the improvement in enzymatic digestion and intestinal absorption (Duke, 1992; Amerah et al., 2007).

Today, different feed processing techniques have been introduced to improve nutrient digestibility in the poultry industry. For instance, physical and chemical changes by steam conditioning have been reported to affect nutrient utilization positively (Silversides and Bedford, 1999). Earlier studies have shown that the moderate steam conditioning of broiler feed can positively affect their nutritional value through a) starch gelatinization, b) destruction of heat-sensitive anti-nutrients, c) destruction of cell walls, d) denaturation of digestive enzyme inhibitor proteins, and e) improvement of availability of nutrients. In contrast to moderate conditioning, high processing temperatures may impair the nutrient digestibility of broiler feed by, for example, triggering the Millard reaction (Silversides and Bedford, 1999).

The hypothesis of this study was that feeding birds with different corn particle sizes and conditioning temperature may improve their performance potential and enhance their gastrointestinal development. The present experiment was evaluated whether fine or coarse ground corn and different conditioning temperatures would have influence growth performance, carcass traits, intestinal morphology and immune system in broiler chickens.

Materials and methods

Experimental Design and processing

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received (In line with the Animal Research Ethical Committee of the Animal Science Department of University of Tabriz under the ethical approval number of IR-TU-AEC 1344/PD/3). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes [and feed legislation, if appropriate]. This experiment was conducted to investigate the effects of corn processing in broiler diets at different CT and PS on their growth performance, carcass characteristics, immune responses, and small intestine morphology in a completely randomized design with a 2×3 factorial arrangement including two corn PS: fine corn PS (PS_F) (357±15μm) and coarse corn PS (PS_C) (737±19μm) and three different CT of corn (unconditioned (CT_U), 75°C (CT_{75}) and 90°C (CT_{90})).The corn grain used in the current study was obtained from the nearest local feed supplier in West Azerbaijan, Iran. First, the fine and coarse particle sizes of corn were obtained using a hammer mill with a 2 and 8 mm hole screens, respectively. Particle size was determined using an eight-sieve stack with the sieve numbers 4, 6, 10, 18, 35, 60, 120, 200, and a pan. Approximately 100 g of the ground corn samples were used to sift for 10 min. The weight of particles retained by each sieve was expressed as the proportion of initial sample weight. The geometric mean diameter (GMD) and the geometric standard deviation of corn particle size were then determined as described by Baker and Herman (2002). After preparing the corn with a specific PS, each of the fine and coarse particles was divided into three equal aliquots. First part was remained unprocessed, the other two parts were conditioned separately at 75 and 90°C for 60s by adjusting the steam flow rate in a commercial double conditioner (Eghtesadgostare Salem Poultry Feed Co, Urmia, Iran). The steam pressure used in this conditioner was 2 bars. The steam entered through 6 steam injection valves for better and more uniform distribution, *i.e.*, in the beginning of the conditioner. After applying heat treatment to ground corn, it was dried and cooled at 25°C for eight min in the cooler. The 6 differently processed corn batches were used to develop dietary treatments in mash form.

Diets and Bird Management

A number of 360, one-day-old male Ross 308 broilers were purchased from the nearest commercial hatchery and settled at the experimental farm from one to 42 days of age. The birds were allocated into six treatments with five replicates and 12 chicks per each in floor pens with the dimensions of 120×120 cm^2. The experimental diets were fed in three phases, namely starter (1 - 10d), grower (11 - 24d), and finisher (25 - 42d). The feed was provided in mash form and the birds had free access to fresh water throughout the study. The ingredients and nutrient compositions of the experimental diets are shown in Table 1.

Table 1. Ingredients and calculated chemical composition (%) of the experimental basal diet.

Ingredients	Starter (0-10d)	Grower (11-24d)	Finisher (25-42d)
Corn (7.80% CP)	48.90	52.64	57.74
Soybean meal (43.88% CP)	42.75	38.74	33.31
Soybean oil	4.50	5.20	5.81
Dicalcium phosphate	1.13	0.92	0.75
Calcium carbonate	1.07	0.99	0.92
Common salt	0.33	0.33	0.33
Sodium bicarbonate	0.15	0.13	0.13
Mineral premix [a]	0.25	0.25	0.25
Vitamin premix [a]	0.25	0.25	0.25
DL-Methionine	0.33	0.28	0.26
L- Lysine HCL	0.21	0.16	0.16
L- Threonine	0.07	0.05	0.03
Choline Chloride	0.05	0.05	0.05
Phytase (1000FTU/kg)	0.01	0.01	0.01
Calculated chemical composition			
Apparent metabolizable energy (Kcal/Kg)	3000	3100	3200
Crude protein (%)	23.00	21.50	19.50
Calcium (%)	0.96	0.87	0.79
Available phosphorus (%)	0.48	0.435	0.395
Potassium (%)	0.98	0.92	0.83
Chlorine (%)	0.25	0.25	0.24
Sodium (%)	0.18	0.18	0.18
Digestible Lysine (%)	1.28	1.15	1.03
Digestible Methionine (%)	0.63	0.57	0.53
Digestible Methionine + Cystine (%)	0.95	0.87	0.80
Digestible Threonine (%)	0.86	0.77	0.72
Digestible Tryptophan (%)	0.25	0.24	0.21
Digestible Arginine (%)	1.52	1.42	1.26
Digestible Isoleucine (%)	0.92	0.86	0.77
Digestible Leucine (%)	1.77	1.68	1.55
Digestible Valine (%)	0.98	0.93	0.84

a Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 20 mg; cholecalciferol, 4500 IU; cyanocobalamin, 0.017 mg; folic acid, 2.0 mg; menadione, 3.6 mg; niacin, 65 mg; pyridoxine, 4 mg; trans-retinol, 12000 IU; riboflavin, 8 mg; thiamine, 4.0 mg; all-rac-α-tocopheryl acetate, 80 IU; Cu, 16 mg; Fe, 20 mg; I, 1.25 mg; Mn, 120 mg; Se, 0.3mg; Zn, 120 mg. b Aviagen (2019) Ross 308 nutrients requirements tables were used for calculation.

The stocking density of 12.5 birds/m^2 was considered during the study. The brooding temperature was maintained at 32°C for the first day and gradually decreased by 1°C every three days until 21°C and maintained to the end of study. The lighting program, relative humidity, and

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temperature followed the Ross 308 management guide (Aviagen, 2018) and the diets were formulated to meet the nutrient recommendations for Ross 308 (Aviagen, 2019).

Sample and Data Collection

Growth Performance

Growth performance traits, including ADWG (Average Daily Weight Gain) and ADFI (Average Daily Feed Intake), were measured at the end of each feeding phase, and FCR was calculated by dividing the ADFI by ADWG. Mortality was recorded daily. FCR values were corrected for the body weight of any bird that died during the course of the experiment.

Carcass traits

On d 42, 10 chickens with similar BW from each treatment (two bird per each replicate) were selected, weighed, and euthanised by cervical dislocation. Then, breast muscle, thigh, wing, heart, liver, gizzard, proventriculus, pancreas, small intestine segments, abdominal pad fat, thymus, spleen and bursa of Fabricius were taken out and weighed. After digesta depletion, the length and weight of small intestine segments including, duodenum, jejunum and ileum, and cecum were measured. The relative empty weight of organs and length of the intestine segments were calculated as follows:

Relative empty weight of organs = (Empty weight of organ (g)/slaughter body weight (g)) × 100

Relative intestinal length (cm/kg body weight) = Intestinal length (cm) / body weight (gr)

Jejunum morphology

At d 42, two birds per each replicate, with body weight closest to the mean weight of the pen were selected, weighed and euthanised by cervical dislocation. Briefly, 1.5 cm from the middle of the jejunal segment was isolated, and following the tissue fixation by 10% formaldehyde, they were dehydrated at graded ethanol and embedded in paraffin wax. Four cross-section cuts with 4-µm thickness were provided and stained with hematoxylin and eosin (H&E). The histomorphometry indices of jejunum (villus height, villus width, crypt depth, villi-to-crypt ratio, crypt depth ratio, and absorption surface area) were analyzed using a light microscope (Olympus Model BX41, Japan) and Digimizer image analysis software (V.6.3.0). Villus absorption surface area was calculated using the formula: Villus absorption surface area = 2π × (average villus width/2) × villus height (Nain et al., 2012; Sohail et al., 2019).

Immune responses

Sheep red blood cell (SRBC), non-specific and T-cell dependent antigen, were administrated to quantify antibody production. Briefly, for primary antibody titers to SRBC, 2 male broiler chickens per each treatment were randomly selected at 28d of age and 1 ml of 5 % SRBCs suspension diluted in the phosphate buffer saline (PBS) were intramuscularly injected into breast muscle. At 35 d of age, a booster inoculation of 1 ml of 5% SRBC were conducted to same birds. For the secondary humoral immune response, the same SRBC solution was injected intramuscularly into the breast muscle of the same birds at 35d of age. Blood samples were collected 7 days after each injection and serum were collected to determine total, IgM and IgG anti-SRBC antibody titers by microhemagglutination assay (Tsiagbe et al., 1987).

At 42d of age, serum antibody titers against Newcastle disease (ND) virus was performed by using standard hemagglutination inhibition test (Meijer et al., 2006)

Statistical Analysis

The data obtained in the present study were analyzed using the GLM procedure of SAS 9.2 software (SAS, 2008) in a two-way factorial arrangement. Pens were considered as the experimental unit for all data. The significance of the differences among means was determined using Duncan's Multiple Range Test, where the $P<0.05$ was considered statistically significant.

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Results

Growth performance

The effect of different corn PS and CT on growth performance of broiler chickens is reported in Table 2. The results showed no significant (P>0.05) interaction between PS and CT on the growth performance of broiler chickens at any growth phases. However, PS_F significantly (P<0.05) improved FCR and ADWG in starter (1-10d) and whole experimental period (1-42d) compared to birds received PS_C diets. During the finisher phase, the FCR in PS_F fed birds was significantly lower (P<0.05) compared to PS_C fed birds. The main effect of CT was significant (P<0.05) for ADWG and FCR in starter phase. In the grower phase, the main effects of CT were significant in ADWG and FCR, where the highest ADWG and lowest FCR were observed in CT_{75} and CT_{90} (P<0.05). In the finisher (25 to 42d) phase, the main effect of different CT was significant on FCR, where the lowest FCR was observed in CT_{75} (P<0.05). In whole grow-out period, the main effect of CT was significant (P<0.05) for ADWG, with the CT_{75} having the highest ADWG.

Table 2. Effect of corn particle size (PS) and conditioning temperature (CT) on performance of broiler chickens.

Treatments		Starter (1-10 days)			Grower (11-24 days)			Finisher (25-42 days)			Total (1-42 days)		
PS*	CT (°C)	ADFI[1] (gr/bird/d)	ADWG[1] (gr/bird/d)	FCR[1] (gr/gr)	ADFI (gr/bird/d)	ADWG (gr/bird/d)	FCR (gr/gr)	ADFI (gr/bird/d)	ADWG (gr/bird/d)	FCR (gr/gr)	ADFI (gr/bird/d)	ADWG (gr/bird/d)	FCR (gr/gr)
F	-	30.9	22.7[a]	1.360[b]	73.7	47.6	1.552	142	89.5	1.592[b]	89.1	57.3[a]	1.554[b]
C	-	29.7	20.8[b]	1.433[a]	74.8	47.4	1.582	143	87.8	1.638[a]	89.8	56.1[b]	1.600[a]
SEM	-	0.404	0.358	0.0187	0.642	0.564	0.0205	0.850	0.823	0.0137	0.495	0.395	0.0116
-	U	29.5	20.6[b]	1.439[a]	73.4	45.0[b]	1.634[a]	142	89.0	1.602[ab]	88.7	55.7[b]	1.594
-	75	30.4	22.4[a]	1.354[b]	75.0	48.2[a]	1.555[b]	142	89.7	1.593[b]	89.7	57.6[a]	1.557
-	90	31.0	22.2[a]	1.396[ab]	74.5	49.3[a]	1.512[b]	143	87.2	1.649[a]	89.9	56.9[ab]	1.580
SEM		0.494	0.439	0.0229	0.787	0.691	0.0252	1.041	1.009	0.0168	0.607	0.484	0.0142
	U	30.4	21.5	1.414	73.3	44.8	1.640	141	90.4	1.567	88.6	56.4	1.571
F	75	31.2	23.5	1.325	73.9	48.3	1.528	142	91.2	1.562	89.3	58.4	1.527
	90	31.0	23.1	1.341	74.0	49.7	1.488	143	86.9	1.647	89.4	57.1	1.564
	U	28.7	19.6	1.464	73.4	45.2	1.628	143	87.5	1.637	88.8	54.9	1.618
C	75	29.6	21.4	1.383	76.1	48.1	1.581	143	88.3	1.624	90.1	56.7	1.588
	90	30.9	21.4	1.452	75.0	48.8	1.536	144	87.6	1.652	90.4	56.6	1.595
SEM		0.699	0.621	0.0323	1.113	0.977	0.0356	1.472	1.427	0.0237	0.858	0.685	0.0200
						P value							
PS		0.06	<0.01	0.01	0.09	0.75	0.31	0.25	0.15	0.02	0.32	0.03	0.0093
CT (°C)		0.14	0.01	0.04	0.23	<0.01	0.007	0.61	0.22	0.05	0.36	0.03	0.2045
PS × CT (°C)		0.47	0.97	0.60	0.45	0.80	0.60	0.96	0.38	0.34	0.90	0.64	0.7767

Abbreviations: PS, particle size; CT, conditioning temperature; ADFI, Average Daily Feed Intake; ADWG, Average Daily Weight Gain; FCR, Feed Conversion Ratio; U, unconditioned; F,Fine particle size of corn grain; C, Coarse particle size of corn grain. [a-b] Means within a column with different superscripts differ significantly (P<0.05).

Relative length of the small intestine and jejunum morphology

The effects of different corn PS and CT treatments on relative length of small intestine and jejunum morphology of broiler chickens are presented in Table 3. As shown, the PS×CT interaction was significant for the cecum relative length, villus height, crypt depth, villus height to crypt depth ratio, muscle thickness, and absorption surface area. The highest relative length of cecum and crypt depth observed in birds fed the PS_F-CT_U diet (P<0.05). The highest villus height and absorption surface area belonged to the birds fed the PS_F-CT_{75} diet (P<0.05). The thickest intestinal muscle was observed in PS_C-CT_U fed birds (P<0.05). The main effect of PS was significant on GIT, where the highest villus height, villus height:crypt depth, and absorption surface area were observed in PS_F (P<0.05). The highest villus width, crypt depth, and intestinal muscle thickness belonged to the PS_C (P<0.05). The main effects of different CT treatments on dietary corn grain were significant in GIT, where the lowest percentages of duodenum, jejunum, cecum, total small intestine and muscle thickness (µm) were observed in CT_{75} (P<0.05). In the meantime, the highest villus width and absorption surface area were belonged to the CT_{75} and CT_{90} (P<0.05) compared with those fed CT_{U}.

Table 3. Effect of corn particle size and conditioning temperature on carcass traits, gastrointestinal organ weights and gizzard pH of broiler at 42d.

PS	CT (°C)	Slaughtered Body Weight (gr)	Carcass yield	Breast	Thigh	Abdominal fat	Gizzard	Liver	Pancreas	Heart	Gizzard pH
			% of live weight								
F	-	2522^{a}	67.8	26.2	20.1	2.036^{b}	1.033	1.906	0.2215	0.459	3.978
C	-	2472^{b}	67.5	25.7	20.3	2.412^{a}	1.171	1.852	0.2002	0.479	3.125
SEM	-	16.8	0.28	0.34	0.27	0.1065	0.0219	0.0568	0.0047	0.0167	0.0640
-	U	2455	67.6	25.7	20.4	2.142	1.230	1.949	0.2318	0.479	3.623
-	75	2524	67.9	26.2	20.1	2.180	1.061	1.783	0.2072	0.454	3.513
-	90	2512	67.4	25.9	20.0	2.320	1.016	1.904	0.1936	0.475	3.517
-	SEM	20.6	0.34	0.42	0.33	0.1304	0.0268	0.0696	0.0058	0.0205	0.0784
	U	2492	67.3^{b}	26.0	20.0	1.950	1.132^{bc}	2.170^{a}	0.2378^{a}	0.454	4.060^{a}
F	75	2562	68.9^{a}	26.3	20.6	1.975	1.071^{bcd}	1.813^{ab}	0.2415^{a}	0.457	3.740^{ab}
	90	2512	67.1^{b}	26.3	19.6	2.184	0.897^{d}	1.733^{ab}	0.1853^{b}	0.467	4.135^{a}
	U	2418	67.9^{ab}	25.5	20.8	2.335	1.328^{a}	1.728^{b}	0.2258^{ab}	0.504	3.187^{bc}
C	75	2485	67.7^{ab}	26.1	19.6	2.455	1.051^{cd}	1.832^{ab}	0.1728^{b}	0.451	3.287^{bc}
	90	2513	66.8^{b}	25.5	20.5	2.456	1.135^{bc}	1.995^{ab}	0.2019^{ab}	0.483	2.900^{c}
SEM		29.2	0.49	0.60	0.47	0.1844	0.0379	0.0984	0.0082	0.0289	0.1109
					P Value						
PS		0.04	0.47	0.37	0.56	0.04	0.0009	0.55	0.01	0.45	<0.0001
CT		0.06	0.70	0.78	0.73	0.69	0.0002	0.31	0.002	0.71	0.61
PS × CT		0.33	0.03	0.92	0.19	0.88	0.0148	0.02	0.0007	0.69	0.019

*Abbreviations: PS, particle size; CT, conditioning temperature; F= Fine particle size of corn grain, C= Coarse particle size of corn grain, U= Unconditioned, Conditioned at 75℃ and 90℃. $^{a-b}$ Means within a column with different superscripts differ significantly (P<0.05).

Immune responses

The effects of experimental treatments on the broiler chickens' response to SRBC and ND titers are shown in Table 4. The interaction between PS×CT were not significant on SRBC and ND titers (P>0.05). The main effects of CT_{75} on SRBC titer of broilers was significant, where the highest IgM and IgT titer in primary response, and IgG and IgT titer in secondary response against SRBC injection were observed in CT_{75} (P<0.05).

Table 4. Effect of corn particle size and conditioning temperature on SRBC and Newcastle disease (ND) titer of broiler.

PS	CT (°C)	Primary responses			Secondary responses			ND titer
		Ig T	IgG	IgM	Ig T	IgG	IgM	
F	-	4.533	1.866	2.666	5.666	2.733	2.933	6.666
C	-	5.066	1.933	3.133	6.333	3.066	3.266	7.066
SEM	-	0.3000	0.1944	0.2963	0.3073	0.3448	0.2186	0.3055
-	U	4.300^{b}	1.700	2.600^{b}	5.200^{b}	2.600	2.600^{b}	6.800
-	75	5.500^{a}	1.900	3.600^{a}	6.600^{a}	2.800	3.800^{a}	7.100
-	90	4.600^{ab}	2.100	2.500^{b}	6.200^{ab}	3.300	2.900^{b}	6.700
-	SEM	0.3674	0.2380	0.3629	0.3764	0.4223	0.2677	0.3742
	U	4.000	1.600	2.400	5.000	2.400	2.600	6.400
F	75	5.400	2.000	3.400	6.200	3.000	3.200	6.600
	90	4.200	2.000	2.200	5.800	2.800	3.000	7.000
C	U	4.600	1.800	2.800	5.400	2.800	2.600	7.200
	75	5.600	1.800	3.800	7.000	2.600	4.400	7.600

90	5.000	2.200	2.800	6.600	3.800	2.800	6.400
SEM	0.5196	0.3367	0.5132	0.5323	0.5972	0.3786	0.5292
				P value			
PS	0.2208	0.8104	0.2764	0.1381	0.5008	0.2916	0.3638
CT(°C)	<0.01	0.5036	<0.01	<0.01	0.4928	<0.01	0.7367
PS × CT(°C)	0.8423	0.7921	0.9750	0.9105	0.5105	0.1573	0.2764

*Abbreviations: PS, particle size; CT, conditioning temperature; F= Fine particle size of corn grain, C= Coarse particle size of corn grain, U= Unconditioned, Conditioned at 75°Cand 90°C; ND, Newcastle disease. a-b Means within a column with different superscripts differ significantly (P<0.05).

Carcass traits

The effects of corn PS and CT on the carcass characteristics of broiler chickens are presented in Table 5. As indicated, significant PS×CT interaction was seen in carcass yield, gizzard, liver, and pancreas relative weight (g per kg body weight), where the highest carcass yield was observed in birds fed PS_F corn conditioned at 75ºC (P<0.05). Moreover, the highest liver relative weight belonged to the PS_F-CT_U-fed birds, and the highest pancreas weight to those fed the PS_F-CT_U and PS_F-CT_{75} diets (P<0.05). The main effect of PS on dietary corn grain on body weight was significant, where the highest value was observed in PS_F (P<0.05). The main effect of PS on the abdominal fat pad, gizzard, pancreas, and gizzard pH was significant where the highest value was seen in PS_C, PS_C, PS_F and PS_F groups, respectively (P<0.05). The main effects of CT_{75} and CT_{90} treatments on gizzard and pancreas relative weight were significantly highest(P<0.05) than CT_U group.

Table 5. Effects of corn particle size and conditioning temperature on relative length (cm/kg body weight) jejunum morphology of the digestive tract of broiler at 42d.

PS*	CT (°C)	Duodenum	Jejunum	Ileum	Total Small intestine	Cecum	Villus height (µm)	Villus width (µm)	Villus height / Crypt depth	Crypt depth (µm)	Muscle thickness (µm)	Absorption surface area (µm)
		(cm/kg body weight)										
F	-	12.6	30.9	31.0	75.3	7.89	1080	100.4^{b}	12.77	87.8	134	344090
C	-	13.3	29.4	31.0	73.1	7.93	833	117.4^{a}	8.55	105.1	150	306872
SEM	-	0.392	0.546	0.810	1.500	0.1550	34.0	4.95	0.710	5.34	6.0	17340
-	U	14.2^{a}	32.7^{a}	31.4	78.4^{a}	8.46^{a}	918	99.4^{b}	10.10	98.2	166	284167
-	75	12.0^{b}	27.8^{b}	30.2	70.1^{b}	7.40^{b}	986	113.5^{a}	10.18	106.3	133	348582
-	90	12.7^{ab}	29.9^{ab}	31.3	74.0^{ab}	7.87^{ab}	965	113.8^{a}	11.71	84.8	128	343695
-	SEM	0.480	0.668	0.992	1.837	0.1899	41.6	6.06	0.870	6.54	7.3	21237
	U	13.9	32.6	30.0	76.6	8.67^{a}	937^{b}	88.3	12.11^{a}	79.1^{b}	116^{c}	257873^{c}
F	75	11.9	25.7	30.0	67.7	6.91^{b}	1244^{a}	110.0	14.05^{a}	94.5^{ab}	156^{b}	430018^{a}
	90	12.0	30.1	32.8	75.0	8.11^{ab}	1057^{ab}	102.8	12.15^{a}	89.8^{ab}	129^{bc}	344379^{b}
	U	14.5	32.9	32.7	80.2	8.25^{ab}	899^{ab}	110.6	8.09^{b}	117.4^{a}	215^{a}	310459^{bc}
C	75	12.0	30.0	30.4	72.5	7.90^{ab}	728^{c}	116.9	6.31^{b}	118.0^{a}	109^{c}	267145^{bc}
	90	13.4	29.8	29.8	73.1	7.64^{ab}	872^{bc}	124.7	11.27^{a}	79.9^{b}	127^{bc}	343009^{b}
SEM		0.680	0.945	1.403	2.598	0.2685	58.9	8.57	1.231	9.26	10.4	30034
						P value						
PS		0.279	0.099	0.995	0.366	0.891	<0.01	<0.01	<0.01	<0.01	<0.01	0.03
CT (°C)		0.027	0.0008	0.697	0.034	<0.01	0.26	0.03	0.12	<0.01	<0.01	<0.01
PS × CT (°C)		0.68	0.086	0.208	0.481	0.042	<0.01	0.35	<0.01	<0.01	<0.01	<0.01

*Abbreviations: PS, particle size; CT, conditioning temperature; F= Fine particle size of corn grain, C= Coarse particle size of corn grain, U= Unconditioned, Conditioned at 75℃ and 90℃
a-b Means within a column with different superscripts differ significantly (P<0.05).

Discussion

Growth performance

According to the findings of the current study, the main effect of the corn PS on the growth performance of broiler chicken was significant, where the PSF treatment improved BWG in comparison to the birds' fed diets containing PSC-treated corn grain. Studies have shown that feeding finely ground diets can increases the growth performance of broiler chickens. Reducing the particle size of grains used in animal feeds means fracturing the outer seed coat and its endosperm layer. Fine grinding grains increases the particle numbers while reducing their size. Therefore, the overall surface area per unit of volume increases, which in turn gives more access to digestive enzymes and increases digestive efficiency (Goodband et al., 2002). Also, easy handling and better mixing with the other feed ingredients are the other advantages of finely ground PS (Koch, 1996). Grain particle size is crucial in feed processing. Nir et al. (1995) suggested that bigger particles slowed down the digesta passage rate in the small intestine, which in turn increased peristaltic movements and improved nutrient utilization. On the other hand, studies showed that grinding feeds influenced nutrient digestion by decreasing surface area, which subsequently may promote feed intake and enhance gut health in broiler chickens (Ge et al., 2017). Nir et al. (1994a) reported that broiler chickens preferred diets containing coarse particles to those containing fine particles. However, it is still not clear whether coarse-sized feeds are beneficial for all ages of birds. The results obtained in the present study clearly demonstrated that finely-ground-corn-grain inclusion in broiler diets improved ADWG and FCR during the starter phase (1-10d); however, it only improved FCR in finisher period (26-42) and increased ADWG in whole experimental period (1-42d). No significant impact observed in the grower period (11-25d) on birds' performance. These findings are in agreement with those obtained by Chewning et al. (2012), who reported no remarkable difference in BWG phases by feeding diets containing coarsely-ground corn grain (600 μm) and finely-ground corn grain (300 μm). Similar results were reported by Get et al. (2017) where they showed that finely ground corn particle size in the starter and growth phases and course one in the finisher phase were beneficial for BWG in broiler chickens. These researchers suggested that grinding the corn grain finely increases the contact surface of the feed with digestive enzymes and ultimately improves the ADWG and FCR.

The conditioning temperature significantly affected the FCR and ADWG in the starter phase, where the lowest FCR and the highest ADWG in the starter period were observed in CT_{75}. Briefly, CT_{75} treatment of corn grain in this study improved the FCR and ADWG in all experimental phases except for the grower (11-24d). Abdollahi et al. (2010b) have reported improved BWG as conditioning temperatures increased from 60 to 75 and 90ºC in broiler chickens. In another study, Selle et al. (2013) reported a linear increase in FCR in broilers fed sorghum-based diets as conditioning temperatures were increased from 65 to 95ºC with no adverse effect on feed consumption. Recently, Teixeria et al., (2019) evaluated the effect of 5 conditioning temperatures, including 50, 60, 70, 80 and 90 ºC on broiler performance from 1 to 21 d of age and reported a quadratic effect of CT on BWG, where conditioning feeds at 60 and 70 ºC improved the broilers BWG at 21d of age in comparison those fed diets conditioned at 90ºC. Those researchers believed that high conditioning temperatures reduced the digestibility of heat labile nutrients (Abdollahi et al., 2010a; Lunddblad et al., 2011; Loar et al., 2014) by contributing to the formation of indigestible starch-protein and starch-lipid complexes, which may potentially deteriorated nutrient absorption in small intestine (Creswell and Bedford, 2006; Netto et al., 2019).

Carcass traits

In the present study, the two factors of corn PS and CT had no significant effect on carcass traits, including carcass weight, breast weight, and thigh weight. Only the finely ground corn led to a significant reduction in abdominal fat pad compared to the diets containing coarse grinded corn grain. Yan et al. (2022) in agreement with the findings of current study reported that abdominal fat increased with increasing feed particle size. Similarly, Rezaeipour and Gazani (2014) found that different feed particle sizes did not affect the breast and thigh relative weights. Also, Massuquetto et al., (2020) reported that the carcass yield, breast, thigh, and drumstick yields were not affected by physical form of diet. Interestingly, present work showed that fine grinded corn grain inclusion in

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broiler diet significantly decreased the abdominal fat pad in comparison to those fed diets containing coarse grinded corn. These results are in contrast to those obtained by Mingbing et al., (2015), where the authors state that the physical from of the diet has significant effects on broilers carcass yield. However, Unni et al. (2014) found that different feed particle sizes can affect the slaughter yield of broiler chickens.

According to the present findings, different CTs of corn grain had no significant impact on carcass characteristics of broiler chickens. However, Rueda et al. (2022) reported that the broiler chickens fed diets conditioned at 82 ºC had heavier body weight compared to those fed diets conditioned at 71 and 77ºC, but similar to broilers fed diet conditioned at 88ºC. Loar et al. (2014) reported that conditioning temperatures of corn grains (74, 85 and 96ºC) and level of fat addition in mixer (1.00 and 2.18%) did not influence carcass traits of broilers. Similarly, Cutlip et al. (2008) reported no differences in breast yield and fat pad of 39d-old broilers fed diets subjected to different conditioning temperatures (82.2 and 93.3 ºC) and steam pressures (20 and 80 psi).

Immune responses

As shown in Table 4, the immune indices in primary and secondary responses against SRBC were increased by conditioning at 75°C at 42d of age. Currently broiler chickens are genetically selected for improved FCR and rapid growth rate. Increased body weight gain has been reported to be negatively correlated with antibody response (total antibody response and SRBC) in broiler chickens. It is well-known that dietary components per se (ingredients, nutrients, and additives) can modulate development and functionality of the gastrointestinal tract including histomorphology, immune and endocrine systems as recently reviewed (Kiarie and Mills ,2019).

In the most simple of descriptions, a chicken can be thought of as a tube with the lumen of the intestinal tract being outside of the chicken. Feed ingested by a bird contains nutrients, nonnutrients, antinutrients, and beneficial and potentially harmful organisms and material. The lumen of the digestive tract of chickens generally contains feed and its constituents, resident and transient microbial populations, endogenous nutrients, and secretions from the gastrointestinal tract (GIT) and accessory organs such as the liver, gall bladder, and pancreas. The primary purpose of the GIT is to digest and absorb nutrients from the ingested feed. The GIT must selectively allow the nutrients to cross the intestinal wall into the bird while preventing the deleterious components of the diet from crossing the intestinal barrier. In addition to simply preventing access to the bird by blocking entrance, immune tissues and cells within the gut actively respond to microbial challenges (Korver, 2006). Gut microflora has been proven to significantly affect boiler nutrition, health, and growth performance (Barrow, 1992) by interacting with nutrient utilization and GIT development in the host (Yang and Choct, 2009). It seems that the use of moderate-temperature processing by eliminating harmful bacteria and removing anti-nutritive factors can be effective in maintaining intestinal microbial balance and preventing dysbiosis, and as a result, it can lead to an increase in the population of beneficial bacteria. And by improving the efficiency of the mucosal immune system, it can be effective in improving the health of the bird's intestines and thus improving the bird's health.

Relative length of the small intestine and jejunum morphology

The results of current study revealed that the duodenum, jejunum and cecum length of birds fed the CT_U-treated corn grains were greater than those fed the CT_{75} and CT_{90}. The influence of particle size on gut development is depended to the effect of structural components (Hetland et al., 2005), where an abundant degree of physical coarseness is required in order to have a discernable positive impact on gut health (Choct, 2009). Yan et al. (2022) reported that coarsely ground corn had significantly positive impacts on gut development. The current study showed that the length of the digestive tract was not influenced by corn PS. However, the CT had a significant effect on the length of the duodenum, jejunum and cecum, so that the chickens that were fed with CT_U diets had the highest length of the duodenum, jejunum and cecum. The average retention time in the digestive tract, excluding the caeca, is probably three to four hours (Svihus 2011). So, it is assumed that the digesta possibly spends only 60 to 90 min in the anterior parts of digestive tract, which gives only a

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limited opportunity for enzyme action (Abdollahi et al., 2019b). It is hypothesized that rapid passage rate reduces the available time for digestion and absorption, while slow passage rate limits the feed intake (Svihus et al., 2002). Several factors are known to affect the passage rate, including the strain of the chicken (Denbow, 2015), age of the bird (Shires et al., 1987), dietary NSP contents (Almirall and Esteve-Garcia, 1994), the fraction of water insoluble NSPs (Hetland and Svihus, 2001), dietary fat level (Sell et al., 1983), and environmental temperature (Denbow, 2000). In general, larger particles retains longer than finer particles in the digestive tract (Nir et al., 1994b; Denbow, 2000). Thus, the proportion of coarse fiber in the gizzard is double that in the feed (Hetland et al., 2005) possibly reflecting selective retention of coarse particles (Hetland et al., 2004; 2005). In fact, not hydro-thermal conditioned diets increased the length of small intestine segments in comparison to those conditioned at 75 and 90 ºC. Also, significant PS×CT interaction was observed, where PS_F-CT_{75} reduced and PS_C-CT_{75} increased cecum length compared to the other experimental groups.

The development of digestive tract in poultry, especially the gizzard, is known to be influenced by feed PS, which is evident in 7d old chicks (Amerah et al., 2007). In current study, the relative weight of the gizzard, liver, proventriculus and pancreas, did not significantly affect by diets containing different PS-treated corn. In the present study, the CT had no significant impact on the gizzard and liver weights. However, the chickens fed with CT_U diets had a higher pancreas weight compared to the other groups (CT_{75} and CT_{90}). Ghobadi and Karimi (2012) evaluated the effects of feed processing (pelleted vs. mash) on broiler chick performance from 1 to 36d of age and reported that feed processing had significant effects on the pancreas weight. Also, orthogonal comparison showed that the relative weight gizzard of birds fed with diets containing hydrothermal processed corn was higher than that of unprocessed. There are conflicting reports comparing the effects of pelleted feeds with unprocessed mash diets on gizzard weight of broilers. Those previous studies showed that pelleting either reduced the weight of the gizzard or had no effect (Kiarie and Mills, 2019).

The current study revealed significant difference in muscle thickness, crypt depth, and villus width of jejunum in broiler chickens fed PS_C-treated corn. However, villus height (VH), VH to crypt depth (CD) ratio, VH to villus width (VW) ratio, and absorption surface area were significantly reduced by feeding coarse particle size, compared to the fine one. The GIT development is very important parameter for modern broiler production because the utilization of nutrients depends on it. Researchers have reported that broilers fed a conditioned diets had lower relative length of the digestive tract segments compared to those fed with mash diet (Abdollahi et al., 2011, 2013). Although several studies showed that birds fed a conditioned corn exhibit a lower GIT segments length, Amerah et al. (2007) and Zang et al. (2009) reported that the VH and CD of jejunum segment were higher in broilers fed a processed diets than those fed an unprocessed mash diet. The VH increases the surface area of the small intestine, which greatly enhanced the absorption of nutrients. The VH increment, increases the total villus absorptive area and result in higher digestive enzyme action and increased transport of nutrients at the villus surface (Naderinejad et al., 2016). Compared to the diet conditioned at 60°C, conditioning at 88°C resulted in a 10.7% reduction in cecal weight. Ceca enlarges as the consequence of increased fermentable material in the diet (Svihus, 2014). As hypothesized by Svihus et al. (2013), viscous digesta in birds offered diets conditioned at 88°C can imped the passage of fermentable material into the ceca resulting in a significant reduction in the relative ceca weight. Feeding diets conditioned at 88°C increased the relative length of duodenum and jejunum by 7.5 and 7.3%, respectively, compared to the diets conditioned at 60°C. In agreement, Abdollahi et al. (2010a) reported a 6.3% longer small intestine in birds fed diets conditioned at 75° and 90°C compared to 60°C. This can be considered as the natural response to reduced availability of nutrients in diets exposed to higher CT (Perera et al., 2021).

Gizzard pH

The gizzard obviously plays a key role in digestive tract of poultry, and thus the issue of diet structure has gained a renewed interest in nutritional effects of diets which stimulate development and function of the gizzard. Due to the close interaction between the secretory proventriculus and the functional gizzard, the efficiency with which pepsin and hydrochloric acid will degrade feed nutrients will be dependent on functionality of the gizzard in terms of contraction intensity and retention time. Also, since the low pH caused by the hydrochloric acid is considered to potentially have a beneficial effect on gut health through the sterilizing properties, functionality of the gizzard may also affect gut health. (Svihus, 2011). The gizzard pH in chickens that were fed with coarse grinded corn was significantly lower than that of chickens fed with fine grinded corn. This can be due to the fact that the coarse particles of corn stimulate the secretion of acid in the gizzard and leads to a lower gizzard pH. This finding is consistent with that of the other studies, where Nir et al. (1994) reported greater gizzard development and lower gizzard pH in seven-day old chicks fed medium or coarse particle size diets compared with those fine particle diets. The pH of gastric juice secreted by the proventriculus has been reported to be around 2. However, the amount, retention time, and chemical characteristics of the feed in the gizzard/proventriculus region will result in a more variable and usually higher pH. In a recent study, pH of gizzard contents from broiler chickens varied between 1.9 to 4.5, with an average value of 3.5 (Svihus, 2011). In this study, the CT did not significantly affect gizzard pH.

Conclusion

The results of the present study confirmed using fine PS of corn improved FCR at starter, finisher and whole period. Also conditioning at 75°C improved weight gain in starter, grower, and finisher periods, while increasing condition temperature had no significant effect on performance compared to the unconditioned diet. However, no significant interaction effect was observed for PS and CT on the performance of broiler chickens. The use of fine PS conditioned at 75°C had remarkable effects on the absorption surface area, the ratio of villi height/crypt depth and villi height. Based on the current data, using fine particle size of corn conditioned at 75°C can improve growth performance, and gastrointestinal tract development of broiler chickens.

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References

Abdollahi, M. R., Ravindran, V., & Svihus, B. (2013). Influence of grain type and feed form on performance, apparent metabolisable energy and ileal digestibility of nitrogen, starch, fat, calcium and phosphorus in broiler starters. *Animal Feed Science and Technology*, *186*(3-4), 193-203.

Abdollahi, M. R., Ravindran, V., Wester, T. J., Ravindran, G., & Thomas, D. V. (2010b). Influence of conditioning temperature on performance, apparent metabolisable energy, ileal digestibility of starch and nitrogen and the quality of pellets, in broiler starters fed maize-and sorghum-based diets. *Animal Feed Science and Technology*, *162*(3-4), 106-115.

Abdollahi, M. R., Ravindran, V., Wester, T. J., Ravindran, G., & Thomas, D. V. (2011). Influence of feed form and conditioning temperature on performance, apparent metabolisable energy and ileal digestibility of starch and nitrogen in broiler starters fed wheat-based diet. *Animal feed science and technology*, 168(1-2), 88-99.

Abdollahi, M. R., Zaefarian, F., & Ravindran, V. (2019b). Maximising the benefits of pelleting diets for modern broilers. *Animal Production Science*, 59(11), 2023-2028.

Abdollahi, M. R., Zaefarian, F., Hunt, H., Anwar, M. N., Thomas, D. G., & Ravindran, V. (2019a). Wheat particle size, insoluble fibre sources and whole wheat feeding influence gizzard musculature and nutrient utilisation to different extents in broiler chickens. *Journal of animal physiology and animal nutrition*, 103(1), 146-161.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 January 2024 doi:10.20944/preprints202401.0841.v1

Abdollahi, M., Ravindran, V., Wester, T., Ravindran, G., & Thomas, D. (2010a). Influence of conditioning temperature on the performance, nutrient utilisation and digestive tract development of broilers fed on maize- and wheat-based diets. *British Poultry Science*, 51(5), 648-657.

Ai, Y., & Jane, J. l. (2016). Macronutrients in corn and human nutrition. Comprehensive Reviews in *Food Science and Food Safety*, 15(3), 581-598.

Almirall, M., & Esteve-Garcia, E. (1994). Rate of passage of barley diets with chromium oxide: Influence of age and poultry strain and effect of β-glucanase supplementation. Poultry Science, 73(9), 1433-1440.

Amerah, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. (2007). Feed particle size: Implications on the digestion and performance of poultry. *World's Poultry Science Journal*, *63*(3), 439-455.

AOAC International. 2006. Official Methods of Analysis of AOAC International. 18th ed. AOAC International,

Aviagen. 2018. Ross 308 Broiler. Performance Objectives. .https://www.winmixsoft.com /files/info /Ross-308-Broiler-PO-2018-EN. pdf. Accessed May 2022.

Baker, S., & Herrman, T. (2002). Evaluating particle size. MF-2051 feed manufacturing. Dept. Grain Sci. Ind., Kansas State Univ., Manhattan.

Barrow, P. A. (1992). Probiotics for chickens. Probiotics: The scientific basis, 255-257.

Chewning, C. G., Stark, C. R., & Brake, J. (2012). Effects of particle size and feed form on broiler performance. *Journal of Applied Poultry Research*, *21*(4), 830-837.

Choct, M. (2009). Managing gut health through nutrition. *British poultry science*, 50(1), 9-15.

Cowieson, A. J. (2005). Factors that affect the nutritional value of maize for broilers. *Animal Feed Science and Technology*, 119(3-4), 293-305.

Creswell, D., & Bedford, M. (2006). High pelleting temperatures reduce broiler performance. In Proceedings of the 18th Australian Poultry Science Symposium, Sydney, New South Wales, Australia, 20-22 February 2006 (pp. 1-6). Poultry Research Foundation.

Cutlip, S. E., Hott, J. M., Buchanan, N. P., Rack, A. L., Latshaw, J. D., & Moritz, J. S. (2008). The effect of steam-conditioning practices on pellet quality and growing broiler nutritional value. *Journal of Applied Poultry Research*, *17*(2), 249-261.

Denbow, D. M. (2015). Gastrointestinal anatomy and physiology. *In Sturkie's avian physiology* (pp. 337-366). Academic Press.

Duke, G. E. (1992). Recent studies on regulation of gastric motility in turkeys. *Poultry Science,* 71(1), 1-8.

Ge, C., Li, J., Duan, H., Yu, J., Yu, Z., Sun, J., & Qin, Y. (2017). Effects of different diet particle size combinations on growth performance of broilers in early and later periods. *Chinese Journal of Animal Nutrition*, *29*(12), 4342-4348.

Ghobadi, Z., & Karimi, A. (2012). Effect of feed processing and enzyme supplementation of wheat-based diets on performance of broiler chicks. *Journal of Applied Animal Research*, 40(3), 260-266.

Goodband, R. D., M. D. Tokach, and J. L. Nelssen. (2002). The effects of diet particle size on animal performance. MF-2050 Feed Manufacturing. *Dept. Grain Sci.* Ind., Kansas State Univ., Manhattan

Hetland, H., & Svihus, B. (2001). Effect of oat hulls on performance, gut capacity and feed passage time in broiler chickens. *British poultry science*, 42(3), 354-361.

Hetland, H., Choct, M., & Svihus, B. (2004). Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poultry Science Journal*, 60(4), 415-422.

Hetland, H., Svihus, B., & Choct, M. (2005). Role of insoluble fiber on gizzard activity in layers. *Journal of Applied Poultry Research*, 14(1), 38-46.

Hetland, H., Svihus, B., & Olaisen, V. (2002). Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broiler chickens. *British poultry science,* 43(3), 416-423.

Kheravii, S. K., Swick, R. A., Choct, M., & Wu, S.-B. (2017). Coarse particle inclusion and lignocellulose-rich fiber addition in feed benefit performance and health of broiler chickens. *Poultry Science*, 96(9), 3272-3281.

Kiarie, E. G., & Mills, A. (2019). Role of feed processing on gut health and function in pigs and poultry: conundrum of optimal particle size and hydrothermal regimens. *Frontiers in Veterinary Science*, *6*, 19.

Koch, K. (1996). Hammermills and rollermills. MF-2048 Feed Manufacturing. Dept. *Grain Sci*. Ind., Kansas State Univ., Manhattan.

Korver, D. R. (2006). Overview of the immune dynamics of the digestive system. *Journal of Applied poultry research*, 15(1), 123-135.

Loar II, R. E., Wamsley, K. G. S., Evans, A., Moritz, J. S., & Corzo, A. (2014). Effects of varying conditioning temperature and mixer-added fat on feed manufacturing efficiency, 28-to 42-day broiler performance, early skeletal effect, and true amino acid digestibility. *Journal of Applied Poultry Research*, *23*(3), 444-455.

Lundblad, K. K., Issa, S., Hancock, J. D., Behnke, K. C., McKinney, L. J., Alavi, S., ... & Sørensen, M. (2011). Effects of steam conditioning at low and high temperature, expander conditioning and extruder processing prior to pelleting on growth performance and nutrient digestibility in nursery pigs and broiler chickens. *Animal Feed Science and Technology*, 169(3-4), 208-217.

Lv, M., Yan, L., Wang, Z., An, S., Wu, M., & Lv, Z. (2015). Effects of feed form and feed particle size on growth performance, carcass characteristics and digestive tract development of broilers. *Animal Nutrition*, *1*(3), 252-256.

Massuquetto, A., Durau, J. F., Barrilli, L. N. E., Dos Santos, R. O. F., Krabbe, E. L., & Maiorka, A. (2020). Thermal processing of corn and physical form of broiler diets. *Poultry Science*, *99*(6), 3188-3195.

Meijer, A., A. Bosman, E. E. van de Kamp, B. Wilbrink, M. D. R. van Beest Holle, and M. Koopmans. 2006. Measurement of antibodies to avian influenza virus A (H7N7) in humans by hemagglutination inhibition test. *J.Virol. Methods* 132:113–120.

Moran Jr, E. (1982). Starch digestion in fowl. *Poultry Science*, 61(7), 1257-1267.

Naderinejad, S., Zaefarian, F., Abdollahi, M. R., Hassanabadi, A., Kermanshahi, H., & Ravindran, V. (2016). Influence of feed form and particle size on performance, nutrient utilisation, and gastrointestinal tract development and morphometry in broiler starters fed maize-based diets. *Animal Feed Science and Technology*, *215*, 92-104.

Nain, S., Renema, R. A., Zuidhof, M. J., & Korver, D. R. (2012). Effect of metabolic efficiency and intestinal morphology on variability in n-3 polyunsaturated fatty acid enrichment of eggs. Poultry science, 91(4), 888-898.

Netto, M. T., Massuquetto, A., Krabbe, E. L., Surek, D., Oliveira, S. G., & Maiorka, A. (2019). Effect of conditioning temperature on pellet quality, diet digestibility, and broiler performance. *Journal of Applied Poultry Research*, *28*(4), 963-973.

Nir, I., Hillel, R., Ptichi, I., & Shefet, G. (1995). Effect of particle size on performance.: 3. Grinding pelleting interactions. *Poultry science*, 74(5), 771-783.

Nir, I., Shefet, G., & Aaroni, Y. (1994). Effect of particle size on performance.: 1. Corn. *Poultry Science*, *73*(1), 45-49.

Perera, W. N. U., Abdollahi, M. R., Zaefarian, F., Wester, T. J., & Ravindran, V. (2021). High steam-conditioning temperature during the pelleting process impairs growth performance and nutrient utilization in broiler starters fed barley-based diets, regardless of carbohydrase supplementation. *Poultry Science*, 100(8), 101166.

Putra, A., Ismail, D., & Lubis, N. (2018). Technology of animal feed processing (fermentation and silage) in bilah hulu village, labuhan batu regency. *Journal of Saintech Transfer*, 1(1), 41-47.

Rezaeipour, V., & Gazani, S. (2014). Effects of feed form and feed particle size with dietary L-threonine supplementation on performance, carcass characteristics and blood biochemical parameters of broiler chickens. *Journal of Animal Science and Technology*, *56*, 1-5.

Ross (2019) 'Ross 308 Broiler: Nutrition Specification, 2019.' (Ross Breeders Ltd: Newbridge, Midlothian, Scotland)

Rueda, M., Rubio, A. A., Starkey, C. W., Mussini, F., & Pacheco, W. J. (2022). Effect of conditioning temperature on pellet quality, performance, nutrient digestibility, and processing yield of broilers. *Journal of Applied Poultry Research*, *31*(2), 100235.

SAS Institute Inc. 2008. Using JMP 9. SAS Institute, Cary, NC

SELL, J. L., EASTWOOD, J.A. and MATEOS, G.G. (1983) Influence of supplemental fat on diet metabolisable energy and ingest a transit time in laying hens. *Nutrition Reports International* 28: 487-495.

Selle, P. H., Liu, S. Y., Cai, J., & Cowieson, A. J. (2013). Steam-pelleting temperatures, grain variety, feed form and protease supplementation of mediumly ground, sorghum-based broiler diets: influences on growth performance, relative gizzard weights, nutrient utilisation, starch and nitrogen digestibility. *Animal Production Science*, *53*(5), 378-387.

Shi, Z. Y., H. C. Deng, M. Wang, Q. S. Guo, J. H. Zheng, J. S. Situ, G. M. Xie, X. Y. Yang, and W. J. Shen. (2017). Effect of corn particle size on performance and gastrointestinal indices of broilers.Chin. *J. Anim. Nutr*. 29:75–79

Silversides, F., & Bedford, M. (1999). Effect of pelleting temperature on the recovery and efficacy of a xylanase enzyme in wheat-based diets. *Poultry Science*, 78(8), 1184-1190.

Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Ijaz, A., Sohail, A., ... & Rehman, H. (2012). Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poultry science, 91(9), 2235-2240.

Svihus, B. (2011). The gizzard: function, influence of diet structure and effects on nutrient availability. *World's Poultry Science Journal* 67, 207–224.

Svihus, B. (2014). Function of the digestive system. *J. Appl. Poul. Res*. 23:306–314

Svihus, B., Choct, M., & Classen, H. L. (2013). Function and nutritional roles of the avian caeca: a review. *World's Poultry Science Journal*, 69(2), 249-264.

Svihus, B., Hetland, H., Choct, M. and Sundby, F. (2002). Passage rate through the anterior digestive tract of broiler chickens fed on diets with ground and whole wheat. *British Poultry Science* 43: 662- 668

Svihus, B., Kløvstad, K., Perez, V., Zimonja, O., Sahlström, S., Schüller, R., Jeksrud, W., & Prestløkken, E. (2004). Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarsenesses by the use of roller mill and hammer mill. *Animal Feed Science and Technology*, 117(3-4), 281-293.

Tsiagbe, V. K., M. E. Cook, A. E. Harper, and M. L. Sunde, 1987. Enhanced immune responses in broiler chicks fed methionine-supplemented diets. *Poultry Sci*. 66:1147–1154.

Unni, D. R., Chacko, B., & Narayanankutty, K. (2014). Effect of feed particle size on slaughter parameters in broiler chicken.

Xu, Y., Lin, Y., Stark, C., Ferket, P., Williams, C., & Brake, J. (2017). Effects of dietary coarsely ground corn and 3 bedding floor types on broiler live performance, litter characteristics, gizzard and proventriculus weight, and nutrient digestibility. *Poultry Science*, 96(7), 2110-2119.

Yan, L., An, S., Lv, Z. Z., Choct, M., Zhou, G. L., Li, Y., ... & Jia, Y. G. (2022). Effects of corn particle size on growth performance, gastrointestinal development, carcass indices and intestinal microbiota of broilers. *Poultry Science*, *101*(12), 102205.

Yang, Y., Iji, P. A., & Choct, M. (2009). Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World's Poultry Science Journal*, 65(1), 97-114.

Zang, J. J., Piao, X. S., Huang, D. S., Wang, J. J., Ma, X., & Ma, Y. X. (2009). Effects of feed particle size and feed form on growth performance, nutrient metabolizability and intestinal morphology in broiler chickens. *Asian-Australasian Journal of Animal Sciences*, *22*(1), 107-112.