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Effects of Dietary Inclusion of Sodium Bentonite on Biochemical Characteristics of Blood Serum in Broiler Chickens

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ABSTRACT

The effects of different levels of sodium bentonite on blood biochemical parameters in broiler chickens were studied. A total of 256 days-old broilers of male Ross 308 strain were assigned to four treatments with four replicates each containing 16 chicks in a completely randomized design. Treatments were as control and 15, 30, 45 g/kg of sodium bentonite. Blood serum biochemical values such as serum proteins, lipids, enzymes, glucose and total bilirubin were measured at 42 days of experimental period. Results showed that total protein, albumin and total bilirubin in dietary treatments with 45 g/kg sodium bentonite and albumin/globulin in dietary treatment 30 and 45 g/kg sodium bentonite and alanine aminotransferase (ALT) significantly ($P < 0.05$) increased compared to the control. Broilers given diet containing 15 g/kg sodium bentonite had significantly ($P < 0.05$) decreased aspartate aminotransferase (AST) and glucose compared to control. Levels of globulin, urea, creatinine, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), alkaline phosphatase (ALP), creatine kinase (CPK), glutamyltransferase (GGT), lactate dehydrogenase (LDH) did not significantly differ among dietary treatments and control. Supplementation of broiler chickens diet with sodium bentonite had not any adverse effects on their blood serum biochemical characteristics. Thus it can be used as a beneficial feed additive in broiler chicken diet.

Key Words: Sodium bentonite; Serum characteristics; Feed; Broiler

INTRODUCTION

Clay is a widely distributed, abundant mineral resource of major industrial importance for an enormous variety of uses (Ampian, 1985). Bentonite is a rock formed of highly colloidal and plastic clays composed mainly of montmorillonite, a clay mineral of the smectite group and is produced by in situ devitrification of volcanic ash. The special properties of bentonite are an ability to form thixotrophic gels with water, an ability to absorb large quantities of water and a high ion exchange capacity which binds deferent cations. The properties of bentonite are derived from the crystal structure of the smectite group, which is an octahedral alumina sheet between two tetrahedral silica sheets. Variations in interstitial water and exchangeable cations in the interlayer space affect the properties of bentonite and thus the commercial uses of the different types of bentonite (Walz *et al.*, 1998; Trckova., *et al*., 2004; Adamis *et al*., 2005).

Some studies showed that the use of sodium bentonite in broiler chickens diet would improve their weight gain (Tauqir *et al.*, 2001; Prvulovic *et al.*, 2008; Safaeikatouli *et al.*, 2010). Salari *et al.* (2006) indicated that chickens fed diets containing 1 and 2% sodium bentonite consumed more feed, had more weight gain and less feed conversion ratio. Ma and Guo (2008) reported villus height and crypt depth for duodenum, jejunum and ileum indicated that treating the diet of broilers with either Cu^{2+} loaded montmorillonite (CM) or montmorillonite improved the mucosal morphology of the small intestine. Also the presence of CM in the diet of broilers significantly increased the activities of maltase, aminopeptidase N and alkaline phosphatase in small intestinal mucosa. Pasha *et al.* (2008) reported birds fed diets containing sodium bentonite treated with either 0.5% or 1.0% acetic acid had significantly increased protein efficiency ratio and protein digestibility, as compared to the control.

Aflatoxin-contaminated diet has adverse effects on the performance of the birds. Sodium bentonite as a toxin binder decreases the adverse effect of aflatoxin (Fairchild *et al.*, 2008; Shi *et al.*, 2009) and causes the improvement of performance (Ahsan-ul-Haq *et al.*, 2000; Magnoli *et al.*, 2007; Pasha *et al.*, 2007) and reduce mycotoxin concentration in the livers of affected birds (Bailey *et al.*, 2006). Adamis *et al.* (2005) discussed the adverse effect of using sodium bentonite in the diet of birds and livestock on their health, yield and consumers. In recent experiments, which assessed the effect of bentonite on blood biochemical

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parameters, levels of (0.25, 0.5 & 1%) of bentonite were used in the diet (Eraslan *et al.*, 2006; Kermanshahi *et al.*, 2009). Although the main purpose of those experiments was to consider the effects of bentonite as a toxin binder on adverse effect of aflatoxin on blood biochemical parameters.

It is well established that the health and performance of birds is influenced by the nutrient and metabolites of blood. Therefore with the understanding of relationship between blood biochemical parameters and production characteristics, one can estimate the health and performance of the birds. The purpose of this study was to evaluate the changes of blood biochemical parameters of broiler chickens when different levels of sodium bentonite were used as a feed additive in their diet.

MATERIALS AND METHODS

Two hundred and fifty six of day old male Ross 308 broiler chickens obtained from a local hatchery were randomly distributed among four treatments with four replicate groups per treatment and 16 chickens per replicate. The temperature was maintained at 32°C during the 1st week and then was reduced by 3°C per week until 18°C was reached and this temperature was maintained until the end of the experiment. In the 1 to 42 days continuous lighting was used. Before beginning this study, the dry matter, crude protein and energy contents of main feed ingredients were determined (AOAC, 2005) in the laboratory to make sure of the presence of sufficient amounts of protein and energy of the ration. The diet was based on corn and soybean meal, containing or exceeding the nutritional requirements recommended by the NRC (1994) for starter (0-21 days) and grower (22-42 days) diets. Birds were distributed into the following four treatments: 0 (control), 15, 30 and 45 (g/kg) of sodium bentonite. The diets of starter phase calculated to contain 20.85% crude protein (CP) and 2900 kcal of metabolizable energy (ME) per kg of diet and also it contained 18.75% CP and 3000 kcal of ME per kg of diet for the grower phase (Table I). All diets, which were isocaloric and isonitrogenous, were fed for 6 weeks. The broilers were allowed ad-libitum access to feed and water.

At 42 day of age, two broilers per pen were randomly selected and 2 mL of blood samples were obtained by wing puncture and centrifuged at 3000 rpm for 20 min. The serum was collected and stored at -20°C until analyzed for total proteins, albumin, globulin, urea, creatinine, albumin/globulin, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL), alkaline phosphatase (ALP), creatine kinase (CPK), aspartate aminotransferase(AST), alanine aminotransferase (ALT), gammaglutamyl transferase (GGT), lactate dehydrogenase (LDH), glucose and total bilirubin were measured on autoanalyzer (Metrolab 2300 plus, Argentina) using commercially available kits. The globulin calculated using difference between total protein and albumin.

Data were analyzed using the general linear model procedure of SAS (2003). Data were subjected to analysis of variance and significant differences ($P < 0.05$) observed in means Subjected to Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Protein and albumin levels increased significantly ($P < 0.05$) in treatments inclusion of sodium bentonite compared to the control. Also albumin/globulin in treatments with 30 and 45 g/kg sodium bentonite increased significantly ($P < 0.05$) compared to the control. However, levels of globulin, urea, creatinine in serum were not affected ($P < 0.05$) by dietary treatments (Table II). Kececi *et al.* (1998) reported that the use of absorbents in broiler diet will cause the subsequent absorption of toxin in their digestive tract and this prevents the reduction of blood serum albumin, globulin and protein in the case of using contaminating diet.

Pasha *et al.* (2008) reported that those birds fed diets containing sodium bentonite treated with either 0.5% or 1.0% acetic acid had significantly increased ($P < 0.05$) protein efficiency ratio and protein digestibility, as compared to the control. The reason for this improvement was due to the presence of bentonite that prolonged feed passage time and improved nutrient metabolism. Aghashahi *et al.* (2006) experimented effects of different levels of bentonite on ammonia concentration, soluble and digestible protein (*In-vitro*) and reported, ammonia nitrogen was not decreased by inclusion of 2% of bentonite to rations ($P < 0.05$), but it was decreased effectively by inclusion of 4% of bentonite to rations ($P < 0.05$).

The differences of this finding of having a positive effect of sodium bentonite on serum protein concentration with the finding of Rosa *et al.* (2001) and Eraslan *et al.* (2006) can be due to the various levels of bentonite were used in their studies. It is worth noting that Rosa *et al.* (2001) and Eraslan *et al.* (2006) were used levels of 0.3 and 0.25, 0.5% of bentonite, respectively.

Effects of the dietary treatments on blood serum lipids are shown in (Table III). Serum concentrations of cholesterol, triglyceride, HDL, LDL and VLDL had no significant differences ($P > 0.05$) between dietary treatments and control, the only significant differences between two levels of 30 and 45 g/kg sodium bentonite in concentration of LDL were found ($P < 0.05$). These finding are in agreement with (Dwyer *et al.*, 1997; Ledoux *et al.*, 1999; Miles & Henry, 2007).

The AST content of blood increased significantly ($P < 0.05$) in treatment with 15 g/kg sodium bentonite compared to the control. Also ALT content in diets with 45 g/kg sodium bentonite compared to the control and other experimental treatments significant deference was observed ($P < 0.05$). The addition of sodium bentonite to the diet did not significantly affected ($p > 0.05$) on levels ALP, CPK,

Table I: Composition of experimental diets (g/kg) with supplemented sodium betonite levels (g) at 0-21 and 22-42 days

Feed ingredients (g)	Starter phase (0-21 days)				Grower phase (22-42 days)			
	0	15	30	45	0	15	30	45
Corn	587.3	556.2	525	493.9	648.1	617.0	585.8	554.7
Soybean meal	360.3	366.3	372.3	378.3	300.9	306.9	313	319
Soybean oil	14.3	24.4	34.6	44.7	17.5	27.6	37.7	47.8
Dicalcium phosphate	14.1	14.1	14.1	14.1	10.5	10.5	10.5	10.5
Limestone	12.5	12.5	12.5	12.5	13.4	13.4	13.4	13.4
Salt	4.3	4.3	4.3	4.3	3.2	3.2	3.2	3.2
Premix*	5	5	5	5	5	5	5	5
DL-Methionine	1.5	1.5	1.5	1.5	0.7	0.7	0.7	0.7
salinomycine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Nutrient composition								
ME, kcal/kg	2900	2900	2900	2900	3000	3000	3000	3000
CP	20.85	20.85	20.85	20.85	18.75	18.75	18.75	18.75

*Premix contained 50% vitamin premix and 50% mineral premix. Each kg of vitamin premix contained: vitamin A, 3,500,000 IU; vitamin D3, 1,000,000 IU; vitamin E, 9000 IU; vitamin K3, 1000 mg; vitamin B1, 900 mg; vitamin B2, 3,300 mg; vitamin B3, 5,000 mg; vitamin B5, 15,000 mg; vitamin B6, 150 mg; vitamin B9, 500 mg; vitamin B12, 7.5 mg; biotin, 500 mg; choline chloride, 250,000 mg and each kg of mineral premix contained: Mn, 50,000 mg; Fe, 25,000 mg; Zn, 50,000 mg; Cu, 5,000 mg; I, 500 mg, Se, 100 mg

Table II: Effect of different levels of sodium bentonite on blood serum proteins

Treatments	Serum proteins					
	Total Protein	Albumin	Globulin	Albumin/Globulin	Urea	Creatinine
	(g/dL)				(mg/dL)	
Control	3.18^{b}	1.09^{b}	2.09	0.52^{b}	2.02	0.17
Sodium bentonite 15 g/kg	3.69^{a}	1.43^{a}	2.26	0.64^{ab}	2.05	0.13
Sodium bentonite 30 g/kg	3.83^{a}	1.54^{a}	2.29	0.67^{a}	2.04	0.12
Sodium bentonite 45 g/kg	3.87^{a}	1.56^{a}	2.31	0.68^{a}	1.89	0.11
SEM	0.12	0.08	0.08	0.04	0.29	0.02

Table III: Effect of different levels of sodium bentonite on blood serum lipids

Treatments	Serum lipids				
	Cholesterol	Triglyceride	HDL	LDL	VLDL
	(mg/dL)				
Control	136.23	103.50	96.38	19.98^{ab}	19.87
Sodium bentonite 15 g/kg	135.25	115.88	90.13	23.33^{ab}	23.18
Sodium bentonite 30 g/kg	138.63	103.13	91.88	25.13^{a}	21.62
Sodium bentonite 45 g/kg	121.45	101.88	92.63	11.48^{b}	19.85
SEM	7.74	18.14	6.92	3.93	3.47

Table IV: Effect of different levels of sodium bentonite on blood serum enzymes

Treatments	Serum enzymes					
	Alkaline phosphatase	Creatine kinase	Aspartate aminotransferase	Alanine aminotransferase	Gammaglutamyl transferase	Lactate dehydrogenase
	(I U/L)					
Control	3432.10	678.10	181.88^{a}	5.74^{b}	20.45	370.15
Sodium bentonite 15 g/kg	3243.90	613.00	148.25^{b}	6.76^{b}	23.15	476.15
Sodium bentonite 30 g/kg	4622.50	625.30	176.88^{ab}	6.91^{b}	20.78	326.05
Sodium bentonite 45 g/kg	4678.90	640.30	178.00^{ab}	11.34^{a}	20.40	321.93
SEM	592.71	71.38	9.36	1.11	2.80	52.00

Means within columns with no common superscripts are significantly different ($p<0.05$), according to multiple range test

GGT and LDH in serum compared with the control (Table IV). Dwyer *et al.* (1997), Bailey *et al.* (2006), Eraslan *et al.* (2006) and Kermanshahi *et al.* (2009) reported that sodium bentonite had no effect on the blood serum enzymes.

The effects of dietary treatments on glucose and total bilirubin are presented in (Table V). The levels of glucose in treatment with 15 g/kg sodium bentonite significantly decreased ($P < 0.05$). The level of glucose in other treatments also compared with control showed a decrease. But it was not significant ($P > 0.05$). The total bilirubin contain in treat with 45 g/kg sodium bentonite showed a significant increase compared with control. This total bilirubin increased was observed in other treatments compared with control. However, this difference was not significant ($P > 0.05$).

When the body metabolism increases the density of blood glucose declined. Therefore the use of sodium bentonite in diet will cause an increase in metabolism and

Table V: Effect of different levels of sodium bentonite on glucose and total bilirubin

Treatments	Glucose	Total bilirubin
	(mg/dL)	
Control	226.50[a]	0.260[b]
Sodium bentonite 15 g/kg	201.50[b]	0.395[ab]
Sodium bentonite 30 g/kg	216.25[a]	0.408[ab]
Sodium bentonite 45 g/kg	218.75[a]	0.600[a]
SEM	4.57	0.080

Means within columns with no common superscripts are significantly different ($p<0.05$), according to multiple range

more efficient digestion and absorption of nutrients. Eraslan *et al.* (2006) reported that adding 0.5% bentonite in the diet will cause a significant ($P < 0.05$) decrease in blood glucose compared with control and treatment containing 0.25% bentonite. Also, did not observe a significant difference in the content of total bilirubin between control and treatment with 0.25 and 0.5% bentonite. Santurio (1999) reported that blood biochemical variables were not affected by dietary natural sodium bentonite and also they emphasized that natural sodium bentonite caused no adverse effects on chickens. Further research into these effects of bentonite on broiler health and performance is warranted.

CONCLUSION

Dietary inclusion of sodium bentonite had not any adverse effects on serum biochemical characteristics and it can also improve the values of serum total proteins and albumin in broiler chickens. Thus, sodium bentonite can be beneficial as a feed additive in the broiler chickens diet.

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