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Influence of grain type and fat source on performance, nutrient utilization, and gut properties in broilers fed pelleted diets

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ABSTRACT The influence of grain type and fat source on the performance, coefficient of apparent ileal digestibility (**CAID**), and intestinal characteristics in broiler starters fed pelleted diets were studied. The experiment included 8 treatments arranged as a 2 × 4 factorial with 2 grains (wheat and corn) and 4 fat sources (soybean oil, fish oil, tallow, and palm oil). In all fat sources, corn-fed birds had a higher weight gain than those fed wheat-based diets. However, improvement in the weight gain of birds fed wheat-based diets supplemented with tallow resulted in a significant ($P < 0.001$) interaction between grain type and fat source. Inclusion of wheat and tallow increased feed intake compared to corn and other fat sources, respectively. Pellets made from wheat were harder ($P < 0.01$) than those based on corn. Broilers fed corn-based diets, had higher CAID of fat, Ca, and phosphorus ($P < 0.01$) than those fed wheat-based diets. Soybean oil inclusion, also increased ($P < 0.01$) fat digestibility compared to other fat sources. An interaction occurred between grain type and fat source where pellets made from corn and soybean oil had higher protein digestibility compared to the other treatments ($P < 0.01$). Feeding wheat-based diets increased pH of gizzard and proventriculus compared to corn-based diets ($P < 0.01$). Highest viscosity value was observed in wheat-diets supplemented with fish oil, and palm oil ($P < 0.01$). The pancrease, gizzard and cecum were heavier in corn-based fed birds compared to those fed wheat-based diets ($P < 0.01$). A significant interaction between grain type and fat source was noted for *Lactobacillus* spp. and the *total anaerobic bacteria* population in the cecum. Overall, the effect of grain type on weight gain, CIAD of protein and cecal microbiota differed depending on the fat sources. Feeding corn and soybean oil resulted in better gut development and growth performance in broilers fed pelleted diets.

Key words: broiler, grain type, fat source, nutrient digestibility, gut property

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INTRODUCTION

Depending on the locations or regions of the world, poultry diets are commonly formulated with corn or wheat as a main cereal grain. Due to the use of corn in other industries such as ethanol production, the global price of corn has increased. Replacing all or some of the corn in diets with wheat can present an interest opportunity to reduce feed cost and maintain profitability. It is well known; wheat has high levels of water-soluble non-starch polysaccharides (**NSP**), including arabinoxylans, increased digesta viscosity (Steenfeldt et al., 1998a, b; Jahanian and Rasouli, 2014).

The poultry industry utilizes a variety of oil sources such as soybean oil, palm oil, canola oil, fish oil, and tallow as energy sources in diet formulation. Digestion and absorption of different fat sources differ widely, and are influenced by their chemical characteristics (Renner and Hill, 1961; Blanch et al., 1995; Lessire et al., 1982; Wiseman and Salvador, 1989), especially, fatty acid (**FA**) composition and, the length and saturation degree of the carbon chain (Wiseman, 1984). It has been reported that vegetable oils (soybean oil) contain high concentrations of unsaturated fatty acids, and can be easily digested by chicks. Whereas, animal fats (tallow) include high proportions of long chain saturated fatty acids which are nonpolar and poorly digested and absorbed (Whitehead and Fisher, 1975; Krogdahl, 1985; Tancharoenrat et al., 2015). Tancharoenrat et al. (2013) reported that inclusion of 40 g/kg tallow, soybean oil, poultry fat, and palm oil and, a 50:50 blend of tallow and soybean oil in maize-based diets, resulted in lower apparent metabolizable energy (**AME**) of tallow than

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those of soybean oil, palm oil and poultry fat. Furthermore, it has been reported that diets supplemented with 60 g/kg tallow had higher feed per gain and lower total tract retention and ileal digestibility of fat than those supplemented with soybean oil (Tancharoenrat et al., 2015). Moreover, dietary replacement of tallow by vegetable oils (soybean oil or linseed oil) improved performance and decreased abdominal fat deposition in broiler chicks (Crespo and Esteve-Garcia, 2002; Ferrini et al., 2008; Wongsuthavas et al., 2008). Viscosity of NSP has more effect on fat digestion in comparison to the other nutrients and this effect is greater when saturated fat is included in the diet as a lipid source (Dänicke, 2001).

Higher viscosity of intestine reduces the rate of intestinal motility and impaired the diffusion and convection transport of emulsified droplet, FA, mixed micelles, bile salts and lipase enzyme (Smulikowska, 1998), and stimulates microbial growth of the small intestine (Annison and Choct, 1991).

Efforts to ameliorate the possible interactions between viscous and nonviscose grains and saturated and unsaturated fats and their effects on gut properties and utilization of nutrients are scant (Dänicke et al., 1997; Tancharoenrat et al., 2014), and can be useful. Moreover, the interaction of fat sources and grain type in pelleted diets without the presence of NSP-ase enzymes, due to thermal processing has not been considered and merit further investigation. Supporting hypothesis for the current study was that the response of broiler starters to fat source will differ in birds fed diets based on viscous and nonviscous grains. The major focus of the present study was to fully evaluate the possible interaction between grain type (corn *vs.* wheat), and saturated and unsaturated oil sources with distinct fatty acid profile on the growth performance, ileal digestibility of nutrients, small intestine characteristics, and bacterial population in the cecum of broilers fed pelleted diet.

MATERIALS AND METHODS

Diets

The experiment was conducted in 2 × 4 factorial arrangements with 2 factors: type of basal grains (corn and wheat); and oil sources (soybean oil, fish oil, tallow, palm oil). All diets were formulated to be isocaloric and isonitrogenous and meet the Ross 308 strain recommendations (Aviagen, 2018) with further nutrients for male broilers starters. Calculated differences in AME between diets including saturated and unsaturated oils were overcome by the inclusion of sand. The beef tallow (abdominal fat) was provided by slaughterhouse (Bisotun, Iran), melted by heat, then its impurities and sediments were separated. The soybean and palm oils were refined forms and of food grade (Nazgol oil factory, Mahidasht, Iran), and fish oil was purchased from local supplier, Gilan, Iran.

The chemical composition, calculated analysis, and analyzed values of the diets are presented in Table 1. Experimental diets were fed in a pellet form.

Whole corn and wheat were prepared from a commercial supplier, and ground in a hammer mill with 1,500 rpm (model: HM40, Asiab Industrial group, Tehran, Iran) to pass through screen size of 2.0, mm. Diets were mixed in a single-screw ribbon mixer (Asiab Industrial group, Tehran, Iran). Oil sources were added to the mixer. Before mixing, palm oil and tallow were turned into liquid by low heating. Following mixing, all diets were steam-conditioned at 85°C for the 30 s in a superconditioner (Stolz, Paris, France), and pelleted using a pellet mill with feeder rate of 4.5 ton/h (PMK 580, Puyesh Machine Khalij Co., Iran) equipped with the die ring with 3-mm holes and 42-mm thickness.

Titanium dioxide (TiO_2, VWR international bvba, Leuven, Belgium) was incorporated into diets as an indigestible marker at a rate of 3 g/kg for the ileal nutrient digestibility. To prepare diets containing titanium dioxide, the required amount of each experimental diets was crushed, mixed with the marker and turned into pellets again with a cold pellet press machine (without conditioning).

Birds and Housing

Experimental procedures were conducted in accordance with the Razi University Animal Ethics Committee guidelines. A total of 480, 1-day-old male broilers (Ross 308), obtained from a commercial hatchery, were individually weighed and assigned to 48-floor pens (100 cm × 150 cm) that the average bird weight per each pen was similar. Each of the 8 dietary treatments was randomly allocated to 6-floor pens, each containing 10 birds. The bedding material of experimental pens was wood shavings. The temperature was maintained at 33°C on the first day and was gradually reduced to 22°C by 21 d of age. The feed was provided *ad libitum* from d 1 to 21, and water was available freely. Bodyweight and feed intake (**FI**) were evaluated on a pen basis at weekly intervals. Mortality was recorded daily. Feed conversion ratio (**FCR**) was corrected for the bodyweight of any bird that died within the experiment.

Determination of Pellet quality, Digesta Transit Time, and Viscosity

Pellet durability index (**PDI**) was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd, North Walsham, Norfolk, UK) using the method described by Abdollahi et al. (2010a). Pellet hardness (**PH**) was evaluated using a Kahl device (Amandus Kahl GmbH and Co. KG, Germany) as reported by Thomas and Van der Poel (1996). PDI and pellet hardness were measured in 6 and 12 samples in the laboratory, respectively, and the average measurements for each diet were reported as footnote in Table 1.

On d 15, after 2 h feed withdraws, diets containing chromic oxide (1.0 g/kg) were fed for 15 min. The time of digesta transit was defined as the time between feed

Table 1. Composition and calculated nutrient content (g/kg as fed) of the basal diets of broiler starters (d 1−21).[1,2]

Ingredient	Corn				Wheat			
	Soy oil	Fish oil	Tallow	Palm oil	Soy oil	Fish oil	Tallow	Palm oil
Corn	548.5	548.4	556.7	551.4	-	-	-	-
Wheat	-	-	-	-	625.3	625.3	630.2	627.2
Soybean meal	346.5	346.7	345	346.2	276.7	276.7	254.4	268.3
Soy oil	30	-	-	–	30	-	-	–
Fish oil	-	30	-	-	-	30	-	-
Tallow	-	-	30	-	-	-	30	-
Palm oil	-	-	-	30	-	-	-	30
Corn Gluten	20	20	20	20	23	23	39.6	29.2
DCP	21.6	21.6	21.6	21.6	18.3	18.3	18.6	18.4
Limestone	9.5	9.5	9.5	9.5	9.9	9.9	9.8	9.9
Sodium Chloride	2.2	2.2	2.2	2.2	1.7	1.7	1.6	1.7
Sodium bicarbonate	2	2	2	2	2.5	2.5	2.8	2.6
Vit Premix[1]	1	1	1	1	1	1	1	1
Min Premix[1]	1	1	1	1	1	1	1	1
DL-Methionine	3	3	3	3	2.4	2.4	2.3	2.3
L-Lysine HCL	3	3	3	3	4.1	4.1	4.6	4.3
L-Threonine	0.9	0.9	0.9	0.9	1.1	1.1	1.1	1.1
Sand	7.8	7.7	1.1	5.2	-	-	-	-
Titanium oxide	3	3	3	3	3	3	3	3
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Calculated analysis								
Apparent metabolizable energy (AME); Kcal/Kg	2,950	2,950	2,950	2,950	2,950	2,950	2,950	2,950
Crude protein	215	215	215	215	215	215	215	215
Linoleic acid	28.7	14.6	14.5	16.2	16.4	2.3	1.9	3.8
Ether extract	54	54	54.2	54.1	45.6	45.6	45.3	45.5
Crude fiber	37.7	37.7	37.8	37.8	43.2	43.2	42	43
Dig, Lysine	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
Dig, Methionine	5.8	5.8	5.8	5.8	5.3	5.3	5.3	5.3
Dig, Methionine + cysteine	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Dig, Threonine	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Dig, Tryptophan	2.1	2.1	2.1	2.1	2.3	2.3	2.2	2.3
Calcium	9.9	9.9	9.5	9.5	9.5	9.5	9.5	9.5
Available. P	4.9	4.9	4.7	4.7	4.7	4.7	4.7	4.7
Analyzed values								
Gross energy (Kcal/kg)	4237	4218	4227	4237	4175	4140	4186	4153
Crude Protein	192	191	192	190	192	190	194	194
Ether Extract	50	56	60	56	38	45	48	48

1-Supplied per kg diet: vitamin A, 11 000 IU; vitamin D3, 5000 IU; vitamin E, 36.75 IU; vitamin K3, 3.4 mg; vitamin B1,1.98 mg; vitamin B2, 5.25 mg; pantothenic acid, 10.5 mg; niacin, 31.5 mg; vitamin B6, 2.87 mg; folic acid, 1.2 mg; vitamin B12, 0.024 mg; biotin, 0.105 mg; choline, 800 mg; manganese, 120 mg; zinc, 100 mg; iron, 50 mg; copper, 12 mg; iodine, 1.3 mg; selenium, 0.3 mg; antioxidant, 100 mg.

[1]The PDI values (newton) for corn-diets containing soybean oil, fish oil, tallow and palm oil was 63.46, 44.7, 47.13, 63.23, respectively, and for wheat-diets containing soybean oil, fish oil, tallow and palm oil was 91.81, 88.85, 92.36, 92.08, respectively.

[2]The hardness (%) of pellets for corn-diets containing soybean oil, fish oil, tallow and palm oil was 3.46, 2.83, 3.03, 3.13, respectively, and for wheat-diets containing soybean oil, fish oil, tallow and palm oil was 3.93, 3.76, 4.06, 4.3, respectively.

ingestion and the first appearance of green-colored droppings (Naderinejad et al., 2016). The relative viscosity of the feed samples was measured at 20°C using a Brookfield viscometer in a model digestion system -(Bedford and Classen, 1993) and the results are expressed relative to the viscosity of water (centipoise).

Determination of Coefficient of Apparent Ileal Digestibility

On day 21, 6 birds per each pen were randomly selected and euthanized by cervical dislocation, and ileal digesta (lower half towards the ileo-caecal junction) was collected- and pooled within a pen, lyophilized (Zibrus, VaCo 2-11, Germany), grounded to pass through a 0.5-mm sieve and stored at 4°C until laboratory analysis. The diets and digesta samples were analyzed for dry matter (**DM**), titanium (**Ti**), nitrogen (**N**), fat, ash, calcium (**Ca**) and phosphorus (**P**) contents.

Apparent ileal nutrient digestibility coefficients (**CAID**) were calculated using the following formula (Naderinejad et al., 2016):

$$CAID\ of diet\ component = [(dietcomponent/Ti)d - (diet\ component/Ti)i]/(diet\ component/Ti)d$$

where (diet component/Ti) d = ratio of diet component to Ti in the diet, and (diet component/Ti)i = ratio of diet component to Ti in the ileal digesta.

Digestive Tract Measurements

On d 21, digestive tract of 2 birds from each replicate pen, were euthanized for ileal collection were used for measurements. Digestive tract from the crop to ceca was carefully excised and adherent fat was removed. The lengths of duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileo-cecal junction) and ceca were recorded as described by Amerah et al. (2008), and expressed as a relative length (cm/kg live body weight).

The empty weights of proventriculus, gizzard, duodenum, jejunum, ileum and ceca, also pancreas and liver in individual birds were determined and reported as g/kg of BW, expressed as a relative weight (g/kg live body weight). The pH of the gizzard and proventriculus were measured by inserting the calibrated pH meter (SCT-PH-PEN-5) directly in 3 parts (proximal, middle, and distal) of the organs and the average of 3 readings were considered as the final pH value.

Determination of Microbial Population of Caeca

On d 21, the cecal content form 1 ceca (right side) per bird (2 birds/pen) that were euthanized for ileal collection, were aseptically removed for microbial population analysis. All microbiological analyses were performed in a duplicate plate for each bird, and the average value of 2 birds per pen were used for statistical analysis. Total viable counts of *Lactobacillus* spp., *Escherichia coli,* and *total anaerobic bacteria* were measured by the culture technique. Each cecum contents (2 g) were homogenized and diluted with distilled water in the ratio of 1:10, followed by serial decimal dilution. The deMan Rogosa Sharpe agar (MRS agar, Quelab Laboratories Inc., Montreal, Quebec, Canada) and Reinforced clostridial agar (Reinforced clostridial agar, Fisher Scientific, Waltham, Massachusetts) were used to enumerate *Lactobacillus* spp. and *total anaerobic bacteria,* respectively. Plates were incubated under anaerobic conditions at 37°C for 48 h. Furthermore, the number of *Escherichia coli* was enumerated on Eosin methylene blue agar (EMB agar, Quelab Laboratories Inc.). The plates for *Escherichia coli* were incubated in aerobic conditions at 37°C for 24 h (Thitaram et al., 2005). All the data regarding bacterial enumeration were transformed to a logarithmic scale before analysis.

Chemical Analysis

Gross energy of diets and oil sources was measured by adiabatic bomb colorimetry (6200 Calorimeter, Parr Instrument Co., Moline, Illinois) standardized with benzoic acid. Dry matter, total ash, crude protein, and fat contents were determined according to AOAC (2005). Calcium and phosphorus contents were determined by titration (method 927.02) and UV spectrophotometry (method 965.17) (UV-150-02, Shimadzu Corp., Kyoto, Japan), respectively. The concentration of Ti was determined by UV spectrophotometry following the method of Short et al. (1996). Fatty acid composition of fat sources was determined according to procedure of Sukhija and Palmquist (1988) using gas chromatography (Shimadzu GC- 17A; Shimadzu Corporation, Tokyo), equipped with a flame ionization detector.

Statistical Analysis

The experimental design was a completely randomized design arranged factorially with 8 dietary treatments and 6 replicates per treatment. The data were analyzed by a 2-way ANOVA to determine the main effects (grain type and oil source) and their interaction using the general linear models' procedure of the SAS (2004). Significant differences between means were separated by the Least Significant Difference test. Probabilities ($\boldsymbol{P}$) of $\leq$ 0.05 were considered statistically significant, whereas values of $0.05 < P < 0.10$ were declared a near-significant trend.

RESULTS

Performance and Pellet Quality

Fatty acid analysis of the 4 fat sources (Table 2) showed that palm oil contained high amount of palmitic acid (C16:0, 39.7%) and oleic acid (C18:1, 42.6%). Soybean oil was high in linoleic acid (C18:2 n6, 52.9%), tallow was rich in oleic acid (C18:1, 40.4%) and palmitic acid (C16:0, 23.8%), and stearic acid (C18:0, 16.3%). Fish oil contained high amounts of oleic acid (C18:1, 37.1%), and palmitic acid (C16:0, 23.4%). The unsaturated/saturated fatty acid ratio of soybean oil, fish oil, tallow and palm oil was 4.85, 1.6, 1.13, and 1.17, respectively.

However, improvement in the weight gain of birds fed wheat-based diets supplemented with tallow resulted in significant ($P < 0.001$) interaction between grain type and fat source (Table 3). The greatest body weight was observed in birds fed diets containing corn and soybean oil and the lowest was in birds fed wheat-based diets containing soybean oil, fish oil, and palm oil. Overall, in

Table 2. Analysis of fatty acid composition of soy oil, fish oil, tallow and palm oil.

	Soy oil	Fish oil	Tallow	Palm oil
Saturated fatty acids (g/kg)				
C12:0 Lauric	-	-	2.0	2.0
C14:0 Myristic	0.9	38.8	27.7	9.5
C16:0 Palmitic	113.9	234.1	238.4	397.8
C17:0 Margaric	1.0	18.3	12.1	1.1
C18:0 Stearic	46.8	55.0	163.2	45.0
C20:0 Arachidic	4.0	4.4	2.3	4.3
C22:0 Behenic	4.3	5.8	-	-
Unsaturated fatty acids (g/kg)				
C16:1 Palmitoleic	0.8	53.5	29.0	2.0
C18:1 Oleic	234.3	371.9	404.4	426.1
C18:2 Linoleic	529.2	51.9	59.1	107.5
C18:3 Linolenic	62.4	23.3	8.1	2.9
C20:1 Eicosenoic	2.0	19.7	2.3	-
C20:2 Eicosadienoic	-	0.0	-	-
C20:5 n-3	-	51.3	-	-
Total fatty acids	999.40	927.6	948.3	998.0
Saturated fatty acids (g/kg)	170.95	384.00	469.84	460.49
Unsaturated fatty acids (g/kg)	829.05	616.00	530.16	539.51
Unsaturated to saturated ratio	4.85	1.6	1.13	1.17

Table 3. Influence of fat source and cereal type on broiler growth performance in broiler starters on d 21.[1]

Fat source	Cereal type	BWG, g	FI, g	FCR[2], g: g
Soybean	Corn	993[a]	1236	1.245[d]
	Wheat	851[d]	1125	1.322[bc]
Fish	Corn	952[bc]	1220	1.281[cd]
	Wheat	836[d]	1133	1.356[ab]
Tallow	Corn	968[ab]	1255	1.299[c]
	Wheat	933[c]	1204	1.291[cd]
Palm	Corn	944[bc]	1209	1.28[cd]
	Wheat	837[d]	1166	1.392[a]
SEM[3]		10.881	16.709	0.014
Main effects				
Cereal type				
Corn		964	1229[a]	1.276
Wheat		864	1157[b]	1.340
SEM		5.440	8.354	0.007
Fat source				
Soybean oil		922	1180[b]	1.283
Fish oil		894	1176[b]	1.318
Tallow		950	1229[a]	1.295
Palm oil		890	1187[b]	1.335
SEM		7.694	11.810	0.010
Probabilities, $P\leq$				
Cereal type		0.0001	0.0001	0.0001
Fat source		0.0001	0.009	0.005
Cereal type × Fat source		0.0001	0.163	0.002

[a-d]Means within a column followed by the different letters are - significantly different at P ≤ 0.05.
[1]Each value represents the mean of 6 replicates (ten birds/replicate).
[2]Feed conversion ratio.
[3]Pooles standard error of mean.

all fat sources, corn-fed birds had higher weight gain than those fed wheat-based diets. Interestingly, in wheat-based diets, the body weight gain (**BWG**) in tallow group was significantly higher than the other 3 fat sources. A similar interaction ($P < 0.01$) between grain type and fat source was noted for FCR (Table 3), in which birds fed corn-based diets supplemented with soybean oil, fish oil, and palm oil exhibited reduced FCR (5.8, 5.5%, and 8.04% respectively, $P \leq 0.01$) compared to broilers fed the wheat-based diets, while birds fed wheat-based diets supplemented with tallow had similar FCR compared to broilers fed corn-based diets with tallow.

No interaction between grain type and fat sources was observed for FI (Table 3). The main effects of grain type and fat source was significant for the FI with feeding wheat-based diets increased FI compared to corn-based diets ($P < 0.01$), also, diets supplemented with tallow had higher FI than those supplemented with soybean oil, fish oil, and palm oil ($P < 0.01$). Mortality was negligible (0.6%) and not associated to any treatment.

Pellet durability index was higher in pellets made from diets based on wheat than those made from corn, the average of PDI values (newton) for corn-diets containing soybean oil, fish oil, tallow and palm oil were 63.46, 44.7, 47.13, 63.23, respectively; and for wheat-diets containing soybean oil, fish oil, tallow and palm oil were 91.81, 88.85, 92.36, 92.08, respectively (Table 1).

Pellets made from wheat were slightly harder than those based on corn. The average of hardness (%) of corn pellets diets containing soybean oil, fish oil, tallow and palm oil were 3.46, 2.83, 3.03, 3.13, respectively; and for wheat pellets diets containing soybean oil, fish oil, tallow and palm oil were 3.93, 3.76, 4.06, 4.03, respectively (Table 1).

Digesta Transit Time, pH of Gizzard and Proventriculus and Viscosity

Digesta transit time was not different ($P > 0.05$) among dietary treatments (Table 4). Feeding wheat-based diets increased pH of gizzard and proventriculus

Table 4. Influence of fat source and cereal type on the digesta transit time, viscosity of jejunum, pH of gizzard and proventriculus of broiler starters.[1]

Fat source	Cereal type	Digesta transit time	Viscosity (centipoise)	Proventriculus pH	Gizzard pH
Soybean	Corn	133	2.25[d]	3.15	2.86
	Wheat	136	4.21[c]	3.87	3.37
Fish	Corn	135	1.32[d]	3.16	2.9
	Wheat	133	5.94[a]	3.49	3.03
Tallow	Corn	138	1.45[d]	3.15	2.61
	Wheat	132	4.89[bc]	3.77	3.22
Palm	Corn	140	1.51[d]	3.59	2.43
	Wheat	129	5.18[ab]	3.75	3.09
SEM		4.353	0.302	0.164	0.175
Main effects					
Cereal type					
Corn		137	1.06	3.26[b]	2.70[b]
Wheat		132	5.05	3.72[a]	3.17[a]
SEM		2.176	0.151	0.082	0.087
Fat source					
Soybean oil		134	3.23	3.51	3.12
Fish oil		134	3.65	3.32	2.97
Tallow		135	3.17	3.46	2.92
Palm oil		134	3.44	3.67	2.76
SEM		3.078	0.213	0.116	0.123
Probabilities, $P\leq$					
Cereal type		0.183	0.0001	0.0003	0.005
Fat source		0.992	0.404	0.224	0.253
Cereal type × Fat source		0.444	0.001	0.313	0.425

Means within a column followed by the same letter are not significantly different at P > 0.05.
[1]Each value represents the mean of 6 replicates (2 birds/replicate).

compared to corn-based diets ($P < 0.01$). There was an interaction between the grain type and fat source ($P < 0.01$) for the digesta viscosity, overall, broilers fed wheat-based diets supplemented with soybean oil, fish oil, tallow and palm oil had increased viscosity in comparison to corn-based diets ($P < 0.01$). The highest viscosity of digesta was observed in birds fed diets containing wheat with palm oil and fish oil, which was significantly higher than birds fed diets containing wheat and soybean oil. There was no significant difference between viscosity of wheat-based diets supplemented with soybean oil and tallow (Table 4).

Digestibility of Nutrients and Digestive Tract Measurements

The main effect of grain type and fat source was significant for the CAID of fat and with soybean oil inclusion, fat digestibility increased ($P < 0.01$) in compared to fish oil, tallow, and palm oil. Corn-based diets, also had greater fat digestibility than wheat-based diets ($P < 0.01$).

A significant grain type × fat source interaction ($P < 0.01$) was detected for the CAID of protein, and ash, in which birds fed wheat-based diets supplemented with soybean oil, fish oil, and tallow had less protein digestibility compared to their corn-based diets counterparts, while broilers fed palm oil had similar protein digestibility, irrespective of the grain type (Table 5).

Inclusion of fish oil in wheat-based diet reduced ash digestibility compared to corn-based diet ($P < 0.05$). No interaction was detected between grain type and fat source for CAID of Ca and P.

Feeding pellets made from corn improved ($P < 0.01$) Ca, and P digestibility than wheat. Birds fed soybean oil, tallow, and palm oil had higher ($P < 0.01$) Ca, and P digestibility than those fed fish oil. Grain type and fat source had no significant effect on the relative length of ileum, small intestine and cecum. Broilers fed soybean oil, fish oil, and palm oil diets had longer duodenum ($P < 0.05$) than those fed tallow (Table 6). An interaction was noted for jejunum length ($P < 0.05$), broilers fed fish oil, tallow, or palm oil had similar jejunum length regardless of grain-based used, while broilers fed wheat-based diets supplemented with soybean oil had longer jejunum compared to the corn-soybean oil diets ($P < 0.05$).

Grain type had significant effects on the relative weight of pancreas (P < 0.01), gizzard ($P < 0.01$) and cecum ($P < 0.05$); with these organs being heavier in birds fed corn-based diets than those fed wheat-based diets. The relative empty weight of proventriculus was not influenced by dietary treatments (Table 7).

A grain type × fat source interactions was observed for the relative weight of liver ($P < 0.01$), and small intestine ($P < 0.01$). The liver weight was increased in birds fed wheat-based diets supplemented with soybean oil, and fish oil compared to their corn-based diets counterparts.

Inclusion of tallow in wheat-based diets decreased weight of small intestine compared to corn-based diet, although, broilers fed soybean, fish oil, and palm oil had similar small intestine weight regardless of grain used (Table 7).

Cecal Microbial Population

A significant interaction between grain type and fat source was observed for *Lactobacillus* spp. ($P < 0.01$) and the *total anaerobic bacteria* ($P < 0.01$) population in the cecum (Table 8), where birds fed wheat-based

Table 5. Influence of cereal type and fat source on the coefficient of apparent ileal digestibility (CAID) of fat, protein, ash, calcium and phosphorus in broiler starters on d 21.[1]

Fat source	Cereal type	Fat	Protein	Ash	Calcium	Phosphorus
Soybean oil	Corn	0.908	0.855[a]	0.650[a]	0.667	0.463
	Wheat	0.828	0.826[b]	0.648[a]	0.650	0.450
Fish oil	Corn	0.880	0.791[c]	0.622[a]	0.620	0.420
	Wheat	0.765	0.725[e]	0.605[b]	0.593	0.387
Tallow	Corn	0.876	0.814[bc]	0.673[a]	0.683	0.487
	Wheat	0.716	0.754[d]	0.675[a]	0.628	0.430
Palm oil	Corn	0.899	0.826[b]	0.662[a]	0.687	0.484
	Wheat	0.738	0.823[b]	0.647[a]	0.621	0.420
SEM		0.017	0.008	0.010	0.013	0.013
Main effects						
Cereal type						
Corn		0.890[a]	0.821	0.661	0.664[a]	0.463[a]
Wheat		0.761[b]	0.781	0.643	0.622[b]	0.421[b]
SEM		0.008	0.004	0.005	0.006	0.006
Fat source						
Soybean oil		0.876[a]	0.840	0.648	0.658[a]	0.456[a]
Fish oil		0.822[b]	0.758	0.633	0.606[b]	0.403[b]
Tallow		0.795[b]	0.784	0.674	0.655[a]	0.458[a]
Palm oil		0.818[b]	0.824	0.654	0.653[a]	0.451[a]
SEM		0.012	0.006	0.007	0.009	0.009
Probabilities, $P\leq$						
Cereal type		0.0001	0.0001	0.014	0.0001	0.0001
Fat source		0.001	0.0001	0.001	0.0007	0.0003
Cereal type × Fat source		0.075	0.001	0.018	0.228	0.217

[a-e]Means within a column followed by the different letters are - significantly different at P ≤ 0.05.
[1]Each value represents the mean of 6 replicates (2 birds/replicate).

Table 6. Influence of cereal type and fat source on the relative length (cm/kg body weight) of intestine parts in broiler starters on d 21.[1]

Fat source	Cereal type	Duodenum	Jejunum	Ileum	Small intestine[2]	Caeca
Soybean oil	Corn	21.87	44.95[ac]	46.5	113.3	10.58
	Wheat	21.75	51.06[a]	50.94	122.1	10.23
Fish oil	Corn	21.44	49.17[ab]	50.87	121.4	10.87
	Wheat	21.33	47.01[abc]	48.3	116.6	10.61
Tallow	Corn	19.39	43.84[c]	46.66	109.9	10.01
	Wheat	19.98	47.65[abc]	46.5	114.1	10.23
Palm oil	Corn	21.15	45.75[ac]	47.17	114.08	10.06
	Wheat	21.5	48.37[abc]	47.12	115.4	9.86
SEM		0.68	1.41	1.51	3.02	0.393
Main effects						
Cereal						
Corn		20.96	45.92	47.80	114.68	10.38
Wheat		21.13	48.52	48.21	117.08	10.22
SEM		0.341	0.708	0.759	1.47	0.201
Fat source						
Soybean oil		21.81[a]	48.00	48.72	117.75	10.40
Fish oil		21.38[a]	48.08	49.59	119.06	10.74
Tallow		19.68[b]	45.74	46.58	112.01	10.12
Palm oil		21.32[a]	47.06	47.15	114.73	9.95
SEM		0.482	0.995	1.04	2.14	0.284
Probabilities, $P\leq$						
Cereal type		0.716	0.013	0.702	0.27	0.597
Fat source		0.020	0.340	0.195	0.107	0.244
Cereal type × Fat source		0.943	0.043	0.141	0.166	0.90

[a-c]Means within a column followed by the different letters are significantly different at $P \leq 0.05$.
[1]Each value represents the mean of 6 replicates (2 birds/replicate).
[2]The small intestine is the sum of the three parts of the duodenum, jejunum and ileum.

diets supplemented with palm oil had the highest cecal population of *Lactobacillus* spp, whereas lowest population was observed in birds fed wheat-based diets supplemented with tallow, and fish oil, also corn-based diets supplemented with soybean oil ($P < 0.01$). In addition, the higher counts of total anaerobic bacteria were observed in birds fed wheat-based diets containing-soybean oil, and tallow, and the least count was observed in birds fed corn-based diets containing soybean oil ($P < 0.01$).

The main effect of fat source was numerically significant for *E.coli* count ($P = 0.06$), birds fed soybean oil, had lower *E.coli* count than those fed fish oil and tallow.

DISCUSSION

The fatty acid profile of the soybean oil, palm oil and tallow in the current experiment was compared to the ranges found in previous studies (Tancharoenrat et al.,

Table 7. Influence of cereal type and fat source on the relative weight (g/kg body weight) of organs in broiler starters on d 21.[1]

Fat source	Cereal	Liver	Pancreas	Proventriculus	Gizzard	Small intestine	Caeca
Soybean oil	Corn	22.65[e]	3.09	4.86	17.45	36.34[ab]	1.92
	Wheat	29.43[a]	2.64	4.42	12.84	36.62[ab]	1.86
Fish oil	Corn	25.44[d]	3.35	4.8	16.52	36.42[ab]	1.88
	Wheat	29.17[ab]	2.74	5.17	13.66	34.9[bc]	1.74
Tallow	Corn	27.12[bcd]	3.05	4.33	14.25	37.18[ab]	1.87
	Wheat	27.93[abc]	2.69	4.64	13.57	32.52[c]	1.63
Palm oil	Corn	25.23[d]	3.32	4.88	15.52	35.59[abc]	1.95
	Wheat	26.91[cd]	2.79	4.55	14.07	38.55[a]	1.75
SEM		0.71	0.133	0.23	0.834	1.06	0.11
Main effects							
Cereal type							
Corn		25.10	3.20[a]	4.71	15.93[a]	36.38	1.90[a]
Wheat		28.35	2.71[b]	4.69	13.53[b]	35.64	1.74[b]
SEM		0.351	0.066	0.118	0.415	0.531	0.553
Fat source							
Soybean oil		26.04	2.86	4.64	15.14	36.47	1.88
Fish oil		27.30	3.05	4.98	15.09	35.66	1.81
Tallow		27.52	2.87	4.48	13.90	34.85	1.75
Palm oil		26.07	3.05	4.71	14.80	37.06	1.85
SEM		0.504	0.094	0.168	0.590	0.751	0.078
Probabilities, P≤							
Cereal type		0.0001	0.0001	0.888	0.0002	0.333	0.046
Fat source		0.078	0.304	0.232	0.425	0.200	0.636
Cereal type × Fat source		0.0007	0.819	0.213	0.119	0.009	0.839

[a-c]Means within a column followed by the different letters are - significantly different at $P \leq 0.05$.
[1]Each value represents the mean of 6 replicates (2 birds/replicate).

Table 8. Influence of cereal type and fat source on cecal microbial population (log_{10} cfu/g) of broiler starters on d 21.[1]

Fat source	Cereal type	*Escherichia coli*	*Lactobacillus* spp.	Total anaerobic bacteria
Soybean oil	Corn	5.17	10.96[d]	8.87[d]
	Wheat	5.77	12.09[abc]	10.19[a]
Fish oil	Corn	6.2	11.71[cd]	9.8[ab]
	Wheat	6.1	11.03[d]	9.16[cd]
Tallow	Corn	6.14	12.7[ab]	9.49[bc]
	Wheat	6.09	11.12[d]	9.95[ab]
Palm oil	Corn	5.71	11.83[bcd]	9.98[ab]
	Wheat	6.13	12.94[a]	10.23[a]
SEM		0.27	0.307	0.202
Main effects				
Cereal type				
Corn		5.80	11.80	9.53
Wheat		6.02	11.79	9.88
SEM		0.135	0.153	0.101
Fat source				
Soybean		5.47	11.53	9.53
Fish		6.15	11.37	9.48
Tallow		6.12	11.92	9.72
Palm		5.92	12.39	10.10
SEM		0.192	0.217	0.143
Probabilities, $P \leq$				
Cereal type		0.271	0.978	0.019
Fat source		0.06	0.009	0.014
Cereal type × Fat source		0.498	0.0001	0.002

[a-d]Means within a column followed by the different letters are not - significantly different at $P \leq 0.05$.
[1]Each value represents the mean of 6 replicates (2 birds/replicate).

2014; Lindblom et al., 2019). Fish oil also contained a moderate amount of palmitic acid (23.4%) and high amount of oleic acid, which the last one was unexpected and probably indicated contamination with vegetable oils. The analyzed ether extract of corn-diets contained tallow was slightly higher than those supplemented with soybean oil (60, and 50 g/kg, respectively), also in wheat-diets, the ether extract content was higher in tallow and palm oil compared to soybean oil (48, 48, and 38 g/kg, respectively), the reason for which is not clear.

The objectives of the present study was to investigate whether (i) the growth response of broilers chickens to commonly used cereal grains (viscouse or nonviscouse grains) is influenced by the type of fats with widely differentiating fatty acid compositions (ii) the nutrient digestibility and microbial population of broilers are affected by the type of grain and fat.

Differences in corn and wheat nutrient composition, particularly arabinoxylan concentrations, suggest that the magnitude of broiler responses may vary with supplemental fat, as the growth response of young broilers to grain type is interacted by source of fat in the present study. These observed effects are more likely representative of a grain type effect rather than an interactive effect. In general, broilers fed corn diets supplemented with soybean oil, fish oil, tallow, and palm oil, had a 14.3%, 11%, 3.6%, and 11.33% improvement, respectively, in BWG compared to those fed wheat-based diets. One possible reason for poor growth performance in broilers fed wheat-based diets might have contributed to the lower AMEn of wheat-diets, as reported by Njeri et al. (2023), and Amerah et al. (2008) and increased intestinal digesta viscosity due to water-soluble and viscous NSP (Marquardt et al., 1994; Jia et al., 2009; Rodriguez et al., 2012). According to Amerah et al. (2008), pelleted corn diets having a greater AMEn than pelleted wheat diets (3140, *vs.* 2920 kcal/kg). Moreover, it has been hypothesized that pelleting leads to reduce the metabolizable energy content of wheat diets (Abdollahi et al., 2011). Amerah et al. (2007) also reported a decrease in AMEn of wheat-diets from 2995 to 2820 (kcal/kg) due to pelleting. Wheat contained more than double concentration of soluble NSP relative to corn (Kiarie et al., 2014), reflected in the higher jejunal digesta viscosity, which was confirmed in birds fed wheat-diets in the present study. The enhanced feed intake of birds fed wheat diets could be, to a major extent, attributed to the inhibition of assimilation of nutrients due to higher viscosity and lower AMEn (Amerah et al., 2008; Abdollahi et al., 2011) and that birds need to consume more feed to compensate the poor absorption of nutrients (Annison, 1993; Tancharoenrat et al., 2015). These findings indicating that the broiler chicken does indeed adjust the FI according to its energy requirement (Matthiesen et al., 2021). In addition, increased load of feed-origin viscous components such as soluble NSP and gelatinised starch (although minor) into the gut as a result of pelleting induced higher feed intake (Abdollahi et al., 2013). This finding is consistent with the results of study by Amerah et al. (2008), and Tancharoenrat et al. (2015), showing a higher feed intake of birds fed wheat-diets than those fed corn-diets.

There are apparently few studies where the inclusion of different fat sources has been monitored in chickens fed diets containing viscous and nonviscous grains (Dänicke et al., 1997; Tancharoenrat et al., 2015). Concentration of soluble NSP fiber and deleterious effects of increased intestinal viscosity are important considerations in poultry feed formulations, the present data suggest that type of fat and fatty acid profile might also be relevant, particularly in younger birds. In the present study, feeding corn-based diets made with soybean oil produced best responses in terms of weight gain and feed efficiency. Unexpectedly, the combination of tallow and corn produced notable results in pelleted diets. Under the conditions of the current study, the BW of birds fed tallow in corn-diets was similar to those fed soybean oil, this finding may be explained by the greater feed intake in tallow-fed birds to compensate for the compromised fat digestibility. Feed intake is the major factor driving body weight gain (Abdollahi et al., 2013). Supplemental tallow in wheat-diets improved FCR compared to fish oil, and palm oil. One possible reason is that, viscosity of digesta in wheat-based diets supplemented with soybean oil, was similar to those supplemented with tallow. In general, viscosity data of wheat-based diets support the hypothesis that the water-extractable part of the NSP can increase the viscosity of the intestinal contents. Although, heat treatment of wheat has also been suggested to increased solubilization a portion of insoluble NSP, without actual degradation of polymers, and increase soluble NSP concentration (de Vries et al., 2012). Zimonja et al. (2008) reported doubling of intestinal digesta viscosity of broiler chickens when wheat-diets were steam-pelleted at 90°C. In spite of observing the interaction between fat sources and grain type, the data of the present experiment does not confirm the exacerbating effect of saturated fatty acids in increasing the viscosity of digesta, as reported by Tancharoenrat et al., (2015).

Due to high levels of gluten proteins and pentosan/hexosan hemicelluloses in wheat, it has a higher dough-forming capability than corn, and works as good pellet binders (Jensen, 2000), resulted in higher PDI observed in the current study. However, Amerah et al. (2008) reported a better pellet durability in corn diets than wheat-diets, and Abdollahi et al. (2011) found a similar PDI between corn and wheat-diets.

The results of the current study clearly demonstrated the effects of formulating diet based on wheat throughout the GIT in pelleted-fed birds, including increased pH of gizzard and proventriculus, were associated with higher digesta viscosity, lower gizzard weight, and reduced pancreatic weight. The gizzard-stimulating effect of corn vs. wheat might have contributed to particle size difference due to grinding. Abdollahi et al., (2013) reported a greater reduction in proportion of particles larger than 2 mm due to pelleting in wheat- than maize-based diets. Also, in their study, the proportion of fine particles <0.075 mm in pelleted corn-diets was 25.69% compared to 35.28% in pelleted wheat-diets. It seems that pelleting-induced particle size reduction (Amerah et al., 2007; Abdollahi et al., 2011) is more pronounced in wheat-diets than corn-diets and is probably attributed to the narrow gap between the pellet rolls and the pellet die and the frictional force inside the die holes. These findings correspond with results of studies reported by Njeri et al. (2023), and Amerah et al., 2008, they found heavier gizzard in broilers fed pelleted corn-diets compared to wheat-diets.

Increased pancreatic weight due to feeding corn-based diets in the current study may improve the digestion process through enhancing enzyme secretion capacity (Williams et al., 2008; Moradi et al., 2021). The reduction of cecum size in wheat-based diets could be attributed to increase in intestinal viscosity, which could have limited the flow of fermentable substrates into the ceca (Choct et al., 1996, 1999) and variations in resistant starch concentrations between corn (25.2%), and wheat (13.65) (Bednar et al., 2001). Resistant starch is unavailable to endogenous amylase, and readily fermented by microbiota in the distal large intestine (Zijlstra, 2018), which reflected in higher ceca weight in corn-diets in the current study.

In general, wheat-feeding resulted in the highest relative length of jejunum in all fat sources. It seems that, increased length of jejunum can be a natural response of birds to enhanced viscosity and reduced nutrient digestibility in birds fed wheat-diets in the present study (Abdollahi et al., 2010b).

Ileal nutrient digestibility data showed formulating pelleted diets based on corn was effective on ileal fat, Ca, and P digestibility which corresponded with the increased gizzard weight (17.7%), and with lower gizzard and proventriculus pH compared to wheat. The lower pancreas and gizzard weights may, in part, account for the reduced CIAD of nutrients in birds fed wheat-based diets (Abdollahi et al., 2013). Also, the cage effect of water-unextractable NSP in the cell walls can impair nutrient availability by blocking the access to the cell contents for endogenous digestive enzymes (Bedford, 2002).Well-developed gizzard may reduce pH through providing more time for the secretion of hydrochloric acid (Moradi et al., 2021), by enhancing reverse peristalsis that serve to re-expose the digesta to hydrochloric acid and digestive enzymes, and increased levels of cholecystokinin release (Svihus et al., 2004), ultimately improved nutrient digestibility. Highest CAID of protein was observed in birds fed diets made from corn and soybean oil, due mainly to decreased pH in foregut, may stimulate pepsin activity (Yokhana et al., 2016) and enhances the denaturation and hydrolysis of dietary proteins. Moreover, changes in microbiota population due to presence of NSP in wheat-based diets may account for reduced digestibility of fat in wheat-based diets. Changes in bacterial population in the lower intestinal tract may increase the deconjugation of bile acids, and lowering fat retention. Soluble NSP may also interfere with the glycocalyx of the intestinal brush border and thicken the unstirred layer, thereby providing an improved habitat for microbiota (Campbell et al., 1983). Similar to this finding, Tancharoenrat et al. (2015) found a lower fat retention coefficient for wheat and soybean oil than maize and tallow, respectively. In contrast, Kiarie et al. (2014), reported a higher ileal digestibility of fat and CP in birds fed wheat-diets. Inclusion of corn improved P digestibility, a finding which is consistent with that of Kiarie et al. (2014) who reported an increase in P retention of corn diets from 0.831 to 0.922 compared to wheat diets.

The higher fat digestibility of soybean oil compared to fish oil, tallow and palm oil (0.876 vs. 0.822, 0.795, and 0.818 respectively) is in agreement with the findings by Tancharoenrat et al. (2014), who reported greater coefficients of total tract retention (0.840 vs. 0.685) and ileal digestibility of fat (0.892 vs. 0.692) in birds fed soybean oil compared to tallow. Linoleic acid was absorbed throughout the intestinal tract starting from the duodenum, whereas the absorption of palmitic, stearic and oleic acids started only in the jejunum (Tancharoenrat et al., 2015). Beef tallow has a lower fat digestibility and a lower AME content than vegetable oils, and these have been attributed to its higher content of long-chain saturated fatty acids (Blanch et al., 1995), Palm oil due to the high saturated fatty acid palmitic acid (C16:0) content are less digestible than fats high in medium chain fatty acids or unsaturated fatty acids (Smink et al., 2008). It is believed that saturated fatty acids (Palmitic and stearic acids) are less digestible than unsaturated fatty acids (oleic and linoleic acids) (Dänicke et al., 2000; Smink et al., 2008). The nonpolarity, less incorporation into micelles and the requirement to be emulsified before absorption lowered the digestibility of palmitic and stearic (Tancharoenrat et al., 2014), as indicated by lower fat digestibility in tallow and palm oil compared to soybean oil in the present study. Fat digestibility of fish oil was lower than soybean oil, tallow, and palm oil, may indicate oxidative spoilage in fish oil as a result of conditioning temperature, which contains high amounts of unsaturated fatty acids. The lowest value of protein digestibility was observed in wheat treatment supplemented with tallow, may partly account for high intestinal viscosity caused by soluble NSP and exacerbated by high concentrations of saturated fatty acids in tallow, which reduced gut motility and decreased rate of diffusion and transportation of emulsion droplets, lipase, mixed micelles, bile salts and fatty acids in the gut lumen (Dänicke, 2001).

Broilers fed wheat-based diets containing soybean oil and palm oil showed the highest count of total anaerobic bacteria, indicating that there was more substrate for fermentation. According to Apajalahti et al., (2004), the NSP composition of feed ingredients can bring about significant changes to the microbial ecology of the gut. The lower ileal digestibility of fat, calcium and phosphorus in wheat-diets in the current experiment suggests that lower absorption of nutrients in the proximal gut, results in an enhancement in the quantity of nutrients in the terminal ileum and ceca that are available as substrates for anaerobic bacteria. Moreover, heat treatment of wheat has also been suggested to solubilize a portion of insoluble NSP and increase soluble NSP concentration. The reduction of the population of *Lactobacillus* spp. in cecal digesta of broilers fed wheat-based diets containing soybean oil, and palm oil in the present study is consistent with the observation of Rodriguez et al. (2012), who found higher number of *Lactobacilli* and *Escherichia Coli* in the digesta of broilers fed wheat and barley versus those fed corn diets.

In summary, the effect of grain type on weight gain, FCR and CIAD of protein and cecal microbiota depends on the oil sources in the diet and that use of corn and soybean oil advantageous in terms of broiler performance. Feeding pelleted corn-based diets improved performance and development of gut (gizzard and pancreas), and lowered gizzard pH, and increased digestibility of fat, Ca, and P. The observed changes in cecal microbial population imply that it is worthwhile to measure the grain source effects on cecal microbiota development and investigation the fermentative patterns may be beneficial for optimizing cecal VFA production in broilers.

DSICLOSURES

There is no conflict of interest.

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