BAP -34

Intestinal Microbial Population and Serum Lipid Profile of Broiler Chickens Fed Diets Supplemented with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* Cultures.

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Abstract
A study was conducted to determine the effects of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* cultures on intestinal microbial population and lipid profile of broilers. Five dietary treatments consisting of basal diet (control), basal diet + L. *bulgaricus*, basal diet + S.*thermophilus*, basal diet + combination of L. *bulgaricus* and S.*thermophilus* and basal diet + antibiotics (Oxytetracycline) were fed to 150 arbor acre broiler chicks from 1-35 d of age (DOA). The chicks were randomly assigned to 5 dietary treatments each having 3 replicates with 10 birds per pen. The intestinal microbial population and serum lipid profile were determined at 35 DOA. The results showed that serum cholesterol level, triglyceride and LDL were significantly lower ($p<0.05$) in broiler fed diets containing the probiotic organisms. The HDL level were significantly higher ($p<0.05$) in broiler fed diets containing probiotic organisms than those of the antibiotics and control diets. Lactic acid bacteria (LAB) counts and Total microbial counts (TMC) in the ileum were significantly higher ($p<0.05$) in broiler chickens fed diets containing probiotic organisms than those of antibiotics and control fed diets while the coliforms counts in the ileum were significantly higher ($p<0.05$) in broiler fed diets containing antibiotics and control than those of probiotic organisms. Addition of probiotic organisms in broilers diets improved high-densitylipo-proteinthat enhances product quality and health status of broiler chickens.

Keywords: *L.bulgaricus*, *S. thermophilus* , microbial population, serum lipid profile and broiler chickens

Introduction

Many feed additives have been used to improve the performance of birds in terms of their daily weight gain, feed efficiency, nutrient utilization and immunity against infections in intensive poultry production. Commonly used additives include probiotics, antibiotics, organic acids, essential oilsand enzymes. Antibiotics usage has facilitated the efficient production of poultry and enhanced the health and well-being of poultry by reducing the incidence of diseases. They exact their core activity as growth promoters at sub-therapeutic concentrations (50 – 100mgkg-^{1}) in feed and or water by suppressing the harmful microorganisms (Zulkifli *et al*., 2000). However, in spite of these advantages, concerns have been raised of the aftermath effects of indiscriminate use of antibiotics which include antibiotics resistance resulting from the ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agentsand 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 (EC, 2003a).

Recently, modern growth promoters such as orgacizeic acids, herbal agents, prebiotics and probiotics are being used or investigated. Probiotics are defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Most probiotics belong to a large group of bacteria empirically designated as lactic acid bacteria, they include; lactobacillus, baccilus, bifidobacterium, streptococcus, enterococcus and lactococcus. The value of probiotics in improving growth, feed conversion efficiency and its competitive ability against pathogens have been documented (Zulkifli *et al*., 2000; Khaksefidi *et al*., 2005). The modes of action include: competitive exclusion of pathogenic bacteria, intestinal probiotics, particularly bacteria, play an important role in determining the digestive mechanism and general health of all animals (Salminen *et al*., 1996). In all, little is known of the effects of Lactic acid bacteria (*Lactobaccillus bulgaricus* and *Streptococcus thermophilus*) on poultry.

Materials and Methods

Lactobacillusbulgaricus and *Streptococcus thermophilus*were obtained from a yoghurt starter (Yógourmet™), the starter culture. The organisms were cultured using the process of Jin *et al.*(1998). After culturing the organisms, the colonies obtained was then counted to confirm that the bacterial make up to 60,000cfu. 100g of skimmed milk (Marvel®) were obtained to sustain and stabilize the bacteria count in the diet.

As such for *Lactobacillusbulgaricus*, 3ml were measured while *Streptococcus thermophilus* solution of 1.5ml was measured and mixed with 100g of skimmed milk respectively.

A total of 150day old Arbor acre broiler chicks procured from Chi® Farms Ibadan were used in this study. The chicks were randomly divided into 15 groups in a completely randomized design and each group was randomly assigned to a cage unit that had raised wire floors and contains both feeders and drinkers. Chicks were brooded with a 60-watt bulb for 5 days consecutively, thereafter the intensity of light were reduced gradually from 14-16 hrs/day. The chicks were divided into five treatment groups and 3 replicates each. 1.0 g of the skimmed milk culture was mixed with 1kg of the diet. Five dietary treatments formulated. Five dietary treatment diets were formulated (Table 1.0). Treatment A: Control, Treatment B: Antibiotic (oxytetracycline 50mg/kg), Treatment C: *Lactobacillus*bulagaricus (2.5 x 10^6cfu), Treatment D:*Streptococcus thermophilus*(2.5 x 10^6cfu) and Treatment E: *Lactobacillus*bulagaricus + *Streptococcus thermophilus*(1.25x 10^6cfu each)

Table 1.0: Composition of experimental diet (%)

Ingredients	Composition %
Maize	47.3
Wheat bran	11.0
Soyabean	22.0
Fishmeal	3.0
Groundnut cake	13.0
Bone meal	2.0
Oyster shell	1.0
Methionine	0.25
Vitamin/ mineral premix	0.25
Salt	0.20
Totalcalculated value	100
Crude protein	
ME (Kcal/kg)	23.07
Methionine	2876.30
Lysine	0.60
	1.16

At the end of the experiment (35 days), 15 chickens of similar body weight to the group average were selected from the treatment group (1 chicken per replicate), blood samples (5ml) were taken from the jugular vein with a sterile needle and 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 (Jin *et al*., 1998). The lipids constituents analysed includes total cholesterol, high-densitylipo-protein, low-densitylipo-protein, and triglyceride.The bacteriological examinations were carried out using approximately 1g of the crop and ileum content respectively was mixed with 9ml of prepared sterile dilution blank solution (Bryant and Burkey, 1953), and then inoculated according to the process of Jin *et al*. (1998).

All the data analysis was 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 (Duncan, 1995) at 5% level of significance.

Results and Discussion

The effects of *lactobacillusbulgaricus and streptococcusthermophilus* on lipid profile of broiler chickens are presented in Table 2. The result of the cholesterol showed that broiler chickens fed control diet were significantly higher ($p<0.05$) than the remaining treatment groups but those fed diet containg L. *bulgaricus* and S. *thermophillus* were not significantly different ($p>0.05$) from each other, while the triglycerides showed that broiler chickens fed control diet and diet containing antibiotics were not significantly different ($p>0.05$) from each other but they were significantly higher ($p<0.05$) than the remaining treatment groups.

The result of the low-densitylipo protein showed that broiler chickens fed control diet were significantly higher ($p<0.05$) than the remaining treatment groups but those fed diet containing L. *bulgaricu*s, S. *thermophillus* and combination of the two were not significantly different ($p>0.05$) different from each other. The supplementation of *L. bulgaricus* and *S.thermophillus* in diets of broiler chickens was observed to produce a decrease in the concentration of cholesterol compared to both the antibiotics and control treatment. This result is

in agreement with Jin *et al.* (1998) . It also agrees with Ashayerizadeh *et al.*(2009) when he compared antibiotics (flavomycin) with strains of lactobacillus. The results from the present study indicate that *L. bulgaricus* and *S. thermophillus* possesses a hypocholesterolemic effects on broilers in contrast to antibiotics.

Table 2.0. The Effects of *Lactobacillus bulgaricus and Streptococcus thermophilus* on lipid profile of broiler chickens

Parameters mmol/L	T_1 Control	T_2 Antibiotics	T_3 L. *bulgaricus*	T_4 S. *thermophillus*	T_5 L. *bulgaricus* + S. *thermophillus*	S.E.M
Cholesterol	3.63^{a}	3.13^{b}	2.53^{cd}	2.60^{c}	2.40^{d}	0.12
Triglyceride	0.73^{a}	0.73^{a}	0.33^{b}	0.27^{b}	0.33^{b}	0.06
LDL	2.34^{a}	1.80^{b}	1.10^{c}	1.21^{c}	0.91^{c}	0.15
HDL	0.63^{a}	0.69^{a}	0.41^{b}	0.39^{b}	0.37^{b}	0.04

^{a,b,c,d} Means across rows with different superscripts differ significantly at $p<0.05$; S.E.M: Standard Error of the Mean; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein.

Fukushima and Nakano (1995) suggested thatprobiotics influenced the cholesterol blood levels byinhibition of cholesterol synthesis. The triglyceride level in the above results implies that diet supplemented with *S. thermophillus*, *L. bulgaricus* and combination of the two produced the least concentration of triglyceride, this result is inline with kalavathy*et al*.(2008) who reported that serum triglyceride was lower in broiler fed Lactobacillus strains than those fed control or oxytetracycline diet.Ologhobo *et al*.(2008) reported that Garlic hada reducing effect on LDL level. Also, high-densitylipo-protein which is called the 'good' cholesterol because it helps remove excess cholesterol out of the cells, including cells in the arteries and it's evident in this study that the supplementation of *L. bulgaricus* and *S. thermophillus* had the highest concentration of HDL which is higher than the control treatment and antibiotics.

Table 3.0:The effects of *L. bulgaricus and S.thermophilus* on the lactic acid bacteria, total microbial counts and coliforms in the ileum of broiler chickens.

Parameters cfu/ml	T_1 Control	T_2 Antibiotics	T_3 L. *bulgaricus*	T_4 S. *thermophillus*	T_5 L. *bulgaricus* + S. *thermophillus*	S.E.M
LAB	60.50^{c}	81.00^{bc}	130.50^{ab}	179.50^{a}	146.50^{a}	15.32
TMC	92.50^{b}	99.50^{b}	114.50^{ab}	148.50^{a}	101.00^{b}	7.52
Coliforms	140.50^{a}	133.00^{ab}	99.50^{bc}	100.50^{bc}	67.00^{c}	9.39

^{a,b,c,d} Means across rows with different superscripts differ significantly at $p<0.05$; SEM: Standard error of the mean; LAB: Lactic acid bacteria; TMC: Total microbial counts.

The effects of *Lactobacillus bulgaricus and Streptococcus thermophilus* on the lactic acid bacteria, total microbial counts and coliforms in the ileum of broiler chickens is presented in table 3.0. The LAB of the ileum showed that there were no significant differences ($p>0.05$) between broiler chickens fed *L. bulgaricus, S. thermophillus* and combination of the two but those fed *S. thermophillus* and combinations were significantly higher ($p<0.05$) than those fed control diet and diet containing antibiotics, while the TMC showed that there were no significant differences ($p>0.05$) between broiler chickens fed L. *bulgaricus* and S. thermophillus but those fed S. *thermophillus* were significantly higher ($p<0.05$) than the remaining treatment groups. The result of the coliforms showed that broiler chickens fed control diet were significantly higher ($p<0.05$) than the remaining treatment groups except those fed diet containing antibiotics.

The results agreed with that of Jin *et al*. (1998) who noted an increase in the population of lactobacillus in the ileum of broiler chickens fed lactobacillus organisms. The use of lactobacillus in animals has been established to create a strong ability to attach to the walls of the intestinal epithelial cells of chicken *invitro*, this could be interpreted that their adherence and ability to survive in the intestine could have led to an increase in the population. The increased coliforms population observed in the ileum agrees with Watkins and Kratzer (1983) which indicated that chicks supplemented with *L. bulgaricus*, *S. thermophillus* and combination of the two had lower population.

Conclusion and Recommendation

This study showed that supplementation of probiotic organisms to diet of broiler chickens was beneficial in improving serum lipid profile and beneficial microbial population. Thus, supplementation of this aorganisms is

an essential practice that can enhance survivability of broiler chickens and ensures product quality thereby solving the problem of cholesterol synthesis in broiler chickens.It is recommended that the cost implication of using probiotic organisms is to be ascertained just like antibiotics in order to encourage acceptability by farmers.

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