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An Evaluation on the Effects of Dietary Kaolin and Zeolite on Broilers Blood Parameters, T4, TSH and Growth Hormones

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Abstract: This study was conducted to evaluate the effects of dietary kaolin and zeolite on blood biochemical parameters, thyroxine (T4), thyrotrophin (TSH) and Growth Hormone (GH) in broiler chickens. A total of 320 one-day-old male Ross 308 broiler chicks were divided into 1 control and 4 experimental groups each containing 64 birds. Each group was contained four replicates with 16 chickens and rose for a period of 42 days. The experiment was carried out in a completely randomized design. Treatments were 1) control 2) diet included 15 g/kg kaolin 3) diet included 30 g/kg kaolin 4) diet included 15 g/kg zeolite 5) diet included 30 g/kg zeolite. Results showed that blood total protein for treatments containing 15, 30 g/kg kaolin and 30 g/kg zeolite and albumin, albumin/globulin in diets inclusion 15 g/kg kaolin and 30 g/kg zeolite significantly ($p<0.05$) increased compared to the control. Also glucose content in diet containing 15 g/kg kaolin and total bilirubin in treatment containing 30 g/kg zeolite and Growth Hormone (GH) in diets containing 30 g/kg kaolin and zeolite significantly increased compared to the control ($p<0.05$). Broilers were fed diet containing 15 g/kg kaolin and zeolite had shown a significant ($p<0.05$) decrease at triglyceride. There was no significant ($p>0.05$) differences were observed at levels of globulin, urea, creatinine, cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), Alkaline Phosphatase (ALP), Creatine Kinase (CPK), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Gammaglutamyl Transferase (GGT), Lactate Dehydrogenase (LDH), thyroxine (T4), thyrotrophin (TSH) between dietary treatments and control. The use of kaolin and zeolite as a beneficial feed additive in broiler chicken diet is recommended.

Key words: Kaolin, zeolite, serum biochemical, hormones, feed, broiler

INTRODUCTION

The name kaolin is derived from the word Kau-Ling, or high ridge, the name given to a hill near Jau-chau Fu, China, where kaolin was first mined (Sepulveda *et al*., 1983). Kaolin or china clay is a mixture of different minerals. Its main component is kaolinite; in addition, it frequently contains quartz, mica, feldspar, illite and montmorillonite. Kaolinite is made up of tiny sheets of triclinic crystals with pseudohexagonal morphology. It is formed by rock weathering. It has some cation exchange capacity (Adamis *et al*., 2005). The structure of kaolinite is a tetrahedral silica sheet alternating with an octahedral alumina sheet. These sheets are arranged so that the tips of the silica tetrahedrons and the adjacent layers of the octahedral sheet form a common layer. The molecular formula that is common for the kaolinite group (kaolinite, nacrite, dickite) is Al2Si2O5(OH)4 (Grim, 1968; Trckova *et al*., 2004). Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations that posses three-dimensional structures with interconnecting channels and large pores, capable of trapping molecules of proper dimensions (Mumpton, 1999). Each zeolite has its own unique chemical composition, crystalline structure (similar to honeycomb) and therefore, possesses its own set of adsorption properties. Exchangeable cations maintain electrical neutrality within the structure. The pore size in commercially available zeolite ranges from approximately 0.3-0.8 nm (Tiwari, 2007).

Mechanisms of the effects of mineral silicates in the animal performance are not clear enough. There have been various studies and different reasons have been reported for this subject. Various researchers found that animal diets containing kaolin and zeolite has been shown to improve body weight gain and feed conversion ratio chicken (Cabuk *et al*., 2004; Hesham *et al*., 2004; Incharoen *et al*., 2009), pig (Papaioannou *et al*., 2002; Prvulovic *et al*., 2007; Prvulovic *et al*., 2009), sheep (Stojkovik *et al*., 2005; Ghaemnia *et al*., 2010) and goat

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(Smith *et al*., 1994) to improve the quality of animal products such as eggs (Keshavarz and McCormick, 1991; Nassiri Moghaddam *et al*., 2008; Altiner *et al*., 2009) or wool (Eady *et al*., 1980), to reduce bacterial contamination of the gut (Varel *et al*., 1987; Trckova *et al*., 2009) and the addition of kaolin and zeolite to the aflatoxin-containing diet reduced the adverse effects of aflatoxin and should be helpful in a solution to the aflatoxicosis problem in poultry (Kececi *et al*., 1998; Oguz *et al*., 2000; Hesham *et al*., 2004). Among many properties attributed to zeolites, two most characteristics that relates to their effectiveness in poultry nutrition are their ability to lose and gain water reversibly and being capable of exchanging selectively a variety of cations in their structure without much major changes of structure (Shariatmadari, 2008).

Adamis *et al*. (2005) discussed the adverse effect of using mineral silicate in the diet of birds and livestock on their health, yield and consumers. 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 aim of the investigation was the assessment of the effects of dietary inclusion different levels of kaolin and zeolite on broilers blood characteristics, T4, TSH and growth hormones.

MATERIALS AND METHODS

Three hundred and twenty of one-day-old male Ross-308 broiler chicks were obtained from a commercial hatchery. Chicks were divided randomly into five groups; each group was further divided into four replicates with 16 chickens. The experiment was conducted in a completely randomized design of 5 dietary treatments with 4 replicates in each treatment. Birds were reared up to 6 weeks of age. The temperature of the room was 32°C in the first week and was then reduced by 3°C weekly until 18°C and lighting was provided for 24 h per day. Treatments were 1) control 2) diet inclusion 15 g/kg kaolin 3) diet inclusion 30 g/kg kaolin 4) diet inclusion 15 g/kg zeolite 5) diet inclusion 30 g/kg zeolite. The chicks were fed a commercial feed starter (corn and soybean based, 20.85% CP and 2900 kcal ME/kg) up to 21 days and there after a grower diet (18.75% CP and 3000 kcal ME/kg) up to 42 days. Chickens were allowed access to the diets and water ad libitum. The starter and grower basal diets were both supplemented with amino acids, minerals and vitamins as recommended by National Research Council (NRC, 1994).

Prior to being slaughtered, birds were individually weighed and a blood sample of 2 mL was taken from wing vein of each bird. The blood samples were immediately centrifuged at 3000 rpm for 20 min with a centrifuge (Hettich; Germany) and the serum was stored at -20°C in sealed container till analyzed. The serum samples were 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 and serum samples were analyzed for thyroxine (T4), thyrotrophin (TSH) and Growth Hormone (GH) levels with radioimmunoassay method. Data were analyzed for variance analysis using general linear model procedures of the SAS (SAS Institute, 2003). Significant differences ($p<0.05$) among treatment means were determined using Duncan's new multiple range test (Duncan's, 1955).

RESULTS AND DISSCUSION

The effects of dietary treatments on blood serum protein are presented in (Table 1). Total protein in treatments containing 15, 30 g/kg kaolin and 30 g/kg zeolite was significantly higher in compared to control treatment. Concentration of albumin and albumin/globulin significantly increased in treatments containing 15 g/kg kaolin and 30 g/kg zeolite compared to control diet ($p<0.05$). Serum concentrations of globulin, urea, creatinine had no significant differences ($p>0.05$) between dietary treatments and control. In our experiment the concentration of total serum protein and albumin increased and the concentration of globulin was unaffected by the dietary supplementation of kaolin and zeolite. These results are in agreement with previous studies in which silicate mineral significantly improved blood serum protein in broiler chicks (Bailey *et al*., 2006; Prvulovic *et al*., 2008). The mechanism of this effect is not clear. The reason for this improvement in serum protein observed in the present study could have been due to the presence of kaolin and zeolite in the diet, which might have increased feed retention time in the gut of the chicks, thus subjecting the nutrients to enzymatic action for quite a long time, or could have been due to the action of kaolin and zeolite on the enhanced digestibility of certain nutrients. Mumpton and Fishman (1977) suggested that zeolite can increase digestibility of feeds as well as broilers performance. Tatar *et al*. (2008) observed that the amount of ileal digestibility of protein in diets containing zeolite increased with compared to control. The interference of kaolin in the blood serum parameters is not reported in the literature.

Table 1: Effect of different levels of kaolin and zeolite on blood serum proteins

	Serum proteins					
Treatment	Total protein	Albumin	Globulin	Albumin/Globulin	Urea	Creatinine
	(g/dL)				(mg/dL)	
Control	3.27^{b}	1.13^{b}	2.14	0.53^{b}	2.01	0.17
Kaolin 15 g/kg	3.84^{a}	1.58^{a}	2.26	0.70^{a}	2.41	0.13
Kaolin 30 g/kg	3.74^{a}	1.38^{ab}	2.36	0.59^{ab}	2.18	0.14
Zeolite 15 g/kg	3.57^{ab}	1.37^{ab}	2.20	0.63^{ab}	2.04	0.27
Zeolite 30 g/kg	3.82^{a}	1.61^{a}	2.21	0.73^{a}	1.92	0.14
SEM	0.14	0.10	0.09	0.05	0.16	0.06

Means within columns with no common superscripts are significantly different ($p<0.05$)

Table 2: Effect of different levels of kaolin and zeolite on blood serum lipids

	Serum lipids*				
Treatment	Cholesterol	Triglyceride	HDL	LDL	VLDL
	(mg/dL)				
Control	137.75	104.00^{a}	97.50	19.45^{ab}	20.80
Kaolin 15 g/kg	125.88	69.13^{c}	81.54	30.51^{a}	13.83
Kaolin 30 g/kg	133.50	94.75^{ab}	91.25	23.30^{ab}	18.95
Zeolite 15 g/kg	138.50	76.25^{bc}	83.38	27.75^{a}	27.40
Zeolite 30 g/kg	128.25	111.13^{a}	90.63	15.40^{b}	22.23
SEM	10.15	7.94	7.11	3.69	4.58

Means within columns with no common superscripts are significantly different ($p<0.05$). *HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; VLDL = Very Low Density Lipoprotein

Table 3: Effect of different levels of kaolin and zeolite on blood serum enzymes

	Serum enzymes					
Treatment	Alkaline phosphatase	Creatine kinase	Aspartate aminotransferase	Alanine aminotransferase	Gammaglutamyl transferase	Lactate dehydrogenase
	(IU/L)					
Control	3527.30	689.30	180.63^{ab}	5.64	20.95	369.40
Kaolin 15 g/kg	3463.60	505.10	177.63^{ab}	8.45	21.35	335.95
Kaolin 30 g/kg	4760.90	720.80	186.50^{a}	6.27	24.75	388.23
Zeolite 15 g/kg	4187.60	699.40	166.00^{ab}	5.69	22.30	321.83
Zeolite 30 g/kg	4368.00	775.50	155.88^{b}	8.87	21.27	499.58
SEM	509.30	99.89	8.26	1.38	4.06	65.29

Means within columns with no common superscripts are significantly different ($p<0.05$)

Triglyceride level for dietary treatments containing 15 g/kg kaolin and zeolite decreased significantly compared to control group and also triglyceride differences between diets with 15 and 30 g/kg of zeolite was significant ($p<0.05$). LDL content was significantly different among those treatments containing 15 g/kg kaolin and zeolite with treatment containing 30 g/kg zeolite. Levels of cholesterol, HDL and VLDL in serum were not affected ($p<0.05$) by dietary treatments (Table 2). The results of the present study are in agreement with previous findings (Bailey *et al*., 2006; Lotfollahian *et al*., 2004; Miles and Henry, 2007).

The addition of kaolin and zeolite on diets did not significantly affected ($p>0.05$) levels of AST, ALP, CPK, GGT and LDH in serum compared to the control. Only a significant difference was observed in treatments containing 30 g/kg kaolin and 30 g/kg zeolite for AST (Table 3). The serum ALP and ALT activity was higher in broilers fed 30 g/kg kaolin and zeolite but remained in the normal range for broiler. This could be an indication of tissue damage if it was accompanied by the increased activity of other serum enzymes, especially AST. However, the activities of all other measured enzymes were within the normal range for broilers and similar to the control group. This is in agreement with the results of other authors (Harvey *et al*., 1993; Santin *et al*., 2002).

Effects of the dietary treatments on glucose and total bilirubin are shown in Table 4. Diet containing 15 g/kg kaolin had a significantly ($p<0.05$) lower level of glucose compared to control. Total bilirubin in treatment containing 30 g/kg zeolite significantly increased compare to those treatments with 15 g/kg zeolite and control ($p<0.05$). Miles and Henry (2007) using of hydrated sodium calcium aluminosilicate in broiler diet and reported that there was no difference in glucose components due dietary treatments. When the body metabolism increases the density of blood glucose declined. Therefore the use of silicate mineral in diet will cause an increase in metabolism and more efficient digestion and absorption of nutrients.

Table 4: Effect of different levels of kaolin and zeolite on glucose and total bilirubin

Treatment	Glucose (mg/dL)	Total bilirubin (mg/dL)
Control	229.00[a]	0.08[b]
Kaolin 15 g/kg	209.75[b]	0.16[ab]
Kaolin 30 g/kg	211.50[ab]	0.14[ab]
Zeolite 15 g/kg	218.75[ab]	0.10[b]
Zeolite 30 g/kg	221.75[ab]	0.23[a]
SEM	5.72	0.04

Means within columns with no common superscripts are significantly different ($p<0.05$)

Table 5: Effect of different levels of kaolin and zeolite on some hormones

Treatment	T4 (µg/dL)	TSH (µg/dL)	GH (ng/mL)
Control	2.87	1.50	2.02[b]
Kaolin 15 g/kg	2.90	1.87	2.44[ab]
Kaolin 30 g/kg	3.05	1.97	2.64[a]
Zeolite 15 g/kg	2.74	1.56	2.51[ab]
Zeolite 30 g/kg	2.99	1.74	2.98[a]
SEM	0.22	0.20	0.18

Means within columns with no common superscripts are significantly different ($p<0.05$)

Growth hormone in treatments containing 30 g/kg kaolin and zeolite significantly ($p<0.05$) increased compare to control treatment. GH content in other dietary treatments was higher compare to control. However this difference was not significant ($p>0.05$). Plasma T4 and TSH levels were not affected by the dietary treatments (Table 5). Eraslan *et al.* (2006) use of hydrate sodium calcium aluminosilicate in the quail's diet and in TSH content no significant ($p>0.05$) differences were observed among dietary treatments, but observe a significant difference in the content of T4 between control and dietary treatment. Ledoux *et al.* (1999); Oguz *et al.* (2000) and Safaeikatouli *et al.* (2010) reported that silicate mineral had no adverse effect on the blood serum parameters in broiler chickens. It is important to note, however, that the dietary addition of kaolin and zeolite has no adverse effects on serum biochemical parameters and does not affect the normal physiological homeostasis of birds. Further research into these effects of kaolin and zeolite on broiler health and performance is warranted.

Conclusion: In conclusion, the existence of kaolin and zeolite in broiler diet had not showed any adverse effects on blood serum biochemical parameters, T4, TSH and GH hormones. Therefore the use of kaolin and zeolite as a beneficial feed additive in broiler chicken diet is recommended.

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