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Original Article

The Effects of Dietary Clinoptilolite Supplementation on Fattening Performance, Some Blood and Visceral Organ Parameters in Japanese Quails

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ABSTRACT

This study was aimed at determining the effects of dietary zeolite (clinoptilolite) supplementation on fattening performance, slaughter characteristics, and some blood and visceral organ parameters in Japanese quails. For this purpose, 140 (4x35) four-day-old Japanese quail chicks (*Coturnix coturnix japonica*) were randomly assigned to 4 groups with 4 replicates (9+9+9+8), and the study was continued for a period of 31 days. The groups were fed on a basal diet supplemented with 0% of clinoptilolite (control group), 1.5% of clinoptilolite (Z1.5), 3% of clinoptilolite (Z3) and 6% of clinoptilolite (Z6). For the female quails, the best blood TOS and liver TAS levels were detected in Z3 and Z1.5, respectively. For the male quails, the best blood TAS and liver TOS levels were determined in Z1.5. Histopathological examination demonstrated that dietary supplementation with 1.5% of zeolite had increased the height of the intestinal villi in both male and female quails, and had reduced hepatic lipidosis in female quails. Female quails displayed significantly increased levels of hepatic lipidosis in Z3 and Z6, and liver hepatitis in Z3. In result, it is suggested that zeolite, a hydrated aluminosilicate, in its form containing 90.2% of clinoptilolite, can be incorporated as a feed supplement into quail basal diets at a rate of 3%, owing to its positive effect on fattening performance. Furthermore, the use of zeolite may also contribute to maintaining animal health by reducing humidity in poultry houses and feed.

INTRODUCTION

With an ultimate goal of reducing the high feed costs challenging livestock and poultry production, research is ongoing on natural and synthetic feed additives that may reduce feed intake while increasing live weight (Kocaoglu Guclu & Kara, 2009; Celebi & Kaya, 2012; Demirel *et al.,* 2010; Kutlu & Sahin, 2017). Improved feed conversion rates reduce the feed costs and increase the productivity and profitability of holdings. To date, zeolite has been used in the poultry sector to extend the economic life of bedding material/litter, eliminate the bad odour of manure, and reduce both leg and breast wounds and carcass defects (Kaygisiz & Corekci, 2003; Eleroglu *et al.,* 2011; Gulen *et al.,* 2012; Nikolakakis *et al.,* 2013; Bintas *et al.,* 2014). There are nearly 20 different types of zeolite, including ammonioleucite, analcime, leucite, pollucite, wairakite, chabazite, willhendersonite, erionite, amicite, garronite, gismondine, gobbinsite, clinoptilolite, heulandite, laumontite, gonnardite, mesolite, natrolite, paranatrolite and scolecite (Melenová *et al.,* 2003). The most popular zeolite species are clinoptilolite, chabazite, analcime, erionite, heulandite, laumontite, mordenite and phillipsite. In Turkey, zeolite was first discovered in 1971 in the vicinity of the Gölpazarı-Göynük region (Soylu & Gokkus, 2017). Owing to its high absorption capacity, cation exchange and catalysis and dehydration capacities, clinoptilolite is the

most commonly used zeolite species (Gezen *et al.,* 2004; Yeter & Gokce, 2018). In nature, clinoptilolite is found in pure form and may exist in large reserves. As it does not contain any harmful element, this natural zeolite species is considered to be of high quality and is used as a feed additive in animal nutrition (Melenová *et al.,* 2003).

Natural zeolite species, which are known to bind toxins, eliminate bad odour, increase performance and improve eggshell quality, and do not have any adverse effect, are safely used in animal production. In view of its low cost, zeolite can replace residue-forming toxin-binding feed additives, and can be incorporated into laying hen and broiler chicken diets with an aim to increase yields, and improve eggshell and litter quality (Ozturk *et al.,* 1998; Fendri *et al.,* 2012; *Gilani et al.,* 2016). On the other hand, Yeter and Gokce (2018) reported that the incorporation of natural zeolite (clinoptilolite) into broiler rations had no effect on live weight gain and feed conversion rate.

Having found use in poultry nutrition as a feed additive for broiler chickens and laying hens, zeolite needs to be further investigated for rates at which it can be incorporated into quail rations for the improvement of fattening characteristics. This is of particular significance not only because quails, among all poultry species, have the lowest live weight and hatching weight and the shortest fattening period, but also because female quails weigh heavier than males. Eventually, quails are an important alternative poultry species. Thus, the present study was aimed at determining the effects of the incorporation of 0%, 1.5%, 3% and 6% of clinoptilolite into the starter feed of quail chicks on fattening performance, carcass and slaughter characteristics, blood serum parameters, and the intestines and liver.

MATERIAL AND METHODS

Animals, Feeds and Experimental Design

This study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Board (Approval no: 2020/06-7). The quail chicks used in the present study were obtained by incubating fertilized eggs for a period of 17 days. Newly hatched chicks underwent physical examination and those with no body deformity were transferred to a 4-storey brooder and were fed on a commercial starter feed for 3 days. On day 4 post-hatching, the chicks were weighed individually and those with similar average live weights were randomly assigned to four groups. In total 140 four-day-old Japanese quail chicks (*Coturnix coturnix japonica*) were used in this study and these chicks were assigned to four groups, each comprising 35 animals. Each group was established with four randomly distributed repetitions (9+9+9+8). The study groups were fed on a commercial starter feed (basal diet) and powdered zeolite (clinoptilolite), of a particle size of 0.3-0.7 mm. Accordingly, the control group and Groups Z1.5, Z3 and Z6 received the commercial starter feed supplemented with 0%, 1.5%, 3% and 6% of powdered zeolite (clinoptilolite), respectively. The quails were fattened until 35 days of age. Feed was prepared on a weekly basis and provided to the study groups ad libitum.

The composition and nutritional values of the feed are presented in Table 1. The dry matter, crude ash, crude protein and crude fat content of the feedstuffs was determined with the analytical methods of the AOAC (1990). The crude cellulose content was determined as described by Crampton & Maynard (1983), and the metabolic energy level (kcal/kg) was

Table 1 – Composition and contents nutrients of the basal diet (%).

Ingredients	%
Maize	49
Soybean Meal	33.4
Full Fat Soybean	4
Boncalit	4
Sunflower Seed Meal	2.5
Chicken Meal	2.5
Marble Powder	1.45
Soybean Oil	1.08
Monocalcium phosphate (M.C.P)	0.7
Lysine Sulphate	0.34
Methionine	0.27
Salt	0.16
Sodium Sulphate	0.11
Toxin Binder	0.1
Cholin Chloride	0.07
Threonine	0.07
Vitamin-Mineral premix*	0.25
Calculated composition (%)	
Dry Matter	88
Crude Ash	4.96
Crude Fat	6.17
Crude Protein	23.7
Crude Fiber	4.1
Metabolizable energy kcal/kg	2859.3

*1 kg of the premix provided: 15.000.000 IU of Vitamin A, 5.000.000 IU of Vitamin D3, 100.000 mg of Vitamin E, 3000 mg of Vitamin K3, 5000 mg of Vitamin B1, 8.000 mg of Vitamin B2, 60.000 mg of niacin, 15.000 mg of D-calcium pantothenate, 5000 mg of Vitamin B6, 20 mg of Vitamin B12, 200 mg of D-biotin, 2000 mg of Folic acid,100.000 mg of Vitamin C, 0.02 mg of Cyanocobalamin, 74 mg of Mn (from MnO), 45 mg of Zn (from ZnO), 4 mg of Cu (from CuO), 12.5 mg of Fe (from FeSO4), 0.3 mg of I (from KI), 0.15 mg of Se (from NaSe).

calculated as described by Larbier & Leclercq (1994). The powdered zeolite (with a particle size of 0.3-0.7 mm), incorporated into the basal diet, was supplied from a commercial company (Rota Mining Corp., Turkey). The morphological and chemical features of clinoptilolite are listed in Table 2.

Table 2 – Components of the zeolite (clinoptilolite) mineral.

Mineral Components[1]	Rate (%)
Clinoptilolite	90.2
Cristobalite	3.88
Tridimite 4.45	4.45
Chemical Components[2]	Rate (%)
SiO_2	65.8
Al_2O_3	10.45
CaO	2.42
K_2O	2.57
Fe_2O_3	0.78
MgO	0.96
Na_2O	0.11
MnO	0.08
Cr_2O_3	0.01
P_2O_5 0.02	0.02
SiO_2/Al_2O_3 5.432	5.432

[1]A semi-quantitative whole rock analysis (stack mineralogy) was performed with the X-ray diffraction method (Gundogdu and Yilmaz, 1984)

[2]Analysed with an XRF spectrometer (Vendemiatto and Enzweiler, 2001)

The zeolite incorporated into the diets was supplied from a commercial company (Rota Mining Corp., Turkey) (Table 2) and analysed with XRF spectrometry, using the X-ray diffraction method (Gundogdu and Yilmaz, 1984; Vendemiatto and Enzweiler, 2001). The particle size of zeolite ranged between 0.3–0.7 mm.

Determination of The Fattening Performance

The quails included in each study group were weighed individually on a weekly basis with a scale accurate to 0.01 g. The difference between the live weight values measured by two consecutive weightings was considered as the weekly live weight gain. Weightings were finalized on day 35 post-hatching.

Determination of Feed Intake and The Feed Conversion Rate

The feed intake of each study group was determined on a weekly basis. Weekly feed intake was calculated by subtracting the amount of feed remaining in the feeders by the end of the week from the amount of feed provided at the beginning of the week. The feed conversion rate was calculated by dividing the feed intake by the average live weight gain.

Determination of The Slaughter Characteristics

Based on the average live weight at 35 days of age, in total 64 quails (32 females and 32 males), including 8 females and 8 males from each group, were slaughtered with an aim to determine slaughter and carcass characteristics. The live weight of the animal at the time of slaughter (slaughter weight), non-eviscerated hot carcass weight, eviscerated hot carcass weight, breast weight, thigh weight, neck+back+wing weight, heart weight, liver weight, gizzard weight and abdominal fat weight were determined as carcass and slaughter characteristics.

The percentage values of the slaughter and carcass characteristics of the study groups were calculated using the following formulae:

Non-eviscerated hot carcass yield (%) = (Non-eviscerated hot carcass weight/Slaughter weight) x 100

Eviscerated hot carcass yield (%) = (Eviscerated hot carcass weight/Slaughter weight) x 100

Thigh percentage (%) = (Thigh weight/Non-eviscerated hot carcass weight) x 100

Breast percentage (%) = (Breast weight/Non-eviscerated hot carcass weight) x 100

Wing percentage (%) = (Wing weight/Non-eviscerated hot carcass weight) x 100

Neck+back+wing percentage (%) = (Neck+back+wing weight/Non-eviscerated hot carcass weight) x 100

Liver percentage (%) = (Liver weight/Non-eviscerated hot carcass weight) x 100

Heart percentage (%) = (Heart weight/Non-eviscerated hot carcass weight) x 100

Gizzard percentage (%) = (Gizzard weight/Non-eviscerated hot carcass weight) x 100

Determination of The Biochemical Parameters

In the present study, blood and liver samples were taken from the slaughtered 32 female and 32 male (in total 64) quails. The total antioxidant status (TAS) and total oxidant status (TOS) analyses of the blood and liver samples were performed using a Rel assay commercial kit, as described by Kucukgul & Erdogan (2014).

Determination of The Pathological Findings

At slaughter, tissue samples were taken from the liver and ileum, and were fixed in 10% buffered formalin solution for histopathological examination. In line with routine protocols, the samples were passed through graded series of alcohol and xylol, embedded in paraffin and cut into 5-µm-thick sections on a rotary microtome. These sections were deparaffinized in xylol,

passed through a series of 100%, 96%, 80% and 70% alcohol, and finally stained with haematoxylin-eosin (H&E). The preparations were examined under a light microscope (Olympus CX31), and microphotographs were taken using an Olympus DP12 camera attached to the microscope.

The histopathological findings determined in the liver tissue samples were scored according to the following criteria: Grade 0: Histopathological changes at a level below 5%; Grade 1: Mild histopathological changes observed in 5% to 33% of the total area; Grade 2: Moderate histopathological changes observed in 33% to 66% of the total area; Grade 3: Severe histopathological changes observed in more than 66% of the total area.

Statistical Analyses

Data pertaining to the fattening performance, slaughter characteristics, and blood and tissue samples were analysed using the IBM SPSS Statistics 22 software package. One-way analysis of variance (ANOVA) was used to determine whether there was any difference between the average values of the study groups for the parameters investigated. Differences between the groups were determined with Duncan's multiple comparison test.

RESULTS

Results obtained for the fattening performance of the study groups are presented in Table 3. While the differences observed between the groups for the average live weights determined at the beginning of the study and on days 32 and 35 were statistically insignificant ($p>0.05$), the differences observed between the groups for the live weights determined on days 11, 18 and 25 were statistically significant ($p<0.05$). The groups significantly differed for the average live weight of the female quails on days 18, 25 and 32, excluding day 35 ($p<0.001$; $p<0.01$). On the other hand, the differences observed between the groups for the average live weight of the male quails on days 18, 25 and 32 were statistically insignificant ($p>0.05$). The average live weights of both the male and female quails measured between days 18-35 were highest in Z3. Accordingly, the average live weights of the male and female quails on days 18, 25, 32 and 35 were ascertained to be highest in Z3.

Table 3 – The body weight values of the quails in study group (g).

Day	Sex	Control	Z1.5	Z3	Z6	F	*p*
Initial body weight	Both Sexes	15.91	16.46	16.92	16.12	1.250	0.294
11 days	Both Sexes	53.53^{ab}	54.11^{a}	55.25^{a}	50.98^{b}	3.465	0.018
18 days	Both Sexes	103.43^{ab}	103.21^{ab}	107.14^{a}	98.68^{b}	4.425	0.050
	Male	100.98	102.78	104.80	102.20	0.422	0.738
	Female	105.24^{ab}	103.51^{b}	110.25^{a}	95.36^{c}	8.115	0.000
25 days	Both Sexes	152.42^{ab}	153.70^{ab}	158.85^{a}	146.89^{b}	3.755	0.013
	Male	147.38	149.93	156.62	150.77	1.333	0.272
	Female	156.14^{a}	156.35^{a}	161.83^{a}	141.23^{b}	4.556	0.006
32 days	Both Sexes	191.18	189.36	196.04	183.06	2.419	0.069
	Male	181.94	181.02	189.34	181.03	0.925	0.434
	Female	197.99	195.21	204.98	183.98	2.649	0.056
35 days	Both Sexes	196.62	205.70	208.15	200.17	1.190	0.316
	Male	180.93	189.96	196.93	192.87	1.512	0.220
	Female	208.18	216.72	223.12	207.06	1.187	0.321

^{a,b} Differences between the average values shown with different superscripts in the same row are statistically significant ($p<0.05$).

Feed Intake and Feed Conversion Rate

The weekly live weight gain, feed intake and feed conversion rate values of the study groups are presented in Table 4. The weekly live weight gain values of the study groups between days 11-18 were highest in the control group and Z3 ($p<0.05$). The comparison of the study groups for their weekly feed intake, daily feed intake and feed conversion values demonstrated no statistically significant difference ($p>0.05$). According to the assessment of the feed conversion rates between days 4-35, the best rate was determined in Z1.5, and the worst rate was determined in the control group.

Slaughter and Carcass Characteristics

The slaughter and carcass characteristics of the study groups are presented in Table 5 and Table 6. For the slaughter characteristics determined in the present study, the average values of Z3 were observed to be higher than those of the other study groups ($p>0.05$). Neck+back+wing weight ($p<0.05$), liver weight

Table 4 – The body weight, feed intake and feed conversion rate values of the quails in study group.

Day	Characteristics					
	Control	Z1.5	Z3	Z6	F	*p*
Live weight gain (g)						
11-18	49.89ab	49.09b	51.88a	47.80b	4.153	0.031
18-25	48.79	50.38	51.74	48.21	0.549	0.658
25-32	41.84	35.85	36.98	36.15	1.657	0.229
4-35	180.20	189.10	190.99	184.14	0.343	0.795
Feed Consumption (g)						
11-18	147.43	140.77	135.28	134.72	0.733	0.552
18-25	171.39	168.33	169.59	162.22	0.638	0.605
25-32	209.72	195.63	200.70	206.04	0.890	0.474
4-35	720.48	679.58	688.98	678.66	1.704	0.219
Feed conversion ratio, g/g						
11-18	2.96	2.87	2.61	2.82	0.905	0.467
18-25	3.51	3.34	3.28	3.37	0.503	0.687
25-32	5.01	5.46	5.43	5.70	0.683	0.579
4-35	4.00	3.59	3.61	3.69	1.407	0.289

(p<0.01) and liver percentage (p<0.05) values were highest in the male quails included in Z3 and Z6, and non-eviscerated carcass yield (p<0.05) and eviscerated carcass yield (p<0.05) were highest in Z1.5 and Z3. Excluding the breast percentage (p<0.05), the slaughter and carcass characteristics of the female quails showed statistically insignificant differences between the study groups (p>0.05).

Table 5 – The slaughter and carcass characteristics of the study groups (g).

Characteristics	Sex	Control	Z1.5	Z3	Z6	F	*p*
Slaughter weight	Both Sexes	211.56	203.72	215.75	211.53	0.866	0.464
	Male	193.41	190.14	201.96	201.32	1.517	0.232
	Female	229.71	217.30	229.55	221.75	0.840	0.483
Non-eviscerated carcass weight	Both Sexes	158.85	153.57	163.07	159.28	0.846	0.474
	Male	144.09	143.55	153.80	150.70	1.792	0.172
	Female	173.61	163.59	172.34	167.86	0.720	0.549
Eviscerated carcass weight	Both Sexes	128.36	125.99	134.49	127.35	1.537	0.214
	Male	118.38	119.40	128.06	124.39	2.243	0.105
	Female	138.34	132.59	140.92	130.31	1.477	0.242
Breast weight	Both Sexes	52.61	51.54	54.44	50.59	1.238	0.304
	Male	47.23	48.91	50.39	49.45	0.575	0.636
	Female	57.49	54.17	58.49	51.73	2.509	0.079
Neck+back+wing weight	Both Sexes	45.69	44.39	48.52	46.76	2.485	0.069
	Male	43.14^{ab}	41.53^{b}	46.61^{a}	45.51^{a}	3.309	0.034
	Female	48.25	47.25	50.42	48.02	0.826	0.491
Thigh weight	Both Sexes	30.00	29.85	31.08	29.74	0.858	0.468
	Male	27.95	28.74	30.42	29.27	1.840	0.163
	Female	32.06	30.96	31.74	30.21	0.778	0.516
Heart weight	Both Sexes	2.12	2.03	2.26	2.11	1.689	0.179
	Male	1.97	1.88	2.14	2.10	1.712	0.187
	Female	2.27	2.19	2.38	2.12	1.311	0.290
Liver weight	Both Sexes	6.23	5.48	6.34	5.92	0.705	0.553
	Male	4.81^{ab}	4.13^{b}	5.35^{a}	5.13^{a}	4.719	0.009
	Female	7.64	6.82	7.32	6.72	1.496	0.744
Gizzard weight	Both Sexes	4.78	4.56	4.76	4.60	0.329	0.804
	Male	4.16	4.14	4.49	4.08	0.844	0.481
	Female	5.40	4.97	5.06	5.12	0.525	0.669
Abdominal fat weight	Both Sexes	1.67	1.60	2.11	1.59	1.471	0.232
	Male	1.61	1.43	1.50	1.34	0.263	0.851
	Female	1.73	1.77	2.72	1.84	2.532	0.094

^{a,b}: Differences between the average values shown with different superscripts in the same row are statistically significant (p<0.05).

Table 6 – Characteristics of the carcass parts of the study groups (%).

Characteristics	Sex	Control	Z1.5	Z3	Z6	F	*p*
Non-eviscerated carcass yield	Both Sexes	75.03	75.38	75.58	75.26	0.639	0.593
	Male	74.48[b]	75.52[ab]	76.11[a]	74.86[b]	3.894	0.019
	Female	75.59	75.24	75.04	75.66	0.540	0.659
Eviscerated carcass yield	Both Sexes	60.73[ab]	61.97[ab]	62.42[a]	60.28[b]	3.146	0.032
	Male	61.18[b]	62.85[a]	63.40[a]	61.81[ab]	3.502	0.028
	Female	60.29	61.09	61.44	58.75	1.807	0.169
Breast percentag	Both Sexes	33.12ab	33.65a	33.38a	31.81b	2.783	0.049
	Male	33.15	34.10	32.79	32.83	0.773	0.519
	Female	33.10	33.19	33.97	30.79	4.412	0.012
Neck+back+wing percentag	Both Sexes	28.87	28.92	29.80	29.45	1.384	0.256
	Male	29.92	28.93	30.31	30.23	1.916	0.150
	Female	27.82	28.91	29.28	28.66	1.487	0.239
Thigh percentag	Both Sexes	19.00	19.51	19.14	18.69	1.185	0.323
	Male	19.45	20.04	19.79	19.40	0.911	0.448
	Female	18.55	18.98	18.48	17.99	0.774	0.518
Heart percentag	Both Sexes	1.34	1.32	1.39	1.33	0.537	0.659
	Male	1.37	1.31	1.39	1.39	0.571	0.639
	Female	1.31	1.34	1.39	1.27	0.628	0.603
Liver percentag	Both Sexes	3.84	3.53	3.83	3.71	0.497	0.686
	Male	3.34[a]	2.89[b]	3.47[a]	3.41[a]	3.203	0.038
	Female	4.34	4.17	4.20	4.01	0.188	0.903
Gizzard percentag	Both Sexes	3.01	2.97	2.92	2.91	0.199	0.896
	Male	2.89	2.89	2.88	2.72	0.572	0.638
	Female	3.13	3.06	2.96	3.09	0.157	0.924
Abdominal fat percentag	Both Sexes	1.05	1.03	1.28	0.98	1.358	0.264
	Male	1.15	0.98	0.98	0.87	0.546	0.655
	Female	0.99	1.07	1.59	1.10	2.534	0.077

[a,b]: Differences between the average values shown with different superscripts in the same row are statistically significant ($p<0.05$).

While blood TAS and TOS levels were ascertained not to have altered in the groups that included both sexes, when compared to the hepatic TAS and TOS levels of the control group (0.704 and 8.655, respectively), the group that had received 1.5% clinoptilolite in feed displayed a significantly higher hepatic TAS level (0.723) and a lower hepatic TOS level (7.698). In the groups that had received higher levels of dietary clinoptilolite supplementation (3% and 6%), comparison with the hepatic TAS and TOS levels of the control group (0.704 and 8.655, respectively) demonstrated the exact opposite (TAS 0.622 and 0.381, respectively; TOS 9.248 and 9.945, respectively). Based on these data, it was determined that while 1.5% of clinoptilolite acted as an active concentration, higher concentrations of dietary clinoptilolite proved to be toxic. In the present study, the blood and liver TAS and TOS levels

Table 7 – Blood and liver analysis results of the study groups.

Characteristics	Sex	Control	Z1.5	Z3	Z6	F	*p*
Blood TAS level	Both Sexes	1.781	1.750	1.691	1.443	2.138	0.105
	Male	1.773[a]	1.729[a]	1.565[ab]	1.163[b]	2.954	0.050
	Female	1.788	1.771	1.817	1.722	0.108	0.954
Blood TOS level	Both Sexes	0.300	0.349	0.190	0.161	1.827	0.152
	Male	0.340	0.181	0.282	0.187	0.798	0.505
	Female	0.260[ab]	0.518[a]	0.098[b]	0.134[b]	4.269	0.013
Liver TAS level	Both Sexes	0.704	0.723	0.622	0.381	2.515	0.074
	Male	0.691	0.714	0.691	0.816	1.281	0.315
	Female	0.718[a]	0.732[a]	0.553[a]	0.035[b]	7.528	0.002
Liver TOS level	Both Sexes	8.655	7.698	9.248	9.945	2.710	0.059
	Male	8.660[a]	5.835[b]	8.920[a]	9.585[a]	3.839	0.030
	Female	8.650	9.560	9.575	10.305	1.407	0.277

[a,b]: Differences between the average values shown with different superscripts in the same row are statistically significant ($p<0.05$).

of the female quails in the different study groups were compared with each other. Accordingly, when compared to the blood TAS and TOS levels of the control group (1.788 and 0.260, respectively), the group that had received 3% of clinoptilolite displayed an increased blood TAS level (1.817) and a significantly decreased blood TOS level (0.098). On the other hand, the assessment of the liver TAS and TOS levels of the male quails demonstrated that, compared to the control group (TAS 0.691 and TOS 8.660), a similar situation was observed in the group that had received 1.5% of dietary clinoptilolite (TAS 0.714 and TOS 5.835).

The characteristics of the intestinal villi in the different study groups are presented in Table 8. Villus height ($p<0.05$) and crypt depth ($p>0.05$) were greatest in Z1.5, and villus width ($p>0.05$) was greatest in the control group. In the male quails, villus height ($p<0.05$) and villus width ($p<0.05$) were greatest in Z1.5 and Z3, whilst crypt depth ($p<0.05$) was greatest in the control group and Z1.5. In the female quails, villus height ($p<0.05$) was greatest in the control group and Z3, and villus width ($p<0.05$) was greatest in the control group and Z6.

Table 8 – Intestinal traits of the quails in the study groups.

Characteristics	Sex	Control	Z1.5	Z3	Z6	F	*p*
Villus height (µm)	Both Sexes	280.52^{ab}	319.76^{a}	278.76^{ab}	247.70^{b}	3.631	0.018
	Male	249.20^{ab}	278.85^{a}	285.43^{a}	214.31^{b}	3.244	0.037
	Female	311.84^{ab}	360.66^{a}	272.08^{b}	281.09^{b}	3.710	0.023
Villus width (µm)	Both Sexes	88.21	81.89	75.91	74.00	1.200	0.318
	Male	80.88^{b}	102.51^{a}	82.60^{ab}	70.86^{b}	3.600	0.026
	Female	95.54^{a}	61.27^{b}	69.22^{b}	77.14^{ab}	3.564	0.027
Crypt depth (µm)	Both Sexes	53.96	54.80	48.85	47.87	1.489	0.227
	Male	58.70^{a}	51.77^{ab}	45.88^{b}	43.12^{b}	3.363	0.033
	Female	49.22	57.84	51.82	52.63	0.775	0.518

^{a,b}: Differences between the average values shown with different superscripts in the same row are statistically significant ($p<0.05$).

Macroscopically, the liver of the female quails was swollen, whitish and lighter in colour, in comparison to that of the male quails. The hepatic lipidosis (fatty changes) and mononuclear cell infiltration (MCI) scores of the study groups are presented in Tables 9 and 10. The numerical difference determined between the groups for hepatic lipidosis level was statistically insignificant ($p>0.05$). In the female quails, the highest level of hepatic lipidosis was detected in Z6 ($p<0.05$). Fatty changes which are characterized by large empty vacuoles of sharp borders, which replaced the cytoplasm was noticed (Figure 1a, 1b, 1c, 1d, 1e).

The assessment of the MCI scores of the study groups (Table 10) demonstrated statistically significant differences between the groups ($p<0.01$). In the female quails, the highest MCI level was detected in Z6 ($p<0.01$). It was ascertained that, dietary supplementation with 6% of clinoptilolite caused foci of inflammatory cell infiltration, which mainly comprised of lymphocytes, in the female quails (Figure 1).

Table 9 – An assessment of the hepatic lipidosis scores for the different study groups.

Characteristics		n	Median(min-max)	X^2	*p*
Hepatic lipidosis level	Control	14	1.00(0-3)	4.294	0.231
	Z1.5	13	1.00(0-3)		
	Z3	14	1.50(0-3)		
	Z6	13	2.00(0-3)		
Male					
Hepatic lipidosis level	Control	7	1.00(0-1)	1.889	0.596
	Z1.5	6	0.00(0-1)		
	Z3	7	1.00(0-1)		
	Z6	6	1.00(0-2)		
Female					
Hepatic lipidosis level	Control	7	2.00(1-3)	9.016	0.029
	Z1.5	7	1.00(1-3)		
	Z3	7	2.00(2-3)		
	Z6	7	3.00(2-3)		

Table 10 – An assessment of the MCI scores for the different study groups.

Score	Characteristics	n	Median(min-max)	X^2	*p*
MCI	Control	12	0.00(0-0)	12.776	0.005
	Z1.5	12	0.00(0-0)		
	Z3	12	0.00(0-1)		
	Z6	12	0.00(0-2)		
Male					
MCI	Control	6	0.00(0-0)	3.000	0.392
	Z1.5	6	0.00(0-0)		
	Z3	6	0.00(0-1)		
	Z6	6	0.00(0-0)		
Female					
MCI	Control	6	0.00(0-0)	16.674	0.001
	Z1.5	6	0.00(0-0)		
	Z3	6	0.00(0-0)		
	Z6	6	1.00(0-2)		

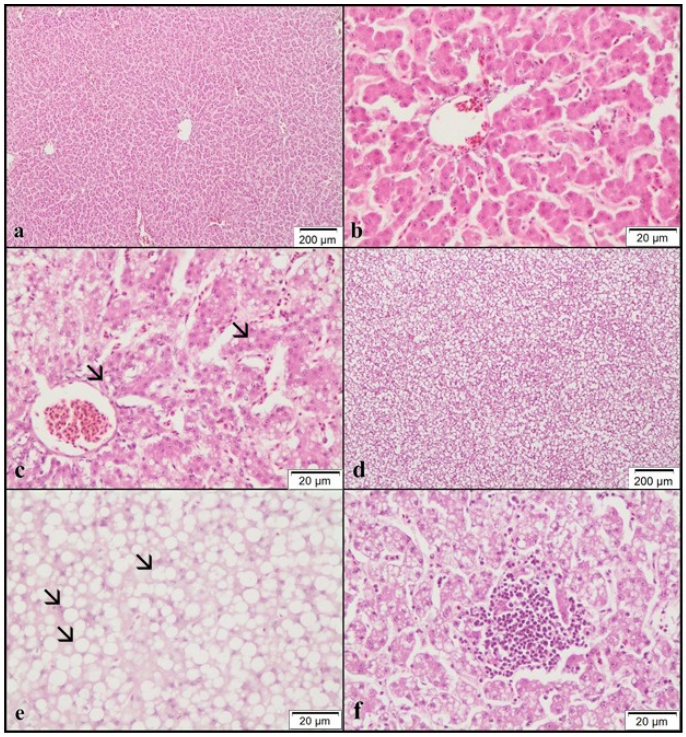

Figure 1 – Histopathological findings in the liver a) Male control subject, normal histological structure in the liver, H&E. b) Male control subject, normal histological structure in the liver, H&E. c) Female control subject, mild fatty changes of hepatocytes (arrows), H&E. d) Female G6, severe fatty changes of hepatocytes, H&E. e) Female G6, severe fatty changes of hepatocytes (arrows), H&E. f) Female G6; focal mononuclear cell infiltration in the liver, H&E.

DISCUSSION

While average live weight values were lowest, excluding day 35, in Z6, which had received 6% of dietary zeolite, these values were highest on days 11, 18, 25, 32 and 35 in Z3, which had received 3% of dietary zeolite (Table 3). Similarly, in their study on meat-type quails, Tufan *et al.* (2014) reported that the weekly average live weight was lowest in the group that had received 6% of dietary zeolite. As clinoptilolite is known to strengthen the bone structure of animals, it is incorporated into both feed and litter in broiler chicken production to reduce the occurrence of leg problems. Previous research, in which zeolite (clinoptilolite) was incorporated into broiler chicken feed at different levels (0.5%, 0.75%, 1%, 2% and 2.5%), has shown a positive impact on live weight (Suchý *et al.,* 2006; Nikolakakis *et al.,* 2013; Sacakli *et al.,* 2015). On the other hand, some other studies have reported that while dietary zeolite supplementation increases the antioxidant capacity, its impact on growth performance is statistically insignificant (Gezen *et al.,* 2004; Wu *et al.,* 2015; Yeter & Gokce, 2018).

In the present study, while the average live weights of the male and female quails on days 18, 25, 32 and 35 were highest in Z3, on the same days, the average live weights of the males were highest in the control group and Z1.5, and the average live weight of the females was highest in Z6. On the contrary to the results of the present study, in their study on the effects of dietary supplementation with 1%, 3% and 5% of zeolite, Eleroglu *et al*. (2011) reported that at the end of 5 weeks of fattening, while the average live weight of female and male quails was highest in the control group and the group that had received 5% of dietary zeolite, it was lowest in the group that had received 1% of dietary zeolite. In general, research on fattening quails has shown that the live weight of female quails is greater than that of male quails. This difference has been attributed to the differences between the male and female reproductive organs. However, in the present study, in the group that had received 6% of dietary zeolite, the live weight of the male quails was greater than that of the females on days 18 and 25, and was similar to that of the

females on days 32 and 35. Given that zeolite, owing to its high silicate content, supports bone growth, and particularly improves the growth of long bones, it is suggested that the difference between the reproductive organs of male and female quails could be partly compensated by this positive impact of zeolite on bone development.

In the present study, between days 4-35, the average live weight was highest in Z3 that had received 3% of dietary clinoptilolite, the weekly feed intake and daily average feed intake were lowest in Z6 that had received 6% of dietary clinoptilolite, and the feed conversion rate was best in Z1.5 that had received 1.5% of dietary clinoptilolite (Table 4). On the other hand, in their study in which they administered meat-type quails with 2%, 4% and 6% of dietary zeolite, between days 1-42, Tufan *et al.* (2014) reported to have determined the highest live weight gain in the groups that had received 2% and 4% of zeolite, the lowest feed intake in the group given 2% of dietary zeolite and the best feed conversion rate in the group that had received 4% of dietary zeolite. Furthermore, in another study by Durak *et al.* (2017), in which meat-type quails were provided with 2.5% and 5% of dietary zeolite, higher levels of dietary supplementation were reported to have a positive impact on live weight gain, and the lowest feed intake and best feed conversion rate were determined in the group that had received 5% of dietary clinoptilolite. In their study on the dietary supplementation of broiler chickens with zeolite (0.5%, 1% and 1.5%) Amad & Al-ansi (2018) reported that live weight gain increased with higher levels of dietary supplementation (Mallek *et al.,* 2012), reduced feed intake and improved feed conversion rate with dietary zeolite supplementation (Eleroglu *et al.,* 2011). The present study was in agreement with these previous studies in terms of live weight gain and feed intake results, but differed in terms of feed conversion rate results.

While the highest values for the slaughter and carcass characteristics investigated in this study were determined in Z3, which had received 3% of dietary zeolite, the lowest slaughter weight was determined in Z1.5, which had received 1.5% of dietary zeolite (Table 5 and Table 6). Similarly, Durak *et al.* (2017) reported to have determined the lowest quail slaughter weights in the group that had received the lowest level of dietary zeolite. On the other hand, Tufan *et al*. (2014) reported that higher levels of dietary zeolite (2%, 4%, 6%) decreased the slaughter weight of quails. In the present study, the neck+back+wing weight and liver weight of the male quails were significantly higher in Z3 and Z6 that had received 3% and 6% of dietary zeolite, respectively, whilst the slaughter and carcass characteristics of the female quails were numerically similar in the control group and Z3 that had received 3% of dietary zeolite. The live weight values and slaughter characteristics of the male quails in the groups given 3% and 6% of dietary zeolite differed from those of the male control subjects. When compared to the control group, the positive impact of higher dietary zeolite levels on the growth and carcass characteristics of the male quails was attributed to the positive effect of zeolite on bone development.

In the present study, while the study groups showed numerical differences for blood and liver TAS and TOS levels, the blood TAS and liver TOS levels of the male quails and the blood TOS and liver TAS levels of the female quails displayed statistically significant differences (Table 7). The assessment of blood TAS and TOS levels demonstrated that the levels of the control group and Z1.5 that had received 1.5% of dietary zeolite were similar, and thereby proved that dietary supplementation with 1.5% of zeolite was effective. Higher levels of dietary zeolite were determined to show toxic effect. Furthermore, in the female quails, the best blood TOS and liver TAS levels were determined in Z3 that had received 3% of dietary zeolite and Z1.5 that had received 1.5% of dietary zeolite, respectively. In the male quails, the best blood TAS and liver TOS levels were detected in Z1.5 that was given 1.5% of dietary zeolite. Oxidative stress is evaluated on the basis of TAS and TOS levels, and TAS levels are considered to be best when higher, whilst TOS levels are best when lower. In this context, in order to ensure animal health, zeolite is recommended to be incorporated into the diet at levels of 1.5% and 3% for female quails and at a level of 1.5% for male quails. In previous research, dietary zeolite supplementation at a level ranging from 1% to 2% was reported to increase the total antioxidant capacity, and this range is similar to the levels of dietary zeolite tested in the present study (Wu *et al.,* 2015; Hcini *et al.,* 2018).

Histopathological examination revealed that, 1.5% of dietary zeolite increased the height of intestinal villi in both the male and female quails and reduced the level of hepatic lipidosis in the female quails. In the female quails, while 3% and 6% of dietary zeolite were determined to have significantly increased hepatic lipidosis, 6% of zeolite was ascertained to have also caused hepatitis (Table 9 - Table 10). Previous studies carried out in humans and animals have demonstrated

that while zeolite has both antioxidant and detoxifying effects, it also positively affects intestinal absorption and the immune system (Andronikashvili *et al.,* 2009; Pavelič *et al.,* 2018). In the present study, the incorporation of zeolite into quail diets at a level of 1.5% was determined to have improved villus measurements and to have reduced hepatic lipidosis in the female quails, and in result, was shown to have caused no adverse effect. Similarly, Miazzo *et al.* (2000) reported that 1% of dietary zeolite was safe for quails. Furthermore, the present study revealed that, when incorporated into quail diets at levels of 3% and 6%, zeolite had increased hepatic lipidosis, and 6% of dietary zeolite had also caused hepatitis.

CONCLUSION

Previous research has mainly focused on the addition of varying levels of zeolite to litter material used in poultry houses with an aim to reduce foot plantar wounds, carcass defects and ammonia levels. The present study demonstrated that zeolite, containing 90.2% of clinoptilolite, when incorporated into the basal diet at levels of 1.5%, 3% and 6%, increased the live weight of Japanese quails throughout the fattening period, in comparison to the control subjects. Of the different levels of dietary zeolite tested, 3% was observed to have positively affected live weight and the other performance traits. The assessment of biochemical and histopathological data showed that 6% of dietary zeolite caused damage to the liver. However, in the group that had received 6% of dietary zeolite, compared to the other study groups, the live weights of the male and female quails were closer to each other. This result could contribute to the assessment of the profitability of the fattening of non-breeder male quails, separate from female quails. The use of zeolite as a feed additive in quail production with an aim to improve fattening performance values, would also contribute to reducing the humidity of feed and faeces and improving the quality of ambient air, owing to the water-holding capacity of zeolite.

Overall, based on the biochemical and histopathological data obtained in the present study, a high level of dietary clinoptilolite (6%) was determined to adversely affect the liver, and in this context, all findings were consistent. The poor values determined for the animals that had received 6% of dietary zeolite, including low live weight gain, were considered to be partly related to the liver damage that had occurred in this group.

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