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Effect of mash and pellet diets containing different sources of fiber on the growth performance and cecal microbial population of broiler chickens

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Abstract The effects of different sources of fiber in mash or pellet diets on growth performance and cecal microbial population of broiler chickens were studied for 42 days. The experimental design was completely randomized with 10 treatments arranged as a 2 × 5 factorial with 2 feed forms (mash vs. pelleted) and 5 diets consisted of 4 feeds containing 4 different fiber sources (i.e., sugar-beet pulp (SBP), wheat bran (WB), sunflower hull (SFH), all of which in 3% of diet and cellulose (CEL) in 0.5% of diet) and a control diet. The results showed that in the starter phase, all diets in pellet form resulted in a higher BWG of birds compared to those fed mashed forms of fiber sources and control diet (P<0.05). Also, feed conversion ratio (FCR) was improved in birds offered diets containing fiber, except for SBP, in the pelleted form, compared to the birds fed fiber in mashed forms, in the starter phase (P<0.05). Birds fed pelleted diets containing WB and SFH had lower (P<0.05) *Coliforms* and *E. coli* populations in the cecum, compared to the other treatments. Dietary inclusion of SFH and CEL in the pellet form reduced blood triglycerides. In summary, pelleting the diets containing WB, SFH, and CEL was more beneficial for improving FCR than the inclusion of SBP at starter phase. Also, the results suggested that the inclusion of 3% of natural fibers or 0.5% of CEL in the diets, based on corn-soybean meal, can improve the growth performance, with effects being more pronounced in pellet-fed birds than in mash-fed ones.

Keywords: broiler, feed form, sugar beet pulp, sunflower hull, wheat bran

Introduction

The broiler feed is commonly in the form of either crumble or pellet. The advantages of pelleting include reduction of feed wastage (Serrano et al., 2013; Jim´enez-Moreno et al., 2016) and increase in feed intake (FI) of the bird (Serrano - et al., 2012; Jim´enez- Moreno et al., 2016). As a result, p-elleting can improve the growth rate and feed efficiency (B-rickett et al., 2007). Part of this improvement could be rela-ted to the steam and pressure applied during the pelleting - which can gelatinize some portions of the starch and can facilitate nutrient utilization (Abdollahi et al., 2010, 2013). On the contrary, there is also evidence of little benefits and even reduced digestibility of certain nutrients including - starch, when the feed is pelleted (Abdollahi et al., 2011; Serrano et al., 2013). Also, it has been shown that inso-luble dietary fiber (DF) increases the weight and size of gizzard (Gonz´alez-Alvarado et al., 2008), nutrient dige - stibility (Gonz´alez-Alvarado et al., 2010; Jim´enez-Mor eno et al., 2010), and gastrointestinal tract (GIT) health (Jim´enez-Moreno et al., 2011; Kalmendal et al., 2011). Strong muscular contractions of a well- developed gizz-ard ensure complete grinding of the feed and help regu-gulate flow of the digesta from the gizzard to the small -

intestine. This facilitates the mixing of chime and gastric juices, and may prevent attachment of the pathogenic bacteria to the small intestinal mucosa, and thus, reduces the risk of enteric disorders (Bjerrum et al., 2005). The extent of these benefits varies depending on different factors, including the physico-chemical characteristics and the level of inclusion of the fiber source (Gonz´alez-Alvarado et al., 2007; Jim´enez-Moreno et al., 2013). Additionally, the effects of purified dietary fiber on intestinal structure and mucus properties still need to be evaluated. According to JRS (J. Rettenmaier and Sohne, Rosenberg, Germany), the lignocellulose Arbocel is used as a source of insoluble dietary fiber. To make Arbocel, natural plant fibers need to undergo various processes, such as micronization and detoxification, to eliminate factors interfering with nutrition, including high soluble fiber content, mycotoxin contamination and intestinal lumen erosion (Knudsen et al., 2008). JRS specifies that this product has an extremely high dietary fiber content of 970.0 g/kg DM, consisting of high levels of crude fiber (approximately 750.0 g/kg) and moderate levels of lignin (approximately 250.0 g/kg), and therefore, is insoluble. It has a high swelling and water-binding capacity. To our knowledge, there is no investigation on the comparison between purified and natural fibers and their effects on GIT development and performance in broilers fed mash or pelleted diets. We hypothesized that the benefits of insoluble fiber, compared to soluble fiber, on GIT development and performance could be more pronounced in the pelleted than in mash diets. Here, we aimed to evaluate the effects of different sources of fiber [sugar beet pulp (SBP), wheat bran (WB), sunflower hulls (SFH), and cellulose (CEL)] on performance, and cecal microbial population in broilers fed diet either in mash or pellet forms for 42 days.

Materials and methods

Sunflower hull (SFH), sugar beet pulp (SBP) and wheat bran (WB) were obtained from Isfahan sunflower seed processing (Iran), Khuzestan sugarcane industry (Iran) and Khuzestan flour factory (Iran), respectively. The chemical composition of fibers was analyzed according to AOAC (2000) methods (Table 1). Crude fiber (CF) was measured by sequential extraction with dilute acid and alkali (Method 978.10; AOAC, 2000). Dry matter (DM) and crude protein (CP) were determined using Methods 930.15 and 990.03, respectively (AOAC, 2000). Crude fat was analyzed by *Soxhlet* after acid hydrolysis (Method 954.02; AOAC, 2000). Neutral detergent fiber (NDF), and acid detergent fiber (ADF) were determined sequentially as described by Van Soest *et al*. (1991), and expressed on an ash-free basis. The moisture and ash contents were determined based on the methods reported by Debon and Tester (2001). For determination of moisture content, samples (10 g) were placed in a petri-dish and dried in a previously heated laboratory oven at 105 ºC to a constant weight. Ash content was determined by weighing 5 g of the ground sample into porcelain crucible in triplicates and decarbonized in a Bunsen burner for 4 hours at 550 ºC.

Table 1. Chemical composition of dietary fiber sources (%)

Items	SBP[1]	WB[2]	SFH[3]	CEL[4]
Dry matter	94.48	93.43	96.34	92.30
Ash	6.85	6.08	3.66	0.50
Crude protein	10.40	17.30	6.86	1.00
Ether extract	2.00	5.00	5.00	0.30
Crude fiber	9.00	12.00	45.00	70.00
Acid detergent fiber	25.00	16.54	51.17	46.00
Neutral detergent fiber	42.00	40.00	73.22	78.00
Nitrogen free extract[5]	66.23	53.05	35.82	20.50
None fiber carbohydrates[6]	33.23	25.05	7.60	-
Starch[7]	00.0	19.80	00.0	0.00
Total dietary fiber[8]	64.70	43.40	80.10	70.20
Insoluble dietary fiber[8]	54.10	36.10	70.00	69.86
Soluble dietary fiber[8]	10.60	7.30	10.10	0.34
AME_n (MJ/kg)[9]	3.76	3.51	1.42	-

[1]Sugar beet pulp, [2]Wheat bran, [3]Sunflower hull, [4]cellulose (Data are reported by J. RETTENMAIER and SOHNE, Rosenberg, Germany), [5]Nitrogen free extract (NFE) = 100 - (CP + Ash + CF + EE), [6]None fiber carbohydrate (NFC) = 100 - (CP + Ash + EE + NDF), [7] Data from Ponter (2004), [8]Data from Bach Knudsen (2014), and Kheravii et al. (2017a), and Guzman et al. (2015), [9]Calculated values (Janssen, 1989).

Experimental design and treatments

All procedures were approved by the Animal Ethics Committee at Agricultural Sciences and Natural Resources University of Khuzestan, Ahvaz, Iran. A total of 400 one-day-old male broiler chicks (Ross 308) (initial BW of 41.4±3.1 g) were obtained from a commercial hatchery, weighed on arrival, and randomly allotted to 10 treatments and 4 replicates (10 chicks) per treatment. The experiment was conducted as a completely randomized design with 10 treatments arranged as a 2 × 5 factorial with 2 feed forms (mash vs. pellet) and 5 diets. Diets consisted of a control diet and 4 extra feeds that resulted from the combination of 4 fiber sources (SBP, WB, SFH, in 3% of diet), and (CEL in 0.5% of diet, Arbocel RC Fine, JRS Co. Inc., Rosenberg, Germany). The diets were manufactured by diluting (wt: wt) the basal diet with 3% SBP, WB, SFH, or 0.5% CEL, respectively. The company recommends that Arbocel® RC Fine (CEL) should be incorporated in broiler diets at a rate of 0.5% of diet. The commercial batches of the fiber sources were first ground with a hammer mill provided with a 2-mm screen and then were added to the experimental diets. The resulting batches of these 5 diets were divided into two equal portions; the first portion was fed as such, whereas, the second portion was steam conditioned first and then passed through a pellet press equipped with a 2-mm die, and an effective thickness of 35 mm for starter period (1-21d) and 4-mm die and an effective thickness of 60 mm for grower period (22-42 d). The temperature of the feed at the exit of the pellet press was 79 ± 3 °C.

The feeding regimen consisted of a starter and grower diet. The basal diet was formulated to meet the nutrient requirements of broilers according to NRC (1994). The ingredients and chemical composition of the diets are shown in Tables 2, 3, 4, and 5. The chickens were reared for 42 d on cemented floor pens with chaff as the bedding material. Each pen contained one suspended drinker and one feeder. Feed and water were provided ad libitum throughout the trial. Lighting consisted of a period of 23 h light and 1 h darkness. The ambient temperature was set at 32 ˚C and subsequently was reduced by 2 ˚C/wk.

Table 2. Starter feed ingredients (d 1-21) (%, unless stated otherwise; as fed basis)

		Fiber source			
Ingredients	Control	Sunflower hulls	Sugar beet pulp	Wheat bran	Cellulose
Corn	54.34	52.69	52.69	52.69	54.04
Soybean meal (44% cp)	33.80	32.79	32.79	32.79	33.63
Gluten meal (60% cp)	3.57	3.46	3.46	3.46	3.55
Vegetable oil	4.00	3.88	3.88	3.88	3.98
Sunflower hulls	-	3.00	-	-	-
Sugarbeet pulp	-	-	3.00	-	-
Wheat bran	-	-	-	3.00	-
Cellulose	-	-	-	-	0.50
Limestone	1.40	1.35	1.35	1.35	1.39
Dicalcium phosphate	1.70	1.65	1.65	1.65	1.69
Sodium chloride	0.30	0.29	0.29	0.29	0.30
Sodium bicarbonate	0.18	0.17	0.17	0.17	0.18
Mineral and vitamin premix[1]	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.21	0.2	0.2	0.2	0.21
L-lysine HCl	0.03	0.02	0.02	0.02	0.03

[1] Provided the following (per kg of diet): Fe, 60 mg; Mn, 100 mg; Zn, 60 mg; Cu, 10 mg; I, 1 mg; Co, 0.2 mg; Se, 0.15 mg; retinyl acetate, 1.55 mg; cholecalciferol, 0.025 mg; α-tocopherol acetate, 20 mg; menadione, 1.3 mg; thiamine, 2.2 mg; riboflavin, 10 mg; calcium pantothenate, 10 mg; choline chloride, 400 mg; nicotinamide, 50 mg; pyridoxine HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B12 (cobalamin), 1.013 mg.

Table 3. Starter feed chemical composition (d 1-21) (%, unless stated otherwise)

Item	Control	Sunflower hulls	Sugar beet pulp	Wheat bran	Cellulose
Calculated analysis					
AME_n (MJ/ kg)	12.68	12.34	12.41	12.40	12.62
Crude protein	21.77	21.32	21.43	21.64	21.67
Calcium	1.00	0.96	0.96	0.96	0.99
Available phosphorus	0.45	0.43	0.43	0.43	0.44
Sodium	0.20	0.19	0.19	0.19	0.20
Arginine	1.33	1.29	1.29	1.29	1.33
Lysine	1.10	1.06	1.06	1.06	1.09
Methionine	0.57	0.55	0.55	0.55	0.56
Methionine + Cystine	0.90	0.87	0.87	0.87	0.89
Determined analysis					
Dry matter	90.07	89.87	89.09	89.21	89.90
Crude protein	21.70	21.30	21.37	21.56	21.66
Crude fiber	3.84	5.07	3.99	4.08	4.17
Ether extract	6.47	6.42	6.33	6.43	6.44
Neutral detergent fiber	9.89	11.83	10.86	10.87	10.24
Acid detergent fiber	3.92	5.33	4.57	4.30	4.13

Table 4. Grower feed ingredients (d 22-42) (%, unless stated otherwise; as fed basis)

Ingredients	Control	Fiber source			
		Sunflower hulls	Sugar beet pulp	Wheat bran	Cellulose
Corn	59.42	57.63	57.63	57.63	59.14
Soybean meal (44% cp)	28.85	27.98	27.98	27.98	28.70
Gluten meal (60% cp)	3.00	2.91	2.91	2.91	2.98
Vegetable oil	5.00	4.85	4.85	4.85	4.97
Sunflower hulls	-	3.00	-	-	-
Sugarbeet pulp	-	-	3.00	-	-
Wheat bran	-	-	-	3.00	-
Cellulose	-	-	-	-	0.50
Limestone	1.42	1.37	1.37	1.37	1.41
Dicalcium phosphate	1.25	1.21	1.21	1.21	1.24
Sodium chloride	0.25	0.24	0.24	0.24	0.25
Sodium bicarbonate	0.11	0.11	0.11	0.11	0.11
Mineral and vitamin premix[1]	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.10	0.10	0.10	0.10	0.10
L-lysine HCl	0.10	0.10	0.10	0.10	0.10

[1]Provided the following (per kg of diet): Fe, 60 mg; Mn, 100 mg; Zn, 60 mg; Cu, 10 mg; I, 1 mg; Co, 0.2 mg; Se, 0.15 mg; retinyl acetate, 1.55 mg; cholecalciferol, 0.025 mg; α-tocopherol acetate, 20 mg; menadione, 1.3 mg; thiamine, 2.2 mg; riboflavin, 10 mg; calcium pantothenate, 10 mg; choline chloride, 400 mg; nicotinamide, 50 mg; pyridoxine HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B12 (cobalamin), 1.013 mg.

Table 5. Grower feed chemical composition (d 22-42) (%, unless stated otherwise)

Item	Control	Sunflower hulls	Sugar beet pulp	Wheat bran	Cellulose
Calculated analysis					
AME_n (MJ/ kg)	13.18	12.83	12.90	12.89	13.11
Crude protein	19.70	19.31	19.42	19.63	19.59
Calcium	0.90	0.87	0.87	0.87	0.89
Available phosphorus	0.35	0.34	0.34	0.34	0.35
Sodium	0.15	0.15	0.15	0.15	0.15
Arginine	1.19	1.15	1.15	1.15	1.18
Lysine	1.02	1.00	1.00	1.00	1.02
Methionine	0.43	0.42	0.42	0.42	0.43
Methionine + Cystine	0.72	0.71	0.71	0.71	0.73
Determined analysis					
Dry matter	90.23	88.85	89.39	89.88	90.10
Crude protein	19.63	19.32	19.35	19.58	19.55
Crude fiber	3.56	4.8	3.72	3.81	3.89
Ether extract	7.60	7.52	7.41	7.53	7.56
Neutral detergent fiber	982	11.72	10.80	10.73	10.15
Acid detergent fiber	3.69	5.11	4.33	4.07	3.90

Weekly body weight gain (BWG) and feed intake (FI) of each pen were recorded. Feed conversion ratio (FCR) was adjusted for mortality and it was calculated by dividing FI with BWG.

At the end of the experiment, 2 birds from each replicate (which were close to the mean BW of the replicate) were selected and killed by cervical dislocation to evaluate the weights (relative to BW) of the breast, thigh, gastrointestinal tract, gizzard, proventriculus, pancreas, liver, and abdominal fat. The length of intestinal segments including duodenum, from the pylorus to the distal portion of the duodenal loop; jejunum, the segment between the point of entry of the bile ducts and Meckel's diverticulum, ileum, from Meckel's diverticulum to the ileocecal junction and cecum (left and right) were also measured separately (Sadeghi et al., 2015).

On d 42, two birds per replicate and 8 birds per treatment were selected and cecal digesta (1 g) from each bird were aseptically transferred into 9 mL of sterile saline solution and serially diluted. *Lactobacilli* was enumerated on de Man–Rogosa–Sharpe (MRS) agar and *E. coli* and *Coliforms* were counted on Mac Conkey (MC) agar after incubation at 37 ˚C for 48 h in an anaerobic chamber and for 24 h in an aerobic chamber, respectively (Guban et al., 2006). All samples were plated in duplicate.

Blood samples were collected, at the end of the study (42 d), via the brachial vein of two birds per replicate. Serum was separated after centrifugation at 4500 g at 4 ˚C for 10 min, and kept frozen at −20 ˚C until further biochemical analysis. Concentrations of glucose, low-density lipoprotein (LDL), high-density lipoprotein (HDL), cholesterol and triglycerides (TG) were analyzed using standard kits (Zist Shimi, Tehran, Iran) with an autoanalyzer (Autolab PM 4000; Medical System, Rome, Italy).

Statistical methods

Data were analyzed as a 2 × 5 factorial arrangement using PROC GLM of SAS (2002). We considered the pen of birds as the experimental unit for performance parameters and the individual chicken as the experimental unit for the rest of the parameters. The treatment means were compared by the Duncan's multiple range test (Duncan, 1955) at P<0.05. Microbiological counts were subjected to base-10 logarithm transformation before analysis. All data were analyzed by a 2-factorial design, according to the following general model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_{ij}$$

Where Yij is the observed dependent variable, μ is the overall mean, αi is the effect of feed form, βj is the effect of fiber type, (αβ)ij is the interaction between feed form and fiber, and δij is the random error.

Results

Growth performance

The results of FI, BWG, and FCR of birds are given in Table 6. As shown, there was a relationship between the feed form and the type of fiber such that birds fed WB in pellet form had a higher FI than birds fed fibers in mash form in starter phase, grower phase, and the entire period of the experiment (P<0.05). Also, a higher BWG was observed for birds fed fiber in pellet form than the birds fed diets in mash form from d 1 to 21 of age (P<0.05). Similar observations of BWG improvements were achieved from d 22 to 42 and for the entire duration of experiment (P<0.05). An improvement in FCR was observed for birds fed fiber, excluding SBP, in pellet form than the birds fed diets in mash form from d 1 to 21 of age (P<0.05). Pelleting the diet improved FCR from d 22 to 42 and also for the whole period of the experiment (P<0.05).

Table 6. Effect of treatments on growth performance traits of broilers at different phases.

		Feed intake (g)			Body weight gain (g)			Feed conversion ratio (g:g)		
Feed Form	Fiber Source	1 to 21 d	22 to 42 d	1 to 42 d	1 to 21 d	22 to 42 d	1 to 42 d	1 to 21 d	22 to 42 d	1 to 42 d
Mash	Control	976.75^{d}	2453.22^{ef}	3429.97^{de}	731.71^{d}	1270.22	2002.03	1.33^{def}	1.93	1.71
	SBP[1]	996.58^{cd}	2517.43^{ed}	3514.01^{d}	693.98^{e}	1340.61	2034.59	1.39^{ab}	1.88	1.72
	WB[2]	1000.00^{cd}	2493.60^{def}	3493.60^{de}	720.08^{d}	1320.42	2040.49	1.38^{abc}	1.89	1.71
	SFH[3]	990.33^{cd}	2396.33^{f}	3386.66^{e}	695.65^{e}	1305.06	2000.71	1.42^{ab}	1.83	1.69
	CEL[4]	1012.65^{c}	2477.70^{ef}	3490.35^{de}	736.04^{d}	1276.77	2012.81	1.37^{bcd}	1.94	1.73
Pellet	Control	1123.50^{a}	2673.03^{bc}	3796.53^{b}	843.63^{ab}	1448.20	2291.33	1.33^{def}	1.84	1.65
	SBP	1143.54^{a}	2663.32^{c}	3775.82^{bc}	792.92^{c}	1481.62	2274.54	1.44^{a}	1.79	1.66
	WB	1144.58^{a}	2852.39^{a}	3996.96^{a}	866.17^{a}	1491.01	2357.18	1.32^{def}	1.91	1.69
	SFH	1092.82^{b}	2763.65^{ab}	3856.48^{b}	836.33^{b}	1494.17	2330.49	1.30^{ef}	1.85	1.65
	CEL	1078.72^{b}	2598.60^{cd}	3677.32^{c}	852.64^{ab}	1456.24	2308.88	1.26^{f}	1.78	1.59
	SEM	9.43	35.69	34.46	8.16	36.25	36.61	0.018	0.044	0.027
	Fiber Source									
	Control	1050.12^{b}	2563.12^{b}	3612.25^{b}	782.67^{ab}	1309.21	2146.68	1.34^{bc}	1.97	1.68
	SBP	1050.05^{a}	2574.86^{b}	3644.91^{b}	743.45^{c}	1411.12	2154.56	1.41^{a}	1.83	1.69
	WB	1072.28^{a}	2672.99^{a}	3745.28^{a}	793.12^{a}	1405.71	2198.83	1.35^{bc}	1.90	1.70
	SFH	1041.57^{b}	2579.99^{b}	3621.56^{b}	765.98^{b}	1374.61	2140.60	1.36^{b}	1.88	1.69
	CEL	1045.68^{b}	2538.15^{b}	3583.84^{b}	794.34^{a}	1366.50	2160.85	1.32^{c}	1.86	1.66
	SEM	6.67	25.23	27.19	5.77	25.63	25.88	0.013	0.031	0.019
	Feed Form									
	Mash	995.26^{b}	2467.65^{b}	3462.91^{b}	713.49^{b}	1272.61^{b}	2018.10^{b}	1.39^{a}	1.94^{a}	1.71^{a}
	Pellet	1116.62^{a}	2703.99^{a}	3820.62^{a}	838.33^{a}	1474.25a	2312.58^{a}	1.33^{b}	1.83^{b}	1.65^{b}
	SEM	4.22	15.96	17.20	3.65	16.21	16.37	0.008	0.020	0.012
					P-Value					
	Feed Form	0<0.001	0<0.001	0<0.001	0<0.001	0<0.001	0<0.001	0<0.001	0.005	0<0.001
	Fiber Source	0.004	0.008	0.002	0<0.001	0.061	0.089	0<0.001	0.059	0.178
	Feed Form× Fiber Source	0.003	0.001	0.001	0.048	0.341	0.210	0.008	0.061	0.120

a-f: Within columns, mean values with common superscript (s) are not different (P>0.05).

[1]Sugar beet pulp, [2]Wheat bran, [3]Sunflower hull, [4]Cellulose (RC Fine, JRS Co. Inc., Rosenberg, Germany).

The relative weight of organs and the length of intestine

The relative weights of organs and the length of various parts of the intestine are presented in Tables 7 and 8. There was a significant dependency between type of fiber and feed form for the relative GIT weight. Pelleting of WB decreased the GIT weight in comparison to other treatments (P<0.05), while pelleting of SBP increased the breast weight compared to other treatments, excluding for SFH, in mash form. The thigh weight increased in birds fed WB, SFH, and SBP in pellet form while the weight of gizzard decreased in birds fed fibers in pellet form compared to those fed with mash form.

There was no interaction between the type of fiber and feed form for abdominal fat percentage (P>0.05). The inclusion of fiber in the diet independently decreased the weight of abdominal fat. In contrary, pelleting increased the relative weight of abdominal fat (P<0.05). Pelleting the diets, except for CEL, decreased the length of jejunum compared to the mash form (P<0.05).

Cecal microbial population

The effect of dietary treatments on the cecal microbial population is given in Table 9. The cecal lactobacillus population was not affected by any of the main factors-nor by the feed form or type of fiber (P>0.05). However, there was significant interaction between type of fiber and feed form for cecal population of Coliform, TAB (total aerobic bacteria), and E. coli on d 42 (P<0.05). Birds fed pellet diets containing WB and SFH had lower (P<0.05) populations of Coliforms and E. coli in the cecum than other treatments on d 42. Adding SBP, SFH, and CEL to the pellet diet decreased the cecal TAB population on d 42 compared to other treatments.

Table 7. Effect of treatments on carcass traits[1] of broiler chickens on d 42 (g/100 g body weight of bird).

Feed Form	Fiber Source	Gastrointestinal tract	Breast	Thigh	Gizzard	Abdominal fat
Mash	Control	11.43^{ab}	25.39^{ed}	18.02^{f}	1.50^{c}	1.18
	SBP[1]	12.47^{a}	26.03^{bcde}	18.91^{f}	2.31^{a}	0.82
	WB[2]	11.79^{ab}	26.77^{bcd}	19.01^{de}	1.98^{b}	1.06
	SFH[3]	11.99^{ab}	27.47^{ab}	19.58^{cd}	1.82^{b}	0.88
	CEL [4]	11.94^{ab}	25.07^{e}	19.75^{bc}	1.55^{c}	0.84
Pellet	Control	10.87^{b}	27.03^{bc}	19.27^{cde}	1.11^{de}	1.63
	SBP	11.54^{ab}	28.26^{a}	19.77^{bc}	1.00^{de}	1.35
	WB	9.08^{c}	25.71^{cde}	20.61^{a}	1.06^{de}	1.28
	SFH	10.97^{b}	26.11^{bcde}	20.30^{ab}	1.21^{d}	1.56
	CEL	10.69^{b}	26.65^{bcd}	19.61^{cd}	0.87^{e}	1.40
	SEM	0.495	0.476	0.207	0.077	0.083
Fiber Source						
	Control	11.15^{ab}	26.21^{b}	18.73^{c}	1.30^{bc}	1.40^{a}
	SBP	12.14^{a}	27.35^{a}	19.34^{b}	1.65^{a}	1.08^{b}
	WB	10.44^{b}	26.24^{b}	19.81^{a}	1.52^{ab}	1.17^{b}
	SFH	11.48^{ab}	26.79^{b}	19.94^{a}	1.51^{ab}	1.22^{b}
	CEL	11.31^{ab}	25.86^{b}	19.68^{b}	1.21^{c}	1.12^{b}
	SEM	0.350	0.337	0.146	0.054	0.059
Feed Form						
	Mash	11.92^{a}	26.14	19.09^{b}	1.83^{a}	0.95^{b}
	Pellet	10.63^{b}	26.75	19.92^{a}	1.05^{b}	1.44^{a}
	SEM	0.221	0.213	0.092	0.033	0.037
			P-Value			
	Feed Form	0.041	0.228	0<0.001	0<0.001	0<0.001
	Fiber Source	0.029	0.033	0<0.001	0<0.001	0.006
	Feed Form× Fiber Source	0.007	0.002	0.005	0<0.001	0.100

a-e: Within columns, mean values with common superscript (s) are not different (P>0.05).

[1] Data represent the mean of 4 pens (2 broiler chickens/pen) per treatment.

[2]Sugar beet pulp, [3]Wheat bran, [4]Sunflower hull, [5]Cellulose (RC Fine, JRS Co. Inc., Rosenberg, Germany)

Blood metabolites

As shown in Table 10, there was a significant interaction (P<0.05) between fiber type and feed form on serum concentrations of glucose, cholesterol, TG and LDL. The inclusion of SBP, WB and SFH reduced blood TG concentration compared to the control in the mash diets (P<0.05). Inclusion of SFH and CEL in the pelleted diet reduced TG concentration compared to the control diet. Dietary inclusion of fiber decreased blood cholesterol concentrations in comparison to the control treatments on d 42 (P<0.05).

Table 8. Effect of treatments on length (cm) of the different parts of the intestine[1] of broiler chickens on d 42.

Feed Form	Fiber Source	Duodenum	Jejunum	Ileum	Cecum
Mash	Control	31.00^{a}	87.25^{a}	93.50^{a}	43.25^{a}
	SBP[2]	26.25^{c}	73.75^{c}	86.00^{b}	35.25^{b}
	WB[3]	27.25^{bc}	77.25^{b}	80.50^{c}	30.25^{c}
	SFH[4]	27.75^{bc}	79.00^{b}	88.50^{b}	35.50^{b}
	CEL [5]	30.25^{ab}	87.75^{a}	96.25^{a}	36.50^{b}
Pellet	Control	30.00^{ab}	80.50^{b}	84.00^{bc}	41.50^{a}
	SBP	25.50^{c}	66.00^{d}	74.50^{d}	32.25^{bc}
	WB	26.25^{c}	71.50^{c}	77.50^{cd}	29.25^{c}
	SFH	22.50^{d}	73.25^{c}	78.00^{cd}	38.50^{b}
	CEL	29.50^{b}	85.25^{a}	88.25^{b}	37.25^{ab}
	SEM	0.91	1.86	1.35	1.44
Fiber Source					
	Control	30.50^{a}	83.87^{a}	88.75^{a}	42.37^{a}
	SBP	25.87^{b}	69.87^{c}	80.25^{b}	33.75^{bc}
	WB	26.75^{b}	74.37^{b}	79.00^{b}	29.75^{c}
	SFH	25.12^{b}	76.12^{b}	83.25^{b}	37.00^{b}
	CEL	29.87^{a}	86.50^{a}	92.25^{a}	36.87^{b}
	SEM	0.64	1.32	1.35	1.02
Feed Form					
	Mash	28.50^{a}	81.00^{a}	88.95^{a}	36.15^{a}
	Pellet	26.75^{b}	75.30^{b}	80.45^{b}	35.80^{b}
	SEM	0.40	0.83	0.85	0.64
		P- Value			
	Feed Form	0.039	0.003	0.005	0<0.001
	Fiber Source	0<0.001	0<0.001	0<0.001	0<0.001
	Feed Form× Fiber Source	0.024	0<0.001	0<0.001	0<0.001

a-f: Within columns, mean values with common superscript (s) are not different (P>0.05).
[1]Data represent the mean of 4 pens (2 broiler chickens/pen) per treatment.
[2]Sugar beet pulp, [3]Wheat bran, [4]Sunflower hull, [5]Cellulose (RC Fine, JRS Co. Inc., Rosenberg, Germany).

Table 9. Effect of treatments on the cecal microbial population (log cfu g^{-1})[1] of broiler chickens on d 42.

Feed Form	Fiber Source	*Lactobacilli*	*Coliforms*	Total aerobic bacteria	*E. coli*
Mash	Control	9.40	8.68^{d}	9.41^{ab}	9.37^{a}
	SBP[2]	9.42	9.16^{abc}	9.41^{ab}	9.24^{a}
	WB[3]	9.42	8.93^{cd}	9.11^{bc}	8.96^{b}
	SFH[4]	9.32	9.37^{ab}	9.14^{bc}	9.37^{a}
	CEL [5]	9.35	9.40^{a}	9.45^{a}	9.43^{a}
Pellet	Control	9.40	9.28^{ab}	9.33^{ab}	9.36^{a}
	SBP	9.30	9.08^{cd}	8.35^{d}	9.37^{a}
	WB	9.39	8.38^{e}	8.99^{c}	8.45^{c}
	SFH	9.34	7.49^{f}	8.61^{d}	7.49^{d}
	CEL	9.38	9.25^{ab}	8.52^{d}	7.33^{d}
	SEM	0.082	0.090	0.303	0.079
	Fiber Source				
	Control	9.40	8.98^{b}	9.37^{a}	9.36^{a}
	SBP	9.36	9.12^{b}	8.88^{b}	9.30^{a}
	WB	9.40	8.65^{c}	9.05^{b}	8.70^{b}
	SFH	9.33	8.43^{d}	8.87^{b}	8.43^{c}
	CEL	9.36	9.32^{a}	8.98^{b}	8.38^{c}
	SEM	0.058	0.064	0.067	0.055
	Feed Form				
	Mash	9.38	9.10^{a}	9.30^{a}	9.27^{a}
	Pellet	9.37	8.69^{b}	8.76^{b}	8.40^{b}
	SEM	0.036	0.040	0.042	0.035
P-Value					
	Feed Form	0.268	0.002	0<0.001	0.002
	Fiber Source	0.140	0<0.001	0<0.001	0<0.001
	Feed Form× Fiber Source	0.120	0<0.001	0<0.001	0<0.001

a-d: Within columns, mean values with common superscript (s) are not different (P>0.05).
[1] Data represent the mean of 4 pens (2 broiler chickens/pen) per treatment.
[2]Sugar beet pulp, [3]Wheat bran, [4]Sunflower hull, [5]Cellulose (RC Fine, JRS Co. Inc., Rosenberg, Germany).

Table 10. Effect of treatments on serum lipid metabolites[1] (mg/dL) of broilers at d 42.

Feed Form	Fiber Source	Glucose	Cholesterol	Triglyceride	HDL	LDL
	Control	205.00[bc]	154.00[a]	91.75[a]	88.26	45.50[a]
	SBP[2]	206.25[bc]	141.25[b]	77.00[bc]	78.30	33.25[b]
Mash	WB[3]	208.00[bc]	129.00[cd]	80.00[b]	82.90	31.75[bc]
	SFH[4]	209.50[b]	123.25[efd]	64.25[e]	75.50	26.35[cd]
	CEL [5]	204.00[bc]	117.50[ef]	89.00[a]	83.75	37.50[b]
	Control	219.25[a]	136.75[bc]	72.00[c]	88.32	45.25[a]
	SBP	202.75[c]	124.00[ed]	71.50[c]	83.95	45.25[a]
Pellet	WB	209.50[b]	114.25[f]	77.00[bc]	80.33	27.25[cd]
	SFH	204.50[bc]	127.25[d]	59.25[d]	89.85	26.25[cd]
	CEL	206.50[bc]	120.25[edf]	53.25[d]	86.05	24.25[d]
	SEM	1.83	2.92	3.93	3.90	1.94
	Fiber Source					
	Control	212.12[a]	145.37[a]	81.87[a]	85.29	45.37[a]
	SBP	204.50[c]	132.62[b]	74.25[b]	81.12	39.25[b]
	WB	208.75[ab]	121.62[cd]	78.50[ab]	81.66	29.50[c]
	SFH	207.00[bc]	125.25[c]	61.75[c]	82.67	26.30[c]
	CEL	205.25[bc]	118.87[d]	71.12[b]	84.90	30.87[c]
	SEM	1.30	2.07	2.78	2.76	1.37
	Feed Form					
	Mash	206.55	133.00[a]	80.40[a]	81.74	34.85
	Pellet	208.50	124.50[b]	66.60[b]	85.70	33.65
	SEM	0.82	1.30	1.76	1.74	0.86
			P-Value			
	Feed Form	0.104	0<0.001	0<0.001	0.088	0.336
	Fiber Source	0.001	0<0.001	0<0.001	0.091	0<0.001
	FeedForm × Fiber Source	0<0.001	0<0.001	0<0.001	0.559	0<0.001

a-d: Within columns, mean values with common superscript (s) are not different (P>0.05).
[1]Data represent the mean of 4 pens (2 broiler chickens/pen) per treatment.
[2]Sugar beet pulp, [3]Wheat bran, [4]Sunflower hull, [5]Cellulose (RC Fine, JRS Co. Inc., Rosenberg, Germany).

Discussion

Growth performance

Mortality during the experiment was negligible and did not depend on any dietary treatment. Significant relationships were observed between feed form and fiber on FI in all phases, and between BWG and FCR in the starter phase. For BWG and FCR, in grower phase and whole period of experiment, main effects are discussed separately. Feeding pellets increased BWG and improved FCR in grower phase and for the entire period of experiment, in agreement with most of the published literature (Abdollahi and Ravindran, 2013; Serrano et al., 2013, Jim´enez-Moreno et al., 2016). The improvements observed for BWG and FCR were consistent with the greater feed intake. It has been shown that pelleting can increase the number of fine particles in the feed, which in turn, can increase the voluntary feed intake, because of the faster emptying of the upper GIT (Svihus et al., 2010). Our results showed that BWG and FCR were not affected by the inclusion of fiber in the diet in the grower phase or the entire duration of experiment. These results, together with the findings of Hetland et al. (2003) and Gonz´alez-Alvarado et al. (2008, 2010), suggest that the response to increase DF in broilers varies depending on factors, such as the level and fiber types, composition of the basal diet, age, and the growth potential of the birds. In this respect, previous studies (Jim´enez-Moreno et al., 2011, 2013) reported that the inclusion of 7.5% pea hulls, or 7.5% OH, can reduce broiler performance as compared with the inclusion of 2.5% or 5% of fiber; however, the broiler performance was still similar to that of broilers fed the control diet. In our study, the FI value in broilers fed pellets was higher than those fed the mash diets. Also, over the whole experimental period, the pellet diet containing 0.5% CEL decreased the FI value compared with the diets containing 3% WB and 3% SFH. The FCR was improved in the pellet diets by the inclusion of fiber, excluding SBP, as compared with mash diets from 1 to 21 d. In this respect, it was shown previously that the processing of raw fiber can have positive effects, such as removal of soluble fiber fraction and anti-nutritional factors. Consequently, it can improve the digestion and absorption of other nutrients (Market and Backers, 2003). Sellers et al. (1980) reported that, in 56 d old birds, increasing the RH levels in the diet from 2.5% to 5% decreased the feed intake by 2.4% without affecting FCR. The data reported herein suggest that insoluble fibers that do not contain any anti-nutritional factor might be more beneficial for broiler performance when the diets are fed as pellets. Sadeghi et al. (2015) reported sugar beet pulp increased the feed intake in the early period but decreased the growth as well as during the entire period of experiment. Rezaeipour and Gazani (2014) reported pelleting the diets significantly increased FI in the starter, growth, and the whole period of experiment, which is in agreement with our results regarding the main effect of the feed form. The favorable effects of pellets on metabolizable energy and nutrient intake can be due to changes during pelleting that can lead to improved nutritional efficiency, digestion, and absorption. For example, the combined effects of heat and pressure during pelleting break down the cell wall structure and lead to chemical changes in the feed that can ultimately increase the digestibility of the feed in the digestive tract (Zatari and Sell, 1990). Also, insoluble dietary fiber has been reported to increase the rate of passage of digesta to the distal end of the gastrointestinal tract, which in turn may increase the feed intake (Hetland et al., 2003). The higher FCR in SBP-birds in mash and pellet diets are predictable due to less nutrient utilization because of high viscosity of the digesta (Smits et al., 1997).

The relative weight of organs and the length of intestine

The birds fed WB in pellet diets exhibited lower GIT relative weight than those fed the other diets. The birds fed WB, SBP, and SFH in pellet form had higher weight of thigh and the birds fed various sources of fiber in pellet form had lower weight of gizzard, compared to the mash form. Many studies have shown that pelleting can increase the size and the storage capacity of the crop, reduce the weight and digesta contents in gizzard, and increase the gizzard pH. We also observed similar results on the gizzard weight. During pellet processing, dietary ingredients are finely ground to improve the pellet quality. Moreover, the conditions applied during the process, including the passing of the feed through the pellet die, results in further decreases in feed particle size (Svihus et al., 2004; Abdollahi et al., 2013). Consequently, retention of digesta in the gizzard is reduced due to pelleting, which results in decreased gizzard weight, lowered feeling of satiation, and increased FI (Mateos et al., 2012). Hughes (2008) and Svihus (2014) suggested that the increase in voluntary FI caused by pelleting could result in hypertrophy of the proventriculus, overload of nutrients in the small intestine, and a reduction in the utilization of the energy of the diet. In contrast, the coarser particles of the mash diets will reduce the rate of feed passage from the gizzard to the small intestine, stimulating the contractions and the development and weight of the muscular layers of the gizzard (Svihus, 2011; Lv et al., 2015), as well as the relative weight of the full GIT (Svihus and Hetland, 2001; Parsons et al., 2006). In our study, fiber inclusion in mash diets, excluding CEL, increased the gizzard weight and the effect of SBP was greater than other fiber types. Because of its processing, the size of CEL particles does not affect the gizzard weight. However, compared to CEL particles, natural fibers are retained for a longer period in the gizzard. Consequently, compared to the CEL fiber source, SBP, WB, and SFH can exert a greater stimulus on the muscular layers and on the development of the gizzard, consistent with the results reported herein. The SBP is less lignified and more resistant to grinding than SFH and WB. Consequently, SBP particles can accumulate in the gizzard to a greater extent than SFH particles do. As a result, the gizzard can be heavier when birds are fed mash diets containing SBP compared to SFH or WB. In the current study, the relative weight of abdominal fat was not influenced by the fiber sources nor feed forms. But, dietary inclusion of fiber decreased the abdominal fat weight. In agreement with our findings, Rahmatnejad and Saki (2016) reported that inclusion of 1% or 2% of CEL, as insoluble fiber, reduced the abdominal fat in broiler chickens at 21 d of age. Also, Mohiti-Asli et al. (2012) showed that using 3% cellulose resulted in a decrease in broiler breeders' abdominal fat. Mourao et al. (2008) reported that birds fed diets containing insoluble fiber had a lighter carcass with a lower level of abdominal fat. In the current research, birds fed the pellet diet had higher percentage of abdominal fat possibly resulting from the higher energy intake due to increased FI, which was deposited as abdominal fat. Several articles have also reported a higher percentage of abdominal fat in broilers consuming pellet diets (Maiorka et al., 2005; Corzo et al., 2011). In the current research, pelleting the diets, except CEL, decreased the length of jejunum when compared to the mash form which is in agreement with the findings by Gonzalez-Alvarado et al. (2007), who reported that the inclusion of insoluble fiber in the diet reduced the relative length of the small intestine. A shorter small intestine in birds fed on diets containing fiber may be explained by the lower nutrient density, which reduces the surface area required for absorption. Alternatively, it may be that the diameter of the intestine increased and that the surface area was similar or greater with fiber inclusion. Santos et al. (2006), however, reported that intestinal length was not affected by dietary inclusion of wood shavings in turkeys. Some possible reasons for these discrepancies could be the type of the control diet (purified, semi-purified, or practical) and the level of used fibers. In the current research, pelleting of diet independently decreased the length of duodenum, jejunum, ileum, and ceca, compared to the mash form. On the other hand, Jimenz Moreno et al. (2019) reported that pelleting increased the absolute length of the small intestine and ceca, probably due the faster rate of passage of fine particles in the intestines. Mirghelenj and Golian (2009) showed that pelleting reduced the relative length of ceca. Abdollahi et al. (2011) found a heavier ceca weight in mash-fed compared to pellet-fed birds. A lower weight of ceca may increase water excretion relative to feed intake (Maisonnier et al., 2001).

Gut microflora

Adding SBP, SFH, and CEL to the pellet diet decreased the TAB population in the cecum on d 42, compared to other treatments. We also found that birds fed with WB and SFH had lower *Coliform* and *E. coli* populations in the cecum on d 42. This observation agrees with that of Bogusławska-Tryk et al. (2015) who found a higher population of *Lactobacillus* spp. in the ileum and a lower population of *Escherichia coli* and *Clostridium* spp. in the ileum and cecum of birds fed diets supplemented with lignocellulose. In agreement with our findings, it has been shown that the inclusion of wheat bran, oat hull, sugarcane bagasse, and cellulose can improve cross-feeding among bacteria and change the total bacterial counts such that the beneficial species (e.g., *Bifidobacteria, Eubacteria, Lactobacilli* spp.) can become the dominant in the cecum and ileum (Vermeulen et al.,2018), Jiménez Moreno et al. (2011), Kheravii et al. (2017b), Cao et al. (2003). These observations indicated that different sources of fiber could be used in the diet to modulate the indigenous bacteria, and thus, health, immune status, and performance in birds. Dietary fiber can increase the acidity of mucin, thus enhancing the resistance of mucus against pathogenic bacterial enzymes (Rhodes, 1989). Similarly, Bogusławska-Tryk et al. (2015) reported that supplementing broiler diets with dietary lignocellulose resulted in an increased number of *Lactobacillus* spp. in the ileum and *Bifidobacterium* spp. in the ileum and ceca, and a decreased presence of ileal and cecal *E. coli* and *Clostridium* spp. The current and former studies imply that some sources of fiber, by reducing the total count of *Coliforms*, might be beneficial to the microbiota balance and GIT health. However, the effect of dietary fiber on the gastrointestinal microbiota in poultry can vary depending on the source, chemical, and physical composition of fiber. Engberg et al. (2002) reported that pellet-fed birds had lower counts of *Lactobacilli* spp. and C. *perfringens* and higher counts of *Coliforms* and *Enterococci* spp. in the ileum, compared to mash-fed birds. Undigested fine particles in pelleted diets can enter the ceca and become available for microbial fermentation. Microbial fermentation in terms of volatile fatty acid concentrations was found to be lower in the ceca of mash fed birds. Data on the effect of feed form on gut microbiota profile are scanty, and more research is warranted. Engberg et al. (2002) indicated that mash-fed broilers had higher cecal *Clostridia perfringens* population compared to those fed pellet diets. Feeding pellet diets significantly increased cecal spore forming bacteria spp. because pelleting is able to reduce only non-spore forming bacteria population in diets (Boroojeni et al., 2016). However, further research is needed for illucidating the mechanism of cecal spore-forming bacteria proliferation in response to feed form changes. Setlow (2014) indicated that spore-forming bacteria need some nutrient germinants such as amino acids and specific bile salts for germination. More efforts are needed to understand fiber structure in general and how it interacts with bacteria within a highly competitive environment. In this study, dietary inclusion of fibers in pellet form decreased TAB and *E. coli* population in the cecum, compared to mash diets. However, SBP increased the cecal population of *E. coli* in both mash and pelleted diets, which could have possibly resulted from the viscosity of SBP. It has been shown that diet rich in water soluble NSP could potentially influence the gut microbiota because these are not typically digested and not absorbed by the host, and therefore, can serve as substrates for intestinal bacteria, particularly pathogenic ones. For instance, wheat, barley, or rye-based diets that contain high levels of indigestible, water-soluble NSP, favor the proliferation of C. *perfringens* and predispose young chicks to necrotic enteritis. Conversely, diets low in NSP, such as corn-based diets, do not instigate such diseases (Annett et al., 2002; Jia et al., 2009). It has been suggested that high levels of water soluble NSP, such as arabinoxylan and glucan, in the diet can increase the digesta viscosity, which, in turn, may reduce digestive and absorptive capacity in the gut (Classen, 1996; Choct et al., 1996). This is partly because digesta with higher viscosity can lead to a thicker unstirred water layer covering the mucosa cells (Johnson and Gee, 1981), reducing the interaction between nutrients and pancreatic enzymes and bile acids (Johnson and Gee, 1981; Edwards et al., 1988). As a result, more undigested starch and protein can reach the hindgut and can act as substrates for undesirable bacteria, such as *Clostridium perfringens* (Bedford and Cowieson, 2012). Examples of this were reported by Shakouri et al. (2006) where they showed that *Enterobacteriaceae* spp. counts increased in the ceca in response to an increase in intestinal viscosity caused by inclusion of 3% methylated citrus pectin, as a source of soluble NSP.

Blood metabolites

Our results showed that the fiber type and feed form significantly affected the serum concentrations of glucose, cholesterol, TG, and LDL. Fiber inclusion in the pelleted diets decreased glucose concentration compared with the mash diets. In the mash diets, inclusion of fiber, except CEL, reduced TG concentration, compared to control. Also, inclusion of SFH and CEL the pellet diets significantly reduced TG concentration. Dietary inclusion of fiber in both the pellet and mash diets decreased blood cholesterol concentration on d 42. Consistent with our findings, Rahmatnejad and Saki (2015) reported that the inclusion of 1% or 2% of CEL, as insoluble fiber, reduced the concentration of cholesterol in blood plasma. Also, Mohiti-Asli et al. (2012) showed that 3% cellulose resulted in a decrease in plasma concentration of cholesterol in broiler breeders. The decreased serum triglyceride concentration in broilers fed various sources of fiber in our experiments may be related to the role of fiber, as dietary fiber content is known to reduce fat utilization by deconjugation of bile salts. This could be the reason for the reduced fat absorption in the gut, if the body fat (liver fat) is utilized for the metabolic needs, and therefore, it causes the triglyceride concentration in serum to decrease. A similar trend was observed in the experiments of Rama Rao et al. (2004; 2006) in which the serum concentrations of LDL and triglycerides decreased in birds that received high-fiber diets. In contrast, Shirzadegan and Taheri (2017) reported higher concentrations of serum LDL at 40 days of age in chickens fed with 30 g/kg of wood shavings. Taheri et al. (2016) showed birds that received 12% wheat bran in barley-based diets had lower total cholesterol concentrations compared to the birds fed with corn-based diet. Serum triglyceride concentration significantly decreased in broiler chickens that received 0.75% of insoluble fiber. High serum concentration of HDL and lower concentrations of VLDL were observed in broiler chickens that received 0.5% and 0.75% insoluble fibers (Sarikhan et al., 2009). It was shown that the consumption of soluble fiber reduced blood glucose, and increased insulin sensitivity (Meyer et al., 2000). This can be the cause of reduced serum glucose concentration in the SBP group. Many studies in humans have shown that wheat bran consumption can reduce the fasting blood glucose concentration in a dosage-dependent manner (Manhire et al., 1981; Hollenbeck et al., 1986).

In conclusion, feeding fibers in the pellet forms could improve FI, BWG, and FCR in the starter phase. In addition, dietary inclusion of WB and SFH decreased the *Coliforms* and *E. coli* populations in the cecum and cholesterol concentrations in blood in pellet and mash diets.

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