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Effects of feed form (pellet or mash), corn particle size, and *Bacillus*-based probiotic supplementation on performance traits and digestive tract health of broiler chickens

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Abstract

A total of 720 Ross 308, male broiler chicks were used to study the effect of feed form, corn particle size, and probiotic supplementation as a completely randomized block design with 2×2×2 factorial arrangement (eight treatments and five replications). The factors included the type of feed (pellet or mash), the average size of corn particles (590 and 1220 µm), and the addition or absence of a probiotic (DIPro) to the diet. At the end of the experiment, relative weights (% of BW) of abdominal fat and gizzard were calculated as a percentage of live weight. Using pellets increased the body weight gain (BWG), feed intake (FI), and improved feed conversion ratio (FCR). Feeding coarse corn particles substantially reduced the body weight gain and increased the feed conversion from 1–10, 25–42, and 1–42 days of age. Feed intake reduced from 1–42 d and 42–25 d by consuming coarse corn particles. The use of probiotics increased feed intake and body weight gain from 25–42 and 1–42 d. The pelleted diet increased gizzard and proventriculus pH, whereas coarse corn particles reduced pH. The pelleted feed decreased the relative weight of the gizzard and increased the abdominal fat percentage. Coarsely ground corn increased the abdominal fat and gizzard percentage. Probiotic supplementation improved feed intake, body weight gain, and feed conversion from 1–42 d. Pelleted feed plus probiotics and a 590-µm particle size produced the best performance in broilers.

Keywords: digestive tract; feed form; growth performance, particle size; probiotics
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Introduction

Over the past years, nutritionists have been seeking the best feed formulation to improve growth performance in livestock and poultry (Chewning *et al*., 2012). In recent years, feed particle size and feed form have been identified as important factors in poultry performance, gut development, and health (Svihus, 2011; Mateos *et al*., 2012). It is believed that a reduction in feed particle size increases the influence of enzymes; pelleting increases the contact surface, but may have a negative effect on the development of the gastrointestinal tract, especially the gizzard (Kheravii *et al*., 2017). The status of the gizzard is important in increasing feed efficiency and improves the health of the gastrointestinal tract. Coarse feed particles lead to a reduction in digesta passage rate, an increase in pancreatic secretions, and thus increase the digestibility of substances, such as nitrogen and phosphorus (Abdollahi *et al*., 2013).

Like particle size, the feed form (mash or pellet) also affects the performance of broiler chickens. Many studies have shown that the use of pelleted feed reduces the development of the

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gastrointestinal tract, including the development of the gizzard, pancreas, and small intestine (Abdollahi *et al*., 2011; Aguzey *et al*., 2018). Various studies have evaluated the effect of pelleting on feed availability, but no positive effect of pelleting on protein availability has been found (Abdollahi *et al*., 2013). Huang *et al*. (2006) studied the effects of feed particle size and feed form (mash and pellet) on the intestinal *Salmonella typhimurium* population. They found that pelleted diets increased the death of *S. typhimurium* in the gizzard and decreased the death of *S. typhimurium* in the caeca. The gizzard weight of birds fed pelleted diets was lower than mash diets, and gizzard pH was higher in pelleted diets. Pelleted diets can increase feed consumption and subsequently cause undeveloped gizzards. Undeveloped gizzards reduce the digestibility of nutrients, increase the pH of gizzard contents, and possibly increase the incidence of necrotic enteritis. Researchers and consumers are focused on poultry rearing without the use of antibiotic growth promoters and are looking for effective and non-antibiotic alternatives to control diseases such as necrotic enteritis. In addition to appropriate feed form and ingredient particle size, other suggested strategies for this improvement in poultry performance include proper vaccination programs, coccidiosis control, use of suitable probiotics, prebiotics, organic acids, plant extracts, or essential oils.

Bacillus-based probiotics are resistant to heat and widely resistant to pelleting temperature or low digestive pH, so that most of the swallowed bacteria remain intact in the small intestine (Lee *et al*., 2010; Harrington *et al*., 2015). These qualitative parameters have increased the tendency to use *Bacillus* species in the poultry industry. Although *Bacillus*-based probiotics are known for their high temperature resistance, there is no consistent information on their ability to survive at conventional pelleting temperatures used in the feed manufacturing industry. The interaction of *Bacillus* supplementation at different temperatures on the performance and immune response of broiler chickens is not known; there is a possibility of physical and chemical changes caused by different thermal conditions (Amerah *et al.,* 2013). Although there are several studies on the effects of feed form and feed particle size on broiler performance, none of them has investigated the use of probiotics in diets of different feed form and particle size.

Material and Methods

To conduct this experiment, 720 male, Ross 308 broilers with initial weights of 45 ± 1.43 g were used in a 2×2×2 factorial arrangement as a completely randomized block design with eight treatments and five replications. The factors studied in this experiment included the feed form (pellet and mash), the type of screen size (3 and 8 mm; the geometric mean of 3-mm screen size was 590 µm and for the 8-mm screen size was 1220 µm) and the addition or absence of a probiotic (250 g DiPro probiotic/ton feed). The probiotic (commercial name, DiPro) was made by Takgene, Iran, and contained equal ratios of *B. subtilis* and *B. licheniformis* (each 6 × 10^{12} cfu/kg). The treatments were:

1- mash feed with 3-m mill screen size, without probiotic
2- mash feed with 3-mm mill screen size, probiotic supplementation
3- mash feed with 8-mm mill screen size, without probiotic
4- mash feed with 8-mm mill screen size, probiotic supplementation
5- pelleted feed with 3-mm mill screen size, without probiotic
6- pelleted feed with 3-mm mill screen size, probiotic supplementation
7- pelleted feed with 8-mm mill screen size, without probiotic
8- pelleted feed with 8-mm mill screen size, probiotic supplementation.

Diets were formulated based on corn and soybean, according to Ross 308 nutritional recommendations (Aviagen, 2014) (Table 1). Performance traits, including BWG, FI, and FCR, were recorded from 1–10, 11–24, 25–42, and 1–42 days of age. Feed intake and feed conversion ratio data were corrected based on mortality and weight. At the end of experiment (42 d of age), one bird was selected randomly from each pen, weighed, and then slaughtered according to the regulations approved by the Animal Protection Committee of the Iranian Institute for Animal Research (04-02-2012; Protocol 10938-5-16-17). Relative weights (% of BW) of bursa, spleen, breast, thighs, and abdominal fat were calculated.

Immediately after slaughter, the digesta contents of the gizzard, proventriculus, and various parts of the intestine were taken and centrifuged at 13000 × *g* at 14.4–15.5 °C for 10 min to homogenize (Boros *et al*., 1998). The pH values of samples were measured by putting the electrode of a digital pH meter (Testo205 model, Germany) into samples for 3 min. After each measurement, the electrode was washed before the next measurement was carried out (Morgan *et al*., 2014).

The data from this experiment were analysed using SAS 9.1 software (2004). Treatment means were compared using Duncan's multiple range test at a significance level of P <0.05.

Table 1 Ingredient composition and nutrient composition of the basal diets (DM basis)

Ingredients %	**Starter (1–10 d)**	**Grower (11–21 d)**	**Finisher (22–42 d)**
Ground corn	47.43	54.27	59.27
Soybean meal (45%)	39.50	32.75	28.16
Soybean oil	1.09	1.00	1.00
Wheat	7.00	7.00	7.00
DL-methionine	0.35	0.27	0.25
L-Lysine hydrochloride	.027	0.20	0.16
L-Threonine	0.14	0.09	0.07
Choline chloride 60%	0.02	0.02	0.02
D-calcium phosphate	1.99	1.57	1.35
Calcium carbonate	1.16	1.12	1.06
Sodium bicarbonate	0.27	0.17	0.22
NaCl	0.22	0.17	0.16
Mineral supplement[1]	0.25	0.23	0.20
Vitamin supplement[2]	0.25	0.23	0.20
Bentonite	-	0.85	0.80
Phytase5000 (BiochemGermany)	0.01	0.01	0.01
Salinomycin	0.05	0.05	0.05
Total	100	100	100
<u>Calculated composition of diets</u>			
Crude protein (%)	22.64	20.00	18.8
Metabolizable energy (kCal/kg)	2877	2936	2995
Calcium (%)	1.03	0.91	0.83
Available phosphorus (%)	0.51	0.44	0.39
Sodium (%)	0.18	0.14	0.14
Crude fibre (%)	3.08	3.84	3.68
Digestible methionine (%)	0.65	0.54	0.50
Digestible methionine + cysteine (%)	0.95	0.82	0.76
Digestible lysine (%)	1.3	0.95	0.98
Digestible threonine (%)	0.89	0.75	0.67

Vitamin and mineral supplements per kilogram of diet provide the following amounts:
[1]Minerals: I, 0.43 mg; Cu, 13.56 mg; Zn, 29.3 mg; Se, 6.57 mg ; Mn, 88.51 mg; Fe, 17.28 mg;
[2]Vitamins: vitamin A, 15600 IU; vitamin D3, 6750 IU; vitamin E, 120IU; vitamin K3, 4.8 mg; vitamin B1, 3.84 mg; vitamin B2, 10.32 mg; vitamin B3, 72 mg; vitamin B5, 20.4 mg; vitamin B6, 6.48 mg; vitamin B12, 0.021 mg; vitamin B9, 2.75 mg; biotin 0.36 mg

Results

In all periods, pelleted feed increased body weight gain compared to mash (P <0.05) (Table 2). In all periods, coarse feed particles reduced body weight gain in comparison to fine particles, except from 11–24 d (P <0.05). Probiotic supplementation increased body weight gain from 25–42 and 1–42 d (P <0.05). There was a triple interaction on body weight gain in all periods (P <0.05). The dual effect of feed form × feed particle size was marked from 1–10 d and feed form × probiotic supplementation from 11–24 d (P <0.05).

In all periods, pelleting increased feed intake compared to mash (P <0.05) (Table 3). Increasing feed particle size reduced feed intake from 25–42 d and 1–42 d (P <0.05). The probiotic supplementation increased feed intake from 1–10, 11–24, 25–42, and 1–42 d (P <0.05). The interaction of feed form × feed particle size on feed intake was considerable from 11–25 d (P <0.05). The interaction of feed form × probiotic supplementation on feed intake was substantial from 1–10, 11–24, and 1–42 d (P <0.05). In all periods, pelleting reduced the feed conversion ratio (P <0.05). In all periods, the coarse feed particles increased the feed conversion ratio (P <0.05). The addition of probiotics to feed caused a reduction in feed conversion ratio, except from 11–24 d (P <0.05). The interaction of feed form × feed particle size on feed conversion ratio was substantial from 1–10 d (P <0.05). The interaction of feed form × probiotic supplementation on feed conversion ratio was substantial from 1–10 and 11–24 d (P <0.05). The interaction of feed particle size × probiotic supplementation on feed conversion ratio was substantial from 1–10 d (P <0.05). The triple interaction on feed conversion ratio was substantial in all periods (P <0.05). From 1–10 d, the interaction of mash feed and 8-mm screen size at both probiotic levels increased feed conversion ratio (P <0.05). From 11–24 and 1–42 d, the interaction of mash feed at 8-mm screen size and without probiotics increased the feed conversion ratio (P <0.05).

Table 2 The main and interaction effects of feed form, feed particle size, and probiotic supplementation on body weight gain in male Ross 308 broiler chicks from 1–42 d

1–42 d	25–42 d	11–24 d	1–10 d	Probiotic	Screen size	Feed form
2901.0^{b}	1756.6^{b}	892.9^{ab}	251.5^{a}	-	3 mm	pellet
3036.2^{a}	1862.8^{a}	929.4^{a}	244.0^{bc}	+	3 mm	
2734.3^{bc}	1655.9^{c}	843.4^{b}	235.0^{cd}	-	8 mm	
2915.1^{b}	1762.8^{b}	906/7^{ab}	245.6^{ab}	+	8 mm	
2543.5^{cd}	1564.7^{d}	753.5^{c}	225.3^{de}	-	3 mm	
2588.8^{c}	1600.6^{cd}	740.5^{cb}	247.7^{ab}	+	3 mm	mash
2436.1^{e}	1457.3^{e}	768.1^{c}	210.7^{ef}	-	8 mm	
2495.1^{de}	1570.8^{d}	727.8^{d}	196.5^{f}	+	8 mm	
34.62	26.62	16.48	2.81			SEM
						Main effect
						Feed form
2899.8^{a}	1759.5^{a}	893.1^{a}	244.7^{a}			pellet
2520.4^{b}	1548.3^{b}	747.5^{b}	220.1^{b}			mash
17.31	13.31	8.24	1.403			SEM
						Screen size
2768.3^{a}	1696.2^{a}	829.1	242.9^{a}			3 mm
2651.9^{b}	1611.7^{b}	811.5	221.9^{b}			8 mm
17.31	13.31	8.24	1.403			SEM
						probiotic supplementation
2652.3^{b}	1608.6^{b}	814.5	230.6			-
2767.9^{a}	1699.3^{a}	826.1	233.5			+
17.31	13.31	8.24	1.403			SEM
						P value
0.0001	0.0001	0.0001	0.0001			Feed form
0.0005	0.0002	0.230	0.0001			Screen size
0.002	0.0001	0.473	0.252			Probiotic level
0.960	0.452	0.0804	0.009			Feed form×Screen size
0.418	0.675	0.0017	0.0188			Feed form× Probiotic
0.759	0.1005	0.767	0.690			Probiotic ×Screen size
0.0001	0.0001	0.0001	0.0001			Feed form×Screen size× Probiotic

^{a,b,c,d} Means within a column with no common superscript differ significantly (P <0.05)

Table 3 The main and interaction effects of feed form, feed particle size, and probiotic supplementation on feed intake in Ross 308 male broiler chicks from 1 to 42 days

1–42 d	25–42 d	11–24 d	1–10 d	Probiotic	Screen size	Feed form
4824.9^{a}	3240.4^{a}	1291.9^{a}	292.6^{a}	-	3 mm	pellet
4877.0^{a}	3310.0^{a}	1275.3^{a}	291.7^{a}	+	3 mm	
4546.1^{b}	3043.5^{b}	12.17.4^{b}	285.2^{a}	-	8 mm	
4819.8^{a}	3237.5^{a}	1293.5^{a}	288.8^{a}	+	8 mm	
4243.1^{c}	2853.7^{c}	1118.2^{d}	271.3^{b}	-	3 mm	mash
4320.8^{c}	2926.7^{bc}	1140/4^{cd}	253.7^{c}	+	3 mm	
4294.1^{c}	2857.5^{c}	1166.6^{c}	270.0^{b}	-	8 mm	
2469.1^{c}	2891.5^{bc}	1119.7^{d}	258.0^{c}	+	8 mm	
45.21	35.48	16.15	3.19			SEM
						Main effect
						Feed form
4767.4^{a}	3207.8^{a}	1269.5^{a}	289.6^{a}			pellet
4283.7^{b}	2882.3^{b}	1136.2^{b}	263.3^{b}			mash
22.61	17.74	8.074	1.60			SEM
						Screen size
4561.3^{a}	3082.7^{a}	1206.4	277.3			3 mm
4489.8^{b}	3007.5^{b}	1199.3	275.5			8 mm
22.61	17.74	8.074	1.60			SEM
						probiotic supplementation
4476.9^{b}	2998.8^{b}	1198.5	279.8^{a}			-
4574.2^{a}	3091.4^{a}	1207.2	273.1^{b}			+
22.61	17.74	8.074	1.60			SEM
						P value
0.0001	0.0001	0.0001	0.0001			Feed form
0.137	0.0168	0.863	0.683			Screen size
0.0366	0.0032	0.769	0.0387			Probiotic level
0.0359	0.19	0.0438	0.298			Feed form×Screen size
0.0480	0.674	0.0437	0.0058			Feed form× Probiotic
0.0780	0.599	0.384	0.316			Probiotic ×Screen size
0.0001	0.219	0.0001	0.0001			Feed form×Screen size× Probiotic

^{a,b,c,d} Means within a column with no common superscript differ significantly (P <0.05)

Table 4 The main and interaction effects of feed form, feed particle size, and probiotic supplementation on feed conversion ratio in Ross 308 male broiler chicks from 1 to 42 days

1–42 d	25–42 d	11–24 d	1–10 d	Probiotic	Screen size	Feed form
1.66^{c}	1.85^{b}	1.45^{bc}	1.16^{c}	-	3 mm	pellet
1.61^{d}	1.78^{b}	1.37^{d}	1.18^{bc}	+	3 mm	
1.66^{c}	1.84^{b}	1.45^{bc}	1.21^{b}	-	8 mm	
1.65^{c}	1.84^{b}	1.43^{cd}	1.19^{bc}	+	8 mm	
1.67^{bc}	1.83^{b}	1.49^{ab}	1.20^{bc}	-	3 mm	mash
1.67^{c}	1.83^{b}	1.54^{a}	1.02^{d}	+	3 mm	
1.76^{a}	1.96^{a}	1.52^{a}	1.28^{a}	-	8 mm	
1.71^{b}	1.84^{b}	1.54^{a}	1.31^{a}	+	8 mm	
0.018	0.03	0.021	0.014			SEM
						Main effect
						Feed form
1.65^{b}	1.83^{b}	1.42^{b}	1.19^{b}			pellet
1.70^{a}	1.86^{a}	1.52^{a}	1.20^{a}			mash
0.0089	0.015	0.0107	0.0072			SEM
						Screen size
1.65^{b}	1.82^{b}	1.46	1.14^{b}			3 mm
1.70^{a}	1.87^{a}	1.48	1.25^{a}			8 mm
0.0089	0.015	0.0107	0.0072			SEM
						probiotic supplementation
1.69^{a}	1.87^{a}	1.48	1.22^{a}			-
1.66^{b}	1.82^{b}	1.47	1.18^{b}			+
0.0089	0.015	0.0107	0.0072			SEM
						P value
0.0001	0.0305	0.0001	0.0013			Feed form
0.0013	0.0174	0.114	0.0001			Screen size
0.0349	0.0086	0.6600	0.0212			Probiotic level
0.0792	0.148	0.660	0.0001			Feed form×Screen size
0.754	0.380	0.0042	0.0214			Feed form× Probiotic
0.875	0.226	0.149	0.0709			Probiotic ×Screen size
0.0001	0.0123	0.0001	0.0001			Feed form×Screen size× Probiotic

[a,b,c,d] Means within a column with no common superscript differ significantly (P <0.05)

Table 5 shows the main and interaction effects of feed form, feed particle size, and probiotic supplementation on the pH of different parts of gastrointestinal tract at the end of experiment. Pelleting the diet caused an increase in gizzard pH (P <0.05) but did not affect the pH of other parts of the gastrointestinal tract (P >0.05). The addition of probiotics did not affect the pH of different parts of the gastrointestinal tract (P >0.05). The interaction between screen size and probiotic level and a triple effect on gizzard pH was important (P <0.05). Triple interaction effects on the pH of gizzard and proventriculus were substantial (P <0.05). The use of mash feed ground with 8-mm screen size, with and without probiotic, reduced gizzard pH compared to other feeds, except the pelleted feed with ground corn of 8-mm screen size, with and without probiotic supplementation (P <0.05).

Table 5 The main and interaction effects of feed form, feed particle size, and probiotic supplementation on the pH of different parts of the gastrointestinal tract in Ross 308 male broiler chicks at 42 days

Ileum pH	Jejunum pH	Duodenum pH	Proventriculus pH	Gizzard pH	Probiotic	Screen size	Feed form
6.32	6.11	6.20	4.01^{ab}	3.67^{a}	-	3 mm	pellet
6.21	6.24	6.15	4.02^{ab}	3.40^{a}	+	3 mm	
6.48	6.19	6.26	3.63^{bc}	2.75^{cd}	-	8 mm	
6.47	6.19	6.15	3.42^{c}	2.77^{bcd}	+	8 mm	
6.62	6.11	6.36	4.30^{a}	3.25^{abc}	-	3 mm	mash
6.49	6.02	6.16	3.69^{c}	3.28^{ab}	+	3 mm	
6.34	6.19	6.35	3.39^{c}	2.23^{d}	-	8 mm	
6.45	6.05	6.20	4.41^{a}	2.73^{d}	+	8 mm	
0.149	0.097	0.064	0.147	0.176			SEM
							Main effect
							Feed form
6.40	6.18	6.19	3.77	3.15^{a}			pellet
6.47	6.09	6.27	3.95	2.87^{b}			mash
0.074	0.048	0.032	0.074	0.088			SEM
							Screen size
6.43	6.12	6.22	4.01^{a}	3.40^{a}			3 mm
6.43	6.15	6.24	3.71^{b}	2.62^{b}			8 mm
0.074	0.048	0.032	0.074	0.088			SEM
							probiotic supplementation
6.47	6.15	6.29	3.83	2.98			-
6.40	6.12	6.16	3.88	3.04			+
0.074	0.048	0.032	0.074	0.088			SEM
							P value
0.587	0.152	0.157	0.1008	0.0322			Feed form
0.711	0.8001	0.2903	0.0092	0.0001			Screen size
0.929	0.923	0.0085	0.629	0.6004			Probiotic level
0.132	0.588	0.836	0.068	0.975			Feed form×Screen size
0.751	0.413	0.119	0.159	0.131			Feed form×Probiotic
0.349	0.609	0.869	0.0022	0.1404			Probiotic ×Screen size
0.0367	0.893	0.729	0.0001	0.0001			Feed form×Screen size×Probiotic

^{a,b,c,d} Means within a column with no common superscript differ significantly (*P* <0.05)

Using pelleted feed compared to mash caused to a reduction in the percentage of gizzard and an increase in the percentage of abdominal fat (*P* <0.05). The coarse feed particles increased gizzard and abdominal fat percentage (*P* <0.05). However, probiotic supplementation did not have any effect on these parameters (*P* >0.05).

Table 6 The main and interaction effects of feed form, feed particle size, and probiotic supplementation on gizzard and abdominal fat percentage in Ross 308 male broiler chicks at 42 days

Gizzard	Abdominal fat	Probiotic	Screen size	Feed form
0.89[c]	1.06[b]	-	3 mm	pellet
0.96[c]	1.07[b]	+	3 mm	
1.16[abc]	1.73[a]	-	8 mm	
1.40[ab]	1.31[ab]	+	8 mm	
1.26[abc]	0.84[b]	-	3 mm	
1.05[bc]	0.97[b]	+	3 mm	Mash
1.58[a]	1.53[a]	-	8 mm	
1.60[a]	1.39[ab]	+	8 mm	
0.098	0.14			SEM
				Main effect
				Feed form
1.10[b]	1.30[a]			Pellet
1.37[a]	1.13[b]			Mash
0.049	0.071			SEM
				Screen size
1.04[b]	0.99[b]			3 mm
1.44[a]	1.44[a]			8 mm
0.049	0.071			SEM
				probiotic supplementation
1.22	1.29			-
1.25	1.13			+
0.049	0.071			SEM
				P value
0.0005	0.0307			Feed form
0.0001	0.0001			Screen size
0.726	0.86			Probiotic level
0.625	0.549			Feed form×Screen size
0.101	0.389			Feed form× Probiotic
0.131	0.0434			Probiotic ×Screen size
0.0001	0.0017			Feed form×Screen size× Probiotic

[a,b,c,d] Means within a column with no common superscript differ significantly (P <0.05)

Discussion

Birds fed pelleted feed compared to mash had higher average weight, body weight gain, feed intake, and a better feed conversion ratio in different periods. In agreement with these findings, Amerah & Ravindran (2008) reported that birds fed pelleted diets had better feed conversion than those fed mash diets. Rezaeipour & Gazani (2014) also reported that feed form had an effect on broiler feed intake and body weight in all experimental periods. Several studies investigated the positive effects of pelleted feeds on performance. Using pelleted feeds can increase the feed intake and body weight gain in broiler chicks (Aguzey *et al*., 2018). Abdollahi *et al*. (2011) reported that the use of pellets in the starter period (1–21 days) increased the feed intake of broiler chickens by 14%. The improvement in feed conversion ratio as a result of pelleted feed is possibly due to decreasing feed wastage (Jensen, 2000). When using pellets, broiler chickens spend less time consuming feed, which reduces the maintenance energy requirement (Abdollahi *et al*., 2018; Aguzey *et al*., 2018). The effect of using pellets on increasing the net energy of the diet is probably due to this. Zang *et al*. (2009) showed that pelleted diets increased daily body weight gain, feed intake, and improved feed efficiency during the starter, grower, and total phases. In their study, regardless of the age, pelleted diets improved the apparent metabolizable energy and apparent crude protein and organic matter digestibility. In the pelleting process, the particle size of the feed becomes smaller and more uniform as it passes through the steam and then undergoes pressure in the die holes (Svihus *et al*., 2004; Abdollahi *et al*., 2018; Aguzey *et al*., 2018). Some studies have found that particle size in pelleted diets has no effect on the performance of broiler chickens, which is related to the uniformity of feed particles during pelleting process (Svihus *et al*., 2004; Peron *et al*., 2005). In agreement with the results of the current study, Benedetti *et al*. (2011) reported that the pelleted diets could increase feed intake and body weight gain in broiler chickens. In the current study, the birds fed diets containing coarse particles (1220 µm geometric mean diameter) compared to diets containing finely ground corn (590 µm geometric mean diameter) had lower body weight gain from 1–10 and 25–42 days of age, whereas feed intake was reduced using coarse corn particles from 25–42 days of age. Coarse corn particles increased the feed conversion ratio from 1–10 and 25–42 d, which is consistent with Lott *et al*. (1992). They stated that increasing the geometric mean of feed particles from 716 to 1196 µm in crumble diets led to a reduction in daily body weight gain and an increase in feed conversion ratio. In agreement with the present study, Chuiung *et al*. (2012) showed that broiler chickens fed a diet with a mean particle size of 300 µm had higher daily weights and better feed conversion than those fed a diet with a particle size of 600 µm. Singh *et al*. (2014) observed that increasing the pre-pelleting inclusion of whole corn in diets caused a decrease in body weight gain.

Reducing the particle size can increase the effect of enzymes on their substrates (Amerah & Ravindran, 2008; Goodband *et al*., 2002); performance reduction by increasing the geometric mean of feed particles can be due to the slower effects of enzymes on these particles. According to the results in the current study, the average body weight gain decreased by consuming coarse particles from 1–11 d but no difference was observed from 11–24 d; an increase in body weight gain was observed from 25–42 d. This difference can be attributed to the effect of fine particles on increasing starch digestibility and metabolizable energy (Peron *et al*., 2005). As more energy is needed due to increased maintenance requirements in the final days, increasing the dietary energy by using fine particles can reduce the use of dietary amino acids to provide energy. If the dietary energy is high in the final phase, this can increase body weight gain. In this regard, Kilburn & Edwards (2001) reported that finely ground corn particles increased the metabolizable energy of diets. Zang *et al*. (2009) reported that reducing the feed particle size increased the metabolizable energy from 19–21 d. They stated that a combination of reducing feed particle size and gelatinization of starch could improve enzymatic digestion, and as a result, they observed improved apparent metabolizable energy, crude protein, and organic matter in their study.

In another study, increasing the size of corn particles (781, 950, 1042, and 2242 µm) improved performance and nitrogen retention in growing chickens (Parsons *et al*., 2006). However, when the particles were larger than 1042 µm, the apparent metabolizable energy was negatively affected. The researchers concluded that coarse particles could increase gizzard size; increasing the energy requirements for gizzard maintenance can affect broiler performance negatively. This justifies the loss in growth improvement using coarse particles, despite the increase in gizzard size, in the current study. However, if corn particles are very fine or very coarse, the use of nutrients by birds may be reduced (Benedetti *et al*., 2011). In contrast to our study, Benedetti *et al*. (2011) showed that increasing the geometric mean of the particle diameter from 460–870 µm resulted in an increase in the feed conversion ratio and a decrease in body weight gain from 1–21 d. Dozier *et al*. (2006, 2009) investigated the effects of increasing the size of corn particles (1500 GMD) from 80 to 235 g/kg from 18–41 d and 2691 GML µm from 150 to 280 g/kg from 18–56 d and observed that body weight gain, feed intake, and feed conversion ratio were not affected. It seems that the combination of diet, the type of grain (Kilburn & Edwards, 2004), the feed form (Mirghelenj & Golian, 2009), the bird's age, the availability of bedding material, the type of feed, type of birds (Mtei *et al*., 2019), and further processing of the feed (pelleting or grinding) (Goodblad *et al*., 2002) determine the effect of the particle size of the diet on growth performance. Some researchers suggest that to achieve good feed quality for broiler chickens, 650–700 µm corn particles are required (Dozier, 2003). Oppong-Sekyere *et al*. (2012) showed that increasing the geometric diameter of the feed particles from 713–1462 µm resulted in an increase in feed conversion ratio and a decrease in body weight gain and final body weight.

Based on our results, the effect of using a probiotic reduced feed intake and increased body weight gain, which is consistent with the study of Apata (2008). This has been reported to be due to the positive effects of using probiotics on the intestinal morphology (Chichlowski *et al*., 2007). Competitive exclusion is another mechanism that can be mentioned for the positive effect of probiotics on body weight gain. The harmful microorganisms compete with intestinal villi to absorb nutrients from the intestines, and since probiotics control the population of harmful bacteria, they can increase the nutrient utilization of birds (Ng *et al*., 2009). Although the exact mode of action for the effects of probiotics is still unknown, some mechanisms, such as the production of antibacterial agents (bacteriocins and bacteria inhibitors), the secretion of non-specific antimicrobials, the induction of antimicrobial compound production (diphenin) by the host, direct enzymatic activities of probiotics in the intestinal lumen, the reduction of luminal pH, inhibition of bacterial adhesion, competition for nutrients, and immunity protection have been proposed (Fooks & Gibson, 2002; Gillor *et al*., 2008). There is increasing evidence that the same effects may be obtained with dead bacteria or inseparable components of a bacterial cell, such as peptidoglycans or DNA fragments (Oelschlaeger, 2010). In the starter phase, birds fed diets containing probiotics had a lower body weight gain than those fed diets without the probiotic. This result may be due to effects of probiotics on bile salt deconjugation and a reduction in fat digestion in starter diets because of the low production of bile in young birds (Lin, 2014).

In the current study, the coarse feed particles decreased the gizzard and proventriculus pH compared to fine particles. In agreement with the present study, other researchers have shown that the use of coarse feed particles reduces the pH of the gizzard (Engberg *et al*., 2002; Naderinejad *et al*., 2016) and proventriculus (Engberg *et al*., 2002). Naderinejad *et al*. (2016) also showed that finely-ground ingredients in pelleted diets increased the pH of the gizzard contents. In a study by Engberg *et al*. (2004), the addition of whole wheat increased gizzard weight and decreased the pH of the gizzard contents.

It has been reported that the consumption of coarse particles by broilers can cause gizzard development. The gizzard development can result in longer retention times and decreasing gizzard and proventriculus pH. Improvements in the size of the gizzard and a marked reduction in pH did not improve the performance traits in the current trial, and are acceptable when not affecting feed intake. Using coarse corn particles (1145 µm) compared to fine particles (271 µm) caused an improvement in feed conversion ratio, feed intake, body weight gain, and gizzard size and a reduction in gizzard pH (Xu *et al*., 2017). Parsons *et al*. (2006) reported that the inclusion of coarsely ground corn in the feed improved the nitrogen and lysine retention in relation to the reduction in gizzard pH. In the current study, the reduction in gizzard pH had no impact on performance. This could be because the pancreas produces a large amount of bicarbonate and alkaline materials to alkalize the intestinal environment and creates an optimal environment for enzyme activity, resulting in an electrolyte imbalance in the blood. The production of hydrochloric acid in the proventriculus requires a large amount of Cl^-, which can also contribute to the electrolyte imbalance in the blood and a loss in performance. More coarse particles substantially increase the gizzard percentage and decreased gizzard pH, which is consistent with other researchers (Abdollahi & Ravindran, 2013). The feed particles do not exit the gizzard until reaching a certain size, thus a longer retention time of coarse particles can stimulate proventriculus acid secretion, which in turn can make the gizzard more acidic in these birds (Nir *et al*., 1994; Svihus *et al*., 2004). They also found that the physical properties of the feed affected the intestinal pH, *Enterobacter* lactose, and *Clostridium perfringens*.

In the present study, the gizzard percentage was reduced by pelleting the diet; this concurs with Huang *et al*. (2006) and Rezaeipour & Gazani (2014), who reported that the pelleting process reduced gizzard and proventriculus weight. Hamedani *et al*. (2015) found that the use of pelleted feed compared to mash feed reduced the size of gizzard. In this regard, we can say that the main cause of the increase in weight and size of the gizzard is the increase in contractions of the gizzard (Röhe *et al*., 2014). According to Svihus (2004), pelleting can reduce particle size; small particle sizes are retained less in the gizzard, resulting in an under-developed gizzard.

In the present study, the use of pelleted feed increased the percentage of abdominal fat, which is consistent with Salari *et al*. (2006). According to Massuquetto *et al*. (2019), pelleted diets can increase feed intake, and subsequently, abdominal fat in poultry. Increasing the fat percentage is probably due to the increased metabolizable energy supply as a result of the pelleting process. Studies have been conducted to improve the use of dietary energy during pelleting (Yang *et al*., 2010) and this excess energy is probably stored as fat. The effect of increasing the corn particle size on increasing the percentage of abdominal fat in the present study concurs with the results of other researchers (Jones and Taylor, 2001; Nahas and Lefrancois, 2001; Parsons *et al*., 2006). However, in contrast to this study, Wu & Ravindran (2004) reported that a coarse size had no effect on abdominal fat; Amerah & Ravindran (2008) observed a reduction in abdominal fat percentage. These differences may be due to the level of dietary energy provided. The effect of increasing particle size was shown to increase the gizzard percentage in the present study, which is consistent with other studies (Svihus *et al*., 2010). Parsons *et al*. (2006) evaluated the effects of fine (781 µm), small (950 µm), medium (1042 µm), and large (1109 µm) corn particles on the performance of broiler chickens from 3–6 weeks of age. They found that feeding larger corn particles substantially reduced the feed efficiency compared to other sizes. By increasing corn particle size, the weight of gizzard (as a percentage of body weight) was increased. The consumption of coarse feed particles, in addition to gizzard weight, also increased pancreas weight, which indicates an increase in the activity and secretion of the enzymes by the pancreas (Williams *et al*., 2008).

Conclusions

The particle size and feed form are very important in the production process. According to the results of this experiment, the use of probiotics in feed has a positive effect on bird performance. Although the coarse feed particles reduced the pH and increased the gizzard size, it is thought that feed intake was reduced due to a decrease in the passage rate of feed from the gastrointestinal tract, leading to a reduction in performance traits. In this experiment, although the gastrointestinal condition in bords fed a particle size of 1220 µm was better than that at 590 µm, the reduced feed intake in this treatment caused the best performance of the birds to be achieved by using 590 µm particle size. Considering that the use of pelleted feed resulted in increased carcass fat, a reduction in the maintenance requirement of bird can be expected, which concurs with the presented references. The energy level in pelleted feed can therefore be reduced compared to mash feed.

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