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Effect of supplementation of broiler diets with benzoic acid as antibiotic replacer on the gut micro flora and haematological indices

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Abstract

An experiment was conducted to evaluate the effect of supplementation of broiler diets with benzoic acid on intestinal micro flora and haematological indices. One hundred and twenty (120) birds were used which was divided into five treatments of three replicates with eight birds per replicate arranged in a completely randomized design (CRD). The levels of benzoic acid inclusion were 0, 0.6, 1.2, 1.8 and 2.4%. Commercial feed containing benzoic acid was given every morning and evening. Water was given ad *libitum*. The experiment lasted for 49 days. Data on microbial load, pH and haematological indices were collected at the end of the experiment. Blood samples were collected through the Jugular vein. Chyme samples were collected from the intestine lumen and analyzed. The result showed that there was no significant difference ($P<0.05$) between the pack cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration MCHC). The pH of the crop and colorectal were not significantly different, while the pH of the gizzard, duodenum, ileum and caecum were significantly different ($P<0.05$) from each other across the levels of treatment. The pH value of gizzard was the least on T_4, while the highest value of pH was recorded in colorectal T_4. The microbial load decreased significantly ($P<0.05$) with increased inclusion of benzoic acid in the feed. The least microbial load was recorded in duodenum and ileum at T_4, while the gizzard and caecal microbial load were 5.01×10^5 in T_4. This study showed that supplementing broiler diets with benzoic acid as antibiotic substitute reduced the intestinal microbial load with a corresponding increase in their pH without a significant effect of the haematological indices.

Keywords: Benzoic acid supplementation; broiler diets; guts micro flora haematological indices; antibiotic replacer

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Introduction

Poultry is vulnerable to potentially pathogenic microorganisms such as *Escherichia coli, Salmonella spp.* and *Clostridium*. These pathogenic micro floras present in the gut compete with host for nutrients and essential vitamins thereby depresses growth performance and increases incidence of diseases (Enberg et al., 2000). Antibiotic growth promoters (AGP) have been used for the past five decades to improve the performance of the poultry. Sub-therapeutic levels of antibiotics in poultry feed have increased feed efficiency and growth (Dibner and Richards, 2005). The use of AGPs have been under scrutiny for years (Retcliff, 2000), and have been removed from the market by regulatory authorities in many countries. With the declining use of antibiotic growth promoters due to the development of antibiotic resistant strains of pathogenic microorganisms, especially gram-negative, there is an ongoing need to identify effective replacements (Waldroup et al., 2003). Numerous products are considered and among them,

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the organic acids appear to offer a promising alternative to antibiotics.

Several studies demonstrated that supplementation of organic acids to broiler diets increased growth performance, reduced diseases and management problems (Jin, et al., 1998; Gunal, et al., 2006; Islam et al., 2008; Ao et al., 2009). Organic acids in undissociated forms are able to pass through the cell membranes of bacteria, where the acids dissociate in an alkaline medium to produce H^+ ions which lower the pH of the cell, causing the organism to use its energy to restore to the normal balance. The RCOO anions produced from the acid can disrupt DNA and protein synthesis, putting the organism under stress, so that it is unable to replicate rapidly (Nursery, 1997). The supplementation of feed with benzoic acid resulted in significantly lower counts of lactic acid bacteria, lactobacilli and yeast throughout the entire gastrointestinal tract (Russel and Diez-Gouzalez, 1998). The knowledge of haematological values is useful in diagnosing various pathological and metabolic disorders, which can adversely affect the productivity and reproductive performance of animals, hence, resulting in great economic losses to poultry farmers (Okoli et al., 2008). Therefore, the objective of this study was to determine the effect of benzoic acid as an antibiotic substitute on the gut micro flora and haematological indices of broiler chickens.

Materials and Methods

Birds and Experimental Designs

A total number of 120 unsexed one day old (Anak 2000) broiler chicks were used in this study. The chicks were individually weighed and allocated to 15 cages of 8 birds each, so that average initial body weight of birds of each cage did not vary significantly ($P>0.05$). The experiment was arranged in a completely randomized design (CRD). Birds were randomly allotted to five treatment groups, each with three replicated of eight birds. Control (c) birds were given a standard basal with 0% of benzoic acid; Treatment 1 (T_1) was a diet with 0.6% of benzoic acid; Treatment 2 (T_2) was 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. The control treatment included a starter diet (0 to 4 weeks) and a finisher diet 4-9 weeks). After thorough mixing of ingredients, the benzoic acid; which was in powder form, was mixed at the stated concentrations. The starter and finisher diets were formulated to meet the nutrient requirements of the birds. Ingredient and composition of basal diets are presented in Table 1.

Housing and Management

Chicks were housed in deep litter system which was cleaned thoroughly with formaldehyde and potassium permanganate solution three days prior to arrival of the birds. The day old chicks were offered an electrolyte solution upon arrival. The birds were maintained on a 24 hours constant light schedule. The brooding temperature was maintained close to their requirement, first by a heating device (hover) for the first three days. Health management practice included administration of New Castle disease vaccine 1/0 at day one, infectious Bursal disease on day 7 (1^{st} dose) and day 17 (2^{nd} dose) and New Castle disease vaccine (Lasota) at day 28. Medications like anticoccidal drugs and anthelmintics were also given. Feed and water were supplied *ad libitum*. The experiment lasted for 49 days.

Table 1: Proximate composition of the experimental 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 diets contained benzoic acids at different level of inclusions. Treatment 1 (0.6%), Treatment 2 (1.2%), Treatment 3 (1.8%), Treatment 4 (2.4%) and control (0.0%).

Housing and Management

Chicks were housed in deep litter system which was cleaned thoroughly with formaldehyde and potassium permanganate solution three days prior to arrival of the birds. The day old chicks were offered an electrolyte solution upon arrival. The birds were maintained on a 24 hours constant light schedule. The brooding temperature was maintained close to their requirement, first by a heating device (hover) for the first three days. Health management practice included administration of New Castle disease vaccine 1/0 at day one, infectious Bursal disease on day 7 (1^{st} dose) and day 17 (2^{nd} dose) and New Castle disease vaccine (Lasota) at day 28. Medications like anticoccidal drugs and anthelmintics were also given. Feed and water were supplied *ad libitum*. The experiment lasted for 49 days.

Data Collection and Analysis

On the 49^{th} day, two birds from each replicate were sacrificed, followed by complete bleeding. Within 20 minutes after death, the contents from crop, gizzard, duodenum, ileum, caecum and colorectal were collected aseptically for pH determination (Al-Natour and Alshwabkeh, 2005). Samples from the crop, gizzard, duodenum, ileum and caecum were also cultured for viable counts of bacteria (Quinn et al., 1992).

Blood were collected through the jugular vein using sterile needles and syringes into labeled sterile universal bottles containing ethylene diamine tetracetic acid (EDTA) powder as anticoagulant. This was used for the determination of haematological indices of interest such as size of red blood cell, white blood cell, haemoglobin concentration and packed cell volume. Values obtained were used to calculate the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). All data collected were subjected to analysis of variance (Snedecor and Cochran, 1980); while mean separation was carried out using Duncan Multiple Test (1955).

Results and Discussion

Table 2 showed the effect of benzoic acid on pH of digester from the crop, gizzard, duodenum, ileum, caecum and colorectal. It showed that benzoic acid did not have significant (P>0.05) effect on the pH of the crop and colorectal. However, there was a consistent decrease in the pH of the crop with an increase in the concentration of benzoic acid applied (T_0 = 5.21, T_1 = 5.22, T_2 = 5.08, T_3 = 5.05 and T_4 = 5.02). These findings are consistent with the observation of Matthew et al. (1991) and Hernandez et al. (2006) who reported no effect on intestinal pH with the use of propionic acid and formic acid. This is because of the strong buffering action of crop and colorectal in poultry. The presence of bacteria and protozoa in the crop and colorectal makes them to serve as a fermentation vat which helps to keep the pH at an equilibrium. Also, the pH of the gizzard, duodenum ileum and caecum were significantly (P<0.05) influenced by application of benzoic acid. This result is in line with the findings of Jozefiak et al. (2010) that the caecal pH decreases following benzoic acid supplementation.

Table 3 showed the effect of benzoic acid on gut microbial load. The table showed that benzoic acid supplementation significantly decreased microbial loads in all the parts of the gut studied i.e., crop, gizzard, duodenum, ileum and caecum. Microbial load decreased from 12 x 10^5 at 0% supplementation to 4 x 10^5 at 2.4% supplementation in crop. From 15 x 10^5 to 5 x 10^5 in gizzard, 11 x 10^5 to 4 x 10^5 in the duodenum; also from 13x 10^{10} to 4 x 10^5 in the ileum and 14 x 10^5 to 5 x 10^5 in the caecum. This result conformed the results of Maribo et al. (2000) who reported that supplementation of feed with benzoic acid resulted in significantly lower counts of lactic acid bacteria, lactobacilli, coli forms and yeast throughout the entire GIT. Furthermore, Cherrington et al. (2003) documented that benzoic acid plays an important role in lowering numbers of many pathogenic bacteria.

Benzoic acid has antimicrobial properties because of its inhibiting effect on several microbial enzymes like α-ktoglutoric acid dehydrogenase. This result indicates that when benzoic acid is used as an acidifier is as effective as the antibiotics in reducing *Escherichia coli* and clostridium counts in gut content. Thus the acidifier was shown to have both anti mould and antibacterial properties. It means that benzoic acid can be use as a substitute to antibiotic growth promoter when added in the feed or water of poultry birds. A similar observation was also recorded by Izat et al. (1990) and Byrd et al. (2001), who reported that the addition of lactic acid, formic acid and propionic acid to the diet and water effectively reduced *Escherichia coli,* coli forms and salmonella in poultry.

The haematological parameters of the broiler chicken fed diets supplemented with benzoic acid are presented in Table 4. There was no significant difference (P<0.05) in the haematological indices although there were slight differences in the numerical values among the blood parameters examined. This showed that supplementation of broiler diets with benzoic acid did not affect the physiological status of birds. According to Rose et al. (2008) haemalogical parameters are good indicators of the physiological stressful situation and diseases. For PCV, the highest

Table 2: Effect of benzoic acid on pH of digester

Treatment	Crop	Gizzard	Duodenum	Ileum	Caecum	Colo-rectum
T_0	5.21±0.01	4.15±0.15^{ab}	5.95±0.15^{a}	4.74±0.04^{ab}	6.23±0.08^{c}	7.05±0.05
T_1	5.22±0.01	4.24±0.04^{ab}	5.93±0.03^{a}	4.87±0.07^{b}	5.78±0.08^{b}	7.05±0.01
T_2	5.08±0.04	4.53±0.01^{bc}	6.66±0.06^{b}	4.90±0.10^{b}	5.63±0.13^{b}	7.13±0.02
T_3	5.05±0.05	4.54±0.03^{c}	7.03±0.13^{c}	4.71±0.02^{ab}	5.12±0.02^{a}	7.10±0.10
T_4	5.02±0.05	4.05±0.05^{a}	7.03±0.13^{c}	4.55±0.05^{a}	4.96±0.06^{a}	7.15±0.05

Table 3: Effect of benzoic acid on gut microbial load (x 10^5)

Treatment	Crop (10^5)	Gizzard (10^5)	Duodenum (x10^5)	Ileum (x10^5)	Caecum (x10^5)
T_0	12.01±0.01^{e}	15.01±0.01^{e}	11.01±0.01^{e}	13.01±0.01^{e}	14.01±0.01^{e}
T_1	10.00±0.00^{d}	12.01±0.01^{d}	10.01±0.01^{d}	11.01±0.01^{d}	12.01±0.01^{d}
T_2	8.01±0.01^{c}	9.01±0.01^{c}	9.01±0.01^{c}	10.00±0.00^{c}	8.01±0.01^{c}
T_3	6.01±0.01^{b}	8.01±0.01^{b}	6.01±0.01^{b}	7.01±0.01^{b}	6.01±0.01^{b}
T_4	4.01±0.01^{a}	5.01±0.01^{a}	4.01±0.01^{a}	4.01±0.01^{a}	5.01±0.01^{a}

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

Table 4: Effects of benzoic acid on haematological indices

Treatment	PCV (%)	Hb (g/dl)	WBC(µl)	RBC x 10^{-6}	MCV (FI)	MCH (pg)	MCHC
T0	35.00±5.00	7.20±2.20	944000.0±48.0	2.53±1.33	176.95±73.06	33.04±8.63	20.08±3.42
T1	41.00±1.00	9.70±2.10	42884.27±20.0	2.23±0.18	188.97±59.81	44.61±12.95	23.82±0.70
T2	32.00±4.00	6.60±0.20	475175.76±33.6	1.55±0.05	236.68±24.18	49.57±9.57	20.74±1.92
T3	34.00±4.00	6.20±1.00	497803.170±0.2	2.00±0.35	171.76±10.06	31.07±0.44	18.14±0.18
T4	34.00±1.00	6.70±0.50	339411.25±24.0	1.85±0.05	183.77±0.44	36.32±3.69	19.76±2.05

value (41%) was found in T_1, while the least values were in T_3 and T_4 (34%). For haemoglobin values, T_1 had the highest value for Hb g/gL (9.70). However, the haemoglobin values observed in T_2 (6.60), T_3 (6.20) and T_4 (6.70) were below the range (7-15gldL) as reported by Daramola et al. (2008). According to Rose et al. (2008), low concentrations of Hb g/dL suggest the presence of toxic factors in the diets.

The red blood cell (RBC) values lies within the range as reported by Daramola et al. (2005). Birds on T_0 has the highest values of white blood cell (9.44×10^6 µL) when compared with other treatments. A higher WBC count observed in T_0 is associated with microbial infection, the presence of "foreign" body or antigen in the circulatory medium. Furthermore, the supplementation of the diets in T_1, T_2 and T_3 and T_4 with benzoic acid brought the WBC count to normal.

This shows that the benzoic acid was as effective as antibiotic in reducing microbial infection and antigens present in the gastro intestinal tract.

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