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Effects of dietary supplementation of betaine and sepiolite on performance and intestinal health in broilers

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Abstract: The aim of the study was to explain the effects of betaine and sepiolite in diets on performance, carcass characteristics, some blood parameters and some intestinal health parameters in broilers. A total of 192 daily Ross 308 male broiler chicks were divided into one control group and three experiment groups each containing 48 chicks. Betaine and sepiolite was added as top dressed to the diets of experimental groups at the level of 0.15% betaine (1st group), 1.5% sepiolite (2nd group) and 0.15% betaine + 1.5% sepiolite (3rd group). Sepiolite and betaine were not added to the diet of control group. The experimental period was 6 weeks. No differences were observed in body weight, overall body weight gain, feed intake, feed conversion ratio, carcass yield and the relative weights of gizzard, heart, liver, spleen and bursa of Fabricius among groups. The relative weight of abdominal fat decreased with betaine usage. Supplemental betaine increased relative percentage of kidney weight. Dietary supplementation of betaine with sepiolite had some positive effects on intestinal histomorphology characteristics. The level of serum triglyceride was reduced with 0.15% betaine inclusion in the diet. Albumin, cholesterol, protein, uric acid, AST, ALP and ALT levels of blood serum were not affected by betaine with and without sepiolite supplement. Blood serum IgG level was increased by dietary supplementation of betaine with and without sepiolite. It is concluded that the dietary usage of betaine with sepiolite in broilers can be useful in the field due to having some improvements in intestinal histomorphology and immunity.

Keywords: Betaine, broiler, intestinal histomorphology, performance, sepiolite.

Broyler rasyonlarına betain ve sepiyolit ilavesinin performans ve bağırsak sağlığı üzerine etkileri

Özet: Bu araştırmanın amacı, broyler karma yemlerine betain ve sepiyolit ilavesinin performans, karkas özellikleri, bazı kan parametreleri ve bazı bağırsak sağlığı parametreleri üzerine etkilerini belirlemektir. Toplam 192 adet günlük Ross 308 erkek broyler civciv her biri 48 adet içeren bir kontrol ve üç deneme grubuna ayrılmıştır. Betain ve sepiyolit deneme grubu yemlerine dökme olarak %0.15 betain (1. grup), %1.5 sepiyolit (2. grup) ve %0.15 betain + %1.5 sepiyolit (3. grup) düzeylerinde ilave edilmiştir. Kontrol grubu konsantre yemine betain ve sepiyolit ilave edilmemiştir. Deneme süresi 6 haftadır. Betain ve sepiyolit ilavesi canlı ağırlık, canlı ağırlık kazancı, yem tüketimi, yemden yararlanma, karkas randımanı ile karaciğer, kalp, taşlık, dalak ve bursa Fabricus relatif ağırlıkları bakımından farklılık yaratmamıştır. Abdominal yağ relatif ağırlığı betain katkısı ile düşmüştür. Betain katkısı böbrek relatif ağırlığını artırmıştır. Betain ve sepiyolit katkısının bağırsak histomorflojisi özellikleri üzerine pozitif etkisi gözlenmiştir. Betainin %0.15 düzeyinde ilavesi kan serumu trigliserit değerini düşürmüştür. Kan serumunda protein, albumin, ürik asit, kolesterol, AST, ALT ve ALP düzeyleri betain ve/veya sepiyolit ilavesinden etkilenmemiştir. Kan serum IgG düzeyi betain ve/veya sepiyolit ilavesi ile artmıştır. Sonuç olarak bağırsak histomorfolojisi ve immunite üzerine olumlu etkilerinden dolayı betain ve sepiyolitin broyler rasyonlarında kullanımının sahada yararlı olabileceği kanısına varılmıştır.

Anahtar sözcükler: Bağırsak histomorfolojisi, betain, broyler, performans, sepiyolit.

Introduction

Betaine, a trimethylglycine, is created by choline in combination with the glycine. Betaine has been shown to have potential benefits for protecting intestinal cells and thus counteracting performance losses during heat stress and coccidiosis. Betaine is considered to be a methyl donor and osmoprotectant to improve, growth performance, nutrient digestibility and feed conversion ratio of poultry (29).

Betaine supplementation improved performance and carcass characteristics in broilers (29, 50). The increase in mineral absorption and retention affected water retention capacity of muscle tissue (13). High water retention capacity could be explained by higher meat/fat ratio in the body.

Sepiolite, one of the clay minerals is a magnesium silicate having fibre structure and internal crystal ducts ($Si_{12}Mg_8O_{30}(OH_2)_4(OH)_4.8H_2O$). Sepiolite has amorf, compact and lump types in the nature. Sepiolite has been approved as binding, anti- caking agent and coagulating agent for all animal types in European Union as E562 (10). Sepiolite is an ideal carrier for vitamins, minerals and other feed additives. Also it shows homogenizing characteristics by preventing seperation of feed components. Sepiolite has a low cation change capacity thus it is a good premix tranporter. Being an inert material it refers to have high chemical stability (16). It has a high water absorption capacity and prevents loss of dust. Avcılar et al. (5) reported that the addition of sepiolite at the levels of 25% and 50% in the broiler litter may use to improve the litter quality. Sepiolite increases growth performance due to increasing digestion and absorption of endogen enzymes, fats, carbonhydrate and proteins (33, 41).

Betaine supplementation to methyl group donor-adequate diets improved weight gain, feed conversion, carcass and breast muscle yield by 3-15% in poultry (31, 43). Ismail and Ahmad (20) concluded that the dietary betaine supplementation have beneficial effect on growth performance and some blood parameters without any negative effects on physiological responses in broilers. Some researchers (28, 46) have also reported that dietary betaine had positively affected the breast meat yield but reduced abdominal fat pad in broilers. Transitory inflammatory responses during the pre-starter and starter period was reduced by the presence of betaine in the diet of broilers (37).

Sepiolite can be used as therapeutic agents since it most likely replaces the growth factors in poultry diets (16, 41, 44). Sepiolite inclusion at 1% increased weight gain and reduced abdominal fat percentage and the levels of triglyceride and cholesterol in blood serum (12). Yalçın et al. (49) reported that 1% sepiolite supplementation was an effective additive in broilers due to its beneficial effects on performance, abdominal fat, viscosity of ileal digesta, ileal digestibility and duodenal villus height.

However, a published report regarding the interaction of sepiolite and betaine in poultry was not found. Furthermore, no studies about the effects of 1.5% sepiolite on broiler nutrition were seen. It was assumed that sepiolite and betaine which are given in combination might improve performance and carcass characteristics by influencing the nutrient metabolism and immune system. Therefore, this experiment was aimed to determine the effects of sepiolite and betaine supplementation on performance, carcass characteristics, some blood parameters and some intestinal health parameters in broilers.

Materials and Methods

All study were approved by the Animal Ethics Committee of the Ankara University (2012-16-100).

Animals and diets: A total of 192 daily Ross 308 male broiler chicks were divided to four groups and each group had 8 replicates of 6 chicks each. Each replicates were placed in separate floor pen having 80 cm width x 90 cm length x 80 cm height. As a litter, wood shavings was used. There were two nipples and one hanging suspended feeder in each pen. Water and mash feed were *ad libitum* during 6 weeks. Lighting was permanently applied. Temperature of room was 32±2°C on the first week and then gradually reduced to 24-26°C and this temperature was maintained upto slaughtering. The ingredients and chemical composition of the basal diets were given in Table 1. The diets were formulated to meet or exceed the nutrient requirements of broilers based on the management guide of Ross 308. Basal diets were supplemented with 0 (control), 0.15% commercial betaine (^{TNI}betain96 AN, Trouw Nutrition, Turkey), 1.5% sepiolite (Exal TH, Tolsa Turkey, Turkey) and 0.15% betaine + 1.5% sepiolite. Control group diet consisted of only basal diet. The composition of sepiolite used in this present study was shown in Table 2 (7).

Table 1. The ingredients and chemical composition of the basal diets (as-fed basis).
Tablo 1. Karma yemlerin bileşimi ve besin maddesi içerikleri.

Ingredients (g/kg)	Starter diet 0-21 days	Grower diet 22-42 days
Corn	474.0	493.0
Soybean meal	265.0	190.0
Full fat soya	210.0	237.0
Sunflower seed oil	10.0	40.0
Limestone	13.0	13.5
Dicalcium phosphate	19.0	18.0
Methionine	2.0	2.0
Lysine	1.0	1.0
Sodium bicarbonate	1.0	1.0
Salt	3.0	3.0
Vitamin mineral premix[1]	1.5	1.5
Salinomycine	0.5	-
Chemical composition (Analyzed)		
Metabolizable energy[2] (MJ/kg)	12.96	13.85
Crude protein (g/kg)	234.8	204.0
Calcium (g/kg)	11.3	10.4
Total phosphorus (g/kg)	7.1	6.4

[1]: Provides 1.5 kg of premix: 9000000 IU vitamin A, 4000000 vitamin D_3, 50000 mg vitamin E, 2000 mg vitamin K_3, 2000 mg vitamin B_1, 5000 mg vitamin B_2, 40000 mg niacin, 15000 mg calcium D pantothenate, 2000 mg vitamin B_6, 10 mg vitamin B_{12}, 1500 mg folic acid, 100 mg D-biotin, 120000 mg Mn, 40000 mg Fe, 100000 mg Zn, 16000 mg Cu, 1250 mg I, 200 mg Co, 300 mg Se, 125000 mg antioksidant (ethoxyquin, BHA).
[2]: Metabolizable energy content of diets was calculated (27).

Table 2. The composition of sepiolite[1].
Tablo 2. Sepiyolit bileşimi[1].

Sepiolite, %	65
Attapulgite, %	9
Dolomite, %	18
Calcit, %	8
Humidity, %	8.23
Heavy metals	
As, mg/kg	2.6
Cd, mg/kg	<1
Pb, mg/kg	1.16
Hg, mg/kg	0.02

[1]: Burçak and Yalçın (7).

Traits measured: Nutrient composition of basal diet was analyzed (3) for crude protein (CP, Method 968.06), ether extract (EE, Method 920.39), crude fiber (CF, Method 932.09) and ash (Method 967.05). Calcium (14) and total phosphorus (1) were analyzed. Metabolizable energy levels of diets were calculated (27).

Birds were individually weighed at the beginning and weekly to determine weight gain. Feed intake was determined weekly and feed conversion ratio was calculated as kg feed per kg weight gain. To determine mortality birds were followed up daily. The lesions of footpad (24) and breast (2) were evaluated and litter samples were taken to analyze for moisture content (3) on day 42nd of the experiment.

At the end all broilers were individually weighed and 8 broilers from each group were slaughtered by severing the jugular vein, internal organs, head and foot were removed manually after defeathering. Carcasses were weighed to calculate hot carcass yield. Abdominal fat, spleen, liver, heart, gizzard, kidney and bursa of Fabricius were weighed. Relative weights of them were calculated by dividing to slaughtering weight.

Histomorphological analysis were performed on day of 21 and 42 days of experiment, intestinal tissue samples were taken after slaughtering of 8 broilers (one from each replicate) from each group. About 2 cm segments were taken from the duodenum, jejunum and ileum and all divertikulum applications to intestinal segments were done as reported by Yalçın et al. (49). Intestinal sections were analyzed using Cellsens CS-ST-V1.8 software in digital microscope with camera control (Olympus BX51-DP71) (42). Ten measurements for each sample were made. Villus height and crypt depth were measured as mentioned by Yalçın et al. (49). The ratio of villus height to crypt depth was calculated. A geometric model $[2\pi \times villus\ height \times (villus\ width \div 2)]$ was used to calculate villus area as mm^2 (36).

Blood samples were taken from vena brachialis under the wing from eight fed broilers from each group on day 42 and centrifuged at 3.220 x g for 8 min to collect serum. The levels of total protein (ACN 678), uric acid (ACN 700), triglyceride (ACN 781), total cholesterol (ACN 433), alkaline phosphatase (ALP) (ACN 158), aspartate amino transferase (AST) (ACN 687) and alanine amino transferase (ALT) (ACN 685) by Abbott Aeroset autoanalyser with commercial Cobas kits (Roche Diagnostics). Serum IgG levels were determined with ELISA (17).

Statistical analyses: SPSS programme (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Kolmogorov-Smirnov test was applied for the normality of data distribution. One-way ANOVA was used to detect the differences among groups. The significance of mean differences between groups were analyzed using Duncan. Values were shown as mean±standard error in the tables. Statistical significance level was accepted as $P<0.05$ (9).

Results

Effects of dietary sepiolite and betaine supplementation on performance and litter moisture were given in Table 3. Final body weight, total weight gain, feed intake and feed conversion ratio were not affected by the inclusion of sepiolite and/or betaine. No significant interaction between sepiolite and betaine was found in these parameters. Carcass yield and the percentages of heart, spleen, liver, bursa of Fabricius, gizzard and proventriculus were not affected by the treatments (Table 4). No interaction effects were also seen in these parameters. Effects of sepiolite and betaine on duodenal, jejunal and ileal histomorphology on day 21 and 42 were shown in Table 5, Table 6 and Table 7, respectively.

Villus height, crypt depth and surface area were increased by dietary betaine supplementation ($P<0.05$) at day 21 and the ratio of villus height to crypt depth was increased by sepiolite supplementation at day 42 in duodenum ($P<0.05$). However no interaction was seen in these parameters. Jejunal villus height and vilus surface area were increased with only sepiolite or only betaine supplementation at day 21. Significant interactions in villus height ($P=0.035$) and the ratio of villus height to crypt depth ($P=0.021$) were also seen in villus height due to having increasing effect at day 21. There were significant interactions in villus width ($P=0.008$), crypth depth ($P<0.001$) and the ratio of villus height to crypt depth ($P=0.001$) in jejunum at day 42. İleal villus height ($P<0.001$) and villus surface area ($P=0.008$) was increased with only sepiolite supplementation at day 21. Significant interaction was observed in the ratio of villus height to crypt depth at day 21 ($P=0.020$) and at day 42 ($P<0.001$) in ileum and in the crypt depth at day 42 ($P<0.001$).

The levels of total protein, uric acid, albumin, total cholesterol, ALT, AST and ALP in blood serum were not

affected with sepiolite with/without betaine supplementation as given in Table 8. No interaction effects were also observed in these parameters. Serum IgG concentration was increased with sepiolite and betaine (P<0.001). There was a significant interaction of sepiolite and betaine (P=0.035).

Table 3. Effects of dietary sepiolite and betaine supplementation on performance and litter moisture.
Tablo 3. Karma yemlere betain ve sepiyolit ilavesinin performans ve altlık nemi üzerine etkileri.

Sepiolite %	Betaine %	BW day 0	BW day 42	BWG 0-21 day	BWG 22-42 day	BWG 0-42 day	FI 0-21 day	FI 22-42 day	FI 0-42 day	FCR 0-21 day	FCR 22-42 day	FCR 0-42 day	Litter moisture
		g	g	g	g	g	g	g	g	g/g	g/g	g/g	%
0		44.24	3024.61	839.42	2142.08	2981.50	1092.94	3476.64	4569.58	1.30	1.63	1.53	26.90
1.5		43.99	2970.50	819.66	2112.48	2932.13	1096.53	3476.87	4573.40	1.34	1.65	1.56	26.90
	0	44.19	3001.80	826.21	2134.77	2960.98	1100.34	3491.78	4592.12	1.33	1.64	1.55	26.86
	0.15	44.05	2993.32	832.87	2119.78	2952.65	1089.13	3461.73	4550.87	1.31	1.64	1.54	26.94
0	0	44.36	3016.96	830.68	2141.13	2971.81	1090.16	3478.00	4568.16	1.31	1.63	1.54	26.95
0	0.15	44.12	3032.27	848.16	2143.03	2991.19	1095.72	3475.29	4571.01	1.29	1.63	1.53	26.86
1.5	0	44.01	2986.64	821.74	2128.42	2950.16	1110.52	3505.55	4616.08	1.35	1.65	1.57	26.77
1.5	0.15	43.97	2954.36	817.57	2096.53	2914.11	1082.55	3448.18	4530.73	1.32	1.65	1.56	27.02
							P						
Sepiolite		0.403	0.272	0.035	0.485	0.298	0.819	0.996	0.932	0.084	0.416	0.214	0.989
Betaine		0.635	0.860	0.459	0.720	0.858	0.474	0.493	0.356	0.235	0.905	0.631	0.912
Sepiolite*Betaine		0.729	0.622	0.231	0.686	0.552	0.286	0.532	0.323	0.877	0.938	0.917	0.807

BW: body weight, BWG: body weight gain, FI: feed intake, FCR: feed conversion ratio

Table 4. Effects of dietary sepiolite and betaine supplementation on carcass yield and relative weight percentages of internal organs.
Tablo 4. Karma yemlere betain ve sepiyolit ilavesinin karkas randımanı ve relatif iç organ ağırlıkları üzerine etkileri.

Sepiolite %	Betaine %	Hot carcass yield	Heart	Liver	Spleen	Bursa fabricius	Gizzard	Kidney	Abdominal fat	Proventriculus
		%	%	%	%	%	%	%	%	%
0		73.43	0.524	1.972	0.113	0.22	1.245	0.52	1.195	0.337
1.5		73.00	0.505	1.955	0.105	0.21	1.247	0.51	1.192	0.341
	0	73.46	0.524	1.962	0.114	0.22	1.225	0.50	1.261	0.340
	0.15	72.98	0.504	1.964	0.104	0.21	1.267	0.53	1.127	0.338
0	0	73.45	0.536	1.963	0.116	0.23	1.242	0.51	1.390[a]	0.342
0	0.15	73.41	0.511	1.980	0.110	0.21	1.248	0.54	1.001[b]	0.332
1.5	0	73.47	0.513	1.961	0.112	0.22	1.208	0.49	1.131[ab]	0.337
1.5	0.15	72.54	0.497	1.948	0.098	0.20	1.285	0.53	1.253[ab]	0.344
							P			
Sepiolite		0.104	0.425	0.744	0.337	0.310	0.962	0.470	0.975	0.801
Betaine		0.067	0.379	0.972	0.232	0.060	0.294	0.030	0.220	0.898
Sepiolite*Betaine		0.088	0.865	0.771	0.556	0.830	0.369	0.680	0.023	0.532

[a,b]: Means with different letter in the same column are different at P<0.05 in instances with significant interaction.

Table 5. Effects of dietary sepiolite and betaine supplementation on duodenal histomorphology at day 21 and 42.
Tablo 5. Karma yemlere betain ve sepiyolit ilavesinin 21. ve 42. günlerde duodenum histomorfolojisi üzerine etkileri.

Sepiolite %	Betaine %	21 day					42 day				
		VH µm	VW µm	CD µm	VH/CD	VSA mm^2	VH µm	VW µm	CD µm	VH/CD	VSA mm^2
0		1074.51	158.32	109.85	10.15	0.54	1439.80	207.13	153.43	9.64	0.94
1.5		1090.07	159.46	118.29	9.63	0.54	1623.10	212.63	146.13	11.50	1.10
	0	1015.45	156.62	105.81	10.16	0.49	1479.91	208.68	150.56	10.06	0.98
	0.15	1149.13	161.16	122.32	9.63	0.59	1582.99	211.07	149.00	11.08	1.05
0	0	961.42	155.08	100.89	9.96	0.46	1395.12	208.86	149.50	9.55	0.91
0	0.15	1187.59	161.55	118.81	10.35	0.61	1484.49	205.40	157.37	9.73	0.96
1.5	0	1069.47	158.15	110.73	10.35	0.51	1564.70	208.50	151.63	10.58	1.05
1.5	0.15	1110.68	160.77	125.84	8.91	0.56	1681.49	216.75	140.63	12.43	1.14
						P					
Sepiolite		0.778	0.916	0.124	0.518	0.977	0.006	0.678	0.351	0.003	0.076
Betaine		0.020	0.675	0.004	0.513	0.022	0.109	0.856	0.840	0.093	0.444
Sepiolite*Betaine		0.101	0.859	0.794	0.260	0.228	0.828	0.658	0.230	0.163	0.772

VH: villus height, VW: villus width, CD: crypt depth, VSA: villus surface area

Table 6. Effects of dietary sepiolite and betaine supplementation on jejunal histomorphology at day 21 and 42.
Tablo 6. Karma yemlere betain ve sepiyolit ilavesinin 21. ve 42. günlerde jejunum histomorfolojisi üzerine etkileri.

Sepiolite %	Betaine %	21 day					42 day				
		VH µm	VW µm	CD µm	VH/CD	VSA mm^2	VH µm	VW µm	CD µm	VH/CD	VSA mm^2
0		773.71	167.86	132.52	5.98	0.41	1193.42	206.10	160.11	7.84	0.78
1.5		831.48	177.12	134.50	6.37	0.47	1156.30	188.78	153.83	8.19	0.68
	0	768.51	164.97	130.91	6.00	0.40	1175.70	202.38	153.72	8.17	0.75
	0.15	836.68	180.01	136.11	6.35	0.47	1174.02	192.50	160.22	7.86	0.71
0	0	763.28b	158.75	128.20	6.08^{b}	0.38	1176.38	225.96^{a}	180.85^{a}	6.74^{b}	0.85
0	0.15	784.14b	176.98	136.84	5.89^{b}	0.44	1210.46	186.24^{b}	139.38^{b}	8.93^{a}	0.72
1.5	0	773.75b	171.19	133.63	5.93^{b}	0.42	1175.03	178.79^{b}	126.60^{b}	9.59^{a}	0.66
1.5	0.15	889.21a	183.04	135.37	6.81^{a}	0.51	1137.58	198.76^{ab}	181.07^{a}	6.78^{b}	0.70
						P					
Sepiolite		0.011	0.262	0.586	0.091	0.032	0.603	0.109	0.421	0.617	0.143
Betaine		0.003	0.072	0.158	0.130	0.006	0.981	0.354	0.405	0.659	0.518
Sepiolite*Betaine		0.035	0.697	0.345	0.021	0.423	0.616	0.008	< 0.001	0.001	0.206

^{a,b}: Means with different letter in the same column are different at $P<0.05$ in instances with significant interaction.
VH: villus height, VW: villus width, CD: crypt depth, VSA: villus surface area

Table 7. Effects of dietary sepiolite and betaine supplementation on ileal histomorphology at day 21 and 42.
Tablo 7. Karma yemlere betain ve sepiyolit ilavesinin 21. ve 42. günlerde ileum histomorfoloji üzerine etkileri.

		21 day					42 day				
Sepiolite %	**Betaine %**	**VH µm**	**VW µm**	**CD µm**	**VH/CD**	**VSA mm²**	**VH µm**	**VW µm**	**CD µm**	**VH/CD**	**VSA mm²**
0		566.12	161.34	129.88	4.52	0.29	874.57	176.98	145.02	6.24	0,49
1.5		626.08	163.32	136.60	4.72	0.32	903.85	199.60	137.51	6.83	0,57
	0	587.50	160.37	134.04	4.51	0.30	917.80	190.05	142.09	6.7	0,56
	0.15	604.69	164.29	132.45	4.72	0.31	860.62	186.54	140.45	6.37	0,50
0	0	567.17	162.20	126.87	4.65^{ab}	0.29	870.32	174.33	158.09^{a}	5.59^{b}	0,48
0	0.15	565.06	160.48	132.89	4.39^{b}	0.28	878.81	179.63	131.96^{b}	6.89^{a}	0,49
1.5	0	607.83	158.54	141.20	4.38^{b}	0.30	965.27	205.77	126.08^{b}	7.81^{a}	0,64
1.5	0.15	644.32	168.11	132.00	5.05^{a}	0.34	842.43	193.44	148.94^{a}	5.85^{b}	0,51
							P				
Sepiolite		< 0.001	0.741	0.117	0.308	0.008	0.575	0.081	0.185	0.098	0.124
Betaine		0.253	0.515	0.707	0.277	0.227	0.277	0.781	0.770	0.347	0.292
Sepiolite*Betaine		0.201	0.350	0.078	0.020	0.083	0.214	0.487	< 0.001	< 0.001	0.246

^{a,b}: Means with different letter in the same column are different at P<0.05 in instances with significant interaction.
VH: villus height, VW: villus width, CD: crypt depth, VSA: villus surface area

Table 8. Effects of dietary sepiolite and betaine supplementation on blood serum parameters.
Tablo 8. Karma yemlere betain ve sepiyolit ilavesinin kan serum parametreleri üzerine etkileri.

Sepiolite %	**Betaine %**	**Protein g/l**	**Albumin g/l**	**Uric acid mg/dl**	**Cholesterol mg/dl**	**Triglyceride mg/dl**	**ALT IU/l**	**AST IU/l**	**ALP IU/l**	**IgG mg/dl**
0		26.53	10.86	3.86	62.84	61.27	40.71	130.56	794.50	5.14
1.5		27.87	10.66	4.16	58.36	57.39	38.78	131.06	830.94	6.36
	0	26.89	10.46	4.05	61.56	62.65	40.44	126.22	800.89	5.31
	0.15	27.50	11.05	3.97	59.64	56.02	39.05	135.39	824.56	6.19
0	0	26.51	10.26	3.81	65.66	65.93	41.92	123.44	790.44	4.89^{c}
0	0.15	26.54	11.45	3.90	60.02	56.61	39.50	137.67	798.56	5.38^{bc}
1.5	0	27.28	10.67	4.29	57.46	59.36	38.97	129.00	811.33	5.72^{b}
1.5	0.15	28.46	10.65	4.04	59.27	55.43	38.60	133.11	850.56	7.00^{a}
						P				
Sepiolite		0.115	0.783	0.076	0.138	0.173	0.363	0.941	0.268	<0.001
Betaine		0.469	0.417	0.615	0.521	0.023	0.510	0.184	0.470	<0.001
Sepiolite*Betaine		0.494	0.408	0.329	0.215	0.340	0.626	0.459	0.634	0.035

^{a,b,c}: Means with different letter in the same column are different at P<0.05 in instances with significant interaction

Discussion and Conclusion

During the first three weeks, body weight gain was decreased by the usage of sepiolite (P<0.05). However, supplementation of betaine or betaine with sepiolite did not affect weight gain during the first period. In contrast to the present results, some researchers (6, 12, 49) reported that body weight and weight gain were improved by dietary sepiolite. Endogenous digestive enzyme activity in the digestion of fats, proteins and carbohydrates were effective due to the sepiolite usage that increase digesta retention time (41). Body weight and weight gain in this experiment were not affected by 1.5% sepiolite. Some researchers (12, 33, 41) concluded that addition of sepiolite didn't affect feed intake and feed efficiency. Contrary to the present results, Yalçın et al. (49) indicated that 1% and 2% sepiolite supplementation decreased feed

intake by 4.7% and 1.8%, respectively compared to control group. Effects of sepiolite on broiler performance may be affected by many factors such as diet composition, sepiolite quality, water quality, weather and management conditions (49).

Similar to the present results, Schutte et al. (39) (level of 0.04%), Esteve-Garcia and Mack (13) (level of 0.5%) and Santos et al (37) (level of 0.1, 0.3, 0.5%) did not find any effects of betaine usage on body weight, weight gain, feed intake and feed efficiency. Ismail and Ahmad (20) reported that body weight gain and feed conversion ratio were not affected by dietary betaine supplementation during the 6 weeks experimental period. Some researchers (4, 43, 50) observed that daily weight gain and feed efficiency were improved by betaine supplementation. The improvement in performance due to betaine might be due to some factors such as donation of methyl groups (29), increasing growth hormone and insulin like growth factor I (19), enhancing intestinal immunity (25), increasing the nutrient digestibility and absorption ability of gut tract (18) and improving gut health and function (22, 29).

One chick in each group was dead during six weeks experiment. Similarly, some researchers observed that dietary supplementation of sepiolite (6, 12) and betaine (45, 51) didn't cause any differences in mortality in broilers. In the present study, sepiolite and/or betaine addition had no significant effect on litter moisture (Table 3), footpad lesions and breast burn of broilers. Footpad lesions and breast burn didn't seen due to the small number of birds in each pen and due to the optimal management and environmental conditions and proper litter moisture during the experiment. Similar results were observed by Yalçın et al. (49) in litter moisture. Nevertheless, addition of sepiolite may have beneficial effects against low digestible diets, litter quality, breast lesions and footpad dermatitis under conditions having large capacity and poor hygienic conditions (42, 49). Parizadian Kavan et al. (34) reported that the litter moisture in group fed the diet supplemented with 3% clinoptilolite, an aluminum silicate, was lower than that of control group. Olver (32) also showed that dietary zeolite supplementation leads lower litter moisture content. Mostashari-Mohases et al. (30) reported that excreta moisture content decreased with betaine supplementation.

The relative abdominal fat weight decreased with betaine ($P<0.05$) but didn't change with sepiolite or sepiolite with betaine supplementation. There is an interaction in relative abdominal fat weight between sepiolite usage and betaine usage ($P=0.023$). Betaine supplementation increased kidney weight percentages ($P<0.05$). Similar to the present results, some researchers reported that carcass yield (6, 12, 49) and the relative weights of liver (12, 41, 49), spleen (12, 41, 49), heart (49) and bursa of Fabricius (12, 49) were not affected by dietary sepiolite. In some studies (4, 8, 11, 13) carcass yield was increased by betaine supplementation. The improvement in carcass yield may be due to the osmotic effects of betaine that increases water retention (45). An interaction was observed in abdominal fat from betaine and sepiolite supplementation ($P=0.023$) in this present study. Some researchers reported that abdominal fat was decreased by sepiolite supplementation (12, 49) and betaine supplementation (21, 50). Betaine improves quality of lean meat by reducing amount of carcass fat. Zhan et al. (50) and Wang et al. (47) concluded that betaine addition decreased the abdominal fat due to the role in lipid metabolism in the body. However some researchers showed that betaine had no significant effect on the abdominal fat (8, 40, 51). Attia et al. (4) also indicated that there is an increase in abdominal fat by betaine supplementation. Santos et al (37) reported that betaine inclusion at the level of 0.1, 0.3 and 0.5% did not affect liver weight percentage as a proportion of body weight.

From all of the present results in intestinal histomorphology it can be concluded that dietary betaine with sepiolite may be positive effect on intestinal digestion. There was a reverse interaction ($P<0.05$) between sepiolite supplemented group and betaine supplemented group in crypt depth and the ratio of villus height/crypt depth in jejunum and ileum. Betaine affects intestinal development and intestinal functions. Betaine improves water holding capacity in intestinal cells (22) and affects intestinal epithelium. Klasing et al. (25) observed that dietary betaine supplementation could eliminate reduction of villus height due to coccidial infection in chicks. In another studies crpyt/villus ratio was decreased in both healthy and coccidial infected chicks (23) and lesion score was reduced (43) by dietary betaine supplementation. Dietary betaine supplementation with ionophore anticoccidials could help to protect intestinal health (23, 43) and reduce lesion score (39). Santos et al. (37) concluded that betaine supplementation reduced the severity of the apparent inflammatory response, decreased the width and increased the length of the villus and therefore improved the absorptive area of the duodenum. Yalçın et al. (49) stated that 1% and 2% sepiolite supplementation increased duodenal villus height in broilers.

In the present study blood serum triglyceride was decreased with betaine supplementation ($P=0.023$). Sepiolite with and without betaine significantly increased serum IgG concentration. In addition, additive effects were seen with the usage of sepiolite and betaine. In the study of Eser et al. (12), serum levels of cholesterol and triglyceride were decreased with 1% sepiolite supplement. Jahanian and Rahmani (21) reported that plasma

triglyceride was decreased with betaine supplementation but there was no effect on the level of plasma cholesterol. Reduction in triglyceride could be correlated with betaine usage in shynthesis of carnithine and lecithin for fat immobilization. El-Shinnawy (11) showed that betaine supplementation increased serum cholesterol, protein and albumin. Some researchers (8, 15) indicated that betaine supplementation improved the immunity of birds. Latshaw (26) also reported that betaine improves immune response due to increased digestibility and utilization of nutrients such as methionine, carotenoids, lysine, protein and fat (35).

In conclusion sepiolite and betaine supplementation didn't have any negative effects during 6 weeks experimental period. It is concluded that the improvement in intestinal health and immunity due to the dietary sepiolite with betaine supplementation can increase performance under industrial conditions especially under suboptimal conditions in broiler production.

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