BAP -12

On-Farm Evaluation of Three Strains of *Lactobacillus spp.* Based Probiotics on the Hematology and Serum Lipid Profile of Local Turkeys

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Abstract
A 16 week study was conducted to determine the effect of three strains of *Lactobacillus sp.* based probiotics on the hematological indices and serum lipid profile of local toms. A total of 80 day old local male poults were randomly assigned to four treatments (T1, T2, T3 and T4) in a completely randomized design with 20 birds per treatment. Three strains of *Lactobacillus* namely; *L. delbrueckii* sub sp. *bulgaricus, L. acidophilus* and *L. sporogens,* were incorporated in the drinking water of T2, T3, and T4 respectively at 0.5ml/L. T1 served as control. Each treatment was replicated twice with 10 birds per replicate. Feed and water were provided *ad libitum*. Results showed that the effect of the different strains of *Lactobacillus sp.* on hemoglobin, red blood cell count, white blood cell count, serum triglycerides, cholesterol, high density lipoproteins and low density lipoproteins were found to be significant ($p<0.05$). Birds on probiotics inclusion had least values for white blood cell counts, low density lipoproteins, cholesterol and serum triglycerides with T4 recording the most superior values. It was thus concluded that of the three strains of *Lactobacillus sp* based probiotics used in the study, *Lactobacillus sporogens* was observed to support superior hematological values with reduced lipid profiles.

Keywords: Direct-fed microbials, *Lactobacillus sp.,* turkey production, fat accretion, health status

Introduction

Toms are reared for their delicious and high quality meat (Prabakaran, 2003). They have high percentage of protein and low proportion of fat when compared with other poultry species (Nixey and Grey, 1985). Accretion of fats in poultry cut parts and visceral organs signify a waste to consumers who are ever more concerned about the nutritional and health benefit of their food (Oso *et al*., 2011). Diminution of fat and improvement of hematological indices in poultry birds has thus become a main focus of nutritional research as it serves as a genuine tool in disease diagnosis and assessment of health status in animals (Theml *et al.,* 2004).

Probiotics have been defined as mono- or mixed cultures of "live microorganisms primarily *Lactobacillus* (*sp.*) which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). Currently, the best studied probiotics are the lactic acid bacteria, particularly *Lactobacillus sp.* and *Bifidobacterium sp.* Effects of *Lactobacillus sp.* on production and their mode of action in monogastric animals have been reported in poultry (Panda *et al.,* 2007).

This work therefore seeks to determine the effect of three strains of *Lactobacillus sp.* on the hematological indices and serum lipid profile of local toms.

Materials and Methods

Location and duration of study

The study was carried out at Sabugo Farms, Nsukka. The experiment lasted for a period of 16 weeks..The three strains of the *Lactobacillus* – based probiotics used in the study included *Lactobacillus sporogenes* NRRL – 4496 (1×10^8 CFU/mL)*, Lactobacillus delbrueckii* subspecies *bulgaricus* NRRL B – 4527 (1×10^8 CFU/mL) and *Lactobacillus acidophilus* NRRL – 4495 (1×10^8 CFU/mL). They were obtained and constituted in a broth from the Microbial Genomics and Bio-processing Research Unit, National Centre for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, University St., Peoria, Illinois.

Three experimental diets were formulated for the starter (0-8 weeks), grower (9-12 weeks) and finisher (13-16 weeks) phases of the experiment. The percentage compositions of the experimental diets are presented in table 1. The experiment was carried out in accordance with the provisions of the Ethical Committee on the use of animals and humans for biomedical research of the University of Nigeria, Nsukka (2006). A total of 80 day old male poults were randomly divided into 4 groups of 20 birds each. Each group was randomly assigned to four treatments T1, T2, T3, and T4, using a completely randomized design (CRD). Each treatment was replicated twice with 10 birds per replicate placed in deep litter system. Birds on T2, T3 and T4 received probiotics of *L. delbrueckii* subspecies *bulgaricus, L. acidophilus* and *L. sporogenes* respectively at inclusion levels of 0.5ml/L in their drinking water for 3 consecutive days from 1 – 3, 10 – 12 and 21 – 23 days of age to achieve the recommended microbial concentration as stipulated by the supplier (10^6 CFU/mL). In other to protect the probiotic organisms, 0.5kg of powder

milk/1000L of their drinking water was used. T1 served as control with no strain of *Lactobacillus* inclusion. Feed and water were supplied *ad libitum* to the birds.

Table 1: Percentage composition of experimental diets

Ingredients	Starter (%)	Grower (%)	Finisher (%)
Maize	45.00	48.00	50.00
Soybean meal	40.00	37.00	24.10
Fishmeal	7.30	4.50	1.50
Wheat offal	0.00	4.00	17.55
Bone meal	4.30	3.20	3.65
Oyster shell	2.50	2.50	2.50
Vitamin Premix*	0.25	0.25	0.25
Salt	0.25	0.25	0.25
DL-methionine	0.30	0.20	0.10
Lysine	0.10	0.10	0.10
Total	100	100	100
Calculated composition			
Crude protein (%)	26.00	22.40	19.50
Crude fibre (%)	3.90	4.26	4.65
Ether extract (%)	5.00	4.52	4.31
ME (K cal/Kg)	2845.00	3100.00	3150.00

*Each 2.25 kg of vitamin premix contains; 10,000,000 I.U Vitamin A, 2,200,000 I.U Vitamin D3, 10,000 mg Vitamin E, 2000 mg Vitamin K3, 1500 mg Vitamin B1, 5000 mg Vitamin B2, 1500 mg, Vitamin B6, 10 mg Vitamin B12, 15,000 mg Niacin, 20 mg biotin, 125,000 mg Anti-Oxidant, 500 mg, Folic acid, 5000 mg Calpan.

Blood samples (about 2.5ml each) were collected from the wing vein of five toms per replicate at the end of 8th, 12th and 16th week of the study. A set was collected in EDTA bottles for the determination of hematological indices while another set was collected in plain tubes without EDTA. Plasma was harvested subsequently by centrifuging at 3000rpm for 15 minutes as described by Hayat *et al*. (1993).

Data collected were subjected to analysis of variance (ANOVA) using a statistical package (SAS, 2002) windows version 8.0 and mean differences were separated using Duncan's New Multiple Range Test as outlined by Obi (2002).

Results and Discussion

Effect of three strains of *Lactobacillus sp.* on haematological indices of local toms was shown in Table 2. There were significant ($p<0.05$) differences among treatment means on hemoglobin, red blood cell counts and white blood cell counts. The birds on T2, T3 and T4 recorded lower values for the white blood cell count than the birds on the control group with T4 having the least ($p<0.05$) values (19860.00 $cumm^3$, 19853.80 $cumm^3$ and 22650.00 $cumm^3$ for the birds' starter, grower and finisher phases respectively). These values decreased progressively with age. This is an indication that these birds on the probiotics treatment were not under any disease threat, as the normal range of values spans between 19400 – 24000$cumm^3$ (Alberts, 2005).

Table 2: Effect of three strains of *Lactobacillus sp.* based probiotics on hematological indices of local toms

	Parameters	Treatments				
		T1	T2	T3	T4	SEM
8 weeks	Packed cell volume(%)	38.98	38.94	39.00	38.80	0.54
	Hemoglobin (g/dl)	10.71^{c}	11.70^{b}	13.20^{a}	11.59^{b}	0.22
	Red blood cell ($x10^{12}$/l)	2.40^{c}	3.13^{b}	3.81^{a}	2.94^{b}	0.03
	White blood cell ($cumm^3$)	31140.00^{a}	28225.00^{b}	27480.00^{b}	19860.00^{d}	1529.45
12weeks	Packed cell volume(%)	32.10	33.40	32.40	31.01	1.66
	Hemoglobin (g/dl)	9.18^{c}	10.19^{b}	10.90^{a}	10.10^{b}	0.40
	Red blood cell ($x10^{12}$/l)	2.48^{c}	2.59^{b}	3.28^{a}	2.60^{b}	0.10
	White blood cell ($cumm^3$)	26337.10^{a}	24250.00^{b}	20094.10^{c}	19953.80^{c}	709.10
16 we.	Packed cell volume(%)	29.00	31.00	30.00	30.60	1.40
	Hemoglobin (g/dl)	9.19^{c}	9.92^{b}	10.01^{b}	14.00^{a}	0.33

	Red blood cell ($x10^{12}$/l)	2.46^{b}	2.50^{b}	2.47^{b}	3.39^{a}	0.10
	White blood cell ($cumm^{3}$)	26110.00^{a}	23400.00^{b}	22900.00^{b}	22650.00^{b}	701.68

[abc] Means on the same row having different superscripts are significantly different (p<0.05). SEM= Standard Error of Mean, T1= No probiotics inclusion (control), T2= *L. delbrueckii* subsp. *bulgaricus,* T3= *L. acidophilus,* T4= *L. sporogens*

Birds on T2, T3 and T4 also recorded high values for hemoglobin and red blood cell counts with T3 having the highest values at the starter phase. However, birds on T4 recorded highest values during the finisher phase. These high values can also be attributed to the role of these strains of *Lactobacillus sp.* in the synthesis of vitamin B_{12} which is implicated in the production of erythrocytes in the bone marrows, and also vitamin E which acts as an antioxidant to prevent erythrocyte destruction and promote hemoglobin concentration. Fuller (1992) reported that probiotic bacteria produce B vitamins, which are biocatalysts in food metabolism and help to fight stress. These results agree with the work of Cetin *et al.* (2005) who reported significant increase in the erythrocyte count, hemoglobin concentration and hematocrit values of turkeys fed probiotics.

Table 3 shows the effect of three strains of *Lactobacillus sp.* on serum lipid profile of local toms. There were significant (p<0.05) differences among treatment means on cholesterol, triglycerides, high density lipoprotein and low density lipoproteins. The birds on T2, T3, and T4 recorded lower values of cholesterol and low density lipoproteins than the control group as the birds mature, with T4 having the least values for starter, grower and finisher phases. The birds' cholesterol levels during the 8 weeks of age were similar (p<0.05) for birds on probiotics inclusion. But as they aged, their cholesterol values differed significantly (p<0.05) among the treatments. The birds' low density lipoproteins values also differed (p<0.05) among the treatments during the finisher phase, with T4 having the least (p<0.05) value among the treatment groups. The birds on probiotic inclusions also recorded higher (p<0.05) values of high density lipoproteins than the control group with T4 recording the highest (p<0.05) values during the 12th and 16th weeks of age. Birds on T4 recorded least (p<0.05) values for serum triglycerides for starter, grower and finisher phases. This reduction in the level of cholesterol, low density lipoproteins and triglycerides observed in the birds on *Lactobacillus sp.* based probiotics can be attributed to anti-cholesterolemic effect of these bacteria. Tahri *et al.* (1995) reported that lactic acid bacteria possess anti-cholesterolemic and anti-lipidemic factors which aid in cholesterol reduction. This is also in tandem with the report of Panda *et al.* (2007) who observed anti-cholesterolemic effects of *Lactobacillus sp.* when fed to food animals.

Table 3: Effect of three strains of *Lactobacillus sp.* based probiotics on serum lipid profile of local toms

Parameters		Treatments				
		T1	T2	T3	T4	SEM
8 weeks	Cholesterol (mg/dl)	141.50^{a}	131.20^{b}	131.03^{b}	130.90^{b}	0.10
	Triglycerides (mg/dl)	100.50^{c}	120.00^{a}	103.74^{a}	93.30^{d}	2.36
	HDL (mg/dl)	39.33^{c}	39.42	40.62	39.72	0.18
	LDL (mg/dl)	82.80^{a}	78.05^{b}	77.96^{b}	77.70^{b}	0.11
12 weeks	Cholesterol (mg/dl)	194.50^{a}	158.25^{b}	146.81^{c}	124.40^{d}	6.70
	Triglycerides (mg/dl)	120.20^{a}	114.10^{b}	111.01^{b}	106.25^{c}	2.47
	HDL (mg/dl)	40.69^{c}	41.57^{b}	41.44^{b}	45.25^{a}	1.36
	LDL (mg/dl)	122.01^{a}	68.51^{b}	67.50^{b}	67.47^{b}	0.12
16 weeks	Cholesterol (mg/dl)	192.64^{a}	156.48^{b}	141.45^{c}	114.34^{d}	7.20
	Triglycerides (mg/dl)	128.21^{a}	110.19^{b}	109.13^{b}	100.41^{c}	2.74
	HDL (mg/dl)	30.57^{d}	33.90^{c}	37.00^{b}	41.30^{a}	1.10
	LDL (mg/dl)	125.20^{a}	95.45^{b}	87.54^{c}	58.62^{d}	3.43

[abc] Means on the same row having different superscripts are significantly different (p<0.05). SEM= Standard Error of Mean, T1= No probiotics inclusion (control), T2= *L. delbrueckii* subsp. *bulgaricus,* T3= *L. acidophilus,* T4= *L. sporogens*

Conclusion and Recommendation

It is glaring from the results obtained in the current study that birds that received *L. sporogens* recorded a reduced serum triglycerides, low density lipoproteins, and cholesterol values than other treatment groups. *L. sporogens* was implicated in the high values of the red blood cell count and hemoglobin concentration of the toms at the later stage of growth. White blood cells count of birds that received *L. sporogens* was least among the treatment groups throughout the growth phases, which is an indication of the role of *L. sporogens* in modulating immunity of the birds. *Lactobacillus sporogens* based probiotic should therefore be used in local toms' nutrition. Nevertheless, mixed cultures of these strains alongside other strains of *Lactobacillus* or other bacteria based probiotics should be explored by future researchers.

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