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The effects of increasing levels of dietary sodium bentonite on performance, carcass indices, blood chemistry and meat quality in Japanese quails

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Abstract

This study was conducted to determine the effect of the inclusion of sodium bentonite (SB) in Japanese quails' diet on performance, carcass traits, blood biochemical status, and meat quality traits. A total of 120 seven-day-old Japanese quails (*Coturnix coturnix Japonica*) were randomly divided into four groups, with each group containing five replicates, six birds each. SB was mixed with the diet at four concentrations (0, 0.5, 1.0 and 2.0%). Performance indicators were determined for an experimental period of six weeks. At the end of the experiment, 2 quails from each replicate were slaughtered and carcass traits were measured. The blood serum biochemical values, antioxidant status, and meat quality indicators were determined from blood and breast meat samples. The results showed that SB supplementation significantly improved the feed conversion ratio (FCR) in the 2–7 week period, the relative weight of the proventriculus, glucose and total cholesterol (TC) and a* (redness) colour traits in meat. Moreover, the total oxidant status (TOS) in blood serum and malondialdehyde (MDA) values were decreased with SB addition to Japanese quails' diet. Hence, dietary SB supplementation could be used to improve the performance and antioxidant status in Japanese quails.

Blood biochemistry, Japanese quails, performance, sodium bentonite

Clays including aluminum silicate, chlorite, sepiolite, bentonite, zeolite kaolinite, montmorillonite, illite, and hydrate sodium calcium aluminosilicate are commonly used in industry due to their absorption ability (Aydin et al. 2020). These minerals also have been long-known in the nutrition of farm animals due to their promoting effect on the gastrointestinal system by detoxification of anti-nutritional elements and regulation of gut microbiota (Slamova et al. 2011; Banaszak et al. 2021a). Bentonite is a tri-layered aluminosilicate clay mineral with immense ion exchange capacity (Eraslan et al. 2005; Attar et al. 2019). Bentonite clays exist in nature mostly as sodium, potassium, and calcium salts of hydrated aluminosilicates (Pasha et al. 2008). Bentonite is widely used in the feed sector due to its moisture absorption capacity and pellet binding properties, although studies using bentonite as a feed additive in poultry mostly focused on the ability to absorb mycotoxins (Santurio 1999).

Meat quality traits are also important in poultry production along with growth performance indicators since growth performance does not always mirror the physicochemical traits of the carcass and meat of the birds (Banaszak et al. 2021a). Aluminosilicates could be used to improve meat characteristics in broilers. Hashemi et al. (2014) demonstrated that silver nanoparticles coated with zeolite improved meat quality in broiler chickens. According to Banaszak et al. (2020), aluminosilicates enhanced muscle content in carcass and water holding capacity (WHC) in broilers. It is also believed that detoxifying ability of aluminosilicates could be related to their lipid peroxidation reducing capacity by scavenging radical oxidants in tissues. Bentonite was tested before as performance and carcass stimulant in broilers (Pasha et al. 2008; Damiri et al. 2012; Bouderoua et al. 2016;

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Attar et al. 2019) and quails (El-Abd 2014). Although some research has been conducted on the effect of bentonite clay on the meat properties and antioxidant status in broiler chickens (Safaei et al. 2016) and lambs (Aydin et al. 2020), no study has been found on this topic in quails.

The purpose of this study was to determine the effect of different levels of dietary sodium bentonite (SB) on performance, carcass parameters, blood biochemical status, antioxidant values, and meat quality in growing Japanese quails.

Table 1. Ingredients and chemical compositions of the diets.

Ingredients (%)	Sodium bentonite supplementation (%)			
	0	0.5	1.0	2.0
Barley	10.00	9.95	9.90	9.80
Vegetable oil	0.50	0.50	0.50	0.49
Maize	49.27	49.02	48.78	48.27
Corn gluten meal	1.72	1.71	1.70	1.69
Soybean meal	35.28	35.10	34.93	34.57
Dicalcium phosphate	0.73	0.73	0.72	0.72
DL-Methionine	0.15	0.15	0.15	0.15
Coccidiostat	0.08	0.08	0.08	0.08
L-Lysine hydrochloride	0.15	0.15	0.15	0.15
Marble powder	1.37	1.36	1.36	1.34
Sodium bicarbonate	0.10	0.10	0.10	0.10
Salt	0.40	0.40	0.40	0.39
Vitamin and Mineral Premix[1]	0.25	0.25	0.25	0.25
Sodium bentonite	0.00	0.50	1.00	2.00
Total	100.00	100.00	100.00	100.00
Chemical composition (%)				
Dry matter	88.74	89.33	89.04	89.01
Crude protein	23.66	23.61	23.53	23.44
Crude fat	1.92	2.05	2.11	1.96
Crude ash	6.69	7.47	7.40	8.14
Crude fibre	6.95	5.34	6.10	5.32
Calculated composition				
Sodium (%)	0.23	0.23	0.23	0.23
Calcium (%)	0.79	0.79	0.79	0.78
Phosphorus (%)	0.30	0.31	0.30	0.30
Lysine (%)	1.33	1.34	1.33	1.32
Total methionine + cysteine (%)	0.93	0.92	0.92	0.91
ME (kcal/kg)[2]	2.649	2.624	2.566	2.584

[1]Total content of 1 kg Vitamin-Mineral Premix was 8,800 IU vitamin A; 2,200 IU vitamin D_3; 11 mg vitamin E; 44 mg nicotinic acid; 8.8 mg calcium D-pantothenate; 4.4 mg riboflavin; 2.5 mg thiamin; 6.6 mg vitamin B_{12}; 1 mg folic acid; 0.11 mg D-biotin; 220 mg choline; 80 mg manganese; 60 mg iron; 5 mg copper; 60 mg zinc; 0.20 mg cobalt; 1 mg iodine; 0.15 mg selenium.

[2]Metabolizable energy content of diets calculation was conducted according to the equation of Carpenter and Clegg (1956).

Materials and Methods

Ethical statement

All procedures applied to the quails were approved by the Local Animal Ethics Committee of Selcuk University Veterinary Faculty (Protocol No.2021/81).

Animal care and management

One-day-old unsexed Japanese quail chicks (*Coturnix coturnix Japonica*), with 8.33 g mean weight, were housed in multi-storey pens (30 × 80 × 8 cm) with a cement floor. After an adaptation period of seven days to new conditions, 120 chicks with uniform body weights were randomly and equally divided into four groups with five replicates with six quails per pen. In the pens, the quails were reared in cages with an electric heater and 23 h fluorescent lighting. Feed and water were available for *ad libitum* consumption. The trial was conducted for six weeks (8–49 days).

Feeding trial

A corn and soy-bean based diet was formulated to meet the nutrient requirements of the Japanese quails according to National Research Council (1994) (Table 1). The groups received four different dietary treatments. Control fed with the basal diet and treatment groups fed with 0.5%, 1.0%, and 2.0% SB, respectively. Levels were determined according to the study conducted by Aydin et al. (2020). SB was supplied from a local company in Türkiye (Muktibont-B, Sanita, İstanbul, Türkiye). The chemical content of the SB is shown in Table 2.

All performance indicators, body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded at the end of 1, 3, 5, and 7 weeks of age. At the end of the experiment, all feeders were removed overnight, and two quails were randomly selected from each replicate (total 40 birds) and weighed before decapitation. After sacrifice, hot carcass yield (HCY), total digestive tract (TDT), heart, liver, proventriculus, clean gizzard, and hot carcass of the quails were weighed and expressed as a % of the live BW before slaughter.

Table 2. Chemical composition of sodium bentonite.

Component	%
Silicon dioxide (SiO_2)	58.54
Aluminum oxide (Al_2O_3)	14.73
Calcium carbonate ($CaCO_3$)	9.95
Iron (III) oxide (Fe_2O_3)	5.09
Magnesium oxide (MgO)	3.18
Sodium oxide (Na_2O)	1.76
Potassium oxide (K_2O)	1.25
Sulphur trioxide (SO_3)	0.92
Titanium dioxide (TiO_2)	0.72
Other minerals	3.86
Total	100.00

Blood chemistry

Blood samples were harvested from the selected quails' jugular vein in 6 ml serum-separating tubes during slaughtering. The samples were centrifuged at 2,000 × *g* for 10 min to separate blood serum. Commercial kits were used to determine blood urea nitrogen (BUN), glycogen, total protein (TP), sodium (Na), triacylglycerols, total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (Rel Assay Diagnostics, Gaziantep, Turkey). Total antioxidant capacity (TAC) and total antioxidant status (TOS) were analysed spectrophotometrically using the diagnostic kits of the same company. Oxidative stress index (OSI) was found by dividing TAC into TOS.

Meat quality characteristics

Approximately 15 g breast meat samples were taken from the selected birds after slaughter and divided into four pieces. The colour of the breast meat (L* = lightness, a* = redness, and b* = yellowness) was determined immediately after sacrifice using a Minolta colorimeter (CR-200, 123 Minolta Co., Osaka, Japan). Water holding capacity and meat malondialdehyde (MDA) concentration were analysed using the methods described by Morshdy et al. (2021). The remainder of the meat samples were minced and stored at 4 °C for a month. The pH values of the breast meat were measured one and 30 days after slaughter with a digital pH meter (Milwaukee MW102, USA).

Statistical analysis

Data were analyzed using SPSS packaged software (SPSS Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA) method was used to determine the significance of the differences between the statistical calculations belonging to the groups and the mean values of the groups. Orthogonal polynomial contrast and regression analyses were conducted to examine the linear and quadratic trends of the increasing SB doses utilized in this study. In addition, the Duncan test was applied for the significance control of the difference between groups. The effect of dietary SB inclusion was evaluated by means of polynomial contrasts (linear and quadratic responses). Values were shown as mean ± standard error in the tables. Significance was considered at $P < 0.05$.

Results

As shown in Table 3, the FCR between 2–7 weeks significantly decreased in a linear trend with the inclusion of increasing levels of SB in Japanese quails' diets compared to the control ($P < 0.05$). Diets supplemented with 2.0% SB had the lowest FCR during the expressed period. Nonetheless, no difference was observed in the birds for all performance traits except for FCR in the 2–7 weeks period ($P > 0.05$).

Compared to the control group, dietary supplementation of SB did not significantly affect ($P > 0.05$) quail carcass characteristics in terms of percentages of HCY, heart, liver, TDT, and gizzard (Table 4). On the contrary, *P* values of the linear model showed that augmenting the SB supplementation in Japanese quails' diets significantly increased relative weights of the proventriculus ($P < 0.05$).

Table 3. Effect of dietary sodium bentonite (SB) in the diet on live weight gain, feed intake, and feed conversion ratio of Japanese quails.

Indicator	SB supplementation (%)					*P* value[1]	
	0	0.5	1.0	2.0	S.E.M.	Linear	Quadratic
Body weight gain (BWG) (g)							
Weeks 2–3	22.56	22.95	26.78	23.22	1.005	0.530	0.344
Weeks 4–5	60.21	73.11	60.47	68.56	2.039	0.439	0.502
Weeks 6–7	55.09	53.81	55.91	64.86	1.854	0.056	0.152
Weeks 2–7	137.86	149.86	143.15	156.64	3.042	0.065	0.896
Feed intake (FI) (g)							
Weeks 2–3	69.15	74.06	82.53	68.44	2.747	0.792	0.091
Weeks 4–5	194.22	206.83	192.86	171.64	6.586	0.175	0.208
Weeks 6–7	232.43	238.40	224.55	239.00	5.080	0.904	0.697
Weeks 2–7	490.12	494.45	477.97	448.86	10.356	0.150	0.432
Feed conversion ratio (FCR) (g/g)							
Weeks 2–3	3.24	3.22	3.10	2.96	0.106	0.349	0.802
Weeks 4–5	3.22	2.88	3.28	2.51	0.140	0.160	0.430
Weeks 6–7	4.26	4.47	4.04	3.78	0.134	0.129	0.382
Weeks 2–7	3.55^{a}	3.35^{ab}	3.37^{ab}	2.87^{b}	0.102	0.024	0.422

^{a,b} Means within the same row with no common superscript differ significantly ($P < 0.05$) according to Duncan's test.
[1] Data were analysed using linear and quadratic regression models of SPSS.

Table 4. Effect of dietary sodium bentonite (SB) in the diet on the relative weight of carcass, heart and digestive organs (g/kg body weight) of Japanese quails.

Indicator	SB supplementation (%)					*P* value[1]	
	0	0.5	1.0	2.0	S.E.M.	Linear	Quadratic
HCY (%)	56.11	56.42	57.98	56.67	0.562	0.532	0.487
Heart (%)	0.97	0.87	0.95	0.93	0.021	0.813	0.328
GC (%)	11.95	11.64	11.09	11.64	0.235	0.498	0.373
Liver (%)	2.49	2.79	2.77	2.91	0.103	0.182	0.700
Gizzard (%)	2.00	2.00	2.11	2.12	0.045	0.267	0.944
Proventriculus (%)	0.53^{b}	0.55^{ab}	0.57^{ab}	0.66^{a}	0.021	0.029	0.432

^{a,b} Means within the same line with no common superscript differ significantly ($P < 0.05$). according to Duncan's test.
[1]Data were analyzed using linear and quadratic regression models of SPSS.
HCY - hot carcass yield; GC - gastrointestinal organs

Table 5 presents the biochemical and oxidative status of blood serum of Japanese quails fed SB-supplemented diets. The different diets did not produce any significant differences in the levels of BUN, Na, TP, triacylglycerols, LDL, HDL, TAC, and OSI ($P > 0.05$); whereas a significant linear increase in serum glucose and TC values were found with elevated SB levels in the quails' diet ($P < 0.05$). Moreover, the administration of dietary SB as a supplement led to a significant reduction linearly in the overall level of oxidation in the blood serum of Japanese quails ($P < 0.05$).

The meat quality traits for Japanese quails fed with SB are shown in Table 6. The data revealed that augmenting supplementation of SB in quail diets did not differ based on WHC, pH, L^* and a^* values ($P > 0.05$). On the other hand, the MDA level linearly decreased with an increasing rate of SB in diets ($P < 0.05$). Furthermore, the a* value exhibited a positive quadratic trend as SB levels increased ($P < 0.05$).

Table 5. Effect of dietary sodium bentonite (SB) in the diet on serum lipid and protein metabolic status and oxidative status of Japanese quails.

Indicator	SB supplementation (%)				S.E.M.	*P* value[1]	
	0	0.5	1.0	2.0		Linear	Quadratic
Glucose (mg/dl)	309.67^{b}	317.11^{ab}	338.11^{a}	338.00^{a}	4.147	0.003	0.615
BUN (mg/dl)	2.89	3.86	3.22	2.67	0.223	0.190	0.399
Na (mmol/l)	142.60	142.70	141.90	143.70	0.927	0.772	0.660
TP (g/dl)	3.18	3.77	3.24	3.99	0.177	0.232	0.820
Trig (mg/dl)	201.22	235.00	304.78	224.17	42.855	0.733	0.540
TC (mg/dl)	189.89^{b}	262.44^{a}	213.33^{ab}	291.11^{a}	13.435	0.026	0.915
LDL (mg/dl)	72.14	56.96	64.44	64.26	5.092	0.729	0.478
HDL (mg/dl)	92.73	77.72	97.64	90.61	5.578	0.791	0.727
TAC (mmol/l)	1.93	1.82	1.97	1.88	0.107	0.985	0.969
TOS (μmol/l)	7.71^{a}	6.20^{ab}	6.54^{ab}	4.81^{b}	0.466	0.039	0.901
OSI	0.44	0.35	0.37	0.27	0.034	0.112	0.915

^{a,b} Means within the same line with no common superscript differ significantly ($P < 0.05$). according to Duncan's test.
[1] Data were analyzed using linear and quadratic regression models of SPSS.
BUN - blood urea nitrogen; Na - sodium; TP - total protein; Trig - triglyceride; TC - total cholesterol; LDL - low-density lipoprotein cholesterol; HDL - high-density lipoprotein cholesterol; TAC - total antioxidant capacity; TOS - total oxidant status; OSI - oxidative stress index.

Table 6. Effect of dietary sodium bentonite (SB) in the diet on meat properties in Japanese quails.

Indicator	SB supplementation (%)				S.E.M.	*P* value[1]	
	0	0.5	1.0	2.0		Linear	Quadratic
MDA (nmol/l)	6.79^{a}	5.26^{ab}	3.34^{b}	4.25^{b}	0.449	0.014	0.143
WHC (%)	62.64	64.54	62.19	65.04	0.793	0.498	0.769
pH, day 1	6.18	6.10	6.07	6.12	0.023	0.334	0.186
pH, day 30	6.28	6.26	6.30	6.34	0.032	0.456	0.435
Lightness (L*)	32.40	33.53	31.51	32.87	0.663	0.920	0.936
Redness (a*)	5.85^{b}	7.80^{a}	6.61^{ab}	5.58^{b}	0.275	0.365	0.004
Yellowness (b*)	5.64	7.05	6.01	5.24	0.353	0.216	0.389

^{a,b} Means within the same line with no common superscript differ significantly ($P < 0.05$). according to Duncan's test.
[1] Data were analyzed using linear and quadratic regression models of SPSS.
MDA - malondialdehyde; WHC - water holding capacity.

Discussion

In the present study, there was no significant difference for BWG and FI between Japanese quails receiving SB supplementation and the control group for all experimental periods. Similarly, Attar et al. (2019) also reported a lack of differences on BWG, and FI of broilers supplemented with processed sodium butyrate. In contrast, other researchers have found positive effects of bentonite on growth performance in poultry (Pasha et al. 2008; El-Abd 2014; Bouderoua et al. 2016; Prasai et al. 2017). In addition, Damiri et al. (2012) reported that 0.75 and 1.5% SB concentrations in broilers' diets increased body weight, yet the same trait decreased at a 3.75% SB concentration. Feed conversion ratio improved significantly in the 2–7 week period in the current study. Some other researchers also found similar improvements with FCR after inclusion of bentonite in diets of broilers (Pasha et al. 2008; Damiri et al. 2012; Prasai et al. 2017; Attar et al. 2019) and quails (El-Abd 2014). A possible explanation for the FCR improvement in poultry receiving aluminosilicate clay could be the prolonged retention time of digesta in the birds' digestive system, resulting in better enzymatic activity in the intestines and absorption of nutrients such as minerals, fats, proteins, and carbohydrates (Khanedar et al. 2013; Yalçın et al. 2017). In turn, the utilization of nutrients in the intestines could also have enhanced growth performance in the current study.

Addition of SB to growing Japanese quail diets did not have a significant effect on HCY and relative weights of digestive giblets except for the proventriculus. These observations lend support to earlier findings by other researchers who did not find any effect of aluminosilicates on digestive organs in broilers (Eraslan et al. 2005; Eser et al. 2012; Khanedar et al. 2013; Yalçın et al. 2017) or quails (El-Abd 2014). A linear improvement was detected in the relative weight of the proventriculus as SB was supplemented in our experiment. Similar to our findings, Prvulovic et al. (2008) detected an increased proventriculus weight with hydrated aluminosilicate including zeolite and bentonite. It was concluded that feeding broiler with dry feed improved the muscle layer thickness of the proventriculus compared to wet diets (Prvulovic et al. 2008). The water absorbing ability of bentonite could cause a decelerated passage rate and decreased moisture content of the digesta which may also lead to improved weight of digestive system organs in poultry (Damiri et al. 2012; Banaszak et al. 2021a).

In the present study, a significant linear increase was observed in both glucose and TC concentrations with elevated SB supplementation. Like our results, other studies conducted on quails which received SB supplementation (El-Abd 2014) and broilers fed with hydrated aluminosilicate enriched diets (Yasar 1999) also reported increased glucose and cholesterol levels in blood serum. It is believed that the motility of the digesta in the small intestines likely alters the extent of glucose absorption (Müller et al. 2018). Eser et al. (2012) also described that sepiolite addition to broiler diets may increase the effectiveness of enzyme activity in the intestines that improve fat, protein, and carbohydrate absorption. An increase of glucose and cholesterol levels in blood serum of Japanese quails in the current study may be due to a retarding effect of dietary implementation of bentonite on digesta transit in the intestines and this situation may advance pancreatic enzyme activity in the small intestines. Furthermore, several studies have shown that clay supplementation in poultry diets could cause mobilization of abdominal fat in broilers (Eser et al. 2012; Prasai et al. 2017; Yalçın et al. 2017). Although we did not examine the amount of abdominal fat; SB may show a similar effect on mobilization of fat tissue in the current research according to blood serum cholesterol levels.

Poultry meat is very susceptible to oxidation due to the presence of more fatty acids than other livestock and MDA is an important identifier for detection of lipid peroxidation in tissues (Safaei et al. 2016). In the present study, TOS values in the blood serum were reduced in a linear trend with the augmenting amount of SB in Japanese quails' diet. Breast meat malondialdehyde values were also linearly decreased in parallel with lower ROS levels in the current experiment. Studies conducted on antioxidant properties of bentonite clays in livestock are very limited. Aydin et al. (2020) described that SB supplementation to the Tuj breed of lambs increased MDA and antioxidant enzyme levels in blood plasma. On the contrary, several researchers reported that dietary clay minerals improved TAC values in layers (Qu et al. 2018), and reduced MDA levels in sows (Papadopoulos et al. 2016) and broilers (Safaei et al. 2016; Morshdy et al. 2021). Cervini-Silva et al. (2015) explained that fibrous clays such as sepiolite and palygorskite could reduce lipid peroxidation due to their hydroxyl radical species stabilization ability.

Meat colour, tenderness, WHC and pH are essential quality factors for marketing and pricing of the carcass and meat cuts in the poultry sector (Liu et al. 2011; Morshdy et al. 2021; Banaszak et al. 2021a). In the present study, no effect of SB addition in quail feeds on days 1 and 30 on values of pH, WHC, L* and b* in breast meats was found. Similarly, dietary clay supplementation in previous studies did not affect pH (Hashemi et al. 2014; Yalçın et al. 2017; Banaszak et al. 2020), WHC (Hashemi et al. 2014; Banaszak et al. 2021b), L* (Hashemi et al. 2014; Banaszak et al. 2021b), and b* (Hashemi et al. 2014; Biesek et al. 2021; Banaszak et al. 2021b) values of breast meat in broilers. In the current study, the a* (redness) value showed quadratic elevation in groups fed with 0.5 and 1% SB compared with the control and 2% SB group. Banaszak et al. (2020) also found halloysites significantly improved the a* value of both breast and leg meat of broiler chickens. A rise of lipid peroxidation in muscle tissues induces the oxidation of bright red oxygenated myoglobin to metmyoglobin which changes the meat colour to dark brown (Wang et al. 2016). Hence, the lipid peroxidation inhibitor effect of SB may have increased the a* value in breast meat in the current experiment.

In conclusion, under the conditions of this experiment, it was found that 2% SB supplementation improved FCR, relevant weight of the proventriculus, and increased glucose and cholesterol in blood serum of growing Japanese quails, while reducing blood TOS levels. Furthermore, inclusion of 0.5% SB in the quails' diet resulted the highest redness value of breast meat and the treatment group fed with 1.0% SB had the lowest MDA level in our experiment. Further research should focus on the correlation between dietary bentonite supplementation and digestive enzyme activities and nutrient absorption capacity of the intestines.

Availability of Data and Materials

The author declares that the dataset supporting the study findings could be received upon a reasonable request.

Conflict of Interest

There was no conflict of interest throughout the duration of the current investigation.

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