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Research Article

The Effects of Dietary Inclusion of Hydrated Aluminosilicate on Performance and Biochemical Parameters of Broiler Chickens

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Abstract: This study investigated the effects of dietary supplementation with hydrated aluminosilicate (antitoxic nutrient-ATN), based on zeolitic ore (of > 90% clinoptilolite), bentonite (of > 83% montmorillonite), and small amounts of activated charcoal, on performance, hematological, serum, and liver biochemical parameters, as well as organ weights and meat quality in broiler chickens. The study included 300 1-day-old broilers of both sexes, which were assigned at random to 2 groups based on treatment: no dietary supplementation (control) and supplementation with 5 g/kg of ATN (ATN group). Dietary supplementation with 5 g/kg of ATN in broiler diets significantly increased weight gain only during the 1st and 3rd weeks; however, the effect on weight gain and the feed conversion ratio was not significant ($P > 0.05$) for the overall study period. Most hematological, serum, and liver biochemical indices were unaffected by the dietary treatment. Supplemental ATN significantly increased serum amylase and lactate dehydrogenase activity ($P < 0.05$). Spleen, proventriculus, ventriculus, and ileum weights were significantly higher ($P < 0.05$) in the ATN group, whereas the weights of the other measured organs were not affected by the dietary treatment. Significantly higher ($P < 0.05$) protein and ash, and significantly lower fat content was observed in the breast meat of chicks in the ATN group. Ash content was also significantly higher ($P < 0.05$) in drumstick meat. The results of this study demonstrate that supplementation with 5 g/kg of hydrated aluminosilicate influenced serum traits, organ weights, and the chemical composition of broiler chicken meat.

Key Words: Aluminosilicate, broiler, feed, blood, organ weight

Introduction

Phyllosilicate clays are crystalline, hydrated aluminosilicates that contain alkali and alkaline earth cations, and have a layered structure. Phyllosilicates vary in their composition from one phyllosilicate to another, depending mainly on the interchangeable ions that may be contained within their structure (1). The basic building blocks of natural zeolites are electrostatically charged tetrahedra of silica and aluminium, with the net negative charge balanced by cations such as calcium, magnesium, sodium, and potassium. The stacking of these tetrahedra gives rise to various crystal 3-dimensional honeycomb structures containing tunnels or channels of uniform diameter (2).

The dietary clays may slow the passage of ingesta through the digestive tract, increase digestibility because of greater surface exposure, buffer the rumen of ruminant animals, and adsorb ammonia in the digestive tract. Many clays possess active binding sites that are capable of adsorbing a variety of diverse molecules (3,4).

Dietary addition of aluminosilicates has been shown to alter tissue mineral concentrations and have potential value in protecting animals from toxic mineral accumulation in tissues (5,6). Aluminosilicates might also influence calcium and phosphorus utilization (7,8). Many studies have demonstrated that hydrated sodium calcium aluminosilicate clays and zeolites significantly diminish the adverse effects of aflatoxins in poultry (9-13) and other animal species (14). Zeolites are also suitable vehicles for the slow release of some drugs (15). The addition of hydrated aluminosilicates to the diet of animals, at rates between 5 and 50 g/kg, has been reported to improve

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growth and feed utilization, and to reduce the incidence and severity of diarrhea (4,16,17). However, there is also a concern that some dietary aluminosilicates might absorb small nutritional particles in the gastrointestinal tract.

The present study was undertaken to determine the effects of hydrated aluminosilicate (antioxidant nutrient-ATN) on growth performance, meat quality, various serum and liver biochemical parameters, and hematological features in broiler chicks.

Materials and Methods

Animals and Housing

Day-old unvaccinated broiler chicks of both sexes were obtained from a commercial hatchery. In all, 300 individually weighed chicks were divided at random into 2 treatment groups with 3 replicates of 50 chicks in each group. The chickens were maintained in electrically heated batteries under continuous florescent lighting, with feed and water available for ad libitum consumption.

Feeding Trial

The experimental design consisted of 2 dietary treatments: a commercial diet (control group) and ATN supplementation at 5 g/kg feed. ATN is a fine powder that contains mostly zeolitic ore (containing > 90% clinoptilolite) and bentonite (containing > 83% montmorillonite), together with small amounts of activated charcoal (zeolite:bentonite:charcoal ratio of 60:20:1). The chicks were maintained on these treatments until 6 weeks of age. The chicks were fed a non-medicated corn and soybean commercial feed starter until 21 days, then a grower diet until 42 days. The basal diet met or exceeded the National Research Council's recommended levels for critical nutrients (18). Broilers were weighted individually on a weekly basis, feed consumption was recorded weekly, and mortality was recorded as it occurred.

Blood Sampling and Analysis

When the chicks reached 6 weeks of age, the feeding trial was terminated and 36 broilers from each treatment group (12 chicks for each replicate) were selected at random and bled via cardiac puncture for hematological and serum biochemical analyses. Heparin was used as an anticoagulant and non-coagulated blood was tested for hemoglobin (Hb) concentration, total erythrocyte count (TEC), and packed cells volume (PCV) or hematocrit. Blood Hb concentration was determined by the cyanmethemoglobin procedure (19). Micro Wintrobe hematocrit tubes and a hematocrit centrifuge were used to determine PCV. Serum concentrations of total protein, albumin, creatinine, urea nitrogen, glucose, cholesterol, triglycerides, inorganic phosphorus, calcium and chlorides, and γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), α-amylase (AMY), creatine kinase (CK), and L-lactate dehydrogenase (LDH) activity were determined on a clinical chemistry analyzer (Microlab 200, Merck) according to the manufacturer's recommended procedure.

Slaughter

In all, 24 broilers (12 chicks from each treatment) were killed by cervical dislocation and the liver, spleen, pancreas, proventriculus, ventriculus, bursa of Fabricius, esophagus, duodenum, and ileum were removed and weighed. Glutathione S-transferase (GST) activity and protein content were measured in liver homogenates. Protein content was determined according to the method of Bradford (20), using bovine serum albumin as the protein standard. GST activity in liver homogenates was evaluated spectrophotometrically (Jenway 6505 UV/Vis., UK) using 1-chloro-2,4 dinitrobenzene (CDNB) as the substrate, as previously described by Habig et al. (21), and was expressed as U/mg of protein. To examine meat quality, 6 breasts and 6 right drumsticks from each treatment group (2 chicks for each replicate) were used. The pH 24 was determined 24 h after slaughter. Chemical analysis of the meat (moisture, and fat, protein, ash, sodium, and potassium content) was determined using the international procedures of AOAC (22).

Statistical Analysis

All results are expressed as mean ± standard error (SE). Statistical analyses included analysis of variance (ANOVA), and post hoc comparisons were made using Duncan's multiple range test. Statistical significance was set at $P < 0.05$ (23).

Results

Growth Performance

The effects of the dietary treatments on chick growth performance, weekly average body weight gain (BWG), and feed conversion ratio (FCR) data obtained from week 1 to 6 are presented in Table 1. Chicks fed ATN consumed the same amount of feed as chicks in the control group. A trend toward less BWG was observed in the ATN group, as compared to the control group, during the second and last week of the experiment. ATN supplementation increased BWG numerically during weeks 4 and 5, and significantly ($P < 0.05$) increased it during the 1st and 3rd weeks of the experiment; nonetheless, overall, BWG was similar in both groups. Supplementation with ATN did not appear to influence the chickens' appetites or FCR.

Blood Parameters

Analysis of the hematological indices showed that the values of the ATN and control groups were within the reference range for chickens (Table 2). There were no significant interactions of the experimental factors with regards to hematological blood indices in either group. Data presented in Table 2 also show the effects of the dietary treatments on serum biochemical values and enzyme activity of broilers. Supplementation with ATN caused significant increases in serum LDH and AMY activity. There were no differences in the serum concentrations of total protein, albumin, inorganic phosphorus, or calcium between the 2 groups, whereas there were differences between the groups in terms of serum creatinine, urea nitrogen, glucose, cholesterol, triglycerides, and chlorides levels, and GGT, ALP, AST, ALT, and CK activity.

Relative Organ Weight

The relative weight of the liver (major target organ of most toxicants) was not affected by feed supplementation with ATN. The relative weights of the spleen, proventriculus, ventriculus, and ileum were higher in the ATN group (Table 3). There were no differences in the relative weights of the pancreas, bursa of Fabricius, esophagus, or duodenum between the groups.

Chemical Analysis of the Liver

GST (an enzyme involved in detoxification) activity and protein content in the liver homogenate of broilers are also summarized in Table 3. ATN supplementation had no significant influence on GST activity or protein content of liver homogenates.

Chemical Composition of Muscle

The effects of ATN supplementation on pH24, and chemical composition of breast and drumstick muscle are shown in Table 4. In this study meat pH 24 h after slaughter was an average 5.83 for breast meat and 6.09 for drumstick meat. ATN treatment had no significant effect on this variable. Breast and drumstick meat showed significantly higher values for ash content in animals fed supplemental ATN. Supplemental ATN also significantly increased the level of protein and decreased the level of fat in breast meat, but did not affect the level of protein or fat in drumstick meat. Moisture, sodium, and potassium content of breast and drumstick meat were unaffected by dietary treatment.

Discussion

Results of previous experiments concerning the effects of zeolite and clays on animal performance are generally inconsistent. Harvey et al. (11), Kubena et al. (24), and Xia et al. (25) found that weight gain in chickens improved when clays were added to the feed. Contrary to these results, other authors (26,27) found

Table 1. Effects of ATN on weekly average BWG and FCR.

Treatment	BWG						FCR
ATN (g/kg)	(g/day)						(kg/kg)
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	
0	12.42	29.84	45.84	62.02	77.70	76.96	1.91
5	13.07	28.91	55.63	62.34	79.38	69.42	1.93
CFC (%)	+ 5.23	–3.12	+ 31.36	+ 0.52	+ 2.16	–9.80	+ 1.58

BWG: body weight gain; FCR: feed conversion ratio; CFC: change from control.
Values represent the mean of 3 replicate groups of 50 broilers per treatment.

Table 2. Effects of ATN on some hematological parameters, serum biochemical values, and enzyme activity of broiler chicks.

Parameter		Treatment	
		ATN (g/kg)	
		0	5
Hemoglobin (Hb)	(g/l)	82.44 ± 5.05	85.00 ± 3.66
Packed cell volume (PCV)	(%)	33.10 ± 1.13	33.55 ± 0.55
Total erythrocyte count (TEC)	($10^6/mm^3$)	4.65 ± 0.13	4.70 ± 0.07
Total protein	(g/l)	29.00 ± 1.30	30.30 ± 0.80
Albumin	(g/l)	13.90 ± 0.53	15.00 ± 0.33
Creatinine	(mmol/l)	26.30 ± 0.84	28.60 ± 0.73
Urea nitrogen	(mmol/l)	0.49 ± 0.03	0.55 ± 0.03
Glucose	(µmol/l)	14.18 ± 0.27	14.78 ± 0.29
Cholesterol	(mmol/l)	2.85 ± 0.11	3.08 ± 0.08
Triglycerides	(mmol/l)	0.62 ± 0.05	0.59 ± 0.02
Chlorides	(mmol/l)	117.50 ± 2.20	117.50 ± 1.46
Inorganic phosphorus	(mmol/l)	1.74 ± 0.04	1.80 ± 0.03
Calcium	(mmol/l)	1.92 ± 0.06	1.96 ± 0.04
α-amylase (AMY)	(IU/l)	502.09^{a} ± 54.86	738.43^{b} ± 39.01
γ-glutamyltransferase (GGT)	(IU/l)	13.74 ±2.90	16.74 ± 1.55
Alkaline phosphatase (ALP)	(IU/l)	3469.20 ± 143.15	3215.41 ± 248.57
Aspartate aminotransferase (AST)	(IU/l)	283.82 ± 12.30	320.89 ± 17.34
Alanine aminotransferase (ALT)	(IU/l)	6.46 ± 0.78	5.97 ± 0.67
Creatine kinase (CK)	(IU/l)	940.25 ± 58.36	1190.58 ± 114.32
L-lactate dehydrogenase (LDH)	(IU/l)	2095.22^{a} ± 59.98	2338.40^{b} ± 54.21

^{a,b}Values within columns without common superscripts are significantly different ($P < 0.05$).
Values represent the mean ± SE of 3 replicate groups of 12 broilers per treatment.

Table 3. Effects of ATN on relative organ weights, liver protein content, and glutathione S transferase (GST) activity in broiler chicks.

Parameter		Treatment	
		ATN (g/kg)	
		0	5
Liver	g/100 g BW	2.74 ± 0.19	2.48 ± 0.13
Spleen	g/100 g BW	0.14^{a} ± 0.01	0.22^{b} ± 0.01
Proventriculus	g/100 g BW	0.42^{a} ± 0.01	0.48^{b} ± 0.02
Ventriculus	g/100 g BW	1.30^{a} ± 0.04	1.58^{b} ± 0.05
Ileum	g/100 g BW	0.24^{a} ± 0.02	0.32^{b} ± 0.02
Pancreas	g/100 g BW	0.22 ± 0.01	0.24 ± 0.02
Esophagus	g/100 g BW	0.14 ± 0.01	0.16 ± 0.01
Duodenum	g/100 g BW	0.48 ± 0.02	0.44 ± 0.04
Bursa of Fabricius	g/100 g BW	0.20 ± 0.02	0.22 ± 0.01
Soluble proteins	(g/100 g tissue)	6.94 ± 0.64	7.15 ± 0.61
Glutathione S-transferase	(U/mg protein)	473.48 ± 72.71	440.73 ± 36.12

^{a,b}Values within columns without common superscripts are significantly different ($P < 0.05$).
Values represent the mean ± SE of 3 replicate groups of 4 broilers per treatment.

Table 4. Effects of ATN on the chemical composition of chicken breast meat and drumstick meat.

Parameter		Treatment	
		ATN (g/kg)	
		0	5
Protein (%)	breast	$21.90^{a} \pm 0.22$	$22.69^{b} \pm 0.12$
	drumstick	18.12 ± 0.18	18.37 ± 0.13
Fat (%)	breast	$1.14^{a} \pm 0.10$	$0.62^{b} \pm 0.08$
	drumstick	6.96 ± 0.76	7.01 ± 0.40
Moisture (%)	breast	75.73 ± 0.22	75.14 ± 0.17
	drumstick	73.76 ± 0.61	73.26 ± 0.34
Ash (%)	breast	$1.03^{a} \pm 0.02$	$1.27^{b} \pm 0.06$
	drumstick	$0.88^{a} \pm 0.01$	$1.05^{b} \pm 0.05$
pH	breast	5.91 ± 0.02	5.76 ± 0.10
	drumstick	6.12 ± 0.06	6.06 ± 0.04
Sodium (mg/kg)	breast	57.12 ± 6.22	50.06 ± 4.67
	drumstick	75.03 ± 6.06	74.44 ± 3.15
Potassium (mg/kg)	breast	308.78 ± 5.72	301.47 ± 13.20
	drumstick	233.39 ± 32.44	263.73 ± 6.94

[a,b]Values within rows without common superscripts are significantly different ($P < 0.05$).
Values represent the mean ± SE of 3 replicate groups of 2 broilers per treatment.

that body weight gain was unaffected by hydrated aluminosilicates. The type of aluminosilicate and the physical or chemical structural characteristics affect the feed value of hydrated aluminosilicates, as well as does the target species. In the present study dietary supplementation with 5 g/kg of ATN had no clear effect on weight gain or feed utilization. The current results show that ATN had a significant beneficial effect on chick performance during weeks 1 and 3, but that there was only a tendency to reduce BWG during the later phase of experiment; however, there wasn't a significant difference between the 2 grouıps in chick performance during the overall period of the experiment.

The hematological indices (Hb, PCV, and TEC) used in the present study were not affected by supplementation with ATN, which is in agreement with the results of Keçeci et al. (12). Supplementation with 5 g/kg of ATN significantly increased serum AMY and LDH activity. In contrast to our results, Kubena et al. (9,28) reported that supplemental hydrated sodium calcium aluminosilicates did not affect serum LDH activity. High serum LDH activity could be an indication of tissue damage (especially liver) if accompanied by an increase in the activity of other serum enzymes, especially aminotransferases. Activity of all the other measured enzymes was within the reference range in the ATN group and was similar to the control group; however, LDH activity in the serum of the chickens in the ATN group, although higher than in the control group, was within the reference range for chickens and did not indicate pathological change.

GST is a key enzyme in the biotransformation and detoxification of various toxicants, reactive oxygen species, and endo- and xenobiotics (29,30). Studies on the expression and activity of antioxidative enzymes in animals suggest that regulation of pro-oxidant/antioxidant equilibrium affects numerous physiological processes. Some studies have suggested that the detoxification pathways of some toxicants may be connected to antioxidative defense mechanisms (31,32). Our results show that GST activity in the liver was not affected by dietary treatment and that the animals were not exposed to xenobiotics, or pro-oxidants.

It is reported that supplemental clays and zeolites did not significantly affect the relative weights of the liver, pancreas, spleen, proventriculus, or bursa of Fabricius during a 3-week feeding period (10,11,13,24). Differences to our results are possibly due to the longer duration of treatment in our study. Yet, Kubena et al. (9) reported slightly higher relative weights of the spleen and proventriculus in a 3-week trial, though not significantly higher. The higher relative weights of the proventriculus, ventriculus, spleen, and ileum observed in chickens fed supplemental ATN may be associated with slower passage of ingesta through the digestive tract.

At present there is limited information about the quality of meat of animals fed hydrated aluminosilicates. ATN seems to positively and significantly affect the chemical composition of breast meat only, lowering its fat content and increasing its protein content. Fat and protein content of drumstick meat was not affected by dietary treatment. Ash content was higher in the meat of broilers fed ATN, but still within the reference range for chickens.

In conclusion, it could be said that dietary supplementation with 5 g/kg of ATN affected some serum parameters, organ weights, and breast meat chemical composition of broilers.

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