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EFFECT OF SUPPLEMENTED DIETS WITH GARLIC ORGANIC EXTRACT AND STREPTOMYCIN SULPHATE ON INTESTINAL MICROFLORA AND NUTRIENTS DIGESTIBILITY IN BROILERS

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ABSTRACT: *This experiment was carried out to study the effects of garlic organic extract and streptomycin sulphate on intestinal microflora and nutrients digestibility in broilers. Forty eight Hubbard line one day-old chicks with equal numbers of males and females were randomly allocated to eight treatment combinations to conduct a 4 x 2 factorial experiment in a completely randomised design. The diets were supplemented with: no supplement (control), garlic organic extract at 40 ppm/kg (GOE 40 ppm), garlic organic extract at 60 ppm/kg (GOE 60 ppm) and streptomycin sulphate at 30 ppm/kg (SS 30 ppm) administered by oral gavage from day 13 to day 47 of experiment. There were two birds (males or females) per experimental unit, replicated three times in twenty four deep litter pens. The colony forming units of Escherichia coli were significantly reduced (P<0.001) in the ileo-caecal digesta of birds on streptomycin sulphate (3.33 x 10^5) followed by the garlic organic extract treated groups (4.08 x 10^5) compared with the control (8.50 x 10^5). The same observation was made for Staphylococcus aureus (P<0.001). The colonies of Salmonella and Shigella spp were statistically similar between streptomycin sulphate and garlic extract treated groups (1.65 x 10^5), but they significantly (P<0.001) decreased compared with the values obtained in the control group (4.53 x 10^5). Female broilers had higher (P<0.001) colony forming units of enterobacteriaceae, Salmonella and Shigella spp and Staphylococcus aureus in their ileo cecal digesta than the males. Even within the treatment and sex interaction, female birds generally recorded higher number of colony forming units as compared with the males. Only mold fungi were found in the ileo-cæcal digesta of all the groups. Significant improvement in apparent digestibility of nutrients except for the calcium and inorganic phosphorus absorption rates in birds on supplemented diets was observed (P<0.01) compared with those on the control. There were no significant differences (P>0.05) in nutrients absorption between male and female broilers. Treatment and sex interaction significantly (P<0.05) affected all the parameters studied indicating a synergistic effect of the two factors on nutrients absorption. It could be concluded that GOE even at 40 ppm/kg controlled pathogens and improved nutrients digestibility in birds.*

ORIGINAL ARTICLE

Keywords: *Garlic organic extract, ileo- cecal digesta, intestinal microflora, nutrients digestibility, streptomycin sulphate.*

INTRODUCTION

The strongest determinant factor of the gut microbial profile is the host's diet. Factors such as diet composition, nutrient concentration, feed physical traits, feed processing, feed additives and environmental pollutions play significant roles in the dynamics of gut microflora (Hume et al*.*, 2006; Oviedo-Rondón et al., 2006; Parker et al*.*, 2007; Nalian et al., 2009). Microbes have profound effects in some of the physiological processes of their animal host (Lan et al*.*, 2005;

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Uscebrka et al., 2005). Digestive microflora populations affect broiler and layer hen performance and health (Hume et al., 2006; Oviedo-Rondón et al., 2006; Parker et al., 2007). These effects in the host may be due primarily to the complex interactions that influence the intestinal environment, and secondly to the responses of the host immune system against pathogenic and non-pathogenic antigens (Uscebrka et al., 2005; Dl Ines, 2009). Plant organic extracts have been shown to have antimicrobial effects (Komgeum et al., 2005; Tamokou et al., 2008). Their antimicrobial mode of action consists of interactions with the cell membranes of micro-organisms by changing permeability for cations such as H^+ and K^+ (Belguith et al., 2009). Moreover, there are evidences that herbs, spices and various plant extracts have appetizing, digestion-stimulating properties and antimicrobial effects (Zhang et al., 2005). The improvement in feed efficiency achieved with plant organic extracts mixtures could be attributed to their positive effects on nutrients digestibility (Jamroz et al., 2005; García et al., 2007; Loh et al., 2008). Thus, this study was conducted to determine the effects of garlic organic extract and streptomycin sulphate on gut microflora and nutrients digestibility in broilers.

MATERIALS AND METHODS

Animals and experimental design

Twenty four male and twenty four female day-old chicks of *Hubbard* line were selected from the batch kept in a brooding room of Abubakar Tafawa Balewa University Poultry Research Farm, Bauchi state, Nigeria for two weeks and transferred to experimental pens. The brooding room temperature decreased from 32°C during the first week of life to 28°C in the second. In order to boost their immunity, they were vaccinated against Infectious bursal disease on the fourteenth day of the experiment while Newcastle disease vaccine was administered at 21 days of age. Experimental diets and water were given *ad libitum* to birds every day. The entire flock was subject to deworming on the 35th day of age using piperazine. The experiment lasted for five weeks during which feed intake, weekly weight gain and feed conversion ratio were monitored. Two broilers died of coccidiosis in the fourth week of experiment giving a percentage mortality of 4.16% and the whole flock was thereby subject to five days of cure with pure amprolium.

The forty eight chicks were randomly allocated to eight (8) treatment combinations to conduct a 4x2 factorial experiment in a completely randomised design in which garlic organic extract and streptomycin sulphate were supplemented to the basal diet as follows: Control (Water), Garlic organic extract at 40 ppm/kg (GOE 40 ppm), garlic organic extract at 60 ppm/kg (GOE 60 ppm) and streptomycin sulphate at 30 ppm/kg (SS 30 ppm) [(Fraser et al., 1991; Radostits et al., 1997; Group Zhongnuo Pharmaceutical (shijiazhuang) Co., Ltd)]. Oral intubation of birds started when they were thirteen days old till day 47 of experiment. There were two birds (males or females) per experimental unit, replicated three times in twenty four deep litter pens. Weekly weighting of birds were carried out to determine the concentration of treatments to be given to birds. The quantity of garlic extract and streptomycin sulphate administered were calculated taking into account the proportion of the major component in the extract, the minimum recommended oral route dose of the antibiotic and the chicken live weights.

Diets and feeding regimens

Birds were fed with commercial starter and finisher diets (NRC, 1994) formulated to meet their nutrient requirements throughout the experiment (Table 1). As for the supplement, garlic extract was obtained by organic solvent extraction (Soxhlet, 1879) and oil analysis was carried out according to Adams (2001) method (Table 2).

Garlic organic extract and streptomycin sulphate control on gut pathogens

Four males and four females from each treatment were randomly selected, weighted and slaughtered. Faecal samples collected from the ileo-caecal junction of the thirty two eviscerated birds on the farm were put into sterilised vials and conveyed immediately to the laboratory. Then 1 g of digesta taken from each sample was added to 10 ml sterile distilled water and mixed for 1 minute in test tube. A tenfold serial dilution was made. Finally 0.1 ml was pipetted from the 1/1000 dilution test tube of each sample and inoculated on the solid culture medium prepared in Petri dishes the previous day. Dispersion was done using a sterile spreader sterilised after each step over a bunsen flame (AOAC, 1995).

Yeast and mold fungi were cultured on Sabouraud agar medium mixed with 250 mg chloramphenicol in order to inhibit any bacterial growth. They were incubated at 37°C for 24 hours and kept on the media preparation bench up to two weeks for identification. Bacterial counts were performed using *Salmonella/Shigella* agar medium for *Salmonella* and *Shigella* species, MacConkey agar for *Escherichia coli*, then the medium for identification of *Staphylococci spp* was prepared using Nutrient Agar + 12% (w/v) dilution of sodium chloride (NaCl). They were all subjected to incubation at 37°C for 24 hours (Johnston and Booth, 1983; Sinclair and Dhingra, 1995).

Digestibility studies and proximate analyses of feed and dried faecal collections

Four males and four females per treatment were randomly selected and subjected to digestibility study in battery cages for five days. Birds were all fed with the commercial broiler finisher diet. On the first day of digestibility study in battery cages, birds were given only the supplementations in the morning as usual without feed till 3.0 pm. Materials for

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faecal collections were placed under the cages in the morning of day 2 and wet faecal collections started on the third day in the morning before feed distribution coupled with oral supplementations. Dried matter digestibility was calculated for each sample. The sun-dried faecal collections and the basal diet as well were subject to laboratory analyses to determine their contents in crude protein, crude fibre, and ether extract according to the AOAC (1995) procedures. Calcium and inorganic phosphorus rates were obtained by UV absorption spectrophotometry [(Atomic Absorption Electronic Machine, Shimadzu, UV-1201, Japan)].

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Table 1 - Composition of experimental starter and finisher diets

Ingredients	Percent starter	Percent finisher
Maize (7.6.% CP)	51.25	56.80
Rice bran (11.8% CP)	8.00	10.00
Soybean meal (44% CP)	33.50	30.00
Fish meal (72% CP)	3.50	—
Bone meal	3.00	2.50
Vitamin/Mineral Premix[k] (0.25%)	0.25	0.25
Sodium chloride (NaCl)	0.30	0.25
Methionine (99%)	0.20	0.20
Totals in kilogramme	100.00	100.00
Feed nutrients proximate analysis		
Metabolizable Energy (Kcal/kg)	2818.33	2855.37
Crude protein (%)	22.05	19.08
Crude fibre (%)	4.21	4.31
Fats (%)	4.58	6.31
Calcium (%)	1.02	1.00
Available Phosphorus (%)	0.49	0.47
Lysine (%)	1.25	1.03
Methionine (%)	0.58	0.51

[k]each 2.5kg premix contained the followings: Vit A 10,000,000 IU; Vit. D_3 3,000, 000 IU; Vit. E 30,000 IU; Vitamin K_2 3 g; Vit $B_1$1.7g;Vit B_2 5.0 g; VitB_6 3.1g;Vit. B_{12} 16 mg; Biotin 60 mg; Niacin 1.0 g; Pantothenic Acid 8 g; Folic Acid 0.8 g; Manganese 85 g; Zinc 50 g; Iron 25 g; Copper 6 g; Iodine 1.1 g; Selenium 120 mg; Cobalt 220 mg; B.H.T 60 g; Ethoxyquin 65 g; Choline Chloride 200 g

Table 2 - Chemical composition (%) of the garlic organic extract

No	Retention Index	Compound Name	Percent in oil
		Thioethers	**83.67%**
1	660	Allyl methyl sulfide	3.49
2	849	1-propene, 3,3'-thiobis-sulfide	3.99
3	1099	Disulfide, di-2-propenyl	9.78
4	1131	Trisulfide, methyl 2-propenyl	26.82
5	1134	3-vinyl-1,2-dithiacyclohex-5-ene	32.72
6	1350	Trisulfide, di-2-propenyl	6.86
		Fatty acids	**16.33%**
7	1968	n-hexadecanoic acid	6.51
8	2183	Linoleic acid	9.82

Statistical analysis

The data collected were compared using the analysis of variance (ANOVA) option of Minitab (version 11.0) and Compare Means option of Statistical Package for Social Sciences software (version 11.0) as described by Steel and Torrie (1980). Significantly different means among treatments were separated using the Duncan's Multiple Range Test (Duncan, 1955) at ($P<0.05$).

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RESULTS

Effects of garlic organic extract and streptomycin sulphate supplementations on intestinal microflora of chickens

The intestinal microbial counts in ileo-caecal digesta of broilers are given in Table 3. At first glance, it can be noticed that birds in the control group had the highest number of colony forming units (CFU) of enteropathogens studied as compared with those on garlic extract and streptomycin sulphate. We could also observed that these colonies reduced as the dosage of garlic extract increased and the drop in CFU was even better with streptomycin sulphate except for *Salmonella and shigella spp.* Only mold fungi were observed in the ileo-cecal digesta of all the groups.

Sex effects of garlic extract and streptomycin sulphate supplementations on gut microbial population of broilers

The sex effects of garlic extract and streptomycin sulphate supplementations on gut microbiota of broilers are shown in Table 4. Female broilers had higher (P<0.001) colony forming units of *Escherichia coli, Salmonella and Shigella spp* and *Staphylococcus aureus* in their ileo cecal digesta than the males. However, the effect of treatments significantly decreased (P<0.001) the mean values of population of these enteropathogens per gramme of digesta collected from males as compared with those observed in female boilers. Mold fungi alone were found in the digesta of female birds and the males as well.

The treatment and sex interaction (Table 5) also significantly affected (P<0.01) the count of *Staphylococcus aureus,* the counts of *Salmonella and shigella spp*, and that of *E.coli* (P<0.001) per gramme of digesta studied indicating that the two factors contributed synergistically to the decrease in number of these parasites in broiler chickens. Even within the treatment and sex interaction, female birds generally recorded higher number of colony forming units as compared with the males. Only mold fungi were found in the ileo-cecal digesta of all the groups.

Treatments effect of garlic extract and streptomycin sulphate supplementations on nutrients absorption

Table 6 presents the results of nutrients digestibility in broilers. Garlic extract and streptomycin sulphate fortified diets significantly improved digestibility of nutrients in birds compared with the control except for the calcium and inorganic phosphorus absorption rates.

Sex effects on nutrients digestibility

Sex effects on nutrients digestibility (Table 7) in broilers subjected to garlic organic extract and streptomycin sulphate supplementations showed no significant differences (P>0.05) between male and female broilers. However, the mean values of nutrients digestibility in female birds were slightly superior to those of the males for all the parameters studied.

Treatment and sex interaction (Table 8) on nutrients digestibility significantly (P<0.05) affected all the parameters studied indicating a synergistic effect of the two factors on nutrients absorption. Even within the interaction, the nutrients digestibility values in female birds were generally higher as compared with those in males except for the apparent digestibility of crude fiber and the values found in birds on diets fortified with streptomycin sulphate.

Table 3 - Effects of garlic organic extract and streptomycin sulphate supplementations on the gut microbiota expressed in colony forming units per gramme of ileo-cecal digesta of broilers

Parameters	Control	Garlic Organic Extract		Streptomycin	SEM
		40 ppm	60 ppm	30 ppm	
Staphylococcus spp X 10^5	8.36 ^{a}	5.51 ^{b}	4.76 ^{c}	4.51 ^{c}	24138.75
Salm. & shig. spp X 10^5	4.53 ^{a}	2.28 ^{b}	1.65 ^{b}	2.70 ^{b}	42561.27
Escherichia coli X 10^5	8.50 ^{a}	4.97 ^{b}	4.08 ^{c}	3.33 ^{d}	23433.36
Yeast & Mold fungi	Mold (+)	Mold (+)	Mold (+)	Mold (+)	

^{a,b,c,d}Mean values in the same row with different superscripts are significantly different (P<0.001).

Table 4 - Sex effects of garlic organic extract and streptomycin sulphate supplementations on gut microbial counts in broilers

Parameters	Male	Female	SEM	P value
Staphylococcus aureus x 10^5	5.17	6.40	4217.78	<0.001
Salmonella & *Shigella* species x 10^5	2.67	2.91	2645.13	<0.001
Escherichia coli x 10^5	5.01	5.43	2242.17	<0.001
Yeast & Mold fungi	Mold (+)	Mold (+)		

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Table 5 - Treatment x Sex interaction of garlic organic extract and streptomycin sulphate supplementations on gut microflora of broilers

Parameters	Control		GOE 40 ppm		GOE 60 ppm		SS 30 ppm		SEM
	♂	♀	♂	♀	♂	♀	♂	♀	
Staphylococcus aureus x 10⁵	7.81 b	8.91 a	4.90 f	6.12 c	3.95 h	5.58 d	4.03 gh	5.00 ef	8435.57
Salmonella & Shigella spp x 10⁵	4.50 a	4.57 a	2.25 e	2.32 ed	1.30 g	2.00 f	2.65 c	2.75 cb	5290.27
Escherichia coli x 10⁵	8.00 b	9.00 a	4.75 d	5.20 c	4.00 f	4.17 fe	3.32 h	3.35 hg	4484.34
Yeast and Mold fungi	Mold (+)		Mold (+)		Mold (+)		Mold (+)		

a,b, c, d, e, f, g, h Mean values in the same row with different superscripts are significantly different (P<0.001). *GOE: Garlic Organic Extract; SS: Streptomycin Sulphate

Table 6 - Treatments effect of garlic organic extract and streptomycin sulphate supplementations on nutrients absorption

Parameters	Control	Garlic Organic Extract		Streptomycin	SEM	P value
		40 ppm	60 ppm	30 ppm		
ADDM	60.49 b	72.96 a	72.07 a	70.26 a	1.499	0.003
ADCP	51.48 b	65.61 a	64.91 a	60.80 a	1.843	0.001
ADCF	2.45 b	17.34 a	14.94 a	15.01 a	1.990	0.022
ADEE	90.28 b	93.42 a	93.10 a	92.11 a	0.365	0.044
CaAR	19.28 b	45.43 a	42.82 a	32.74 ab	3.023	0.034
PiAR	3.46 b	18.62 a	10.65 ab	7.11 b	1.890	0.020

a, b Mean values in the same row with different superscripts are significantly different. ADDM: Apparent digestibility of dry matter, ADCP: Apparent digestibility of crude protein, ADCF: Apparent digestibility of crude fiber; ADEE: Apparent digestibility of ether extract, CaAR: Calcium Absorption Rate, Pi AR: Inorganic phosphorus Absorption Rate

Table 7 - Broilers' sex effect of garlic organic extract **and streptomycin sulphate supplementations on nutrients digestibility**

Parameters	Males	Females	SEM	P value
ADDM	58.32	62.66	3.540	0.600
ADCP	49.00	53.96	4.694	0.586
ADCF	1.68	3.22	5.124	0.762
ADEE	89.58	90.98	0.838	0.388
CaAR	17.93	20.63	7.042	0.952
PiAR	1.49	5.43	4.820	0.992

Table 8 - Treatment x Sex interaction of garlic organic extract **and streptomycin sulphate supplementations on nutrients digestibility**

Parameters	Control		GOE 40 ppm		GOE 60 ppm		SS 30 ppm		SEM	P value
	♂	♀	♂	♀	♂	♀	♂	♀		
ADDM	58.32c	62.66 cb	71.56 a	74.37 a	72.20 a	71.95 a	71.03 a	69.49ba	3.540	0.032
ADCP	49.00 c	53.96 cb	63.88 ba	67.35 a	64.74 a	65.09 a	61.50 ba	60.11ba	4.694	0.046
ADCF	1.68 b	3.22 ba	13.75 b	20.93 a	15.01ba	14.88 ba	17.07 ba	12.94ba	5.124	0.004
ADEE	89.58 c	90.98 cb	93.01 ba	93.83 a	93.10 ba	93.10 ba	92.17 cba	92.05cba	0.838	0.002
CaAR	17.93 d	20.63dc	42.05 ba	48.82 a	45.03 ba	40.61ba	34.66 cba	30.82dcb	7.042	0.042
PiAR	1.49 d	5.43 dcb	16.44 ba	20.81 a	12.90 cba	8.40 dcba	8.94 dcba	5.28 dcb	4.820	0.020

a, b, c, d Mean values in the same row with different superscripts are significantly different. GOE: Garlic Organic Extract ; SS: Streptomycin Sulphate; ADDM: Apparent digestibility of dry matter, ADCP: Apparent digestibility of crude protein, ADCF: Apparent digestibility of crude fiber; ADEE: Apparent digestibility of ether extract, Ca AR: Calcium Absorption Rate, Pi AR: Inorganic phosphorus Absorption Rate

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DISCUSSION

The intestinal microbial population of *Staphylococcus aureus of* broilers on control was heavier than that of birds on garlic extract which was in turn heavier than the values in birds on diets fortified with streptomycine sulphate. Likewise, Guo (2003) used mushroom and herb polysaccharides as alternative for antimicrobial growth promoters in poultry and observed alteration of gut microbial activities and composition of chikens' caeca. The highest number of colony forming units (CFU) of *Escherichia coli* in ileo-cecal digesta was found in birds on the control and the lowest number in birds on streptomycin sulphate and they were all significantly different ($P<0.001$) from one another even as the dosage of garlic extract used increased. Likewise, Juneja and Friedman (2007) in an in vitro study using carvacrol, cinnamaldehyde, oregano oil, and thymol observed an inhibition of *Clostridium perfringens* spore germination in ground turkey during chilling. The colony forming units of *Salmonella and shigella spp* in ileo-caecal digesta of birds on supplements were significantly reduced ($P<0.001$) compared with the values in birds on control. Ben-Mahdi et al., (2010) studied the effect of the thyme essential oil in the improvement of growth performance and sanitary status of broiler chickens and observed a significant reduction ($P<0.05$) of the number of CFU of *Escherichia coli* in the groups supplemented with thyme essential oil compared with the control.

The effects of garlic organic extract and streptomycin sulphate supplementations on nutrients absorption showed that the values of apparent digestibility of dry matter from birds on garlic extract (40-60 ppm) and streptomycin sulphate did not differ but were significantly greater ($P<0.01$) than that of birds in the control group. This result followed the same pattern in the apparent digestibility of crude fiber, apparent digestibility of fats, except for the calcium and inorganic phosphorus absorption rates. These results tally with the findings of García et al*.,* (2007) who studied the effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers and reported an improvement in apparent ileal digestibility of nutrients in birds on supplemented diets with plant extracts compared with those on the control.

Apparent digestibility of crude protein mean values of birds on garlic organic extract (40-60 ppm) did not differ but were significantly higher ($P<0.05$) than the lowest value observed in birds on the control diet whereas that of birds on streptomycin sulphate supplementation was statistically similar to both. The same observation was made for the calcium absorption rate. Loh et al*.,* (2008) studied the effects of feeding phytogenic substances and phytase on growth performance and nutrient digestibility of young broilers and reported that birds on supplemented diets had better digestibility of nutrients such as crude protein, phosphorus and calcium compared with the control.

CONCLUSION

It was concluded that diets fortified with garlic organic extract at minimum 40 ppm level of inclusion could efficiently control enter pathogens and improve upon nutrients digestibility while boosting the immune system for a good health and carcass yield of broiler chickens.

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