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Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 3 (5): 344-350, 2008
ISSN 1683-9919

Effects of Dietary Humic Acid and *Saccharomyces cerevisiae* on Performance and Biochemical Parameters of Broiler Chickens

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Abstract: One hundred and fifty broilers (1-day-old) were randomly allocated to 4 treatments, each of which had individnal pens of 50 chicks and were used to investigate the effects of humic acid (HA) *Saccharomyces cerevisiae* (SC extract (YE), 2×10^8 cfu g^{-1}) on growth performance and immunity. All animals received the same basal diet based on corn and soybean meal and while HA was added to the basal diet at 0.25% and YE 0.25%, respectively. The following diet treatments were applied: (1) Basal diet + 0 additives, (2) Basal diet 0.25% HA kg of feed (3) Basal diet 0.25% YE kg of feed (4) Basal diet 0.25% HA+0.25% YE kg of feed. Each experimental group was fed *ad libitum* with its own diet for 42 days. During the experiment the chicks were reared at conventional ambient temperature (from 30°C reducing to 21°C by 3°C/week) and relative humidity of 60-70%. Light was provided 24 h in a day. Performance data of each replicate was determined weekly during the experiment. All chicks were slaughtered when end of 6 weeks. Blood samples from 30 birds in each group was collected by branchial vein and were analyzed for serum biochemical values, enzyme activities and performance characteristics were measured. Body weight, body weight gain, feed intake and carcass weight were positively influenced ($p<0.05$) by HA supplementation (0.25%) during the experiment. Difference among the groups in terms of gizzard, liver and biochemical parameters such as WBC, Heterophil, Lymphocyte, Monocyte, RBC, HCT were not statistically important among different treatment groups ($p<0.05$). But, Glucose and BUN levels were significantly decreased in groups HA and YE ($p<0.05$). ALP, ALT, Fe, Ca, P were not statistically significant among groups.

Key words: Humic acid, *S. cerevisiae*, performance, biochemical parameters, broiler

INTRODUCTION

In poultry, novel methods to improve efficiency of meat production must be developed because of antibiotic use decreases. Hence, any improvements which will serve as a replacement for antibiotics in animal feed, must enhance growth, improve feed efficiency, or decrease mortality at no additional cost to the consumer. Several ways have been suggested as strategies to limit the antibiotic usage. Addition of prebiotics, probiotics and some organic acids (Chaveerach *et al*., 2004). Nowadays in poultry production organic acids and live yeasts have mainly been using in order to improve animal performance (Çelik *et al*., 2003; Deuli *et al*., 2003; Ricke, 2003; Madrigal *et al*., 1993). Besides organic acids are metabolic intermediates produced in pathways of central energy production, detoxification, neurotransmitter breakdown, or intestinal microbial activity as well. Humic Acid (HA) have been used for feed preservation, protecting feed from microbial and fungal destruction or to increase the preservation effect of fermented feed (Maslinski *et al*., 1993). HA are naturally occurring decomposed

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organic constituents of soil and lignite that are complex mixtures of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains (Klocking, 1994; Mac Carthy, 2001). The objective of dietary acidification is the inhibition of intestinal bacteria competing with the host for available nutrients and a reduction of possibly toxic bacterial metabolites, e.g., ammonia and amines, thus improving weight gain of the host animal. Furthermore, the growth inhibition of potential pathogen bacteria and zoonotic bacteria, e.g., *E. coli* and Salmonella, in the feed and in the GI-tract are of benefit with respect to animal health. Humic acid has been used as an antidiarrheal, analgesic, immunostimulatory and antimicrobial agent in veterinary practices in Europe (EMEA, 1999). Similarly yeast and yeast cultures have a long standing tradition of use in animal feeds. As well known, before the widespread availability and use of synthetic vitamins, yeast was added to pet food formulations as a natural of B vitamins. Yeast culture has been shown to affect intestinal mucosa development in poultry. More recently, yeast cultures often contain supplemental enzymes which may assist in protein, carbohydrate and fat digestion. have been examined. In addition to growth performance, there are many trials showing that enrichment of diets with yeast could favorably improve the quality of edible meat from broilers. For example, edible meats from broiler chicks fed a diet containing chromium-enriched Sc exhibited increased tenderness and increased water holding capacity (Bonomi and Vassia, 1978). Sc, one of the most widely commercialized types of yeast, has long been fed to animals. Any attribute which can improve gastrointestiual tract health and immunity is extremely valuable in broiler diets. Yeast is also an excellent source of selenium and chromium, two trace minerals which may have positive effects of broiler health (Çelik *et al*., 2001) The objective of this study was to determine if experimental supplementation of HA, SC extract (YE) and mixture of this additives in the young broiler chicken diet may cause improve of performance and affect some serum health parameters.

MATERIALS AND METHODS

Birds and Diets

This study was conducted at Çanakkale Onsekiz Mart University and Animal Science Department in winter of 2007. One hundred and fifty 1-day-old Ross male broiler chickens obtained from a commercial hatchery were individually weighed, wing-banded and randomly distributed to the different treatment groups (3 replicates of 50 chicks per dietary treatment). Humic acid was purchased from Topkim A.Ş and was added to the diets and mixed thoroughly in a graded sequence to specified concentrations in the total period of experiment in the chicks were grouped based on the following dietary treatments: 1) basal feed free additives (Control, 2) basal feed containing 0.25% HA, 3) basal feed containing 0.25% YE and 4) basal feed containing 0.25% YE+0.25% HA. The chickens were reared under uniform management conditions with feed and water available *ad libitum*. Diets were formulated for starter (0-3 week) and grower (4-6 week) phases according to NRC specification (Natioual Research Council, 1994) dnring the experiment (Table 1). Chicks raised in floor pens under constant light period of 24 h and individual BW, FI, FCR, BWG mortality and morbidity of the birds were recorded at the onset of experiments and measured weekly thereafter for each group. 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 experiment.

Collection, Processing and Analysis of Blood Samples

At the end of study (15 males and 15 females) in each treatment were selected randomly and humanely euthanized. Blood was collected in nonheparinized tubes by brachial vein puncture. Serum was separated and stored at -25°C and samples were analyzed for Total Protein (TP), Blood Urea Nitrogen (BUN), Alanine Amino Transferase (ALT), γ-Glutamyl Transferase (GGT) and Aspartate

Table 1: Ingredients and nutrient composition of basal diets

	Period	
Ingredients (%)	0-3 week	4-6 week
Corn	56.50	60.300
Soybean meal	32.80	29.100
Sunflower oil	3.00	4.000
Fish meal	4.00	3.000
NaCl	0.30	0.300
Calcium phosphate	1.50	1.500
Limestone	1.20	1.200
DL-Methionine	0.20	0.100
Vitamin-mineral premix[1]	0.50	0.500
Nutrient level		
ME[2] (MJ kg^{-1})	12.47	12.570
CP (%)	21.40	19.230
Lysine (%)	1.14	0.980
Methionine (%)	0.52	0.390
Cystine (%)	0.35	0.330
Calcium (%)	1.01	0.880
Available phosphorus (%)	0.48	0.340
β-mannan	0.682	0.600

[1]Supplied kg of diet: vitamin A, 1,500 IU; cholecalciferol, 200 IU; riboflavin, 3.5 mg; vitamin E, 10 IU; pantothenic acid, 10 mg; cobalamin, 10, niacin, biotin, 0.15 mg; 30 mg; choline chloride, 1,000 mg; folic acid, 0.5 mg; thiamine 1.5 mg; pyridoxine 3.0 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, selenium, 0.15 mg 0.18 mg; copper, 8 mg; [2]Based on National Research Council (1994) feed composition tables

Amino Transferase (AST) using automatic analyzer according to the recommendation of the manufacturer with a Technicon RA-1000 system (Miles Inc. Diagnostics Division, Tarrytown, NY) according to standard procedures, as described by Technicon-RA Systems (1994). The differential count was done using a Cell-Dyn blood counter and clinical chemistry with a Corning clinical chemistry analyzer (Chiron Corporation, San Jose, CA). Total erythrocyte count (TEC), hemoglobin (Hb), Ca, P and hematocrit were measured using auto analyzer. Liver, kidney, gizzard, intestines were collected, weighed and calculated as a percentage of body weight.

Statistical Analysis

The data were analyzed using the General Linear Models procedure of SAS Institute (1997). Significant differences between treatment means were separated using the Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

HA (0.25%) supplementation to the diet at improved ($p<0.05$) BW, BWG, FI and CW of broilers at 42 days compared with control, YE and HA+ YE groups (Table 2). The current study supports the observations of (Yoruk *et al*., 2004; Kocabaglı *et al*., 2002; Avcı *et al*., 2007). It is well established that the gastrointestinal microflora (normal) plays an important role in the health and well being of poultry. Various pathogenic microbes, such as *E. coli*, have been implicated to reduce the growth of poultry. Possible mechanisms for this reduction of growth are: toxin production, utilization of nutrients essential to the host and suppression of microbes that synthesize vitamins or other host growth factors. Mortality for chickens fed the control diet was not different from that for birds fed HA and YE diets. The mortality was also not different statistically among chickens fed HA and YE diets. Deaths occurred toward the middle of the experiment (Table 2). Shermer *et al*. (2002), similarly, has been observed that humates the inclusion of HA during later stage in broiler diet could included in feed and water of poultry promotes growth more beneficial in respect of performance. Besides the use of

Table 2: Effects of dietary humic acid and *Saccharomyces cerevisiae* on broiler chickens

Attributes	Age (day)	Control	HA (0.25%)	SC (0.25%)	HA+SC (0.25+0.25%)	SEM SEM	p-value
Body weight (g)	0	39.18	39.22	39.45	39.23	0.52	0.125
	21	660.52^{a}	666.32^{a}	661.37^{a}	613.84^{b}	17.21	0.034
	42	2186.30^{a}	2199.60^{b}	2190.50^{a}	2170.20^{b}	15.24	0.026
Body weight (g)	0-21	621.34^{a}	632.10^{c}	620.92^{a}	604.73^{c}	16.54	0.039
	0-42	2147.10^{a}	2164.40^{b}	2152.10^{a}	2129.50^{a}	14.37	0.015
Feed intake (g)	0-21	950.30^{a}	966.60^{b}	955.40^{a}	915.70^{c}	13.25	0.021
	0-42	4012.20^{a}	4065.40^{b}	4041.20^{b}	3946.20^{c}	22.34	0.036
FCR ($g\ g^{-1}$)	0–21	1.43^{a}	1.45^{a}	1.44^{a}	1.49^{b}	0.02	0.015
	0-42	1.83^{a}	1.84^{a}	1.84^{a}	1.81^{b}	0.02	0.027
Carcass (kg)	42	1743.30^{a}	1760.30^{b}	1749.30^{a}	1721.30^{c}		
Gizzard (g)	42	41.10^{a}	42.60^{a}	42.40^{a}	41.90^{a}		
Liver (g)	42	51.00^{a}	49.50^{a}	52.00^{a}	50.90^{a}		
Survivability (%)	0-42	98.00	99.00	99.00	98.00		

Means within row with no common superscripts differ significantly($p<0.05$)

HA in animal feed, leads to an increase in live weight of the excludes of course the possibility of antibiotic residue or animal without increasing the amount of feed given to microbial resistance the animal. The diet digestibility as a result of maintaining optimum pH in the gut increases, resulting in lower levels of nitrogen excretion and less odour in lower levels of nitrogen excretion and less odour as calcium and trace element utilization. On the other hand, *Saccharomyces cerevisiae*, one of the most widely commercialized types of yeast, has long been fed to animals. Results of earlier studies with yeast fed to chickens, however, have not been consistent. It has been reported by Bonomi and Vassia (1978), Eren *et al*. (2000) and Onifade *et al*. (1999) that feeding yeast to chicks improves BW gain and feed/gain ratio. But Zhang *et al*. (2005) failed to observe a positive effect of feeding yeast on BW of broiler chicks. Kanat and Calyalar (1996) reported that active dry yeast effectively increases BWG without affecting feed/gain ratio in broiler chicks in contrast. During starter period, BW, BWG, FI and CW were not significantly ($p<0.01$) affected by SE but mentioned parameters were positively influenced ($p<0.05$) by HA (0.25%) supplementation during the experiment. Because HA stabilize the intestinal flora and thus ensure an improved utilization of nutrients in animal feed. This leads to an increase in live weight of the animal without increasing the amount of feed given to the animal. Moreover, HA is effects to improve protein digestion as well as calcium and trace element utilization. Supplementation with yeast has also been shown to enhance survival by altering gastrointestinal flora (Netherwood *et al*., 1999) to suppress growth of pathogenic bacteria (Ehrmann *et al*., 2002) and by enhancing immune potency (Balevi *et al*., 2001).

In similar studies involving broilers, it was reported that supplementation of humate (Kocabaglı *et al*., 2002) and probiotic (Jin *et al*., 1998) did not alter feed conversion efficiency on 21 day, but improved it on 42 day. It appears that supplementation of humate and probiotic do not improve growth by affecting FI, suggesting that improvement in WG and reduction in FCR by supplemental humate and probiotic could be related to their promoting effects on metabolic processes of digestion and utilization of nutrients (Yeo and Kim, 1997; Belyavin, 1993). Findings from this research showed that measured factors are correlated, because as the, BW, BWG, FI and CW are improved in group HA ($p<0.05$). Islam *et al*. (2005) who reported that HA and YE supplementation to broiler grower diets caused numerical improvements in feed efficiency of broilers. This is consistent with the results from previous studies (Midilli and Tuncer, 2001) that addition of ouly YE to the diet was affected carcass compositions. Hyginus *et al*. (1997), Mohan *et al*. (1996) and Lee *et al*. (2002) suggesting that (SC) affects on the performance of laying and broiler hens compared with controls. The results of present and previous studies as Çelik *et al*. (2001), Deuli *et al*. (2003) and Yeo and Kim (1997) agree with this report showing that good growth performance in YE group. The results in this experiment were similarly. Biochemical parameters such as WBC, Heterophil, Lymphocyte,

Table 3: Effect of HA and SC on blood differential counts of 6-week-old broiler chickens (n = 15), mean ±SEM*

Treatment	WBC (10^3µL)	Heterophil (%)	Lymphocyte (%)	Monocyte (%)	RBC (10^6 µL)	HCT (%)
Control	35.45±4.58	24.220±4.00	66.88±4.82	8.24±1.67	2.56±0.09	34.27±0.68
25.0% HA	45.26±5.38	16.650±2.69	74.67±3.00	7.52±0.76	2.47±0.04	32.10±0.32
2.5% SC	36.88±2.24	17.844±2.33	74.22±3.23	7.12±0.82	2.53±0.04	32.12±0.42
HA+SC	43.18±5.22	17.150±2.33	74.44±3.12	7.00±0.46	2.48±0.04	32.12±0.50

*There were no differences in blood parameters among different treatment groups ($p<0.05$)

Table 4: Effect of Humic acid and *Saccharomyces cerevisiae* on serum chemistry of 6-week-old chickens (n = 13), mean±SEM

Treatment	Protein (g dL^{-1})	Albumin (g dL^{-1})	Glucose (mg dL^{-1})	BUN[1] (mg dL^{-1})	ALP[2] (U L^{-1})	ALT[3] (U L^{-1})	Fe (µg dL^{-1})	Ca (mg dL^{-1})	P (mg dL^{-1})
Control	3.2±0.1^a	1.4±0.04^a	201.8±5.3^a	1.0±0.1^a	740.5^a	8.5±13^a	81±5.2^a	10.4±5.1^a	6.3±0.1^a
HA (025%)	3.2±0.1^a	1.4±0.03^a	196.3±6.5^b	0.9±0.1^b	696.5^a	7.5±10^a	84±5.0^a	11.1±4.7^a	5.8±0.1^b
SC (0.25%)	3.1±0.1^b	1.3±0.03^b	186.7±4.3^b	0.9±0.1^b	737.5^a	8.0±11^a	80±5.0^a	10.9±4.8^a	6.4±0.2^a
HA + SC	3.1±0.1^b	1.2±0.03^b	194.4±4.7^b	1.0±0.1^b	735.5^a	7.0± 9^a	78±4.0^a	11.4±2.0^a	6.2±0.2^a

[1]BUN: Blood Urea Nitrogen, [2]ALP: Alkaline Phosphatase, [3]ALT: Alanine Amino Transferase; [ab]Dissimilar scripts in a column denote significant differences $p\leq 0.05$

Monocyte, RBC, HCT stay on normal levels under the influence of HA and YE in comparison with control groups and there were no differences in blood parameters among different treatment groups ($p<0.05$), (Table 3). ALP, ALT, Fe, Ca, while were not statistically significant among groups ($p<0.05$), but P was different in group HA (Table 4). 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. RBX, WBC, monocyte and HCT values were not affected, but there was a decrease in blood heterophil counts and heterophil to lymphocyte ratio, which was significant in 4 week HA-treated birds.

Overall these results show that HA improved growth more than YE in broiler chicks and it does not have any adverse health effects on chickens. In conclusion, supplementation of HA (25%) and YE (25%) may extend the profitability of a broiler chicks without adverse effects.

ACKNOWLEDGMENTS

This study was supported in part by BANVİT and Scientific Research Commission of COMU (BAP).

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