

ON-FARM EVALUATION OF THREE STRAINS OF *Lactobacillus* sp. BASED PROBIOTICS ON THE HEMATOLOGY AND SERUM LIPID PROFILE OF LOCAL TOMS

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ABSTRACT

A 16-week study was conducted to determine the effect of three strains of *Lactobacillus* sp based probiotics on hematological indices and serum lipid profile of local toms. A total of 80-day-old local male poultts 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 0.5 ml/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 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.

Key words: direct-fed microbials, fat accretion, health status, *Lactobacillus* sp., turkey production

INTRODUCTION

Despite its greater potential than the chicken, turkey production has not been fully exploited in the developing countries (Perez-Lara *et al.*, 2013). Most research works on poultry are focused on the chicken with paucity of attention on turkeys (Speller, 2009). In the tropics, turkey thrives better than the domestic chicken. It manages heat stress better and has superior meat quality than the average chicken (Prabakaran, 2003; Yakubu *et al.*, 2013).

Fat accretion in poultry cut parts and visceral organs is an indication of waste to poultry product consumers. These consumers are ever more concerned about the nutritional and health benefit of their foods (Oso *et al.*, 2011). Fat reduction and improvement of hematological parameters in poultry birds have thus become a focal point for nutritional research as they serve as credible tools in disease diagnosis and assessment of health status in animals (Theml *et al.*, 2004). Several dietary manipulations have been used over the decades for diminution of fats in the blood and serum of poultry, including the use of probiotics (Cetin *et al.*, 2005; Ahmad, 2006; Ashayerizadeh *et al.*, 2009).

Probiotics are mono- or mixed cultures of 'live' microorganisms (Osita *et al.*, 2019). They are mainly *Lactobacillus* sp. and when well administered confer health benefits on the host (FAO/WHO, 2002). The ban on the use of antibiotics in poultry production necessitated the advent and use of probiotics in poultry production as alternatives (Edens, 2003). Currently, the best studied probiotics are the lactic acid bacteria, particularly *Lactobacillus* sp. and *Bifidobacterium* sp. (Vahjen *et al.*, 2002; Bielke *et al.*, 2003; Priyankarage *et al.*, 2004; Berrin, 2011).

Effects of *Lactobacillus* sp. on production and their mode of action in monogastric animals have been reported in poultry (Panda *et al.*, 2007; Apatá, 2008; Willis and Reid, 2008; Ashayerizadeh *et al.*, 2009; Awad *et al.*, 2009). The species currently used in probiotic preparations are varied and many. These are mostly *Lactobacillus bulgaricus*, *L. acidophilus*, *L. casei*, *L. helveticus*, *L. lactis*, *L. salivarius*, *L. plantarum* (Jin *et al.*, 2000; Christensen *et al.*, 2002; Lammers *et al.*, 2003; Rakoff-Nahoum *et al.*, 2004). The current study therefore seeks to determine the effect of three *Lactobacillus* strains on hematological indices and serum lipid profile of local toms.

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
<i>Calculated composition</i>			
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. Vit. A, 2,200,000 I.U. Vit. D3, 10,000 mg Vit. E, 2000 mg Vit. K3, 1500 mg Vit. B1, 5000 mg Vit. B2, 1500 mg Vit. B6, 10 mg Vit. B12, 15,000 mg Niacin, 20 mg biotin, 125,000 mg anti-oxidant, 500 mg, Folic acid, 5000 mg Calpan

MATERIALS AND METHODS

Location and Duration of Study

The study was conducted at the Turkey Unit of Sabugo Farms Limited, Umuakashi Industrial Layout, Nsukka. Nsukka lies within longitude 6° 45'E and 7°E and latitude 7°12.5'N and on the altitude 447m above sea level. The climate of the study area is typically tropical, with relative humidity ranging from 65 to 80% and mean daily temperature of 26.8°C (Agbagha *et al.*, 2000). The study lasted for 16 weeks.

Test Strains

The three strains of *Lactobacillus*-based probiotics used in the study were *Lactobacillus sporogenes* NRRL-4496 (1×10^8 CFU/mL), *L. delbrueckii* subspecies *bulgaricus* NRRL B-4527 (1×10^8 CFU/mL) and *L. acidophilus* NRRL-4495 (1×10^8 CFU/mL). They were obtained and constituted in a broth, from the Microbial Genomics and Bioprocessing Research Unit, National Centre for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, University Street, Peoria, Illinois.

Experimental Diets

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.

Management of Experimental Birds

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. 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 0.5 ml/L (10^6 CFU/mL) 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. In order to protect the probiotic organisms, 0.5 kg of powder milk/1000L was added to their drinking water. T1 served as control with no strain of *Lactobacillus* inclusion. Feed and water were supplied *ad libitum* to the birds.

Collection of Blood Samples

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 weeks of 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 collected subsequently by centrifuging at 3000 rpm for 15 min. as described by Hayat *et al.* (1993).

Statistical Analyses

Data collected were subjected to One way analysis of variance (ANOVA) using a statistical package (SAS, 2006) 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. Based Probiotics on Hematological Indices of Local Toms

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 packed cell volume of the birds was however found to be non-significant ($p > 0.05$) all through the birds' growing phases: starter (8 weeks of age), grower (12 weeks of age) and finisher (16 weeks of age). The birds on the *Lactobacillus* sp. based probiotics inclusion (T2, T3 and T4) recorded lower values for the white blood cell count than the birds on the control group with T4 (*L. sporogenes*) 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 birds on probiotics treatment were not under any disease threat, as the normal range of values spans between 19400 and 24000 cumm^3 (Alberts, 2005). Birds on T2, T3 and T4 also recorded high values for hemoglobin and red blood cell counts with T3 (*L. acidophilus*) having the highest values at the starter phase. However, birds on T4 recorded highest values during the finisher phase. These high values can also

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

	Parameters	Treatments				SEM
		T1	T2	T3	T4	
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 ^c	19860.00 ^d	1529.45
12 weeks	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 weeks	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*

be attributed to the role of these strains of *Lactobacillus sp.* in the synthesis of vitamin B₁₂ 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. It has been reported that probiotic bacteria produce B vitamins, such as folic acid, niacin, riboflavin, B₁₂, B₆ and pantothenic acid (Leblanc *et al.*, 2011), 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.

Effect of Three Strains of *Lactobacillus sp* Based Probiotics on Serum Lipid Profile of Local Toms

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 treatments on cholesterol, triglycerides, high-density lipoprotein and low-density lipoproteins. However, a non-significant difference ($p > 0.05$) was observed among the treatments in the birds' values for high-density lipoproteins during the starter phase (8 weeks of age). The birds on *Lactobacillus sp.* based probiotics inclusion (T2, T3, and T4) recorded lower values of cholesterol and low-density lipoproteins than those

in the control group as the birds mature, with T4 (*L. sporogens*) having the least values for starter, grower and finisher phases. The birds' cholesterol levels during the 8 and 12 weeks of age were statistically similar ($p < 0.05$) for birds on probiotics inclusion despite the difference in their numerical values. 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 (45.25 and 41.30 mg/dl, respectively). 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 anticholesterolemic effect of these bacteria. Chen *et al.* (2016) and Jeun *et al.* (2010) reported that lactic acid bacteria possess anticholesterolemic and antilipidemic factors which aided in cholesterol reduction. This is in tandem with the reports of Xie *et al.* (2011) and Panda *et al.* (2007) who observed anticholesterolemic 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				SEM
		T1	T2	T3	T4	
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 ^b	93.30 ^d	2.36
	HDL (mg/dl)	39.33	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

The results obtained in this study have shown that birds on probiotic inclusions especially those on T4 (*L. sporogens*) recorded reduced values of serum triglycerides, low-density lipoproteins and cholesterol than other treatment groups. *Lactobacillus 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, 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 other researchers.

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