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Microbial population and blood profile of finisher broiler chickens fed diets supplemented with Crina poultry plus®.

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***Target Audience**:* Farmers, Consumers, Health Officers and Nutritionist

Abstract

A study was carried out to determine the effect of Crina Poultry Plus® (a natural blend of essential oils and benzoic acid) on microbial population and blood profile of finisher broiler chickens. Six treatment diets were formulated by the addition of Crina Poultry Plus® (CPP®) at 0, 0.2, 0.3, 0.4, 0.5 and 0.6g/kg of feed. Two hundred and forty day-old chicks were allocated with each treatment having 4 replicates and 10 broilers each in a completely randomized design (CRD). Data were collected on the microbial population (crop and ceca) and serum lipid profile. The result of microbial population showed that lactic acid bacteria counts were significantly (P<0.05) higher in the crop and ceca of birds fed diet containing CPP® compared to the control group. The coliform count was significantly (P<0.05) higher in the crop and ceca of broilers fed control diets compared to diets supplemented with CPP®. The PCV, RBC, Hb and WBC were significantly (P<0.05) higher in broilers fed CPP® compared to the control group, while the cholesterol, triglycerides, low density lipoprotein were significantly (P<0.05) lower compared to birds fed control diet. High density lipoprotein was significantly (P<0.05) higher in birds fed diets containing CPP® than those fed control diet. It was concluded that the addition of 0.6 g CPP® in broiler diets maintained the haematological indices, microbial balance and improved the level of high density lipoprotein.

Keywords: benzoic acid, essential oils, microbial population, blood profile and broiler chickens.

Description of Problem

The production of poultry meat safe for human consumption is dependent on many factors which include suitable environment, breeds/strains of the animal, nutrient requirement and health status of the birds. Among these factors, meeting the nutritional requirement of the animals with the objective to optimize digestibility and absorption of nutrients is certainly one of the most important elements (1). In this respect, the gastrointestinal tract needs special attention because any impairment of the secretion of enzymes or disturbance of the microbiological equilibrium in the gut could negatively affect the digestibility of the feed, resulting in reduced growth rate or impaired feed conversion of the growing birds.

Before now, antibiotics were used to facilitate efficient production of poultry by reducing the incidence of diseases. They exacted their core activity as growth promoters at sub-therapeutic concentrations (50-100mgkg^{-1}) in feed and or water by suppressing the harmful microorganisms. They also counteracted the adverse consequences of

stress responses and improving the gut health (2).

However, in spite of these advantages, concerns were raised of the effects of indiscriminate use of antibiotics which included antibiotics resistance, resulting from the ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agents (3) and accumulation of antibiotic residues in tissues of poultry birds which upon consumption possess risk to human health. In view of these, antibiotics have been banned for use as growth promoters in most developed countries (4, 5). Researchers have continued to search for alternatives to antibiotics growth promoters.

Thus, Eubiotic feed additives (EFA) such as organic acids; essential oils; prebiotics and probiotics are being used or investigated. In recent years, some of these products have been described by the general term 'Eubiotics', which is related to the Greek term 'Eubiosis", referring to an optimal balance of microflora in the gastrointestinal tract. The main purpose of using eubiotics is to maintain the intestinal eubiosis, which will result in an improved health status and performance in farm animals.

Organic acids and their salts are generally considered safe and have been approved by most member states of European Union (EU) to be used as the feed additives in animal production. Organic acids work in animals, not only as a growth promoter but also as a significant tool for controlling intrinsic bacteria, both pathogenic and non-pathogenic (6 and 7) and they exert physiological effects in the proximal sections of the digestive tract (8). Organic acids in non-dissociated (non-ionized, more lipophilic) form can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria (9). Examples of organic acid include fumaric acid, citric acid, formic acid, lactic acid, sorbic acid and benzoic acid.

Essential oil compounds are another group of feed additives that has potential for the replacement of antibiotic growth promoters. These essential oils are made up of active ingredients (e.g. thymol, carvacrol, eugenol, piperine cubeb, cumin) present in various plants and spices. Due to their antibacterial activity. They are able to modify the composition of the intestinal microflora and exert beneficial effects on performance of poultry. Several authors have reported an *in vivo* effect on microflora by specific formulations of essential oil in poultry.

The present study was therefore conceived to evaluate the effect of dietary supplementation of Crina Poultry Plus® on crop, ceca microbial population, haematology and serum lipid profile of broiler chickens.

Materials and Methods

Experimental Site

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The area falls within the tropical rain forest zone, with an annual average rainfall of 2177mm, temperature range between 20^{o} -30^{o}, with relative humidity 50-59% depending on season (10).

Source of Eubiotics

The eubiotics; CRINA POULTRY PLUS®used in this study was purchased from the manufacturer; DSM Nutritional Products, LTD., Wurmisweg, Switzerland. The product was in powder form and contains a blend of essential oil compounds [thymol (≥1%), eugenol (≥0.5%), piperine (≥0.05%), and other flavoring substances (≤0.6%)] with the organic acid; benzoic acid (≥80%).

Experimental Birds and Management

A total of 240 day old Arbor acre broiler chicks procured from Chi® Farms Ibadan were used in this study. The chicks were brooded

and reared for 21 days; thereafter they were randomly divided into 24 groups consisting of six treatment and 4 replicates with 10 birds per replicate. Each group was raised in floor pens with wood shavings litter material and contained feeders and drinkers to provide *ad libitum* access to feed and water for duration of 21 days. Birds were vaccinated against Gumboro disease at 7^{th} and 18^{th} days of life while Newcastle disease was vaccinated at 12^{th} day. Coccidiostats was also administered to the birds during the experiments.

Experimental Diets

Six experimental broiler finisher diets were formulated such that the control Diet A contained no Crina Poultry Plus®. Diet B, C, D, E and F contained 0.2, 0.3, 0.4, 0.5 and 0.6g CPP® in the control diet respectively. The ingredients a composition of the broiler finisher control feeds is shown in table 1.0

Table 1: Percentage compositions of the finisher diet (22 to 42 d) period.

Ingredients	Finisher
Maize	60.55
Soya bean meal	30.70
Palm kernel cake	5.00
Bone meal	3.00
Methionine	0.20
Lysine	0.10
Vitamin/mineral premix	0.25
Salt	0.20
Total	**100**
Calculated composition %	
Crude protein	19.90
ME, Kcal/kg	2957.24
Methionine	0.53
Lysine	1.11
Calcium	1.16
Crude fibre	4.18
Analyzed composition	
Crude Protein %	19.31
Crude Fiber %	6.08
Ether Extract %	1.47
Dry Matter %	91.59
NFE %	60.51

Each 2.5 kg vitamin-mineral premix provided the following: A 8,000,000 iu, D_3 2,000,000 iu, E 5000 mg, K_3 2000 mg, Folic acid 500 mg, Niacin 15,000mg, Calpan 5,000 mg, B_2 8000 mg, B_{12} 10,000 mg, B_1 1,500 mg, B_6 1,500 mg, Biotin 20 mg.

Data Collection

Microbial analysis.

At the end of the experiment (21 days), 24 chickens of similar body weight to the group average were selected from the treatment group (1 chicken per replicate), weighed and slaughtered by severing the jugular vein, they were then thoroughly bled and scalded by dipping in warm water with temperature of $50\text{-}55^0$C before defeathering.

The internal organs were removed. From the eviscerated organs (i.e 4 birds/treatment), a section of the crop and ceca were cut and used for microbial analysis. Approximately 1 g of the crop and cecal contents were mixed with 9 ml of pre-reduced sterile dilution blank solution (11), and homogenized for 3 minutes individually. From the initial 10^1 dilution, 6-fold serial dilutions were made in sterile pre-reduced dilution blank solution for total coliforms, lactic acid bacteria (LAB's) and total microbes. For each dilution, 1 ml was inoculated in mediums which include MRS agar for LAB's, MacConkay agar for coliforms. All the inoculated plates were then incubated at 37-40^{o}C between 24-48 hours. Total number of bacterial colonies was counted at the end of each incubation period using improved bacteria colony counter. (12).

Haematological and serum lipid profile.

At the end of the experiment (21 days), 24 chickens of similar body weight to the group average were selected from the treatment group (1 chicken per replicate) and blood samples (10ml) were taken from the jugular vein with a sterile needle and 5mls was kept in sample bottles containing ethylene diamine tetra acetic acid (EDTA), an anti – coagulant to prevent blood clotting. The blood samples were taken to the laboratory for haematological assessment such as packed cell volume (PCV), red blood cells (erythrocyte) count (RBC), haemoglobin (Hb), White blood cell (leukocyte) count.

The remaining 5mls were kept in sample bottles without coagulant. Serum was isolated by centrifugation at 2,000rpm for 10 minutes and then stored at -70^{o}C until analysis. The lipids constituents analysed include total cholesterol, high density lipo-protein, low density lipo-protein, and triglyceride. (12)

Chemical analysis and data analysis

Proximate composition of the experimental diet was determined according to the methods of A.O.A.C (13).The experiments were laid out in a Completely Randomized Design. Data analysis were done using IBM® SPSS version 20.0. The data were subjected to analysis of variance (ANOVA). Where treatment means were significant, separation of means was done using the Duncan's Multiple Range Test (14) at 5% level of significance.

Completely randomized design model: $Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij}= individual observation

μ = Overall mean

T_i= Treatment Effect

e_{ij}= Random error

Results and Discussion

Microbial Analysis

The microbial population of crop of finisher broilers fed diets containing combinations of essential oils and benzoic acid is presented in Table 2.0. Birds fed 0.5 and 0.6g CPP® had similar total microbial count values that were significantly higher ($P<0.05$) than those of birds fed 0.2, 0.3 and 0.4g CPP® diets respectively. The values for birds fed 0.2 and 0.4g CPP® were significantly higher ($p<0.05$) than that of birds fed the 0.3g CPP® diet. The lactic acid bacteria values were significantly higher ($P<0.05$) in birds fed the 0.5g and 0.6g CPP® diets than the other treatment groups. Birds on the control diet had the lowest LAB values, that differed significantly ($P<0.05$) from the 0.2 and 0.3g CPP® diets.

Table 2: Effect of Crina Poultry Plus® on microbial population of the crop of finisher broilers.

Parameters	T1 (Control)	T2 (0.2g)	T3 (0.3g)	T4 (0.4g)	T5 (0.5g)	T6 (0.6g)	S.E.M
TMC (logcfu/g)	2.41[a]	2.37[b]	2.33[c]	2.35[b]	2.41[a]	2.42[a]	0.01
LAB (logcfu/g)	1.72[e]	1.91[d]	2.00[c]	2.13[b]	2.26[a]	2.27[a]	0.04
Coliforms(logcfu/g)	2.27[a]	2.15[b]	1.99[c]	1.89[d]	1.81[e]	1.82[e]	0.04

[a,b,c] Means across rows with different superscripts differ significantly at P<0.05; S.E.M: Standard Error of the Mean; TMC: Total Microbial Count; LAB: Lactic Acid Bacteria; Logcfu: Logarithm/ Colony Forming Unit

The coliform values were significantly different from each other with birds on the control diet having value. The TMC and LAB showed a similar trend; as the rate of inclusion of the CPP® is increasing, so the microflora increases. The TMC and LAB counts of birds fed diets supplemented with 0.5g and 0.6g were significantly (P<0.05) higher than the remaining treatment groups, while the coliform counts of birds fed diets supplemented with 0.5g and 0.6g CPP® were significantly (P<0.05) lower than the remaining treatment groups.

The microbial population of ceca of finisher broilers fed diets containing combinations of essential oils and benzoic acid is presented in Table 3.0. The TMC of birds fed diet supplemented with 0.5g of CPP® was significantly (P<0.05) higher than those fed diets supplemented with 0.2, 0.3 and 0.4g of CPP® but was similar to the control, while the LAB showed an increase in counts as the rate of inclusion of CPP® increases.

Table 3: Effect of Crina Poultry Plus® on microbial population of the ceca of finisher broilers.

Parameters	T1 (Control)	T2 (0.2g)	T3 (0.3g)	T4 (0.4g)	T5 (0.5g)	T6 (0.6g)	S.E.M
TMC (logcfu/g)	2.44[a]	2.39[c]	2.35[d]	2.41[b]	2.45[a]	2.42[ab]	0.01
LAB (logcfu/g)	1.80[d]	1.97[c]	2.08[b]	2.24[a]	2.29[a]	2.29[a]	0.04
Coliforms (logcfu/g)	2.29[a]	2.15[b]	1.95[c]	1.85[d]	1.81[de]	1.76[e]	0.04

[a,b,c] Means across rows with different superscripts differ significantly at P<0.05; S.E.M: Standard Error of the Mean;
TMC: Total Microbial Count; LAB: Lactic Acid Bacteria; Logcfu: Logarithm/ Colony Forming Unit

Thus, birds fed diets supplemented with 0.4, 0.5 and 0.6g CPP®s had significantly (P<0.05) higher TMC or LAB than those supplemented with 0.2g, 0.3g CPP® and control group. The coliform showed a declining trend in counts as the inclusion rate of CPP® increases.

Blood Profile

Table 4.0 showed the Haematological indices of finisher broilers fed diets containing combination of essential oils and benzoic acid. The PCV and WBC of birds fed diets supplemented with different levels of CPP® and control diet were not significantly (P>0.05) different within the treatment groups, while the RBC of birds fed diet supplemented with 0.6g of CPP® was significant (P<0.05) higher than the control group leaving the remaining treatment groups with similar values. The Hb counts of birds fed diets supplemented with 0.6g of CPP® was significantly (P<0.05) higher than the control group, while the remaining treatment groups had similar values.

Table 4: Effect of Crina Poultry Plus® on haematological indices of finisher broilers.

Parameters	T1 (Control)	T2 (0.2g)	T3 (0.3g)	T4 (0.4g)	T5 (0.5g)	T6 (0.6g)	S.E.M
PCV %	26.77	27.90	27.67	27.50	26.72	27.85	0.40
RBC $x10^{12}$/l	3.36^{b}	3.97^{ab}	4.01^{ab}	3.90^{ab}	3.87^{ab}	4.29^{a}	0.09
Hb g/dl	6.23^{b}	7.47^{a}	7.48^{a}	6.78^{ab}	6.53^{ab}	7.60^{a}	0.16
WBC x 10^{3} /mm^{3}	13.40	10.67	14.20	10.13	11.90	10.28	0.80

^{a,b,c} Means across rows with different superscripts differ significantly at P<0.05; S.E.M: Standard Error of the Mean; PCV: Packed Cell Volume; RBC: Red Blood Cell; Hb: Haemoglobin; WBC: White Blood Cell.

Table 5.0 showed the serum lipid profile of finisher broilers fed diet containing combinations of essential oils and benzoic. The total cholesterol of birds fed diets supplemented with 0.3, 0.5 and 0.6g CPP® and control were significantly (P<0.05) lower to those fed diets supplemented with 0.2 and 0.4g CPP® which are not significantly (P>0.05) different from each other. The triglyceride (Tg) of birds fed diets supplemented with varying levels of CPP® and control diet did not show any significant (P>0.05) different within the treatment groups, while the HDL of birds fed different levels of CPP® and control diet differed significantly (P<0.05).

Table 5: Effect of Crina Poultry Plus® on serum lipid profile of finisher broilers.

Parameters(mg/dl)	T1 (Control)	T2 (0.2g)	T3 (0.3g)	T4 (0.4g)	T5 (0.5g)	T6 (0.6g)	S.E.M
Cholesterol	67.23^{b}	115.47^{a}	66.66^{b}	119.28^{a}	65.27^{b}	63.97^{b}	6.50
Tg	35.04	47.42	29.27	49.19	44.72	45.70	2.86
HDL	27.50^{d}	52.25^{a}	41.00^{bc}	50.50^{a}	35.00^{cd}	44.75^{ab}	2.06
LDL	32.72^{ab}	53.73^{b}	23.44^{a}	58.94^{b}	21.33^{a}	10.08^{a}	4.82

^{a,b,c} Means across rows with different superscripts differ significantly at P<0.05; S.E.M: Standard Error of the Mean;
Tg: Triglycerides; HDL: High Density Lipo-Protein; LDL: Low Density Lipo-Protein.

The LDL of birds fed diet supplemented with 0.6g CPP® was significantly (P<0.05) lower than those supplemented with 0.2 and 0.4g CPP® but not significantly (P>0.05) different from the remaining treatment groups.

Discussion

Microbial analysis

The result obtained from this study showed that as the inclusion of CPP® in the diet is increasing the beneficial microbiota increase while the non-beneficial (Coliform) decreases as the inclusion of CPP® increases. Thus, indicating that CPP® has a positive effect on the growth of beneficial bacteria and this agrees with the work of (15) which stated that dietary supplementation of benzoic acid and essential oils shifted microbiota populations by increasing Lactobacillus loads. It has been reported that lactic acid-producing bacteria may improve gastrointestinal function, feed digestibility and animal performance (16). It is suggested that the establishment of Lactobacillus spp. prevented the colonization of pathogenic bacteria by competitive exclusion (17). Lactobacilli and bifidobacteria compete against potential pathogens for nutrients and binding sites, thereby reducing the intestinal population of pathogens. Furthermore,

lactobacilli and bifido-bacteria produce organic acids and other bactericidal substances (12), all of which can suppress the colonization of the intestine by pathogenic bacteria. It is possible that benzoic acid and essential oils favoured the growth of lactobacilli and bifido-bacteria populations and inhibited that of coli-forms.

Other studies have reported effects on intestinal microflora when herbs and essential oils have been included in broiler diets. The dietary supplementation of XTRACT, an encapsulated product containing capsaicin, carvacrol and cinnamaldehyde, reduced the numbers of *E. coli* and *Clostridium perfringens* in broiler rectal contents to the same extent as avilamycin (18). *Clostridium perfringens* has been reduced in number when the blended essential oil supplement CRINA® poultry, was fed in poultry diets (19; 20). Selective inhibitions in the growth of the hyper-ammonia-producers *Clostridium sticklandii* and *Peptostreptococcus anaerobius* were observed when a blend of dietary terpenes containing thymol, eugenol, vanillin and limonene were fed to ruminants (21). The growth of *E. coli* and *C. perfringens* was reduced in broilers, when blends of essential oils were fed in industry trials (19; 22), while numbers of Lactobacillus spp. increased (22). Thus, essential oils may act differently compared with synthetic antimicrobials, which tend to depress bacterial numbers across species.

(23) demonstrated in the batch culture conditions that benzoic acid and, to a lesser extent, fumaric acid exerted strong bacteriocidal properties towards lactic acid bacteria. These authors suggested pH-dependent effects on coliform bacteria and lactic acid bacteria in stomach content, and on coliform bacteria in the small-intestine content. The pH values used in the batch culture system by (23) were supposed to mimic physiology of the pig and so were lower than those observed in the chicken.

Blood profile

Hematological parameters are usually related to health status and are of diagnostic importance in clinical evaluation of the state of health. Blood parameters are good indicators of physiological, pathological and nutritional status of an animal and changes in hematological parameters have the potential of being used to elucidate the impact of nutritional factors and additives supplied in diet on any living creature. For example, leucocytes are known to increase sharply when infection occurs, as they are one of the first lines of defense of the body (24).

The hematological values obtained in this study indicated no detrimental impact of EFA's on RBC and WBC counts, hemoglobin content and PCV percentage because all the parameters were within range as reported by (25). (26) showed that feeding diets supplemented with oil extract derived from thyme and cinnamon to broilers, which significantly increased RBC, PCV, Hb and WBC values compared with the control group. (27) reported similar results where they used thyme extract on broiler chickens.

Serum biochemistry is a labile biochemical system which can reflect the condition of the organism and the changes happening to it under influence of internal and external factors. Triglyceride concentration tended to decrease by addition of EFA's, similarly, (28) reported that the addition of 1% thyme to broiler diet resulted in a marked decrease in plasma total lipids.
The reduction of triglycerides and cholesterol noticed with thyme in animal studies was attributed to the lowering effect of thymol or piperine on HMG-Co A reductase the rate limiting enzyme of cholesterol synthesis (29; 30). (31) reported that dietary thyme oil

increases plasma level of triglycerides, LDL-cholesterol and HDL-cholesterol in broilers. Also, the findings of serum lipid profile are in agreement with (32), who reported that blood total lipids and cholesterol decreased significantly by dietary acidifiers. The beneficial role of essential oils and organic acid in reducing the blood lipid profile may be interpreted through their influence in decreasing the microbial intracellular pH. Thus, inhibits the action of important microbial enzymes and forces the bacterial cell to use energy to release the acid protons, leading to an intracellular accumulation of acid anions (33). Also, (34), reported that, the observed lower feed consumption during the period of growth and consequently lower fat intake that resulted in fat depletion may also contribute in reducing blood lipid content. Thus, further study is needed to clarify the mechanism of hypo-lipidemic actions of EFA's.

Conclusion and Applications

This study showed that:

1. Supplementation of a blend of essential oils and benzoic acid to dietS of broiler chickens was beneficial in improving gut health, microbial balance, improved haematological and biochemical blood profile.
2. Supplementation of this additive is an essential practice that can increase the growth, survivability of broiler chickens and enhanced product quality thereby solving the problem of accumulation of residues of antibiotics in tissues of chickens, total destruction of the gut microbiota and cholesterol synthesis in broiler chickens.
3. Thus, it is recommended that CPP® could be used in broiler diet at 0.6g/kg since the level had no deleterous effects on blood profile.

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