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THE EFFECT OF DIETARY BENZOIC ACID SUPPLEMENTATION ON GROWTH PERFORMANCE AND INTESTINAL WALL MORPHOLOGY OF BROILERS

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ABSTRACT: *The research was conducted to determine the influence of benzoic acid on growth performance and intestinal wall morphology of broiler birds. The research was carried out using 120 day-old broilers divided into five (5) groups, each having 24 broiler birds, and eight (8) birds per replicate. The levels of inclusion of the benzoic acid was based on control 0%, Treatment 1 = 0.6%, Treatment 2 = 1.2%, Treatment 3 = 1.8% and Treatment 4 = 2.4%. After six weeks, 2 animals from each replicate were killed. The carcasses weights, the pH of the digester and organ proportions were determined. Result showed that the body weight gain of birds in Treatment 1 and Treatment 2 were the highest (T_1=1.44kg, T_2=1.76kg), but T_2 had the best growth performance which was significantly different (P<0.05) in the final body weight of other birds in other treatments. The different segment of the gastro-intestinal tract had different pH concentration which differed significantly (P<0.05) between the control and the treatments. Benzoic acid supplementation improved (P< 0.05) duodenal and jejuna villous height. This study showed that feeding benzoic acid at 1.2% inclusion level in broiler feed improved weight gain and also suppressed some microbes, which compete with the host animal for nutrient, thereby improving the growth performance and gut health of broiler birds.*

ORIGINAL ARTICLE

Key words: *Organic Acid Supplementation, Performance, Intestinal Wall Morphology And Broiler Chickens.*

INTRODUCTION

During the last 50 years, the uses of antibiotics as growth promoters in farm animals have been questioned. Although it is clear that antibiotics merit for growth performance and health in poultry. But antibiotics have been prohibited due to the development of resistant strains of pathogenic microorganisms and possible transmission of these resistant strains from the poultry birds to human consumers (Neu, 1992). The adjustment following the withdrawal of the use of sub-therapeutic antibiotics in poultry production has been difficult and many replacement solutions have been proposed by the feed additive industry. This led to the discovery of organic acid as an important approach, that have the potential to improve performance in poultry production (Patterson and Burkhold, 2003; Ricke, 2003). Organic acids and their salts have been widely used as feed components for poultry and many animal species to inhibit some pathogenic bacteria in their gastro-intestinal tract (Charveorach et al., 2004).

Benzoic acid plays an important role in lowering numbers of pathogenic bacteria like *Campylobecter jujuni,* 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 pigs and broiler birds (Mroz et al., 2000; Buhler et al., 2006). 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 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 mucosal (Gaifa and Bokeri, 1990) which help in efficient feed absorption and assimilation in the broiler birds.

The effect of dietary benzoic acid supplementation as a good substitute of antibiotics growth promoters on growth performance and intestinal wall morphology was investigated.

MATERIALS AND METHODS

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A total of 120 day-old "Anak 2000" broiler chicks were used in the experiment. Chicks were housed in a warmed fumigated brooder house and fed on a starter diet for 4 weeks and finisher diet from 4 to 9 weeks. The chicks were individually weighed and allocated to 15 cages of 8 chicks, each, so that average initial body weight of birds of each cage did not vary significantly ($P>0.05$).

Birds were randomly allotted to five treatment groups, each with three replicates of eight birds. Control (c) birds were given a standard basal diet; Treatment 1 (T_1) was a diet with 0.6% of benzoic acid; Treatment 2 (T_2) a diet with 1.2% of benzoic acid; Treatment 3 (T_3) was a diet with 1.8% of benzoic acid and Treatment 4 (T_4) a diet with 2.4% of benzoic acid.

After thorough mixing of ingredients, the organic acid, which were in powder form were mixed at the stated concentrations. The starter and finisher diets were formulated to meet the nutrient requirements of the birds. Ingredient and chemical composition of the basal diets are presented in Table 1.

Table 1 - Ingredients and chemical composition of broiler starter and finisher diets

Ingredients	Starter phase (%)	Finisher phase (%)
Crude protein	21.00	18.00
Fats/oil	6.00	6.00
Crude fibre	5.00	5.00
Calcium	1.00	1.00
Phosphorus	0.45	0.40
Methionine	1.00	0.35
Salt	0.30	0.35
Metabolic energy	2800kcal/kg	2900kcal/kg

The experiment was designed using complete randomized design (CRD) and analyzed using analysis of variance (ANOVA) as described by Steel and Torrie (1980). The replicates were use as experimental units for studying the effect of benzoic acid on broiler growth performance and gut health. Levels of significance were calculated as per the standard method described by Duncan (1995) wherever any effect was found significant.

Housing and Management

Broiler chicks were housed in pens which were cleaned properly; the floor was covered with fresh saw dust. The day old chicks were weighed to determine their day old weight. The brooding temperature was maintained close to their requirement by heating device. The birds were vaccinated against new castle diseases and infections bursal disease on day 1, 14 and 21. The experiment lasted for 6 weeks. Mortality was recorded as it occurred. Body weight gain, feed conservation ratios and feed intake were obtained by calculation.

Measurement of pH in Different Parts of the Gut

To determine the pH, 10g of gut content from crop, proventriculus gizzard, duodenum, jejunum and ileum were collected aseptically in 90ml sterilized physiological saline (1:dilution) Al-Natour and Alshawabkeh, 2005) and pH was determined.

Morphological Evaluations

Intestinal samples from duodenum, jejunum and ileum were used to measure villus height and the depth of the crypts. Paraffin sections were made from formalin-fixed tissues samples and then were stained with loefflers' haematoxyline and eosin and mounted on Distrene plasticizer xylene. Heights of intestinal villous were measured by ocular micrometer under 10 objective of microscope. The reading was taken from ocular micrometer and the actual villous height and depth of the crypt were obtained by conversion factor derived from stage micrometer (Lillie, 1965). The measurements were expressed as micrometers (μm).

Bacterial Count in Intestinal Contents

Two birds from each replicate were sacrifice at 9 weeks of age. Intestines, including duodenum, jejunum and ileum were removed and legated at both sides. Then tissues chymes were placed in 50ml tubes sterile saline (0.9g sodium chloride in 100ml distilled water) and then kept at 4^0C until used for intestinal sampling. Serial dilutions of collected samples from different parts of intestinal contents were made up to the fifth dilution with sterile saline (0.9g sodium chloride in 100ml distilled water) and different bacterial loads of the gut contents were enumerated by the pour method (Quinn et al., 1992).

Table 2 showed that no mortality was observed in T_2 during the course of the experiment, but T_3 and T_4 had mortality rate of 5.00% and 10.53% respectively. This result is in agreement with Polonen et al, (2000) who reported that overload of benzoic acid in animal diet might be toxic to animals. The initial and final body weight followed the same pattern in Table 2 as well as average body weight gain of broiler. There was an increased body weight gain in T_1 and T_2 which is in agreement with Patterson and Bunkhold, (2003) which reported that inclusion of organic acid helps improve growth in broilers. The ability of benzoic acid to improve growth in broiler birds might be because of its role in lowering the number of pathogenic bacteria like *Campylobacter Jejuni* and *Eschericha coli* that compete with the host animal for nutrients (Friedman et al., 2003). There was no significant difference in the weight gain; with T_3 and T_4 having the lowest weight gain of 1.41kg and 1.30kg respectively, which was probably

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due to the toxicity of the level of benzoic acid in their feed. Treatment 2 had 1.76kg weight gain which was probably the highest weight gain when compared with T_1 and the control group. Although T_4 had the lowest feed conversion ratio of 1.45, this does not justify it to be the best result because T_4 had poor feed intake (2.56g). This is in contrast to Akinmutimi (2004), who stated that the lower, the feed conversion ratio the better the result.

Table 3 showed the pH concentration of digesta collected from different segment of the gastro intestinal tract of broiler birds. It was observed that the caecum had the highest pH across the treatments. This is in agreement with Thompson and Hinton (1997) who observed that most organic acid used in feed and drinking water are absorbed at the upper gastro intestinal segment of poultry birds i.e. crop, proventriculus and gizzard; and only little portion of the organic acids get to the lower digestive tract i.e. caeca (Hummel et al., 1993). The crop of the broiler birds contains microbes that ferment food materials ingested by the animal, thereby causing an increase in the pH of the crop. This was observed with the control group having the highest pH of 7.13, but the actions of benzoic acid on microbes in the crop resulted to lower pH in all the treatment groups (T_1-T_4). The gizzard and ileum recorded the lowest pH level among the segments of gastro-intestinal tract, which was statistically significant ($P < 0.05$) in the GIT of the birds.

The supplementation of feed with benzoic acid resulted in significant lower count of microbes throughout the gastro-intestinal tract. There was a significant difference ($P<0.05$) in the microbial load counts among the various levels of treatments. It was observed that the lowest microbial load was recoded in T_4 (12.01×10^6), while the control group had the highest population of microbes (41.01×10^7). The lowest bacterial load in T_4 was due to the level of inclusion of benzoic acid, but this did not reflect well in the performance of the broiler birds.

Table 2 - Effects of Benzoic acid on growth performance in broilers

Treatment group	Mortality rate	Initial body wt(kg)	Final body wt(kg)	Total wt gain (kg)	Average wt gain (kg)	Total feed intake (kg)	Feed conversion ratio
Control	$0.83{\pm}.00^{b}$	$0.40{\pm}.00^{a}$	$2.05{\pm}.00^{bc}$	$1.63{\pm}.02^{a}$	$1.22{\pm}.00$	$4.89{\pm}.00^{C}$	$2.38{\pm}.00^{b}$
T_1	$1.67{\pm}.00^{C}$	$0.47{\pm}.03^{ab}$	$2.12{\pm}.05^{c}$	$1.66{\pm}.07^{a}$	$1.31{\pm}.02$	$4.77{\pm}.03^{c}$	$2.24{\pm}.06^{c}$
T_2	$0.00{\pm}.00^{a}$	$0.55{\pm}.04^{c}$	$2.31{\pm}.05^{d}$	$1.76{\pm}.07^{a}$	$1.43{\pm}.03$	$4.76{\pm}.07$	$2.06{\pm}.06^{d}$
T_3	$5.00{\pm}.00^{d}$	$0.50{\pm}.01^{bc}$	$1.91{\pm}.05^{ab}$	$1.41{\pm}.05^{b}$	$1.59{\pm}.36$	$2.97{\pm}.08^{a}$	$1.55{\pm}.01^{a}$
T_4	$10.53{\pm}.00^{e}$	$0.44{\pm}.03^{ab}$	$1.76{\pm}.06^{b}$	$1.30{\pm}.05^{b}$	$1.11{\pm}.04$	$2.56{\pm}.05^{b}$	$1.45{\pm}.04^{a}$

a b, c, d, e. means with different superscript and within the same column are significantly different (P<0.05).

Table 3 - pH and Microbial Loads of Digester of Gastro-Intestinal Tract of Broilers

Treatment group	Crop	Gizzard	Duodenum	Ileum	Caecum	Microbial
Control	$7.13{\pm}.00^{c}$	$4.57{\pm}.00^{d}$	$5.37{\pm}.01^{a}$	$5.57{\pm}.01^{e}$	$7.16{\pm}.001^{e}$	$41.01 \times 107{\pm}.01^{e}$
T_1	$5.21{\pm}.01^{b}$	$4.28{\pm}.01^{a}$	$5.87{\pm}.01^{d}$	$4.93{\pm}.01^{b}$	$6.57{\pm}.001^{b}$	$36.01 \times 107{\pm}.01^{d}$
T_2	$5.11{\pm}.01^{a}$	$4.43{\pm}.01^{c}$	$5.76{\pm}.01^{c}$	$5.01{\pm}.01^{c}$	$6.72{\pm}.01^{c}$	$31.01 \times 107{\pm}.01^{c}$
T_3	$5.11{\pm}.01^{a}$	$4.56{\pm}.01^{d}$	$5.54{\pm}.01^{b}$	$5.45{\pm}.01^{d}$	$7.07{\pm}.01^{d}$	$28.01 \times 107{\pm}.01^{b}$
T_4	$5.21{\pm}.01^{b}$	$4.57{\pm}.00^{d}$	$5.16{\pm}.01^{e}$	$4.70{\pm}.00^{a}$	$6.11{\pm}.01^{a}$	$12.01 \times 106{\pm}.01^{a}$

a,b,c,d,e means with different superscript and within the same column are significantly different

Table 4 showed the effect of benzoic acid on the weight of various organs of broiler birds. The organs measured were crop, gizzard, heart, spleen and kidney. There was no significant difference ($P>0.05$) in the weight of spleen across the treatments. Treatment 4 had a decrease in the weight of organs, while Treatment 2 had an increase in the weight of the organs when compared with the control. Thus benzoic acid at various inclusion levels had an effect on the organ size of broiler birds. Therefore, 1.2% inclusion level of benzoic acid is an important approach to improve performance in poultry production (Ricke, 2003). When fed above this dosage it will result to reduction of growth and weight gain which might be due to its toxicity to animals.

Table 4 - Weight of Various Organs in Broiler Binds (Gms)

Treatment	Crops (gms)	Gizzard (gms)	Heart (gms)	Spleen (gms)	Kidney (gms)
Control	$43.11{\pm}0.01^{b}$	$84.13{\pm}2.03^{b}$	$8.88{\pm}0.87^{ab}$	$2.05{\pm}.00$	$47.75{\pm}1.41^{bc}$
T_1	$47.32{\pm}.01^{d}$	$82.48{\pm}0.93^{b}$	$11.81{\pm}1.00^{c}$	$2.65{\pm}0.48$	$51.94{\pm}1.47^{c}$
T_2	$56.11{\pm}.01^{e}$	$101.72{\pm}.41^{d}$	$15.70{\pm}0.72^{d}$	$4.58{\pm}1.57$	$71.15{\pm}1.00^{d}$
T_3	$45.01{\pm}.01^{c}$	$77.92{\pm}0.22^{b}$	$10.66{\pm}0.25^{bc}$	$2.15{\pm}1.03$	$45.59{\pm}1.42^{b}$
T_4	$35.13{\pm}.01^{a}$	$32.62{\pm}6.53^{a}$	$6.76{\pm}0.31^{a}$	$1.97{\pm}1.05$	$37.48{\pm}0.70^{a}$

a,b,c,d,e, means with different superscript and within the same column are significantly different (P<0.05)

Morphological evaluations showed that significant difference ($P< 0.05$) was found in duodenum and jejunum villous among treatments. The average length of villous in the duodenum, jejunum and ileum was 1396, 1165 and 721 μm, respectively. Earlier workers {Pelicano et al (2005); Loddi et al (2004)} also noted higher villous height in the duodenum and jejunum with most organic acidifiers added to broiler diets. The increase in villous height of the different segments of the small intestine may be attributable to the intestinal epithelium acting as a natural barrier against pathogenic bacteria toxic substances that are present in the intestinal lumen. Therefore, benzoic acid reduce the growth of many pathogenic or non- pathogenic intestinal bacteria, therefore, reduce intestinal colonization and reduce infectious processes, ultimately decrease inflammatory processes at the intestinal

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mucosa, which increase villous height and function of secretion, digestion and absorption of nutrients can be appropriately performed by the mucosa (Iji and Tivey 1998; Pelicano et al 2005; Loddi et al 2004).

CONCLUSION

Birds given benzoic acid supplementation in their diet act as a good substitute for antibiotic growth promoter and helped improve the growth performance and gut health of the broilers. It reduced pathogenic microbes that compete with the broiler for nutrient. The best result was achieved at 1.2% inclusion level of benzoic acid in the broiler diet.

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