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The Effects of Sugar Beet Pulp with Tallow and Soybean Oil on Broiler Chicken Performance and Jejunum Morphology

Research Article

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ABSTRACT

This experiment was conducted to assess the effect of three levels of dietary sugar beet pulp (SBP), tallow (T) and soybean oil (SO) on the performance and morphology of the jejunum in the young broilers were applied central composite design (CCD; 3 levels and 3 factors) and response surface methodology (RSM). A total of 420 one-day-old male chicks Ross 308 were appropriated to 60 cages of seven birds each and fed with 15 diets, produced by CCD containing three levels 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 days of age. The results showed that maximum average daily body weight gain (ADG, 31.59 g/bird/d) and minimum feed conversion ratio (FCR, 1.02) were obtained with diets comprising 0.51% SBP, 0.20% T, 0.38% SO and 0.50% SBP, 0.05% T and 0.10% SO, respectively. On day 7 of age, the linear effects for all models, the quadratic and interactions effects for the number of goblet cells (GC) model were significant (P<0.05). On day 14 of age, the linear and quadratic effects for all models and the interactions for the model of villus width (VW) were significant (P<0.05). Generally, aging and subsequent growth and expansion of the gastrointestinal tract can moderate the destructive impacts of soluble fibers to some extent and the RSM could provide an acceptable prediction for the relevance between variables and traits.

KEY WORDS fat, fiber, goblet cells, intestine, response surface methodology.

INTRODUCTION

The efficiency of the poultry industry has increased due to advances in genetics, nutrition, and management practices over the past few decades (Sanchez *et al.* 2021). On the other hand, nutrition, as an important point of profitability in terms of examining the physiology of digestion and metabolism, as well as a more accurate assessment of the quality of raw materials of the diet, has made significant progress (Kiarie and Mills, 2019). Due to the growing conditions of the human population and mutual demand for food, the poultry feed production industry has faced obstacles such as unstable commodity markets, limited access to natural resources, climate change pressure, and food-feed-biofuel competition (FAO, 2017). The aforementioned factors have pressured the monogastric industry to use significant amounts of food that are unacceptable for human consumption, such as sugar beet pulp (SBP) (Rho *et al.* 2018). The remaining product after sugar distillation is called SBP, which is reported to contain comparatively 400 g/kg of neutral detergent fiber (NDF) and 250 g/kg of pectin as soluble fiber (SF) (Voelker and Allen, 2003). One kilogram of dry SBP contains 9.7-11.2 MJ of metabolic energy and 102.7 gram of protein. It contains comparatively high calcium, sodium, magnesium and trace elements. As it possesses approximately no lignin, it has a great digestibility in pigs (Koschayev *et al.* 2019). Owing to the pectin quantity of the SBP, it can yield cohesive and wettish excretion by construct viscosity and water holding capacity (Jozefiak *et al.* 2006). Important sources of energy in the intensive feeding system of poultry production are usually oils and fats. The benefits of using oils and fats in the birds' diet include reducing dust, providing essential fatty acids and fat-soluble vitamins, as well as helping to produce less heat increment (Abedpour *et al.* 2017). 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). Soybean oil (SO) is rich in linoleic acid, the most momentous part of the omega-6 family of fatty acids. Oxidation is greater in SO because of its high measures of unsaturated fatty acids, and it has been said that the amount of lipid peroxidation is smaller in broiler chickens nourished with tallow (Hosseini-Vashan *et al.* 2014). The gastrointestinal tract (GIT) function consists of digestion, absorption, and conservation, and the anatomy of the gut is well adjusted to carry out these actions. The fruitful performance of the GIT and its health are vital agents in defining animal growth performance, meat and egg quality (Jha and Mishra, 2021). In addition, the advancement of the GIT mainly during the early post-hatching period of broiler chicks is a substantial aspect of growth (Jha *et al.* 2019). A high villus height to crypt depth (VH:CD) ratio is an index of further development and operation of the intestinal mucosa (Jha and Mishra, 2021). Therefore, it is urgent to determine the best concentration and source of fiber and fat to aid better performance and GIT growth and development. Novel mathematical-statistical procedures are utilized to optimize trial conditions and reach to more reliable outcomes (Ahmadi and Golian, 2011). In the response surface methodology (RSM), the effects of several factors on one or more outputs are investigated simultaneously (Aziz-Aliabadi *et al.* 2023a). Using this method, the optimal conditions related to the desired response are obtained by performing fewer treatments than conventional full factorial methods (one or two variables at a time).As a result, it will save time and cost and better controls of experiment conditions (Gulati *et al.* 2010).

Various studies have been conducted on the effects of dietary fiber and fat on the performance of birds. However, limited studies have been conducted on the effects of these two nutrients on the development and evolution of the broilers'GIT. Based on our research, there was no study that mathematically expressed the relationship between fiber and dietary fat.

Since the greatest growth of the GIT takes place in the early ages of the bird, the purpose of this study was to investigate the effect of different amounts of SBP, T and SO on the performance and jejunum morphology in 14-day-old broiler chickens using the RSM.

MATERIALS AND METHODS

Experimental design

All procedures were approved by the Animal Care and Use Committee of the Ferdowsi University of Mashhad, Mashhad, Iran (3/30184). A total of 420 day-old Ross 308 male broiler chicks with average weights of 46.45 ± 0.97 g were received from a commercial hatchery and randomly assigned to 60 battery brooder cages of seven birds each. According to the scheme produced by three-levels, three-factors central composite design (CCD), the birds were fed with the 15 diets comprising of three levels of SBP (0.00, 1.75 and 3.50%), T (0.00, 0.50 and 1.00%), and SO (0.00, 0.50 and 1.00%), from 0 to 14 d of age (Tables 1, 2, and 3). 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, 0.50, 0.50%) is repeated 18 times. The ingredients and chemical compounds of the 15 trial diets are shown in Tables 4 and 5 (NRC, 1994). Tables 4 and 5 illustrate the components and nutrient compound of diets. All birds were feed according to the Ross 308 strain requirements (Aviagen, 2014).

Diet samples were analyzed for NDF (Mertens *et al.* 2002), acid detergent fiber (ADF) and insoluble fiber (IF) (AOAC, 2005). The soluble fiber was calculated from the difference of total crude fiber from its insoluble fraction. Fatty acid profiles of T and SO used in diets were characterized by gas chromatography (Table 6).

The rearing house temperature was set at 32 ˚C in the first 2 days, which was reduced by 0.5 ˚C each day to eventually reach 26.0 ˚C at the end of the study on day 14. The light and darkness schedule was considered as 18 h light and 6 h dark all over the study period.

Growth performance

The group weight of birds in every cage were measured at the beginning and end of the study (0 d and 14 d of age). The average daily body weight gain (ADG), feed intake (FI) and feed conversion ratio (FCR) were determined (Aziz-Aliabadi *et al.* 2023b). After running a growth experiment by CCD, a data set, including 60 data lines, was produced and subjected to statistical analyses.

Intestinal morphology

Two broilers from each cage were randomly selected and euthanized by cervical dislocation on days 7 and 14 of age. Intestinal segments collected from the midpoint of the jejunum between the bile duct entry and the Meckel`s diverticulum. Segments were immediately fixed in a 10% neutral buffered formalin solution.

Table 1 Effect of different levels of sugar beet pulp (SBP), tallow (T) and soybean oil (SO) concentration diet according to central composite design with average response of ADG and FCR in 0-14 days of broiler chicks

Treatment numbers	Replications*	Factors (% of diet)			Experimental response (0-14 d of age)			
		SBP	T	SO	ADG (g/bird/d)	±SD	FCR	±SD
1	18	1.75	0.50	0.50	31.25	1.32	1.34	0.19
2	3	3.50	1.00	0.00	16.74	0.89	2.03	0.09
3	3	0.00	0.00	1.00	21.98	0.74	1.36	0.11
4	3	3.50	1.00	1.00	17.38	0.68	2.35	0.23
5	3	0.00	1.00	1.00	27.25	1.55	1.43	0.06
6	3	1.75	0.00	0.50	30.37	1.54	1.04	0.06
7	3	0.00	1.00	0.00	19.84	0.14	1.33	0.05
8	3	0.00	0.50	0.50	30.95	0.70	1.26	0.02
9	3	1.75	0.50	0.00	31.32	1.03	1.06	0.06
10	3	1.75	1.00	0.50	26.15	1.04	1.24	0.02
11	3	3.50	0.00	0.00	17.60	0.31	2.09	0.04
12	3	1.75	0.50	1.00	19.42	0.27	1.28	0.09
13	3	3.50	0.00	1.00	17.65	0.06	2.11	0.15
14	3	0.00	0.00	0.00	27.28	0.70	1.43	0.09
15	3	3.50	0.50	0.50	17.19	0.67	2.27	0.04

* A total of 60 run numbers were provided.
ADG: average daily body weight gain and FCR: feed conversion ratio.
SD: standard deviation.

Table 2 Effect of different levels of sugar beet pulp (SBP), tallow (T) and soybean oil (SO) concentration diet according to central composite design with average response of jejunum morphology of broiler chicks on 7 day of age

Treatment numbers	Replications*	Factors (% of diet)			Experimental response (7 d)							
		SBP	T	SO	VH	±SD	VW	±SD	VH:CD	±SD	GC	±SD
1	18	1.75	0.50	0.50	552.41	78.96	122.93	18.20	4.26	0.36	26.88	0.19
2	3	3.50	1.00	0.00	518.33	35.27	113.17	9.68	3.62	0.15	27.39	0.34
3	3	0.00	0.00	1.00	722.03	76.07	151.63	2.56	5.42	0.25	25.40	1.16
4	3	3.50	1.00	1.00	401.34	95.63	96.54	2.43	3.25	0.84	27.73	0.11
5	3	0.00	1.00	1.00	656.17	42.79	136.93	18.30	4.78	0.74	26.58	1.11
6	3	1.75	0.00	0.50	760.00	52.24	146.57	0.31	5.22	0.18	26.47	0.02
7	3	0.00	1.00	0.00	512.54	19.16	132.23	6.15	4.68	0.15	25.66	0.31
8	3	0.00	0.50	0.50	720.00	76.97	151.50	9.98	6.17	0.04	24.85	0.68
9	3	1.75	0.50	0.00	557.67	52.21	139.28	15.39	4.72	0.20	27.30	0.21
10	3	1.75	1.00	0.50	584.65	41.05	118.05	17.14	4.79	0.41	26.67	0.11
11	3	3.50	0.00	0.00	496.00	10.50	108.29	18.66	4.10	0.07	27.73	0.37
12	3	1.75	0.50	1.00	566.00	72.33	135.64	7.77	4.80	0.19	27.27	0.27
13	3	3.50	0.00	1.00	494.33	30.04	126.69	3.42	4.33	0.21	27.50	0.11
14	3	0.00	0.00	0.00	719.16	54.54	155.28	9.10	5.28	0.13	25.49	0.17
15	3	3.50	0.50	0.50	490.66	11.52	114.56	13.22	3.75	0.36	27.54	0.30

* A total of 60 run numbers were provided.
VH: villus height (μ); VW: villus width (μ); VH:CD: villus height to crypt depth ratio and GC: number of goblet cells in each villus.
SD: standard deviation.

Table 3 Effect of different levels of sugar beet pulp (SBP), tallow (T) and soybean oil (SO) concentration diet according to central composite design with average response of jejunum morphology of broiler chicks on 14day of age

Treatment numbers	Replications*	Factors (% of diet)			Experimental response (7d)							
		SBP	T	SO	VH	±SD	VW	±SD	VH:CD	±SD	GC	±SD
1	18	1.75	0.50	0.50	719.52	64.15	157.88	6.03	5.69	0.37	57.35	4.16
2	3	3.50	1.00	0.00	549.80	53.28	136.50	3.90	3.98	0.52	83.16	3.17
3	3	0.00	0.00	1.00	743.86	52.20	177.00	8.18	5.71	0.36	53.56	9.22
4	3	3.50	1.00	1.00	523.23	35.66	138.00	11.35	3.58	0.30	74.76	10.83
5	3	0.00	1.00	1.00	692.56	82.70	149.32	7.02	5.50	0.48	62.20	1.91
6	3	1.75	0.00	0.50	727.10	29.10	160.00	5.29	5.74	0.13	56.60	2.20
7	3	0.00	1.00	0.00	640.43	17.97	159.31	7.02	5.36	0.17	54.70	7.13
8	3	0.00	0.50	0.50	938.80	77.37	177.00	5.56	6.23	0.12	49.66	1.52
9	3	1.75	0.50	0.00	634.96	68.38	160.16	0.28	5.22	0.58	60.03	4.32
10	3	1.75	1.00	0.50	629.85	52.10	159.18	1.89	5.06	0.39	58.23	4.15
11	3	3.50	0.00	0.00	494.03	8.20	136.00	1.73	3.42	0.28	85.50	0.86
12	3	1.75	0.50	1.00	690.50	76.75	165.35	11.23	5.63	0.61	55.56	5.09
13	3	3.50	0.00	1.00	554.36	68.10	134.86	2.92	4.46	0.55	76.96	7.42
14	3	0.00	0.00	0.00	790.90	14.45	161.62	2.88	5.83	0.14	59.36	0.77
15	3	3.50	0.50	0.50	466.40	6.40	137.37	1.52	3.25	0.12	93.50	5.63

* A total of 60 run numbers were provided.
VH: villus height (μ); VW: villus width (μ); VH:CD: villus height to crypt depth ratio and GC: number of goblet cells in each villus.
SD: standard deviation.

Table 4 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**
Broken rice	69.06	69.14	68.98	66.17	66.82	70.14	69.79	69.38	70.55	67.98	71.30	67.58	68.33	71.95	68.74
Soy protein concentrate, 84% CP	20.07	19.89	20.25	20.18	20.46	19.96	20.17	20.21	19.92	20.17	19.68	20.21	19.97	19.96	19.93
Dicalcium phosphate	1.42	1.41	1.43	1.45	1.46	1.41	1.42	1.42	1.40	1.43	1.39	1.44	1.42	1.39	1.42
Limestone	1.59	1.56	1.61	1.54	1.60	1.59	1.62	1.62	1.60	1.58	1.57	1.58	1.55	1.63	1.56
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	3.52	1.91	5.14	3.57	6.07	3.05	4.41	4.77	2.69	3.99	0.97	4.35	2.64	3.47	2.27
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	0.10	0.10	0.10	0.09	0.09	0.10	0.10	0.10	0.11	0.10	0.11	0.10	0.10	0.11	0.10
DL-methionine	0.57	0.56	0.57	0.57	0.58	0.57	0.57	0.57	0.56	0.57	0.56	0.57	0.57	0.56	0.57
L-threonine	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
$NaHCO_3$	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Salt (NaCl)	0.21	0.21	0.21	0.22	0.22	0.21	0.21	0.21	0.21	0.21	0.21	0.22	0.21	0.21	0.21

[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 (DL-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.

Table 5 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)	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100
Crude protein	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Crude fiber	1.28	1.56	1.00	1.51	0.97	1.29	1.01	1.01	1.30	1.26	1.59	1.26	1.54	1.04	1.55
Insoluble fiber	1.10	2.05	0.15	2.04	0.15	1.10	0.16	0.15	1.10	1.10	2.05	1.10	2.05	0.16	2.05
Soluble fiber	0.22	0.41	0.04	0.41	0.04	0.22	0.04	0.04	0.22	0.22	0.41	0.22	0.41	0.04	0.41
Acid detergent fiber	1.65	2.06	1.25	2.01	1.20	1.67	1.25	1.24	1.67	1.63	2.10	1.62	2.04	1.29	2.05
Neutral detergent fiber	4.38	5.03	3.74	4.87	3.60	4.43	3.76	3.74	4.46	4.32	5.15	4.30	4.99	3.88	5.01
Lysine	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Methionine	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.84	0.85	0.85	0.85	0.85
Met + Cys	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
Threonine	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Tryptophan	0.26	0.26	0.26	0.25	0.25	0.26	0.26	0.26	0.26	0.26	0.26	0.25	0.26	0.26	0.26
Calcium	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Available phosphorus	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.45	0.48	0.48	0.48	0.48
Sodium	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	0.16	0.16
Chlorine	0.17	0.17	0.17	0.17	0.17	0.17	0.18	0.17	0.18	0.17	0.18	0.17	0.17	0.18	0.17
Determined analysis (%)															
Crude fiber	2.32	2.71	2.03	3.05	2.12	2.41	2.14	2.00	2.29	2.24	2.80	2.22	1.67	1.11	1.69
Insoluble fiber	2.15	3.00	1.10	2.11	0.99	2.43	1.00	2.21	2.25	1.12	3.11	2.13	4.03	0.95	2.00
Soluble fiber	0.47	0.69	0.12	1.56	0.50	0.18	0.17	0.20	0.30	0.41	0.75	0.39	1.27	0.11	1.01
Acid detergent fiber	2.71	3.10	2.29	3.25	2.42	2.75	2.22	2.30	3.12	2.60	3.25	2.74	3.30	2.42	4.24
Neutral detergent fiber	5.36	7.11	4.60	5.46	5.23	5.34	4.78	4.80	5.32	5.48	6.14	5.58	6.42	5.01	6.25

[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%).

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

About 24 hours after sampling, the initial formalin solution was drained and the container containing the sample was refilled with fresh 10% formalin solution. In order to saturate the samples with paraffin, paraffinization was performed. In the next step, using a rotating microtome (Pouyan Company, model MK1120), sections with a thickness of 5 to 6 µm were prepared from paraffin molds containing the sample.

Then, the slides were placed on a hot plate (45 ˚C) to dry and melt the excess paraffin. After paraffin removal with xylene, tissues were stained on the slide using hematoxylin and eosin. A computer-connected light microscope was used to examine the morphology of the tissue samples (Olympus model BX51 microscope; magnification 100). Images of desired sections were prepared and finally, the required parameters including villus height (VH), villus width (VW), VH:CD ratio (Garcia *et al.* 2007) and the number of goblet cells (GC) per villus (Wils-Plotz and Dilger, 2013) were measured using the relevant software (DP2-BSW software).

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 variables (k=3).

x_i: input variables (dietary percentage of SBP, T, and SO).

β_0: constant term.

β_i: coefficients of the linear parameters.

B*ij*: coefficients of the interaction parameters.

β_{ii}: coefficients of the quadratic parameter.

ε: residual associated with the experiment.

The experimental data (60 data lines) collected by CCD were fitted to the second order polynomial equation by Minitab 17. Using ANOVA and the corresponding absolute t-value of the model parameters, a procedure of the sensitivity analysis was done on RSM models to discover which model term is considered more notable within the modeling procedure. A more important model term (linear, quadratic, or interaction of obtained model related to dietary SBP, T, and SO concentration) has a higher absolute t-value. Therefore, the input variables may be sorted in the order of importance.

RESULTS AND DISCUSSION

Average response values for ADG and FCR according to CCD are presented in Table 1. The polynomial equation from raw experimental data for ADG (R^2=0.78; root MSE=2.90) and FCR (R^2=0.84; root MSE=0.17) is generated as follows:

ADG (g/bird/d)= 27.77 – 3.61 × SBP – 1.15 × T + 10.71 × SO – 1.68 × SBP × SBP – 3.94 × T × T – 15.50 × SO × SO + 0.14 × SBP × T – 0.20 × SBP × SO + 6.66 × T × SO

FCR= 1.37 – 0.46 × SBP + 0.12 × T – 0.16 × SO + 0.18 × SBP × SBP – 0.24 × T × T – 0.22 × SO × SO + 0.02 × SBP × T + 0.04 × SBP × SO + 0.22 × T × SO

The estimated parameters for SBP, SBP × SBP, SO × SO, and T × SO terms in the ADG model, and SBP, SBP × SBP terms in the FCR model were significant (P<0.05). The contribution of each type of effect in the RSM model (linear, quadratic, and interaction) to the statistical fit (in term of R^2) is shown in Table 7.

In the ADG model the quadratic terms have higher contribution (partial R^2=0.53) to explain existing variation in the response of chickens. It was followed by linear (partial R^2=0.24) and interaction (partial R^2=0.03) terms. In the FCR model the linear (partial R^2=0.47) and quadratic (partial R^2=0.37) terms had contribution to explain existing variation in feed efficiency, and the interaction terms were not significant (Table 7). Maximum ADG (31.59 g/bird/d) and minimum FCR (1.02) were observed with diet containing 0.51% SBP, 0.20% T, 0.38% SO and 0.76% SBP, 0.05% T and 0.10% SO, respectively.

The absolute t-value may indicate to what extent each model term contributed to the statistical fit so that the greater absolute t-value, the more notable the corresponding coefficient. The regression coefficient estimates and corresponding t-value and P-value are illustrated in Table 8. Lack of fit for both ADG and FCR models was significant (P<0.05), indicating that a more complicated modeling method or other testing with extra variables should be made (Table 7).

Jejunum morphology on day 7 of age

Average response values for jejunum morphology according to CCD are highlighted in Table 2. The polynomial equations extracted from raw experimental datafor VH (R^2=0.60; root MSE=72.33), VW (R^2=0.50; root MSE=15.02), VH:CD ratio (R^2=0.60; root MSE=0.48) and number of GC in each villus in jejunum (R^2=0.76; root MSE=0.43) obtained as follows:

VH (μ)= 710.26 – 40.50 × SBP – 389.00 × T + 281.00 × SO – 2.25 × SBP × SBP + 228.00 × T × T – 214.00 × SO × SO + 28.80 × SBP × T – 37.90 × SBP × SO + 12.70 × T × SO

VW (μ)= 153.58 – 9.91 × SBP – 9.60 × T – 9.10 × SO – 0.19 × SBP × SBP – 5.20 × T × T + 15.40 × SO × SO + 1.78 × SBP × T + 0.10 × SBP × SO – 13.30 × T × SO

VH:CD ratio= 5.40 – 0.43 × SBP – 0.81 × T + 0.84 × SO + 0.02 × SBP × SBP + 0.40 × T × T – 0.55 × SO × SO – 0.04 × SBP × T + 0.05 × SBP × SO – 0.32 × T × SO

Number of GC per villus= 25.36 + 1.34 × SBP + 0.92 × T – 2.23 × SO – 0.17 × SBP × SBP – 0.66 × T × T + 2.20 × SO × SO – 0.20 × SBP × T – 0.10 × SBP × SO + 0.79 × T × SO

The estimated parameters for SBP, T, T × T, SO × SO and SBP × SO terms in the VH, SBP and T terms in the VW and VH:CD and SBP, SBP × SBP, SO × SO,T × SO and SBP × T terms in the GC models were significant ($P<0.05$). The contribution 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 Tables 9 and 10. In all models, the linear effects have a premier portion to elucidate existing variability in the response of chicks, while-quadratic and interaction effects displayed short impressions. Maximum (802.66 μ) and minimum (392.82 μ) VH were earned with the diet containing 0.00% SBP, 0.00% T, 0.65% SO and 3.50% SBP, 0.60% T, and 1.00% SO, respectively.

Maximum (159.90 μ) and minimum (100.19 μ) VW were discovered with the diet containing 0.00% SBP, 0.00% T, 1.00% SO and 3.50% SBP, 1.00% T, and 0.71% SO, respectively. Maximum (5.72) and minimum (3.33) VH:CD ratio were acquired with the diet containing 0.00% SBP, 0.00% T, 0.75% SO and 3.50% SBP, 1.00% T, and 1.00% SO, respectively. Maximum (27.90) and minimum (24.80) GC were generated with the diets contained 3.42% SBP, 0.14% T, 0.00% SO and 0.00% SBP, 0.00% T, and 0.50% SO, respectively.

The regression coefficient estimates and corresponding t-value and P-value are shown in Tables 11 and 12. Lack of fit for the VH and VH:CD models was significant ($P<0.05$) (Tables 9 and 10), demonstrating that more complex models or other tests with extra variables should be made. The lack of fit for both the VW and GC models was not significant ($P>0.05$) (Tables 9 and 10), highlighting that the observed data fitproperly with the model.

Jejunum morphology on day 14 of age

Average response values for jejunum morphology according to CCD are shown in Table 3. The polynomial equations extracted from raw experimental datafor VH (R^2=0.64; root MSE=72.86), VW (R^2=0.72; root MSE=7.11), VH:CD ratio (R^2=0.80; root MSE=0.44) and number of GC in each villus in jejunum (R^2=0.77; root MSE=6.18) obtained as follows:

VH (μ)= 795.90 – 76.40 × SBP + 22.00 × T + 280.00 × SO – 3.28 × SBP × SBP – 137.00 × T × T – 200.00 × SO × SO + 32.30 × SBP × T + 4.10 × SBP × SO + 6.10 × T × SO

VW (μ)= 166.43 – 3.23 × SBP + 6.30 × T + 10.90 × SO – 1.97 × SBP × SBP – 14.52 × T × T – 1.85 × SO × SO + 4.81 × SBP × T – 0.71 × SBP × SO – 11.33 × T × SO

VH:CD ratio= 5.74 + 0.21 × SBP + 0.22 × T + 0.62 × SO – 0.24 × SBP × SBP – 0.36 × T × T + 0.27 × SO × SO – 0.05 × SBP × T + 0.09 × SBP × SO – 0.59 × T × SO

Number of GC per villus= 55.67 – 5.27 × SBP + 3.55 × T + 0.40 × SO + 4.25 × SBP × SBP – 4.57 × T × T – 3.04 × SO × SO – 1.21 × SBP × T – 2.66 × SBP × SO + 6.72 × T × SO

The estimated parameters for SBP, and T terms in the VH, SBP, T, and SBP × SBP terms in the VW and VH:CD models and SBP, and SBP × SBP terms in the GC models were significant ($P<0.05$). The contribution 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 Tables 13 and 14. In all models, the linear effects have a more portion to explain variability in the response of chicks, while quadratic and interaction effects showed a short impression. The maximum (851.43 μ) and minimum (487.04 μ) VH were acquired with the diet comprising 0.00% SBP, 0.09% T, 0.52% SO and 3.50% SBP, 1.00% T, and 0.00% SO, respectively. The maximum (175.50 μ) and minimum (130.93 μ) VW were obtained with the diet containing 0.00% SBP, 0.00% T, 1.00% SO and 3.50% SBP, 0.00% T, and 0.00% SO, respectively. The maximum (6.19) and minimum (3.49) VH:CD ratio were earned with the diet containing 0.63% SBP, 0.00% T, 1.00% SO and 3.50% SBP, 0.00% T, and 0.00% SO, respectively. The maximum (89.33) and minimum (49.31) GC per villus were afforded with the diet including 3.50% SBP, 0.00% T, 0.00% SO and 0.92% SBP, 0.00% T, and 1.00% SO, respectively.The regression coefficient estimates and corresponding t-value and P-value are shown in Tables15 and 16.

Table 6 Analysis ofmajor fatty acids in tallow and soybean oil (%)

Fatty Acids(%)	Tallow (T)	Soybean oil (SO)
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
SFA:UFA	60:33	16:84

SFA:UFA: saturated fatty acids:unsaturated fatty acids.

Table 7 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 ADG and 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	541.02	0.24	< 0.0001	4.97	0.46	< 0.0001
Quadratic	3	1162.40	0.51	< 0.0001	3.93	0.37	< 0.0001
Interaction	3	67.64	0.03	0.06	0.12	0.01	0.24
Total model (regression)	9	1771.06	0.78	< 0.0001	9.04	0.84	< 0.0001
Lack of fit	5	359.98		< 0.0001	0.36		0.02
Pure error	45	62.22			1.10		
Total error	50	422.20			1.47		

ADG: average daily body weight gain (g/bird/day) and FCR: feed conversion ratio.
Significant= P < 0.05.

Table 8 Estimated parameters of response surface model for ADG and FCR in broiler chicks from 0 to 14 d of age

Quadratic model term	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	30.45	0.57	52.80	< 0.0001	27.77	1.28	0.03	37.75	< 0.0001	1.37
SBP	-4.07	0.53	-7.69	< 0.0001	-3.61	0.40	0.03	12.80	< 0.0001	-0.46
T	-0.75	0.53	-1.42	0.16	-1.15	0.03	0.03	1.06	0.29	0.12
SO	-0.91	0.53	-1.72	0.09	10.71	0.06	0.03	2.00	0.05	-0.16
SBP × SBP	-5.17	1.01	-5.11	< 0.0001	-1.68	0.57	0.05	9.60	< 0.0001	0.18
T × T	-0.99	1.01	-0.97	0.33	-3.94	-0.05	0.05	-0.94	0.35	-0.24
SO × SO	-3.88	1.01	-3.83	< 0.0001	-15.50	-0.05	0.05	-0.93	0.35	-0.22
SBP × T	0.12	0.59	0.22	0.82	0.14	0.02	0.03	0.73	0.47	0.02
SBP × SO	-0.18	0.59	-0.30	0.76	-0.20	0.03	0.03	1.06	0.29	0.04
T × SO	1.66	0.59	2.81	0.007	6.66	0.05	0.03	1.64	0.10	0.22

SBP: sugar beet pulp; T: tallow and SO: soybean oil.
ADG: average daily body weight gain (g/bird/day) and FCR: feed conversion ratio.
SE: standard error.
Significant= P < 0.05.

Table 9 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 VH and VW in jejunum of broiler chicks on day 7 of age

Source of variation	VH model				VW model		
	df	Sum of squares	R^2	P-value	Sum of squares	R^2	P-value
Linear	3	337862.00	0.49	< 0.0001	11012.00	0.48	< 0.0001
Quadratic	3	37045.00	0.05	0.08	137.30	0.006	0.89
Interaction	3	41886.00	0.06	0.05	325.50	0.01	0.69
Total model (regression)	9	416793.00	0.60	< 0.0001	11474.90	0.50	< 0.0001
Lack of fit	5	76296.00		< 0.01	2071.40		0.09
Pure error	45	185360.00			9214.30		
Total error	50	261656.00			11285.60		

VH: villus height (µm) and VW: villus width (µm).
Significant= P < 0.05.

Table 10 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 VH:CD and GC in jejunum of broiler chicks on day 7 of age

Source of variation	VH:CD model				GC model		
	df	Sum of squares	R^2	P-value	Sum of squares	R^2	P-value
Linear	3	19.06	0.58	< 0.0001	30.35	0.64	< 0.0001
Quadratic	3	0.20	0.008	0.83	3.98	0.08	< 0.0001
Interaction	3	0.24	0.01	0.80	1.92	0.04	0.02
Total model (regression)	9	19.51	0.60	< 0.0001	36.26	0.76	< 0.0001
Lack of fit	5	6.24		< 0.0001	5.32		0.21
Pure error	45	6.01			4.04		
Total error	50	12.25			9.36		

VH:CD: villus height to crypt depth ratio and GC: number of goblet cells in each villus.
Significant= P < 0.05.

Table 11 Estimated parameters of response surface model for VH and VW in jejunum of broiler chicks on day 7 of age

Quadratic model term*	VH model					VW 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	710.40	14.40	40.22	< 0.0001	577.50	153.58	2.98	42.66	< 0.0001	127.20
SBP	-40.50	13.20	-7.01	< 0.0001	-92.50	-9.91	2.74	-6.14	< 0.0001	-16.83
T	-389.00	13.20	-3.93	< 0.0001	-51.90	-9.60	2.74	-3.34	< 0.01	-9.15
SO	281.00	13.20	0.27	0.78	3.60	-9.10	2.74	-0.03	0.97	-0.08
SBP × SBP	-2.25	25.00	-0.27	0.78	-6.90	-0.19	5.23	-0.11	0.91	-0.57
T × T	228.00	25.00	2.27	0.02	57.10	-5.20	5.23	-0.25	0.80	-1.29
SO × SO	-214.00	25.00	-2.12	0.03	-53.40	15.40	5.23	0.74	0.46	3.86
SBP × T	28.80	14.80	1.71	0.09	25.20	1.78	3.07	0.51	0.61	1.56
SBP × SO	-37.90	14.80	-2.24	0.02	-33.10	0.10	3.07	0.03	0.97	0.09
T × SO	12.70	14.80	0.22	0.83	3.20	-13.30	3.07	1.09	0.28	-3.33

SBP: sugar beet pulp; T: tallow and SO: soybean oil.
VH: villus height (μm) and VW: villus width (μm).
SE: standard error.
Significant= P < 0.05.

Table 12 Estimated parameters of response surface model for VH:CD and GC in jejunum of broiler chicks on day 7 of age

Quadratic model term*	VH:CD model					GC 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	5.40	0.09	45.90	< 0.0001	4.51	25.36	0.08	312.24	< 0.0001	26.82
SBP	-0.43	0.09	-8.06	< 0.0001	-0.72	1.34	0.07	12.54	< 0.0001	0.99
T	-0.81	0.09	-3.57	< 0.01	-0.32	0.92	0.07	1.81	0.07	0.14
SO	0.84	0.09	0.19	0.84	0.01	-2.23	0.07	1.16	0.25	0.09
SBP × SBP	0.02	0.17	0.36	0.72	0.06	-0.17	0.15	-3.60	< 0.01	-0.54
T × T	0.40	0.17	0.59	0.55	0.10	-0.66	0.15	-1.10	0.27	-0.16
SO × SO	-0.55	0.17	-0.81	0.42	-0.13	2.20	0.15	3.66	< 0.01	0.55
SBP × T	-0.04	0.10	-0.38	0.70	-0.03	-0.20	0.08	-2.06	0.04	-0.18
SBP × SO	-0.05	0.10	-0.46	0.65	-0.04	-0.10	0.08	-1.03	0.30	-0.09
T × SO	-0.32	0.10	-0.79	0.43	-0.08	0.79	0.08	2.24	0.03	0.19

SBP: sugar beet pulp; T: tallow and SO: soybean oil.
VH:CD: villus height to crypt depth ratio and GC: number of goblet cells in each villus.
SE: standard error.
Significant= P < 0.05.

Table 13 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 VH and VW in jejunum of broiler chicks on day 14 of age

Source of variation	VH model				VW model		
	df	Sum of squares	R^2	P-value	Sum of squares	R^2	P-value
Linear	3	470880.00	0.53	< 0.0001	6277.45	0.57	< 0.0001
Quadratic	3	102371.00	0.10	0.01	1224.29	0.10	< 0.0001
Interaction	3	19586.00	0.02	0.30	628.08	0.05	0.01
Total model (regression)	9	592837.00	0.64	< 0.0001	8128.82	0.72	< 0.0001
Lack of fit	5	117390.00		< 0.0001	871.92		< 0.01
Pure error	45	148107.00			1662.11		
Total error	50	265497.00			2534.03		

VH: villus height (μm) and VW: villus width (μm).
Significant= P < 0.05.

Table 14 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 VH:CD and GC in jejunum of broiler chicks on day 14 of age

Source of variation	VH:CD model				GC model		
	df	Sum of squares	R^2	P-value	Sum of squares	R^2	P-value
Linear	3	30.94	0.58	< 0.0001	5535.78	0.56	< 0.0001
Quadratic	3	10.49	0.21	< 0.0001	2142.51	0.21	< 0.0001
Interaction	3	0.72	0.01	0.32	224.96	0.02	0.13
Total model (regression)	9	42.61	0.80	< 0.0001	7903.25	0.77	< 0.0001
Lack of fit	5	3.64		< 0.01	622.56		< 0.01
Pure error	45	6.61			1291.92		
Total error	50	10.25			1914.48		

VH:CD: villus height to crypt depth ratio and GC: number of goblet cells in each villus.
Significant= P < 0.05.

Table 15 Estimated parameters of response surface model for VH and VW in jejunum of broiler chicks on day 14 of age

Quadratic model term*	VH model					VW 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	795.90	14.50	49.56	< 0.0001	716.80	166.43	1.41	113.25	< 0.0001	160.02
SBP	-76.40	13.30	-9.16	< 0.0001	-121.90	-3.23	1.30	-10.90	< 0.0001	-14.17
T	22.00	13.30	-2.06	0.04	-27.40	6.30	1.30	-2.09	0.04	-2.72
SO	208.00	13.30	0.71	0.48	9.40	10.90	1.30	0.83	0.40	1.08
SBP × SBP	-3.28	25.40	-0.40	0.69	-10.00	-1.97	2.48	-2.44	0.01	-6.05
T × T	-137.00	25.40	-1.35	0.18	-34.20	-14.52	2.48	-1.46	0.14	-3.63
SO × SO	-200.00	25.40	-1.97	0.05	-49.90	-1.85	2.48	-0.19	0.85	-0.46
SBP × T	32.30	14.90	1.90	0.06	28.30	4.81	1.45	2.90	< 0.01	4.21
SBP × SO	4.10	14.90	0.24	0.81	3.60	-0.71	1.45	-0.43	0.66	-0.62
T × SO	6.10	14.90	0.10	0.91	1.50	-11.33	1.45	-1.95	0.05	-2.83

SBP: sugar beet pulp; T: tallow and SO: soybean oil.
VH: villus height (µm) and VW: villus width (µm).
SE: standard error.
Significant= P < 0.05.

Table 16 Estimated parameters of response surface model for VH:CD and GC in jejunum of broiler chicks on day 14 of age

Quadratic model term*	VH:CD model					GC 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	5.74	0.08	62.49	< 0.0001	5.61	55.67	1.23	47.09	< 0.0001	57.84
SBP	0.21	0.08	-12.04	< 0.0001	-0.99	-5.27	1.13	11.90	< 0.0001	13.44
T	0.22	0.08	-2.04	0.04	-0.16	3.55	1.13	0.09	0.92	0.11
SO	0.62	0.08	1.31	0.19	0.10	0.40	1.13	-1.74	0.08	-1.97
SBP × SBP	-0.24	0.15	-4.77	< 0.0001	-0.75	4.25	2.15	6.05	< 0.0001	13.02
T × T	-0.36	0.15	-0.58	0.56	-0.09	-4.57	2.15	-0.53	0.59	-1.14
SO × SO	0.27	0.15	0.43	0.66	0.06	-3.04	2.15	-0.35	0.72	-0.76
SBP × T	-0.05	0.09	-0.51	0.61	0.04	-1.21	1.26	-0.84	0.40	-1.06
SBP × SO	0.09	0.09	0.85	0.39	0.07	-2.07	1.26	-1.84	0.07	-2.33
T × SO	-0.59	0.09	-1.59	0.11	-0.14	6.72	1.26	1.33	0.19	1.68

SBP: sugar beet pulp; T: tallow and SO: soybean oil.
VH:CD: villus height to crypt depth ratio and GC: number of goblet cells in each villus.
SE: standard error.
Significant= P < 0.05.

The lack of fit for all models was significant (P<0.05) (Tables13 and 14), indicating that more complex models and further experiments with other factors are needed.

The current study showed that, the highest ADG and the lowest FCR were observed with diets containing 0.51% SBP, 0.20% T, 0.38% SO and 0.50% SBP, 0.05% T and 0.10% SO, respectively. Recent research with broilers (Jimenez-Moreno *et al.* 2013) and layers (Guzman *et al.* 2015) have illustrated that the use of a temperate quantity of fiber sources in the diet is advantageous for the growth and adequate operation of the GIT and ameliorates the digestibility of nutrients and performance of birds. Sugar beet pulp is rich in pectin and can increase nutrients adhesion to each other and prevent the release and absorption of nutrients in the small intestine. Excess pectin in the diet due to increased SF may reduce feed intake and nutrient consumption (Jimenez-Moreno *et al.* 2013). Jimenez-Moreno *et al.* (2009) in the study of the effect of different sources of dietary fiber and fat on the production performance of broiler chickens stated that the addition of fiber improved body weight gain and FCR. The increase in nitrogen retention, ether extract and apparent metabolizable energy was more noticeable for chickens consuming oat hulls compared to sugar beet pulp. These researchers concluded that adding a moderate amount of fiber to the broilers' diets could improve performance. Several surveys have stated the negative effect of SF sources, however, in our study; the presence of SBP (up to 0.50% of diet) in the diet probably amends the development of the gastrointestinal tract and, as a result, boosts ADG and FCR at 14d of age. It has been reported that the inclusion of fibrous compounds stimulates the development of the GIT and the growth of villus, thereby increasing the level of gastrointestinal absorption, but diluting the diet reduces the energy and nutrient digestibility in broiler chickens and an increase in the VH in the duodenum, which may be related to the bird's attempt to increase nutrient uptake (Adibmoradi *et al.* 2016). The results of the study of Morgan *et al.* (2021) represented that it can be advantageous to use soluble non-starch polysaccharides (NSP) in broilers' diet, because of its remarkable effect on the birds performance, content of excreta moisture and digestibility of nutrient, especially in NSP-poor diets, such as sorghum- and corn-based diets. Few studies have been performed on gastrointestinal development using the RSM. Aziz-Aliabadi *et al.* (2021) have stated that with an increase in bird age due to the development and evolution of the GIT, the bird's ability to use SBP as a SF source mounts. Rezaei *et al.* (2011) observed a dose-dependent relationship between micronized insoluble fiber (MIF) concentration and the number of GC and the ileal VH:CD ratio, so that the highest amount of these parameters was reported in birds fed diets containing 0.50% MIF. Rahmatnejad and Saki (2016) observed that as the SF content of the diet increases, the VH decreases, and the differences in the studies may be related to the type of dietary fiber source. In the present experiment at 7 day of age, a reduction in the VH:CD ratio of chicks fed a diet with higher levels of SBP (as a source of SF) was observed, so that the highest ratio was obtained in birds fed diets with 0.00% SBP. On the other hand, on 14 day of age, the highest VH:CD ratio in the jejunum (main site of absorption)was observed in birds fed diets containing 0.63% SBP, which may be due to the increasing age of the birds and their better ability to utilize nutrients. Dos Santos *et al.* (2019) reported that diets with higher fiber (IF) content increased feed intake and VH but declined the relative weight of the pancreas and had no effect on the body weight gain. These types of responses may be due to the effect of fiber dilution, reducing nutrient uptake and thus stimulating the growth of villus to improve uptake. They stated that chickens receiving higher levels of fiber (7.00% rice bran) showed higher VH, but fiber levels did not affect VW, CD, VH:CD, absorption surface area, and the ratio of the fused villus. Wils-Plotz and Dilger (2013) stated that methoxy pectin consumption increased the number of GC in the intestine, indicating the presence of a thicker mucosal layer, which is related to reduce nutrient availability. This can augment the energy needed for-GIT activities. In accordance with the Wils-Plotz and Dilger (2013), in our experiments, the presence of SBP promotes the number of GC in the jejunum. Chen *et al.* (2019) stated that at the 7 day of age in broilers, the main effect of the treatments showed that 0.05 or 0.075% of lysophospholipid supplementation reduced CD in the duodenum and increased VH:CD ratio compared to those fed diets with no lysophospholipid supplementation. They also observed that the use of 0.025 or 0.075% of lysophospholipid boosted the VH in the jejunum compared to the 0.00% of lysophospholipid. Increasing the VH:CD ratio indicates a decrease in intestinal enterocyte production. As a result, the energy stored from reducing the turnover of the epithelial cells can be used by the bird to produce other tissues, and this can improve bird's growth. The findings of Attia *et al.* (2020) reported that consumption of canola oil significantly increases VH and VH:CD ratio compared to coconut oil. In the present study, the use of SO had a greater role in improving VH:CD ratio in the jejunum, probably due to the higher ratio of unsaturated fatty acids to saturated fatty acids in SO compared to T. Sacakli *et al.* (2023) studied the effects of dietary monoglyceride mixture on the number of goblet cells in jejunum. Their results showed that there were positive dose-dependent alterations in the goblet cells'distribution along the jejunum villus. These results were in agreement with another study reporting changes in the number of goblet cells with the supplementation of fatty acid blends to broilers' diet (Amer *et al.* 2020). In agreement with our study, Faria Filho *et al.* (2008) reported that RSM is effective in predicting the optimal performance point of broiler chickens. They stated that this mathematical method allows more accurate determination of the optimal amount of dietary protein, growth temperature and slaughter age of broilers. It has been reported that artificial neural network models provide more accurate and correct predictions for broiler performance compared to response procedure models (Ahmadi and Golian, 2011). Ghanaatparast-Rashti *et al.* (2018), when using the RSM to study the jejunum morphology of broiler chickens, found that due to insignificant lack of fit for the relevant traits, the data are in good agreement with their models, where as in the current experiment, at 14 day of age, we needed more sophisticated statistical models due to significance of lack of fit.

CONCLUSION

The results of this experiment showed that at the young age due to the growth and development of the digestive system, the negative effects of soluble fibers reduced to some extent and the bird will be able to use fat sources better. Current results illustrated that with increasing age (from 7 to 14 days), the negative effect of sugar beet pulp on the villus height to crypt depth ratio decreased, which is probably due to the increase in chicks' age, GIT development and optimal consumption of nutrients, and soybean oil played a more important role in villus height to crypt depth ratio probably due to its higher unsaturated fatty acids. The number of goblet cells per villus increased with the increment of sugar beet pulp level in the diet. Generally, aging and development of the gastrointestinal tract can modify the negative effects of soluble fibers. Central composite design decreases the number of treatments and the costs of study. In addition, the RSM can be utilized in poultry experiments to characterize the relevance of nutrients to attain the optimized response level.

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