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Effect of Feeding Benzoic acid on Performance of Broiler Chickens

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ABSTRACT

The research was conducted to determine the influence of benzoic acid on growth performance, carcass traits, blood parameters and meat chemical composition of broiler birds. The research was carried out using 90 three weeks old broilers (Ross 308) divided into three groups, 30 per each. The levels of inclusion of the benzoic acid was based on treatment 1 (control) 0%, treatment 2 = 0.4% and treatment 3 = 0.8%. Results showed that, feeding benzoic acid to broilers had no significant on body weight, weight gain, feed intake and feed conversion at the two tested levels. Carcass traits did not show significant differences for the treatments, with the exception of bursa weight significantly increased. The serum total protein and globulin were significantly ($P<0.05$) increased in benzoic acid supplemented broilers. However, no significant differences were observed in serum albumin, triglyceride, cholesterol and uric acid between different experimental groups. No significant differences were observed for hematological parameters among all treated groups. There were no significant differences in chemical composition of broilers meat, including dry matter, protein and ash content. It could be concluded that, dietary inclusion of benzoic acid at both levels improved the immune response by increasing the weight of bursa of Fabricius and elevating blood globulin level but did not affect broiler chickens growth performance.

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Introduction

Organic acids have been used for decades in feed preservation, either for protecting feed from microbial and fungal destruction or to increase the preservation effect of fermented feed, e.g. silage. Organic acids are not antibiotics but, if used correctly along with nutritional, managerial and bio-security measures, they can be a powerful tool in maintaining the health of the gastrointestinal tract of poultry, resulting in improving their performances. Feeding of organic acids may suppress the growth of certain species of bacteria, particularly acid intolerant species such as E. coli, Salmonella sp. and *Campylobacter* sp. (Ricke, 2003; Dibner, 2004). Their principle rule is to lower and supplies the pH in the stomach and intestines so that the gut environment is too acidic for normal bacterial growth. Additionally, they improve protein digestion in young animal by stimulating pancreatic enzyme secretion (Mellor, 2000). Thus, dietary organic acids can suppress the growth of pathogenic bacteria, encourage the growth of beneficial microflora and ensure that, the enzymes function is at maximal capacity (Broek, 2000; Dibner and Winter, 2002; Ricke, 2003; Dibner, 2004).

Practically, organic acids work in poultry not only as a growth promoter but also as a meaningful tool of controlling all enteritis bacteria, both pathogenic and non-pathogenic (Naidu, 2000; Wolfenden *et al*., 2007). Moreover, feeding organic acids is believed to have several beneficial effects such as improving feed conversion ratio, growth performance, enhancing minerals absorption and speeding recovery from fatigue (Gornowicz and Dziadek, 2002) and also provided people with healthy and nutritious poultry products (Patten and Waldroup, 1988).

Benzoic acid plays an important role in lowering numbers of pathogenic bacteria like *Campylobacter jejuni*, which competes with the host animal for nutrient (Friedman *et al*., 2003). It contributes to some certain amount of energy to the host bird (Jamroz *et al*., 2003). Besides bacteriostatic feature, benzoic acid helps in reducing ammonia, thereby stimulates growth in broiler birds. It also helps to increase gastric proteolysis and improve digestibility of protein and amino acid in young broiler birds, thereby improving the feed efficiency and growth performance of broiler birds (Kirchgessner and Roth, 1988). Benzoic acid is an energy source of the epithelia cells

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of the large intestine (Roedigor, 1980) and terminal ileum (Chapman *et al*., 1995). It thereby improves the length of the ileal microvillus and depth of the caecal crypts on intestinal mucos,l which help in efficient feed absorption and assimilation in the broiler birds.

Materials and methods

Birds, housing and feeding

A total number of 90 three weeks old broiler birds (Ross 308) were randomly distributed into 3 equal groups, 30 per each. The birds had free access to water and feed. The climatic conditions and lighting program followed the commercial recommendations. The control diet was formulated to contain approximately crude protein (20%) and metabolizable energy (3200 kcal/kg diet) as recommended by NRC (1994). The first group was fed on control diet without any feed additives, while groups 2 and 3 and (T2 and T3) were fed on basal diets containing 0.4 and 0.8% benzoic acid, respectively (Table 1).

Table 1. Composition and nutrient content of grower (day 22 to 42) basal diets for broiler chicks (%, as fed-basis).

Item	Treatments		
	T1	T2	T3
Ingredients (%)			
Yellow corn	59.60	59.19	58.79
Soybean meal	32.00	32.00	32.00
Sunflower oil	5.10	5.10	5.10
Benzoic acid	---	0.40	0.80
Sodium phosphate dibasic	0.85	0.85	0.85
Limestone	1.84	1.84	1.84
Common salt	0.30	0.30	0.30
Methionine	0.01	0.02	0.02
Premix*	0.30	0.30	0.30
Calculated analysis			
ME (kcal/kg)	3203	3189	3176
Crude protein (%)	20.06	20.02	19.99
Lysine (%)	1.02	1.01	1.01
Methionine (%)	0.33	0.33	0.33
Calcium (%)	0.80	0.80	0.80
Phosphorus, available (%)	0.30	0.30	0.30
Crude fiber (%)	3.35	3.34	3.34

*Mineral and vitamin premix Heromix broilers (Heropharma Co., Egypt). Each 2.5 kg contain: Vit. A, 1200000 IU; Vit. D3, 300000 IU; Vit. E, 700 mg; Vit. k3, 500 mg; Vit. B1, 500 mg; Vit. B2, 200 mg; Vit. B6, 600 mg; Vit. B12, 3 mg; Vit. C, 450 mg; Niacin, 3000 mg; Methionine, 3000 mg; Pantothenic acid, 670 mg ; Folic acid 300 mg; Biotin, 6 mg; Choline chloride, 10000 mg; Magnesium sulphate, 3000 mg; Copper sulphate, 3000 mg; Iron sulphate, 10000 mg; Zinc sulphate, 1800 mg; Cobalt sulphate, 300 mg.

Performance

Performance characteristics including body weight, body weight gain, feed intake and feed conversion ratio were calculated. The proximate analysis of the experimental feeds was performed using procedures detailed by the Association of Official Analytical Chemistry (AOAC, 1990).

Carcass traits

At the end of experiment three birds per treatment were randomly selected and weighed live, slaughtered by neck cut and allowed to bleed. Afterward, the birds were scalded, de- feathered and carcasses were eviscerated. The gizzard, heart, liver, spleen, bursa and thymus were excised and weighed. Dressing percentage was obtained by expressing the dressed carcass weight (with giblet) as percentage of live body weight.

Serum biochemistry

At the end of the experiment, three randomly selected birds from each group were slaughtered after fasting overnight. Blood samples were collected from the selected birds of each treatment, allotted to clot at ambient temperature, centrifuged for 15 minutes at 3000 rpm and serum from each sample was extracted. The serum samples were kept at -20 0C until further analysis. Serum samples were assayed for estimation of total protein and its fractions (albumin and globulin), triglycerides, cholesterol and uric acid by spectrophotometer using commercial test kits (Spectrum, Cairo, Egypt).

Hematological parameters

Three birds from each group were randomly selected and blood samples were taken from brachial vein for blood analysis, including total RBCs, hemoglobin, total white blood cells, Packed cell volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration and percentage of lymphocytes by standard procedures as suggested by (Stoskopf *et al*., 1983a; Stoskopf *et al*., 1983b).

Meat chemical composition

Meat from breast and thigh of the slaughtered birds in all experimental groups were taken, prepared (carefully minced, dried and homogenized) and chemically analyzed for moisture, crude protein, ether extract and ash following AOAC (1990) official method.

Statistical analysis

All experimental data were subjected to statistical analysis with one way ANOVA of (SPSS for windows version 16: SPSS GmbH, Munich, Germany). Least square means were compared by Duncan's multiple range test. All statements of differences were based on significance of $P<0.05$.

Results

The effect of dietary supplementation of benzoic acid on growth performance parameters are summarized in Table 2. There was no significant difference in body weight and body weight gain between experimental groups during the entire period of the experiment. Feed intake of broilers in benzoic acid groups (T2 and T3) was higher than the control group by 27 and 45 gm, respectively. Feed conversion ratio was higher for birds supplemented with benzoic acid (2.22 and 2.24 for T2&T3, respectively) in comparison with control (2.13).

The obtained data in Table 3 revealed that, no significant differences in dressing percentage and the weights of internal organ (liver, gizzard, heart, spleen and thymus) between experimental groups. Birds fed on diet supplemented with benzoic acid at 0.4% recorded significantly (P<0.05) higher weight of bursa than the control one.

Data presented in Table 4 cleared that, birds fed on diet supplemented with benzoic acid at both levels exhibited a significant (P<0.05) increase in serum total protein, globulin and a significant (P<0.05) decrease in albumin/globulin ratio compared with the control one. There were no significant differences in serum albumin, triglyceride, cholesterol and uric acid between different experimental groups.

Effects of feeding benzoic acid on some hematological parameters of broilers (Table 5). The results revealed that there were no significant differences between different experimental groups in hemoglobin concentration, WBCs, RBCs count, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration and lymphocyte percentage.

The results in Table 6 cleared that there were no significant differences in chemical composition, including dry matter, protein and ash content of broilers meat among all treated groups. Birds fed diet supplemented by 0.4% benzoic acid had significantly (P<0.05) higher fat than other treatments.

Table 2. Growth performance of broiler chickens given benzoic acid.

Item	T1	T2	T3
Body weight (g)			
21 d	765±22.83ª	803±27.51ª	800±23.88ª
42 d	2032±63.99ª	2031±90.50ª	2025±94.90ª
Weight gain (g)			
21-42 d	1267±42.75ª	1228±64.53ª	1225±72.12ª
Feed intake (g/bird)			
21-42 d	2699	2726	2744
Feed conversion ratio			
21-42 d	2.13	2.22	2.24

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 3. Dressing percentage and absolute organ weights (g) of broiler chickens

Item	T1	T2	T3
Dressing %	70.07±1.03ª	75.87±4.61ª	76.88±3.00ª
Liver	42.11±2.90ª	53.46±7.76ª	57.92±0.48ª
Gizzard	36.89±2.77ª	41.22±7.03ª	38.64±3.73ª
Heart	9.34±0.94ª	9.73±0.88ª	11.03±0.67ª
Spleen	1.97±0.22ª	2.39±0.40ª	2.31±0.49ª
Thymus	5.38±0.52ª	5.72±0.43ª	6.68±0.75ª
Bursa	1.84±0.39ᵇ	3.35±0.16ª	2.91±0.38ᵇ

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 4. Effect of benzoic acid on some serum constituents in broiler chickens

Item	T1	T2	T3
Total protein g/dl	3.05±0.18ᵇ	5.49±0.36ª	5.08±0.12ª
Albumin g/dl	2.40±0.15ª	2.10±0.04ª	2.07±0.05ª
Globulin g/dl	0.65±0.08ᵇ	3.39±0.32ª	3.01±0.07ª
Alb/Glob ratio	3.80±0.54ª	0.63±0.05ᵇ	0.69±0.01ᵇ
Triglycerides mg/dl	61.54±7.85ª	67.98±6.30ª	54.12±10.12ª
Cholesterol mg/dl	107±4.62ª	95±3.25ª	101±5.67ª
Uric acid mg/dl	50.40±0.85ª	46.48±0.70ª	50.39±2.75ª

Means within the same row with different superscripts are significantly different (P < 0.05).

Table 5. Effect of benzoic acid on hematological parameters in broiler chickens

Item	Dietary Treatment		
	T1	T2	T3
Hemoglobin (g/dl)	7.20±0.06ª	6.87±0.59ª	6.17±0.29ª
WBCs (x10³/mm³)	6.40±0.87ª	9.63±1.21ª	11.13±2.79ª
RBCs (x10⁶/mm3)	4.25±0.03ª	3.73±0.24ª	3.80±0.38ª
PCV%	20.95±0.14ª	20.03±1.66ª	18.10±0.81ª
MCV (fl)	49.35±0.72ª	48.80±1.40ª	48.33±4.60ª
MCH (pg)	16.95±0.26ª	16.60±0.49ª	16.50±1.61ª
MCHC %	34.35±0.03ª	34.27±0.12ª	34.07±0.07ª
Lymphocyte%	82.50±1.44ª	77.33±5.90ª	66.67±8.82ª

Means within the same row with different superscripts are significantly different (P < 0.05).
WBC= White blood cells, RBC= Red blood cells, PCV= Packed cell volume
MCV= Mean corpuscular volume, MCH= Mean corpuscular hemoglobin/
MCHC= Mean corpuscular hemoglobin concentration

Table 6. Chemical composition (%) of broilers meat

Item	Dietary Treatment		
	T1	T2	T3
Dry matter	25.19±0.42ª	26.45±0.45ª	25.10±0.68ª
Crude protein	21.18±0.39ª	21.58±0.37ª	20.96±0.48ª
Ether extract	2.58±0.11ᵇ	3.46±0.11ª	2.80±0.17ᵇ
Ash	0.92±0.07ª	0.97±0.09ª	0.92±0.10ª

Means within the same row with different superscripts are significantly different (P < 0.05).

Discussion

In agreement with our findings, Bonos *et al.* (2011) observed that no effect on body weight of Japanese quail by addition of acidifiers to diets, whereas Amaechi and Anueyiagu (2012) reported that the addition of dietary benzoic acid up to 1.2 % improved body weight of broiler chickens compared with control group. Our results are in accordance with the findings of Talebi *et al.* (2010) who reported that addition of benzoic acid at 0.5% to broilers diet did not show any significant effect on body weight gain. Abdalla *et al.* (2013) found that dietary supplementation of benzoic acid at 0.1% did not affect body weight gain of broilers. In contrast, Jozefiak *et al.* (2010) reported that dietary inclusion of benzoic acid significantly (P<0.05) decreased body weight gain in broiler chickens compared with control group. Sohail *et al.* (2015) found that dietary inclusion of benzoic acid increased body weight gain of broilers significantly.

Concerning the effect of benzoic acid on feed intake. The present data agreed with that reported by Sohail *et al*. (2015) who reported that benzoic acid at level 0.5% in broilers diet increased feed intake. Bagal *et al*. (2016) found that feed intake of broilers did not differ significantly by dietary inclusion of acidifiers.

Our results disagreed with that reported by Abdel-Fattah *et al*. (2008); Chowdhury *et al*. (2009); Bagal *et al*. (2016) who reported improved feed conversion with supplementation of organic acids. Islam *et al*. (2008); Ghazalah *et al*. (2011); Abdalla *et al*. (2013) who reported that, dietary inclusion of organic acids had no significant effect on feed conversion ratio in broiler chickens.

The present data agreed with that reported by Adil *et al*. (2010); Talebi (2010); Sohail *et al*. (2015) who stated that, addition of organic acids had no significant effect on the carcass characteristics (dressing percentage, liver, heart, spleen and gizzard weights) of broiler chickens. However, Amaechi and Anueyiagu (2012) declared that, addition of benzoic acid at 1.2% to the broilers diet was associated with higher gizzard and heart weight ($P<0.05$). Abdel-Fattah *et al*. (2008) observed that supplemental organic acid significantly increased of both primary lymphoid organs (bursa and thymus).

Our results indicated that, supplemental benzoic acid may improve immune response. Globulin level has been use as indicator of immune responses and source of antibody production. This established enhancement of immune response associated with dietary acidification could be account for their inhibitory effects against the pathogenic microorganisms throughout the GI-tract. Griminger (1986) stated that high globulin and low A/G ratio signify better disease resistance and immune response. These results in harmony with Rahmani and Speer (2005) who found higher percentage of gamma globulin in broilers given organic acids than the control one. Adil *et al*. (2010) found no significant effect on serum cholesterol in broiler chicks fed on organic acids. Brzóska *et al*. (2013) reported that blood plasma parameters, including triglyceride and total cholesterol were unaffected significantly by feeding diets containing acidifiers. On the other hand, Abdo (2004) observed that, blood total lipids and cholesterol decreased significantly by dietary acidifiers. Abdel-Fattah *et al*. (2008) found that serum uric acid did not differ significantly by dietary inclusion of citric or lactic acid. Abdalla *et al*. (2013) recorded that a significant decrease in total protein and globulin in birds fed with 0.2% benzoic acid at 6 weeks of age.

The present findings are in conformity with that obtained by Ebru et al (2011) and Khajepour et al (2011). Moreover, Abdalla *et al*. (2013) noted that dietary inclusion of benzoic acid didn't affect the total leucocytic count and differential leucocytic count at days 21and 42 of broilers life.

Our findings agreed with that reported by Brzóska *et al*. (2013) who recorded that addition of acidifier to broilers diet did not show any significant effect on meat composition of broiler chickens.

Conclusion

It could be concluded that, feeding benzoic acid up to 0.8% inclusion level improved the immune response by increasing the weight of the bursa of Fabricius and elevating blood globulin level but had no significant effect on broiler chickens growth performance.

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