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Evaluation of the Effect of Different Levels of Fiber and Fat on Young Broilers' Performance, pH, and Viscosity of Digesta Using Response Surface Methodology

Research Article

F. Aziz-Aliabadi[1], A. Hassanabadi[1*], A. Golian[1], S. Zerehdaran[1] and H. Noruzi[1]

[1] Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

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*Correspondence E-mail: hassanabadi@um.ac.ir

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ABSTRACT

The main purpose of this study was to investigate the effect of different levels of fiber and fat on performance, pH, and viscosity of digesta in young broilers. A total of 420 one-day-old male chicks Ross 308 were assigned to 60 cages of seven birds each. The birds fed with 15 experimental diets produced by central composite design (CCD), containing three levels of sugar beet pulp (SBP: 0.00, 1.75, 3.50%), tallow (T: 0.00, 0.50, 1.00%) and soybean oil (SO: 0.00, 0.50, 1.00%) from 0 to 14 d. The results showed that maximum average daily body weight gain (ADG: 42.10 g/b/d) and minimum feed conversion ratio (FCR: 0.99) were observed with diet containing 0.35% SBP, 0.00% T and 0.51% SO. Maximum (4.64) and minimum (3.01) pH in gizzard were found with diets containing 0.00% SBP, 0.51% T, 0.27% SO and 1.70% SBP, 0.21% T and 0.00% SO, respectively. The highest (6.94) and lowest (5.86) pH in ileum contents were obtained with diets comprising 0.00% SBP, 1.00% T, 0.43% SO and 1.47% SBP, 0.00% T, and 0.00% SO, respectively. Maximum (3.54) and minimum (1.50) viscosity in ileal digesta were observed with diets containing 3.50% SBP, 1.00% T, and 1.00% SO and 0.00% SBP, 0.00% T and 0.53% SO, respectively. This experiment has revealed that, the CCD and response surface methodology (RSM) have the efficiency to describe the relationships between different levels of SBP, T and SO and the ability to predict the optimal point of the level of diet ingredients in order to achieve the best performance. With increasing the amount of SBP and decreasing the level of T and SO in the diets, the pH of digesta in the gizzard and ileum decreased. The ileum digesta viscosity increased with increasing SBP, T, and SO levels.

KEY WORDS chickens, digestion, soybean oil, sugar beet pulp, tallow.

INTRODUCTION

The addition of fiber compounds to the diet may lead to increased digestibility (Gonzalez-Alvarado *et al.* 2007) and improvements in nutrient digestion (Amerah *et al.* 2009), growth performance (Kalmendal *et al.* 2011) and ultimately animal welfare (Van Krimpen, 2009). Dietary fiber has traditionally been considered a dilutor and has anti-nutrients properties. Nevertheless, moderate amounts of fiber have been shown to improve gastrointestinal advancement, secretion of enzymes, and nutrients digestibility in birds (Mateos *et al.* 2012). Nevertheless, along with their natural benefits, the inclusion of indigestible (or poorly digestible) polysaccharides in poultry diets may be associated with negative features, such as impaired energy regulation by feed intake (Williams and Bollella, 1995), reduced mineral availability (Zoppi *et al.* 1982), problems with fat metabolism (Smits *et al.* 1997) due to the impact of fiber on the bile acids and cholesterol (Kongo-Dia-Moukala *et al.* 2011). In the sugar industry, the remaining product after distillation is sugar beet pulp (SBP), according to Voelker and Allen (2003) report, it contains almost 250 g/kg of pectin as soluble fiber and 400 g/kg of neutral detergent fiber (NDF). It has been reported that each kilogram of dried sugar beet pulp (SBP) owns 9.7-11.2 MJ of metabolic energy and 102.7 gram of protein (Koschayev *et al.* 2019). It comprises somewhat great calcium, sodium, magnesium and trace elements. Sugar beet pulp has good digestibility in pigs, due to the negligible amount of lignin (Koschayev *et al.* 2019). Fiber improves the passage of substances throughout the gastrointestinal tract (GIT) and changes the pH of various digestive organs and can affect the microbial growth and production pattern of their products in the GIT (Rochell *et al.* 2012).

The use of fats in poultry feeds has many profits. Fats have high energy, therefore the main way to increase the energy value of the diets is to use them. Poultry fat, tallow (T), yellow grease and vegetable oils such as sunflower oil, soybean oil (SO), or palm oil are important sources of fat (Firman *et al.* 2010). Young chickens cannot efficiently use fat sources, especially animal sources (Atteh and Leeson, 1985). Lipase, along with colipase and bile salts, is involved in the efficient and beneficial breakdown of dietary fats, and deficiency of any of them can impair the metabolism of fats, especially saturated animal fats (Noy and Sklan, 1995). Various studies have shown that non-starch polysaccharides (NSP) in the diet of broilers negatively affect fat retention (Vranjes and Wenk, 1995). In general, the effect of dietary fat on the microbial population of the GIT is probably due to the different effects of different sources of fatty acids on digesta viscosity, pH, time of nutrient transfer in the GIT (Laflamme *et al.* 2011).

The problems and issues of determining the optimal level of essential nutrients in poultry diets are described in detail by Lerman and Bie (1975). Investigating the effect of nutrients on the broilers' performance relies on the type of trial design and statistical analyses (Ahmadi and Golian, 2011). The response surface methodology (RSM) is based on a specific test design in which several factors affecting one or more outputs are studied. The data from the experimental design are fitted to a quadratic polynomial model. Using the response surface methodology (RSM) framework, the optimal conditions corresponding to the desired response are obtained by performing fewer treatments than conventional full factorial methods (one or two variables in time). This saves time and money and better controls experimental conditions and materials (Gulati *et al.* 2010).

The GIT function consists of digestion, absorption, and conservation, and in order to perform these functions, the intestinal anatomy is well adjusted (Jha and Mishra, 2021). Moreover, the improvement of the GIT mainly within the primary post-hatching time of broiler chicks is a considerable status of pick up (Jha *et al.* 2019). There are numerous studies on the effect of dietary fiber and fat on the function and condition of the digestive tract of broilers (Jimenez-Moreno *et al.* 2011; Kimiaeitalab *et al.* 2017). However, there was no study to express the relationship between fiber and fat mathematically. Therefore, this study was conducted to investigate the effect of different amounts of fiber and various amounts and sources of fat on the 14-day-old young broilers' performance, pH, and viscosity of digesta using response surface methodology.

MATERIALS AND METHODS

Birds, diets, and housing

In order to perform the experiment, 420 one-day-old Ross 308 male broiler chickens with the mean weight of 46.45 ± 0.97 g were purchased from a commercial hatchery and haphazardly assigned to 60 cages of seven birds each. The birds were fed with the 15 diets comprising of three amounts of SBP (0.00, 1.75, 3.50%), T (0.00, 0.50, 1.00%), and SO (0.00, 0.50, 1.00%), from 0 to 14 d of age (the arrangement of the treatments was done based on the central composite design (CCD) (Tables 1 and 2)). According to the arrangement of the CCD and the RSM, each treatment is repeated three times and the treatment containing intermediate levels of SBP, T, and SO (1.75, 0.5, 0.5) is repeated 18 times. The nutrient composition of feed ingredients published (NRC, 1994) was utilized for feed formulation. Tables 3 and 4 illustrate the components and nutrient compound of diets. All birds were nourished pursuant to the Ross 308 strain requirements (Aviagen, 2014). Feeds' neutral detergent fiber (Mertens *et al.* 2002), acid detergent fiber and insoluble fiber were determined (AOAC, 2005). By subtracting the amount of insoluble fiber from the total crude fiber, the amount of soluble fiber was obtained. Gas chromatography was applied to determine the fatty acid profiles of T and SO used in diets (Table 5). The rearing place temperature was put at 32 ˚C in the first two days, which declined by 0.5 ˚C each day to reach 26.0 ˚C at the end of the study on day 14. The light and darkness scheme was considered 18 h light and 6 h dark all over the study period.

Performance

Birds were weighed at the beginning and end of the study (0 d and 14 d of age). The average daily body weight gain (ADG) was measured from the birds' weight gain in each group. Feed intake (FI) was determined by deducing the residuary feed from the provided feed in every replicate during the experiment period.

Table 1 Dietary nutrient concentrations used in central composite design response surface methodology (RSM) to feed broiler chicks from 0 to 14 d of age

Item (% of diet)	Level		
	1	0	-1
Sugar beet pulp (SBP)	3.50	1.75	0.00
Tallow (T)	1.00	0.50	0.00
Soybean oil (SO)	1.00	0.50	0.00

Table 2 Sugar beet pulp (SBP), Tallow (T), and Soybean oil (SO) concentrations in experimental diets prepared according to a central composite design (3 levels, 3 factors) and corresponding experimental response values for average daily gain (ADG), feed conversion ratio (FCR), gizzard and ileal digesta pH, and ileal digesta viscosity in broiler chicks

Treatment numbers	Replications[1]	Factors (% of diet)			Experimental response									
					0-14 d of age				14 d of age					
		SBP	T	SO	ADG (g/bird/d)	±SD	FCR	±SD	Gizzard digesta pH	±SD	Ileal digesta pH	±SD	Ileal digesta viscosity	±SD
1	18	1.75	0.50	0.50	28.15	2.30	1.33	0.16	3.62	0.07	6.20	0.07	1.98	0.32
2	3	3.50	1.00	0.00	24.11	1.98	2.01	0.21	3.02	0.07	5.90	0.04	3.11	0.17
3	3	0.00	0.00	1.00	38.23	1.32	0.99	0.08	4.32	0.10	6.84	0.04	1.70	0.24
4	3	3.50	1.00	1.00	22.09	1.50	2.16	0.25	3.03	0.06	5.95	0.01	3.53	0.18
5	3	0.00	1.00	1.00	27.99	1.07	1.20	0.03	3.60	0.15	6.17	0.15	1.93	0.17
6	3	1.75	0.00	0.50	36.76	2.17	1.15	0.08	3.70	0.12	6.23	0.02	1.97	0.01
7	3	0.00	1.00	0.00	31.02	1.78	1.18	0.07	3.61	0.04	6.25	0.07	1.88	0.09
8	3	0.00	0.50	0.50	38.25	2.85	0.99	0.03	4.70	0.09	6.77	0.02	1.52	0.05
9	3	1.75	0.50	0.00	29.35	1.51	1.25	0.14	3.74	0.12	6.11	0.12	2.13	0.07
10	3	1.75	1.00	0.50	28.21	0.41	1.37	0.12	3.62	0.02	6.10	0.05	2.06	0.14
11	3	3.50	0.00	0.00	27.37	1.35	1.49	0.04	3.32	0.19	5.98	0.02	2.81	0.20
12	3	1.75	0.50	1.00	29.12	0.92	1.47	0.08	3.62	0.03	6.11	0.05	2.39	0.42
13	3	3.50	0.00	1.00	27.80	0.34	1.59	0.09	3.26	0.19	5.91	0.01	2.88	0.06
14	3	0.00	0.00	0.00	39.10	0.88	0.95	0.05	4.58	0.37	6.86	0.07	1.77	0.20
15	3	3.50	0.50	0.50	24.95	0.41	1.87	0.10	3.22	0.19	6.06	0.05	3.19	0.60

[1] A total of 60 run numbers were provided.

Table 3 Composition of the experimental diets

Ingredient (%, as-fed basis)	Experimental diets number[1]														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Corn	57.91	57.96	57.87	55.11	55.60	58.99	58.53	58.21	59.39	56.84	60.11	56.38	57.29	60.82	57.62
Soybean meal, 44% CP	27.47	27.07	27.87	27.77	28.26	27.26	27.61	27.76	27.16	27.69	26.61	27.76	27.32	27.22	27.20
Dicalcium phosphate	1.94	1.93	1.94	1.94	1.94	1.93	1.94	1.94	1.93	1.94	1.93	1.94	1.94	1.94	1.93
Limestone	1.02	0.99	1.05	0.99	1.04	1.02	1.04	1.04	1.02	1.01	0.99	1.01	0.99	1.05	0.99
Soybean oil	0.50	0.00	1.00	1.00	1.00	0.50	0.00	0.50	0.00	0.50	0.00	1.00	1.00	0.00	0.50
Tallow	0.50	1.00	0.00	1.00	1.00	0.00	1.00	0.50	0.50	1.00	0.00	0.50	0.00	0.00	0.50
Sugar beet pulp	1.75	3.50	0.00	3.50	0.00	1.75	0.00	0.00	1.75	1.75	3.50	1.75	3.50	0.00	3.50
Sand	2.05	0.70	3.40	1.83	4.30	1.70	3.03	3.20	1.40	2.40	0.00	2.80	1.10	2.10	0.90
Vitamin and mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine HCl	0.49	0.49	0.49	0.48	0.48	0.49	0.49	0.49	0.49	0.49	0.50	0.48	0.49	0.50	0.49
DL-methionine	0.31	0.31	0.32	0.32	0.32	0.31	0.31	0.31	0.31	0.32	0.31	0.32	0.31	0.31	0.31
L-threonine	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.17	0.16
$NaHCO_3$	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Salt (NaCl)	0.25	0.24	0.25	0.25	0.25	0.24	0.24	0.24	0.24	0.25	0.24	0.25	0.25	0.24	0.25

[1] Fifteen diets of central composite design containing: 1 (SBP: 1.75, T:0.50, SO:0.50%); 2 (SBP: 3.50, T:1.00, SO:0.00%); 3 (SBP: 0.00, T:0.00, SO:1.00%); 4 (SBP: 3.50, T:1.00, SO:1.00%); 5 (SBP: 0.00, T:1.00, SO:1.00%); 6 (SBP: 1.75, T:0.00, SO:0.50%); 7 (SBP: 0.00, T:1.00, SO:0.00%); 8 (SBP: 0.00, T:0.50, SO:0.50%); 9 (SBP: 1.75, T:0.50, SO:0.00%); 10 (SBP: 1.75, T:1.00, SO:0.50%); 11 (SBP: 3.50, T:0.00, SO:0.00%); 12 (SBP: 1.75, T:0.50, SO:1.00%); 13 (SBP: 3.50, T:0.00, SO:1.00%); 14 (SBP: 0.00, T:0.00, SO:0.00%) and 15 (SBP: 3.50, T:0.50, SO:0.50%).

[2] Provided the followings per kg of diet: vitamin A (trans-retinyl acetate): 12500 U; vitamin D_3 (cholecalciferol): 5000 U; vitamin E (D L-α tocopherol acetate): 80 U; vitamin K (menadione): 3.20 mg; Riboflavin: 8.6 mg; Pantothenic acid (D-Ca pantothenate): 18.6 mg; Pyridoxine (pyridoxine-HCl): 4.86 mg; Thiamin: 3.2 mg; vitamin B_{12} (cyanocobalamin): 0.02 mg; Biotin: 0.25 mg; Folic acid: 2.2 mg; Nicotinic acid: 62.51 mg; Ethoxyquin (antioxidant): 2.5 mg; Fe: 20.23 mg; Zn: 110 mg; Mn: 120 mg; Cu: 16 mg; I: 1.25 mg and Se: 0.30 mg.

SBP: sugar beet pulp; T: tallow and SO: soybean oil.

Feed conversion ratio (FCR) was calculated as feed used up (gram) by whole broilers apportion by body weight gain (gram). Mortality was noted every day. After operation a experiment by CCD, a data set congaing 60 data lines was obtained and analysed.

Digesta pH in gizzard and ileum

At the end of the experimental period (day 14), two birds were selected from each cage and in order to measure the pH of ileum and gizzard contents, their ileum and gizzard were separated and one gram of digestive material was weighed and it was vortexed with 9 mL of distilled water in a Falcon tube for 5 minutes.

The pH of the solution was measured using a pH meter electrode (WTW Multi 3420 set; Pang and Applegate, 2007).

Table 4 Chemical composition of the experimental diets (calculated and determined analysis, %)

Calculated analysis (%)	Experimental diets number[1]														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
AME (kcal/kg)	2860	2860	2860	2860	2860	2860	2860	2860	2860	2860	2860	2860	2860	2860	2860
Crude protein	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00	21.00
Ether extract	3.07	2.59	3.54	3.49	3.46	3.11	2.56	3.05	2.62	3.03	2.67	3.51	3.57	2.65	3.08
Crude fiber	3.53	3.78	3.28	3.77	3.26	3.54	3.28	3.28	3.54	3.53	3.80	3.52	3.79	3.30	3.78
Insoluble fiber	8.83	9.73	7.93	9.62	7.82	8.88	7.94	7.94	8.89	8.78	9.82	8.76	9.71	8.06	9.72
Soluble fiber	1.90	2.07	1.73	2.06	1.71	1.91	1.73	1.73	1.91	1.90	2.08	1.89	2.07	1.74	2.07
Acid detergent fiber	3.32	3.71	2.94	3.69	2.91	3.34	2.94	2.94	3.34	3.31	3.73	3.31	3.71	2.96	3.71
Neutral detergent fiber	8.55	9.18	7.93	8.96	7.74	8.64	7.97	7.95	8.67	8.47	9.35	8.43	9.13	8.17	9.16
Lysine	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33	1.33
Methionine	0.66	0.65	0.66	0.66	0.66	0.65	0.65	0.66	0.65	0.66	0.66	0.66	0.66	0.65	0.66
Met + Cys	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Threonine	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Tryptophan	0.25	0.25	0.25	0.25	0.26	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calcium	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Available phosphorus	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Sodium	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Chlorine	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Determined analysis (%)															
Crude fiber	3.48	3.82	3.25	3.80	3.24	3.51	3.23	3.25	3.61	3.59	3.82	3.50	3.81	3.28	3.74
Insoluble fiber	8.86	9.70	7.88	9.65	7.86	8.83	7.90	7.91	8.93	8.81	9.75	8.73	9.70	8.00	9.83
Soluble fiber	1.93	2.14	1.66	2.14	1.72	1.70	1.76	1.74	1.95	1.93	2.11	1.80	2.12	1.70	2.15
Acid detergent fiber	3.37	3.66	3.05	3.73	2.87	3.30	2.98	2.95	3.43	3.34	3.77	3.32	3.71	3.00	3.76
Neutral detergent fiber	8.59	9.21	7.90	9.07	7.70	8.52	8.00	7.97	8.60	8.49	9.30	8.50	9.15	8.20	9.24

SBP: sugar beet pulp; T: tallow; SO: soybean oil and AME: apparent metabolizable energy.

[1] Fifteen diets of central composite design containing: 1 (SBP: 1.75, T:0.50, SO:0.50%); 2 (SBP: 3.50, T:1.00, SO:0.00%); 3 (SBP: 0.00, T:0.00, SO:1.00%); 4 (SBP: 3.50, T:1.00, SO:1.00%); 5 (SBP: 0.00, T:1.00, SO:1.00%); 6 (SBP: 1.75, T:0.00, SO:0.50%); 7 (SBP: 0.00, T:1.00, SO:0.00%); 8 (SBP: 0.00, T:0.50, SO:0.50%); 9 (SBP: 1.75, T:0.50, SO:0.00%); 10 (SBP: 1.75, T:1.00, SO:0.50%); 11 (SBP: 3.50, T:0.00, SO:0.00%); 12 (SBP: 1.75, T:0.50, SO:1.00%); 13 (SBP: 3.50, T:0.00, SO:1.00%); 14 (SBP: 0.00, T:0.00, SO:0.00%) and 15 (SBP: 3.50, T:0.50, SO:0.50%).

Digesta viscosity in ileum

On day 14, after euthanizing the birds, the ileum was removed, and its contents were drained into the test tubes. After separating the liquid part, the viscosity of ileal digesta was determined using a viscometer in centipoise (cP) (Garcia *et al*. 2008).

Statistical analyses

The most commonly used model in RSM analysis is the following second-order polynomial equation (Box *et al.* 1978):

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum\sum_{i<j} \beta_{ij} x_i x_j + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \varepsilon$$

Where:

y: response.

k: number of input factors (k=3).

x_i: input factors (SBP, T, and SO%).

β_0: constant term.

β_i: linear parameters' coefficients.

β_{ij}: interaction parameters' coefficients.

β_{ii}: quadratic parameters' coefficients.

ε: residual associated with the experiment.

The empiric data (60 data lines) gathered by CCD were fitted to the second order polynomial equation using Minitab 17 software. A strategy of the sensibility analysis was accomplished on the RSM models to find which model is noticed more substantial within the modeling method by ANOVA and the model parameters' corresponding absolute t-value. A more significant model term (linear, quadratic, or interaction of obtained model related to dietary SBP, T, and SO concentration) has a greater absolute t-value.

RESULTS AND DISCUSSION

Performance

Average daily gain (ADG) and feed conversion ratio (FCR) (0 to 14 d of age)

The resulting polynomial equation from the raw experimental data for ADG (R^2=0.88; root MSE=1.91) and FCR (R^2=0.90; root MSE=0.10) was as follows:

ADG (g/bird)= 40.08 – 5.02 × SBP – 16.49 × T + 7.94 × SO + 0.39 × SBP × SBP + 7.24 × T × T – 7.77 × SO × SO + 1.56 × SBP × T + 0.10 × SBP × SO –2.08 × T × SO

FCR= 0.93 + 0.04 × SBP + 0.36 × T – 0.17 × SO + 0.03 × SBP × SBP – 0.17 × T × T + 0.19 × SO × SO + 0.07 × SBP × T + 0.03 × SBP × SO + 0.08 × T × SO

The estimated parameters for SBP, T, T × T, SO × SO, and SBP × T terms in the ADG model, and SBP, T, SO, SBP×SBP and SBP × T terms in the FCR model were significant (P<0.05). The fit of the RSM model was also represented by the R^2 value, which was 0.88 and 0.90 for ADG and FCR model, respectively, showing that almost 90% of the variations in the responses could be discovered by the model. The quota of each effect (linear, quadratic, and interaction) in the RSM model to the statistical fit (in term of R^2) is given in Table 6. In the ADG model the linear effects have greater role (partial R^2=0.71) to show available variation in the birds' response. It was followed by quadratic (partial R^2=0.11) and interaction (partial R^2=0.07) terms. In the FCR model the linear (partial R^2=0.80) and quadratic (partial R^2=0.07) effects had role to present the available variation in feed efficiency, and the interactions (partial R^2=0.03) had a little quota (Table 6). The highest ADG (42.10) and lowest FCR (0.99) were obtained with dietscomprising0.35% SBP, 0.00% T and 0.51% SO.

The coefficient of regression, t-value and P-value are illustrated in Table 7. The absolute *t*-value shows to what extent each model term contributed to the statistical fit. Thereby, the higher absolute t-value, the more considerable the corresponding factor. Lack of fit for ADG model was notable, representing that a more complex modeling method or other checking with extra factors should be made. Nevertheless, the lack of fit for FCR model was not significant, indicating that the observed data are in suitable accordance with the model (Table 6).

Gizzard digesta pH

Average respond quantities for digesta pH of gizzard according to CCD are shown in Table 2. The polynomial equations exploited from raw experimental data for digesta pH of gizzard (R^2=0.82; root MSE=0.17) was obtained as follows:

Digesta pH of gizzard= 4.61 – 0.59 × SBP – 0.26 × T + 0.26 × SO + 0.06 × SBP × SBP – 0.57 × T × T – 0.49 × SO × SO + 0.16 × SBP × T + 0.03 × SBP × SO + 0.16 × T × SO

The assessment parameters for SBP, T, SBP × SBP, T × T and SBP × T terms were significant (P<0.05). The quota of each type of effect in the RSM model (linear, quadratic, and interaction) to the statistical fit (in terms of R^2) are shown in Table 8. The linear effects have a premier portion (R^2=0.75) to elucidate available variation in the response of chicks, while interaction (R^2=0.04) and quadratic effects (R^2=0.03) had a lesser role. Maximum (4.64) and minimum (3.01) digesta pH of gizzard were observed in diets containing 0.00% SBP, 0.51% T, 0.27% SO and 1.70% SBP, 0.21% T, and 0.00% SO, respectively.

The coefficient of regression and corresponding ***t***- value and P-value are represented in Table 9. The significance of lack of fit for model show that a more twisted modeling method or another test with extra factors should be made (Table 8).

Ileal digesta pH

Average respond quantities for digesta pH of ileum according to CCD are shown in Table 2. The polynomial equations obtained from raw experimental data for digesta pH of ileum (R^2=0.92; root MSE=0.07) was discovered as follows:

Digesta pH of ileum= 6.87 – 0.49 × SBP – 0.38 × T + 0.38 × SO + 0.06 × SBP × SBP – 0.23 × T × T – 0.44 × SO × SO + 0.17 × SBP × T + 0.01 × SBP × SO + 0.03× T × SO

The estimated parameters for SBP, T, SBP × T, SBP × SBP and SO × SO terms were significant (P<0.05). The allotment of each type of trace in the RSM model (linear, quadratic, and interaction) to the statistical fit (in terms of R^2) are displayed in Table 8. In the case of the ileal digesta pH model, linear components (R^2=0.73) had the most participation, followed by interactions (R^2=0.12) and quadratic effects (R^2=0.07).

Maximum (6.94) and minimum (5.86) digesta pH of ileum were found with the diet comprising 0.00% SBP, 1.00% T, 0.43% SO and 1.47% SBP, 0.00% T, and 0.00% SO, respectively. The regression coefficient estimates and respective t-value and P-value are depicted in Table 9. Lack of fit for model was significant, indicating that a more complex models or another test with supplementary factors should be done (Table 8).

Digesta viscosity in ileum

Average respond values for digesta viscosity in ileumconforming to CCD are indicated in Table 2. The polynomial equations adopted from raw experimental data for digesta viscosity (R^2=0.80; root MSE=0.26) was procured as follows:

Digesta viscosity in ileum= 6.87 – 0.49 × SBP – 0.38 × T + 0.38 × SO + 0.06 × SBP × SBP – 0.23 × T × T – 0.44 × SO × SO + 0.17 × SBP × T + 0.01 × SBP × SO + 0.03× T × SO

The estimated parameters for SBP, T and SBP × SBP terms were significant (P<0.05). The portion of each type of effect in the RSM model (linear, quadratic, and interaction) to the statistical fit (in terms of R^2) are demonstrated in Table 8. In the case of the ileal digesta pH model, linear effects (R^2=0.68) had the highest involvement, followed by quadratic effects (R^2=0.11) and interactions (R^2=0.01).

Table 5 Analysis of some major fatty acids (FAs) in tallow and soybean oil (%)

Fatty acids (%)	Tallow	Soybean oil
Stearic acid (C_{18}:0)	29.29	3.79
Palmitic acid (C_{16}:0)	27.34	11.54
Lauric acid (C_{14}:0)	3.04	0.5
Oleic acid (C_{18}:1)	28.51	23.51
Linoleic acid (C_{18}:2)	3.81	52.78
Linolenic acid (C_{18}:3)	0.51	6.95
Saturated FAs/unsaturated FAs ratio	60:33	16:84

Table 6 Analysis of variance on the experimental results along with the contribution of each type of effect (linear, quadratic, and interaction) to the statistical fit in response surface model for average daily gain (ADG) and feed conversion ratio (FCR) in broiler chicks from 0 to 14 d of age

Source of variation	ADG model				FCR model		
	df	Sum of squares	R^2	P-value	Sum of squares	R^2	P-value
Linear	3	1154.41	0.71	< 0.0001	5.32	0.80	< 0.0001
Quadratic	3	61.62	0.11	< 0.01	0.18	0.07	< 0.01
Interaction	3	51.71	0.07	< 0.01	0.14	0.03	0.01
Total model (regression)	9	1267.73	0.89	< 0.0001	5.64	0.90	< 0.0001
Lack of fit	5	128.99		< 0.0001	0.10		0.15
Pure error	45	52.89			0.53		
Total error	50	181.88			0.63		

Table 7 Estimated parameters of response surface model for average daily gain (ADG) and feed conversion ratio (FCR) in broiler chicks from 0 to 14 d of age

Quadratic model term[1]	ADG model					FCR model				
	Estimated parameter from raw data	SE	t- value	P-value	Estimated parameter from coded data	Estimated parameter from raw data	SE	t- value	P-value	Estimated parameter from coded data
Intercept	40.08	0.37	76.69	< 0.0001	29.03	0.93	0.02	59.20	< 0.0001	1.32
SBP	-5.02	0.34	-14.09	< 0.0001	-4.90	0.04	0.02	18.22	< 0.0001	0.37
T	-16.49	0.34	-10.85	< 0.0001	-3.78	0.36	0.02	8.88	< 0.0001	0.18
SO	7.94	0.34	-0.98	0.33	-0.34	-0.17	0.02	3.18	< 0.01	0.06
SBP × SBP	0.39	0.66	1.83	0.07	1.21	0.03	0.03	2.54	0.01	0.09
T × T	7.24	0.66	2.72	< 0.01	1.80	-0.17	0.03	-1.12	0.26	-0.04
SO × SO	-7.77	0.66	-2.93	< 0.01	-1.94	0.19	0.03	1.25	0.21	0.04
SBP × T	1.56	0.38	3.52	< 0.01	1.36	0.07	0.02	2.98	< 0.01	0.06
SBP × SO	0.10	0.38	0.24	0.81	0.09	0.03	0.02	1.41	0.16	0.03
T × SO	-2.08	0.38	-1.34	0.18	-0.52	0.08	0.02	0.88	0.38	0.02

SBP: sugar beet pulp; T: tallow and SO: soybean oil.

Maximum (3.54) and minimum (1.50) digesta viscosity were obtained with the diet including 3.50% SBP, 1.00% T, 1.00% SO and 0.00% SBP, 0.00% T, and 0.53% SO, respectively. The coefficient of regression, relevant ***t***- value and P-value are illustrated in Table 9. The lack of fit for digesta viscosity model was not significant, highlighting that the observed data are in expedient compromise with the model (Table 8).

Different studies with broiler chickens, layers, and turkeys has illustrated that the use of moderate levels of fiber in the birds' diet is advantageous for the development and optimal functioning of GIT and ameliorates the digestibility of nutrients and the birds' performance (Guzman *et al.* 2015; Jimenez-Moreno *et al.* 2013; Roma *et al.* 1999).

Jimenez-Moreno *et al.* (2009) in the study of the effect of different sources of dietary fiber and fat on the broiler chickens' performance stated that adding fiber improved body weight gain and feed conversion ratio. The increase in nitrogen retention, ether extract and apparent metabolizable energy was more noticeable for chickens consuming oat hulls compared to sugar beet pulp. They ascribed the profitable effect of fiber on the growth performance to its useful impact in meliorating the small intestinal histology (Rezaei *et al.* 2012). Since many elements are involved in the response of birds to the inclusion of fiber in the diet, it is difficult to predict the exact respond of the bird to the addition of fiber to the diet and to recommend the expedient quantity of fiber used in the birds' diet (Mateos *et al.* 2012).

Table 8 Analysis of variance on the experimental results along with the contribution of each type of effect (linear, quadratic, and interaction) to the statistical fit in response surface for digesta pH in gizzard and ileum and viscosity of digesta in ileum models on day 14 of age

Source of variation	df	Gizzard digesta pH model			Ileal digesta pH model			Ileal digesta viscosity model		
		Sum of squares	R^2	P-value	Sum of squares	R^2	P-value	Sum of squares	R^2	P-value
Linear	3	9.21	0.75	< 0.0001	3.49	0.73	< 0.0001	14.72	0.68	< 0.0001
Quadratic	3	0.47	0.03	0.01	0.32	0.07	< 0.0001	2.53	0.11	< 0.0001
Interaction	3	0.57	0.04	< 0.01	0.59	0.12	< 0.0001	0.26	0.01	0.35
Total model (regression)	9	10.26	0.82	< 0.0001	4.41	0.92	< 0.0001	17.52	0.80	< 0.0001
Lack of fit	5	1.15		< 0.0001	0.07		0.02	0.40		0.41
Pure error	45	0.78			0.23			3.54		
Total error	50	1.94			0.31			3.89		

Table 9 Estimated parameters of response surface model for digesta pH in gizzard and ileum and viscosity of digesta in ileum on day 14 of age

Quadratic model term[1]	Gizzard digesta pH model					Ileal digesta pH model					Ileal digesta viscosity model				
	Estimated parameter from raw data	SE	t-value	P-value	Estimated parameter from coded data	Estimated parameter from raw data	SE[2]	t-value	P-value[3]	Estimated parameter from coded data	Estimated parameter from raw data	SE	t-value	P-value	Estimated parameter from coded data
Intercept	4.61	0.03	94.44	< 0.0001	3.69	6.87	0.01	394.19	< 0.0001	6.21	1.75	0.05	36.19	< 0.0001	2.01
SBP	-0.59	0.03	-13.94	< 0.0001	-0.50	-0.49	0.01	-21.29	< 0.0001	-0.30	-0.02	0.05	13.27	< 0.0001	0.68
T	-0.26	0.03	-6.37	< 0.0001	-0.22	-0.38	0.01	-10.00	< 0.0001	-0.14	0.25	0.05	2.81	< 0.01	0.14
SO	0.26	0.03	-1.25	0.21	-0.04	0.38	0.01	-0.76	0.45	-0.01	-0.83	0.05	1.55	0.12	0.07
SBP × SBP	0.06	0.06	2.73	< 0.01	0.18	0.06	0.02	7.00	< 0.0001	0.19	0.09	0.09	3.08	< 0.01	0.30
T × T	-0.57	0.06	-2.08	0.04	-0.14	-0.23	0.02	-2.10	0.04	-0.05	-0.21	0.09	-0.55	0.58	-0.05
SO × SO	-0.49	0.06	-1.79	0.08	-0.12	-0.44	0.02	-4.03	< 0.0001	-0.11	0.77	0.09	1.97	0.05	0.19
SBP × T	0.16	0.04	3.63	< 0.01	0.14	0.17	0.01	9.69	< 0.0001	0.15	0.08	0.05	1.23	0.22	0.07
SBP × SO	0.03	0.04	0.65	0.51	0.02	0.01	0.01	0.59	0.55	0.009	0.06	0.05	1.00	032	0.05
T × SO	0.16	0.04	1.02	0.31	0.04	0.03	0.01	0.49	0.62	0.007	0.20	0.05	0.90	0.37	0.05

SBP: sugar beet pulp; T: tallow and SO: soybean oil.
SE: standard error.

The present study showed that, the highest ADG (42.10 g/bird/d) and the lowest FCR (0.99) were observed with diet containing 0.35% SBP, 0.00% T and 0.51% SO. Aziz-Aliabadi *et al.* (2021) reported that with increment age and GIT improvement at 14 d of age, the lowest FCR was obtained at diet including 0.98% SBP, 0.00% T, and 1.00% SO.

In the present study, the SBP attendance (up to 0.35% of diet) in the diets maybe amends the GIT development and, as a result, boosts performance parameters at 14 d of age. In young birds, digestion of triglycerides is less due to limitation in bile secretion and lipase enzyme; meanwhile, with increasing age and the bile secretion and release of fat-digesting enzymes, the bird's potency to digest fats mounts (Freitas *et al.* 2005). Unsaturated oils are more digestible and have more metabolizable energy than fats, which improving broiler chickens' performance (Zulkifli *et al.* 2007). The reason for the better performance in the early period of rearing is due to the better digestion and absorption of fats rich in unsaturated fatty acids by young chickens than saturated fats, which is likely due to limited capacity. It is for the production of bile at a younger age, which causes a decrease in the digestion of saturated fatty acids (Chen and Chiang, 2005).

Studies show that birds respond rapidly to changes in dietary fiber content by altering intestinal length and limb weight, as well as the rate at which food passes through different parts of the gastrointestinal tract (Sklan *et al.* 2003; Jimenez-Moreno *et al.* 2011; Svihus, 2011). Increasing the insoluble fiber content of the diet leads to a decrease in the length of the small intestine (Sklan *et al.* 2003), a decrease in the weight of the proventriculus (Jimenez-Moreno *et al.* 2011), and an increase in the weight and content of the gizzard, which generally indicates an improvement in gastrointestinal function (Svihus, 2011). The type of fiber added to the diet can also affect the development of different parts of the GIT and its pH (Jimenez-Moreno *et al.* 2009).

The use of fiber in diets leads to an increase in the use of nutrients by decreasing the pH of the gizzard (Gonzalez-Alvarado *et al.* 2007), increasing the retention time in the upper parts of the GIT (crop and gizzard) and increasing gizzard function and HCl production in the proventriculus. In addition, low pH in the upper GIT leads to improved solubility and salt absorption (Jimenez-Moreno *et al.* 2009). It has been reported that the inclusion 8% oat hull to the broilers' diet increased the nutrients digestibility and duodenum pH (Mossami, 2011).

Jimenez-Moreno *et al.* (2009) stated that the pH of duodenal digesta was similar in all diets tested, but that feeding chickens with SBP increased the pH of the jejunum, cecum and ileum. These researchers concluded that the type of fiber added to the diet could affect the development of different parts of the GIT and their pH. In contrast, in the present experiment, gizzard and ileum pH decreased with increasing the SBP amount in the diets. Perhaps this is due to the presence of insoluble fiber in corn and soybeans, which to some extent reduces the negative effects of soluble fiber (Saki *et al.* 2011), or possibly the level of SBP used in diets (1.70% SBP for gizzard digesta and 1.47% for ileal digesta). The presence of structural components, coarse particles and fiber in the diet of chickens increases the activity of the gizzard and thus increases the retention time in the upper part of the gastrointestinal tract. This increases bacterial fermentation in the crop (Classen *et al.* 2016) and decreases the pH of the gastrointestinal tract, which in turn increases the activity of pepsin and thus improves the digestibility of proteins. (Naderinejad *et al.* 2016). Stimulating *Lactobacillus* species proliferation in the crop increases lactic acid production, directly lowering intestinal pH and preventing the proliferation of pathogenic bacteria and acid-sensitive microorganisms (Classen *et al.* 2016). Kimiaeitalab *et al.* (2017) concluded that the inclusion of 3% sunflower hull in broilers' diet reduced the gizzard's pH without any negative effect on the performance of chickens. Fermentation of fiber at the end of the GIT produces volatile fatty acids such as acetic, propionic and butyric acids. These volatile fatty acids decrease the cecum's pH, inhibit pathogenic bacteria's growth, increase mineral uptake, and promote enterocyte proliferation (Kumar *et al.* 2012).

It has been shown that fats containing unsaturated fatty acids improve function, microbial population and pH of intestinal contents compared to fats containing saturated fatty acids in the diet of broilers (Poorghasemi *et al.* 2017). Danicke *et al.* (1997) reported higher pH values in different parts of the small intestine and increased host bile acid excretion due to tallow consumption instead of soybean oil in the diet. In the current study, gizzard and ileal digesta pH decreased with the reducing percentage of tallow (0.51 to 0.21% in gizzard and 1.00 to 0.00% in ileum) in the diets. On the other hand, a higher population of pathogenic bacteria and a smaller population of lactic acid-producing bacteria can be expected due to increased intestinal pH, leading to infectious diarrhea and defects in the intestinal immune system (Liopis *et al.* 2005). Therefore, it can be stated that the use of unsaturated fatty acid sources instead of saturated fatty acid sources, improves the health and growth of the birds due to better microbial ecology in the GIT. In addition,it has beneficial effects on the carcass fatty acid composition (Zanini *et al.* 2004). In general, the effect of dietary fat on the microbial population of the GIT is probably due to the different effects of different sources of fatty acids on digestive adhesion, pH, and time of nutrient transfer in the GIT (Laflamme *et al.* 2011). On day 22 of the study, by measuring volatile fatty acids in cecum, it was observed that adding oil does not affect the concentration of these substances in cecum and its pH (Cao *et al.* 2010). It has been suggested that an increase in viscosity may reduce the hydrolysis and solubility of lipids (Yokhana *et al.* 2016). Langhout *et al.* (1999) stated that the anti-nutritive effects of soluble fiber were more observed when tallow was placed in the diet than when the fat source was soybean oil. In our experiment, the highest viscosity values were observed in the groups containing 3.50% of SBP and 1.00% of tallow. In addition, changes in the activity of the intestinal microbial flora are particularly important in lipid metabolism. It has been shown that there is a positive relationship between viscosity and fat droplets' size, which reduces triglycerides' lipolysis. Mechanisms that decrease emulsification reduce activity of pancreatic lipase and the initiation of micelles in the gut, which is more common for saturated fatty acids (McNab and Borman, 2002).

It has been declared that the RSM is efficacious in predicting optimal performance in broilers. This mathematical way allows for more precise designation of optimal amounts of dietary protein, growth temperature, and slaughter age of broiler chickens (Faria Filho *et al.* 2008). Ghanaatparast-Rashti *et al.* (2017) said that due to the linear correlation among the variables, RSM, and neural network model showed similar results. In contrast, Ahmadi and Golian (2011), in investigating the response surface and artificial neural network models concluded that the predictions of artificial neural network models for broilers' performance were more accurate and correct.

CONCLUSION

The results of this experiment showed that at the young age of broilers due to the growth and development of the digestive system, the negative effects of soluble fibers are reduced to some extent and the bird will be able to use fat sources better. Maximum average daily body weight gain and minimum feed conversion ratio was observed with a diet containing 0.35% sugar beet pulp, 0.00% tallow, and 0.51% soybean oil. In addition, digesta pH decreased with the increase of sugar beet pulp (0.00 to 1.70% in gizzard; 0.00 to 1.47% in ileum) and decrease of tallow (0.51 to 0.21% in gizzard; 1.00 to 0.00% in ileum) and soybean oil (0.27 to 0.00% in gizzard; 0.43 to 0.00% in ileum). Regarding ileum digesta viscosity, the results showed that the digesta viscosity increased with increasing sugar beet pulp (0.00 to 3.50%), tallow (0.00 to 1.00%) and soybean oil levels (0.53 to 1.00%), so that the lowest viscosity was observed in diets containing 0.00% sugar beet pulp, 0.00% tallow and 0.53% soybean oil.

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