Research Note

Effects of Humic Acid on Broiler Chickens[1]

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ABSTRACT In view of the alleged effect of humic acid (HA) on growth plate arthrosis in humans, we sought to find if poultry tibial dyschondroplasia (TD) is caused by HA that can occur as a ground water contaminant. In 2 separate trials, broiler chickens were fed different concentrations of HA added to their diet for 4 and 5 wk. The effects of these treatments were measured by general health indices such as growth, feed conversion ratio, relative organ weights, blood differential count, serum chemistry, TD index, and bone biomechanical strength. Humic acid treatment decreased BW but appeared to improve feed conversion ratio. There was no effect on TD index or bone biomechanical strength in HA-treated birds compared with controls. There was no toxic effect of HA that was evident by the absence of any dramatic change in relative organ weights or other telltale signs of serum clinical chemistry that would suggest liver, muscle, or kidney dysfunction. Red blood cell, white blood cell, monocyte, and hematocrit values were not affected, but there was a decrease in blood heterophil counts and heterophil to lymphocyte ratio, which was significant in 4-wk HA-treated birds. Overall these results show that HA slows down growth, but it does not have any adverse health effects on chickens.

Key words: humic acid, chicken, serum chemistry, organ weight, differential count

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INTRODUCTION

Humic acids (**HA**) are naturally occurring decomposed organic constituents of soil and lignite that are complex mixtures of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains (Klocking, 1994; MacCarthy, 2001). Humic acid has been used as an antidiarrheal, analgesic, immunostimulatory, and antimicrobial agent in veterinary practices in Europe (EMEA, 1999). Certain modified forms of humic acid have been shown to possess antiviral activities against herpes simplex and human immunodeficiency viruses (Schneider et al., 1996; Klocking et al., 2002; van Rensburg et al., 2002). Although many experimental studies have shown HA to be largely nontoxic and nonteratogenic (EMEA, 1999; Yasar et al., 2002), it has been implicated as the cause of Kashin-Beck disease that is characterized by arthrosis of joints and growth plate in children and adolescents in certain endemic areas of East Asia (Zhai et al., 1990; Liang et al., 1999). The arthrosis in Kashin-Beck disease results from the death and necrosis of cartilage cells that apparently relates to water pollution associated with high concentrations of humic compounds in drinking water. It has also been cited as a reason for vascular problems associated with black foot disease (Hseu et al., 2002).

In young meat-type poultry, tibial dyschondroplasia (**TD**) is a common cartilage problem in which the growth plate cartilage shows abnormal chondrocyte death and capillary degeneration that lead to the failure of endochondral ossification and the retention of an unresorbed plug of cartilage that causes leg problems (Leach and Lilburn, 1992; Orth and Cook, 1994; Rath et al., 1998, 2004, 2005; Praul et al., 2000). Infrequently, this problem becomes endemic to certain farms with no apparent etiology. Because water in many poultry farms is derived from natural wells, there is a likelihood of occurrence of humic components of the soil, which could lead to cartilage problems such as TD. Therefore, the objective of this study was to determine if experimental supplementation of HA in the young broiler chicken diet may cause growth plate dysplasia and affect growth and associated health parameters.

MATERIALS AND METHODS

Humic acid was purchased from ICN Biochemical (ICN Biomedical Inc., Aurora, OH). Two trials were conducted several months apart using 1-d-old male broiler chicks (Cobb-Vantress, Silom Springs, AR) raised in floor pens under constant light period of 23 h. Corn-soybean-based broiler starter diet was prepared according to NRC speci-

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Table 1. Effect of humic acid (HA) feeding on BW, feed conversion ratio (FCR), and tibial dyschondroplasia (TD) indices of 4- and 5-wk-old chickens[1]

	4 wk (Trial 1) (n = 35)			5 wk (Trial 2) (n = 25)		
Treatment	BW (kg) ± SEM	Gain:feed	TD index ± SEM	BW (kg) ± SEM	Gain:feed	TD index ± SEM
Control	1.21 ± 0.02[a]	0.66	0.23 ± 0.10	1.63 ± 0.03[a]	0.62	0.18 ± 0.12
0.5% HA	1.11 ± 0.01[b]	0.72	0.21 ± 0.09	ND[2]	ND	ND
1.0% HA	1.09 ± 0.03[b]	0.72	0.21 ± 0.10	1.51 ± 0.02[b]	0.66	0.08 ± 0.08
2.5% HA	ND	ND	ND	1.45 ± 0.04[b]	0.71	0.04 ± 0.04

[a,b]Dissimilar scripts in column denote significant differences, $P \leq 0.05$.

[1]Gain:feed = total BW gain/total feed consumed. TD index = incidence × severity score.

[2]ND = not done.

Table 2. Effect of humic acid (HA) on bone biomechanical strength, density, and ash concentration of 5-wk-old chickens (n = 13), mean ± SEM[1]

Treatment	Diameter (mm)	Load at yield (mm)	Stress at yield (kg force/mm^2)	Strain at yield (mm/mm)	Young's modulus (kg/mm^2)	Bone density (g/cc)	Ash (%)
Control	6.38 ± 0.10	31.48 ± 1.60	9.55 ± 0.64	0.07 ± 00	246.28 ± 17.48	1.21 ± 0.05	66.69 ± 0.48
1.0% HA	6.14 ± 0.10	29.71 ± 1.17	10.00 ± 0.49	0.06 ± 00	265.35 ± 19.13	1.22 ± 0.04	66.43 ± 0.46
2.5% HA	6.36 ± 0.11	30.91 ± 1.59	9.37 ± 0.54	0.07 ± 00	242.37 ± 19.0	1.20 ± 0.04	66.44 ± 0.39

[1]There were no differences in biomechanical parameters between different treatment groups.

fication (National Research Council, 1994). The HA was added to the diets and mixed thoroughly in a graded sequence to specified concentrations (Table 1). The birds were given diets with or without supplemental HA and water ad libitum from d 1 through 28 (4 wk, trial 1) and d 1 through 35 (5 wk, trial 2). Trial 1 consisted of 35 birds, and trial 2 had 25 birds per group. Individual BW of the birds were recorded at the onset of experiments and measured weekly thereafter. Weekly mortality and feed consumption were recorded for each group. Prior to euthanasia, the birds were weighed and the blood from 13 birds in each group was collected by cardiac puncture in Vacutainer tubes (BD Bioscience, Franklin Lakes, NJ) containing potassium-EDTA for differential count or clot accelerator for serum clinical chemistry (Rath et al., 2004). The differential count was done using a Cell-Dyn blood counter (Abbott Laboratories, Chicago, IL) and clinical chemistry with a Corning clinical chemistry analyzer (Chiron Corporation, San Jose, CA). All chickens were killed by cervical dislocation to harvest organs and score for the incidence and severity of TD. The TD index was calculated by multiplying incidence with severity score, which was measured as 0 = normal, 1 = moderate, and 2 = severe (Rath et al., 2004). Left and right tibia from 13 birds in each group were also collected for the determination of biomechanical strength, bone density, and bone ash content as described earlier (Rath et al., 1999, 2000). The biomechanical strength (the load and stress at failure, the strain, and the modulus of elasticity) of right tibia from individual birds was determined by a 3-point flexural bending method using an Instron 4502 material testing machine (Instron Corp., Canton, MA). The mid-diaphyseal diameter of the bones at the site of impact was measured using a dial caliper. Bone density and bone ash percentage were measured from 1 cm midsection of bone marrow free left tibia using Mettler kit ME 33340 (Mettler-Toledo Inc., Hightstown, NJ), then drying and ashing the broken bones at 750°C for 22 h in porcelain crucibles (Rath et al., 1999). The percentage of bone ash was calculated relative to weight of bones measured after drying at 110°C for 10 h. The relative weights of selective organs were expressed as percentage of BW. Feed conversion ratio was calculated by dividing the sum of the BW of all birds in a group minus the sum of starting BW with the weight of total feed consumed during the entire trial. The crop and duodenum pH were measured soon after killing using an IQ pH meter (IQ Scientific Inc., San Diego, CA).

Statistics

Data were analyzed by GLM procedure using Duncan's multiple range test with SAS statistical software (SAS

Table 3. Effect of humic acid (HA) on relative organ weights, crop, and duodenal pH ± SEM (n = 25)

Treatment	% Heart	% Liver	% Spleen	% Bursa of Fabricius	% Tibia	Crop pH	Duodenum pH
Control	0.49 ± 0.02	2.84 ± 0.05[ab]	0.15 ± 0.01	0.21 ± 0.01[b]	0.68 ± 0.02	4.38 ± 0.05[b]	5.88 ± 0.03
1.0% HA	0.48 ± 0.01	2.95 ± 0.07[a]	0.18 ± 0.01	0.22 ± 0.01[ab]	0.67 ± 0.03	4.45 ± 0.00[b]	5.86 ± 0.03
2.5% HA	0.48 ± 0.02	2.70 ± 0.05[b]	0.16 ± 0.02	0.25 ± 0.02[a]	0.68 ± 0.01	4.66 ± 0.06[a]	5.92 ± 0.03

[a,b]Dissimilar scripts in a column denote significant differences, $P \leq 0.05$.

Table 4. Effect of humic acid (HA) on serum chemistry of 5-wk-old chickens (n = 12), mean ± SEM

Treatment	Protein (g/dL)	Albumin (g/dL)	Glucose (mg/dL)	Creatine kinase (U/L)	BUN (mg/dL)	Alkaline phosphatase (U/L)	Alanine aminotransferase (U/L)	P (mg/dL)	Ca (mg/dL)	Fe (μg/dL)
Control	3.2 ± 0.1[a]	1.4 ± 0.05[ab]	207.3 ± 5.3[a]	469.6 ± 47.1[a]	1.2 ± 0.1[a]	756.9 ± 104.9[a]	8.9 ± 0.9[a]	6.7 ± 0.3[a]	11.3 ± 0.2[a]	83.0 ± 5.9[a]
1.0% HA	3.2 ± 0.1[a]	1.4 ± 0.03[a]	186.7 ± 6.5[b]	364.1 ± 51.4[b]	0.8 ± 0.1[b]	510.5 ± 85.9[ab]	7.9 ± 1.2[a]	6.2 ± 0.2[ab]	10.9 ± 0.2[a]	80.7 ± 3.9[a]
2.5% HA	2.9 ± 0.1[b]	1.3 ± 0.03[b]	183.7 ± 4.3[b]	280.2 ± 29.0[b]	0.6 ± 0.1[b]	436.0 ± 83.6[b]	4.0 ± 0.6[b]	5.7 ± 0.2[b]	10.3 ± 0.1[b]	68.0 ± 4.7[b]

[a,b]Dissimilar scripts in a column denote significant differences, $P \leq 0.05$.

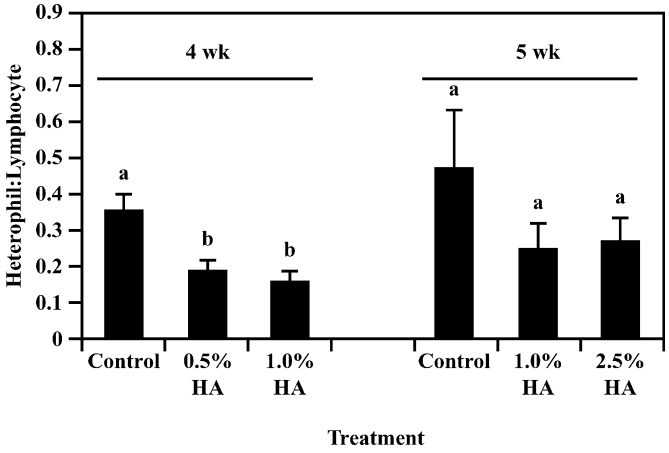

Figure 1. Effect on blood heterophil:lymphocyte of 4- and 5-wk-old control and humic acid (HA)-treated chickens. a,b = significant differences ($P \leq 0.05$) within a trial group.

Institute, 1994). Relative organ weight data were subjected to arc sine transformation, which showed a similar statistical trend. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

In both trials the mortality was negligible with no difference between control and HA-treated groups. However, the HA-treated chickens showed a reduction in BW, and the feed conversion ratio was numerically higher (Table 1). Kocabagli et al. (2002) reported an improvement in feed conversion in birds that were given 0.25% HA either from 0 to 42 d or during grow-out periods only, between d 21 to 42, although the birds did not show improvement in BW gain. A similar conclusion was drawn by Yoruk et al. (2004), who showed a better feed conversion in hens supplemented with 0.1-0.2% HA, and it did not affect BW. The rationale for using a high concentration of HA in our studies compared with other studies in the literature was to find if HA contributed to the development of TD. The results from both trials show that HA had neither an effect on TD index nor did it cause any impairment in growth plate development or bone problems including biomechanical properties (Table 2). In view of the alleged role of HA in Kashin-Beck syndrome in humans (Zhai et al., 1990), it is possible that A) either TD in poultry is not the same as human dystrophic arthrosis or B) that there are other factors that together with HA are responsible for the human disease that was not present in our experiment. Although in our studies HA caused a reduction in the BW of birds, the overall health of the birds appeared to be excellent, which was shown by the relative weights of major organs remaining stable (Table 3). However, the relative weights of the bursa of Fabricius increased in birds given 2.5% HA suggesting a possible immunostimulatory effect that has been suggested to be an effect of HA (EMEA, 1999).

Serum chemistry and blood differential counts after 5 wk of treatment are shown in Tables 4 and 5 and Figure

Table 5. Effect of humic acid (HA) on blood differential counts of 5-wk-old chickens (n = 12), mean ± SEM[1]

Treatment	White blood cell ($10^3/\mu L$)	Heterophil (%)	Lymphocyte (%)	Monocyte (%)	Red blood cell ($10^6/\mu L$)	HCT (%)
Control	35.53 ± 4.52	24.11 ± 4.00	66.88 ± 5.53	8.11 ± 1.62	2.54 ± 0.07	34.07 ± 0.78
1.0% HA	45.26 ± 5.38	16.65 ± 2.69	74.67 ± 3.00	7.52 ± 0.76	2.47 ± 0.04	32.86 ± 0.51
2.5% HA	37.88 ± 3.84	17.82 ± 2.63	74.27 ± 3.38	7.10 ± 0.86	2.51 ± 0.05	32.82 ± 0.64

[1]There were no differences in blood parameters among different treatment groups.

1. The HA affected serum chemistry values at high concentration. Except for cholesterol, triglyceride, creatinine, and lactate dehydrogenase, there was a trend for decrease in protein, albumin, glucose, creatine kinase, blood urea nitrogen, alkaline phosphatase, alanine aminotransferase, Ca, Fe, and P concentrations. Although the decreased values were statistically different than controls, they did not reflect any trend that would suggest any toxic effect of HA on muscle, kidney, heart, or liver. The blood chemistry results were concordant with relative organ weight results, which showed no dystrophic enlargement or atrophy as it could happen under maladaptive conditions. At 2.5% level of HA, the reduction in the serum concentrations of Ca, Mg, Fe, and P may be due to a metal chelating effects of HA, which is affected by large number of carboxylic acid side chains (Klocking, 1994). The HA increased crop pH but had no effect on the duodenal pH (Table 3). Hinton et al. (2000) showed a positive correlation between the adhesion of pathogenic bacteria, *Salmonella* and *Enterobacteriaceae,* to crop epithelium and an increase in crop pH. However, it is not known whether the reverse effect, increasing crop pH, would favor the colonization of the crop by those bacteria. If an increase in pathogen colonization had occurred, that was not reflected by birds being sick. On the contrary, the antimicrobial effects of HA have been described in the literature (Klocking, 1994; EMEA, 1999). In both trials HA did not have any effect on white blood cell, red blood cell, monocyte, and lymphocyte counts, or hematocrit values (Table 5), but at 4 wk trial HA reduced the blood heterophil counts, causing a decrease in heterophil:lymphocyte (Figure 1). A similar numerical, but not statistically significant, trend was seen in the second trial (Figure 1). The differential effect of HA on blood neutrophils is not understood, but in vitro studies show the ability of HA to activate blood neutrophils and increase their adhesibility (Riede et al., 1991; Chen et al., 2002). It is possible that HA may cause nonspecific margination of neutrophils leading to their decrease in the blood. An elevated heterophil to lymphocyte ratio is considered an indicator of stress (Gross and Siegel, 1983); therefore, the results do not reflect that the chickens experienced stress, which was also evident from their overall health and general activity that appeared no different than control birds. Whether HA is beneficial to overall immunity of birds is not known, but several studies have shown HA having immunostimulatory, antiinflammatory, and antiviral effects (Klocking, 1994; EMEA, 1999; Klocking et al., 2002; Schepetkin et al., 2003; Joone et al., 2003; Joone and van Rensburg, 2004).

In conclusion, our studies show that HA produces neither skeletal aberrations nor toxicity in chickens. However, at high concentrations it decreases BW without causing adverse health effects, a property that may be useful in controlling certain production problems related to excess BW gain in breeder males.

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