



Effect of *Bacillus subtilis* and *Lactobacillus acidophilus* as Probiotic Feed Supplements on Growth Performance, Haematology, Biochemistry and Lipid Profile of Starter Broiler Chickens during the Wet Season

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Abstract

The study was conducted to determine the effect of *Bacillus subtilis* and *Lactobacillus acidophilus* as probiotics on growth performance, haematological and serum biochemical and serum lipid profile of starter broiler chickens during the wet season. Three hundred and sixty (360) day-old Cobb 500 broiler chicks were used in this study. The chicks were randomly allotted to six dietary treatments with three replicates consisting of 20 birds each in a completely randomized design. Control diet, *Bacillus subtilis* was included at 6 g, *Bacillus subtilis* 9 g, *Lactobacillus acidophilus* 200 g, *Lactobacillus acidophilus* 300 g and oxytetracycline 60 g per 100 kg diet. Parameters collected were on growth performance, haematology, serum biochemical parameters and serum lipid profile. The results showed that all growth parameters measured on the test materials had significant ($p < 0.05$) influence with the *Lactobacillus acidophilus* 200g being better in the improvement of final weight and total weight gain compared to the control group, but similar to the oxytetracycline group. Feed conversion ratio (FCR) of *Lactobacillus acidophilus* at 200 g had better FCR although similar to the control and the oxytetracycline groups. There were significant ($p < 0.05$) differences in all parameters measured for haematological profile except for red blood cells (RBC) count. Serum biochemical indices showed that there were significant ($p < 0.05$) differences for glucose, total protein, albumin, Alkaline phosphatase (ALP) and creatinine amongst treatment groups with probiotics inclusion groups having higher total protein and albumin when compared to the control and oxytetracycline groups. Lipid profile parameters considered also showed significant ($p < 0.05$) differences among treatment groups with the exception of high density lipo-protein. Therefore, it can be concluded that inclusion of *Bacillus subtilis* and *Lactobacillus acidophilus* as probiotics influenced positively some of the growth performance parameters, haematological, serum biochemical parameters and serum lipid profile of starter broiler chickens in the wet season. Therefore, *Bacillus subtilis* and *Lactobacillus acidophilus* can be used as candidate probiotic for starter broilers.

Keywords: Probiotics, Growth, Haematology, Lipid Profile, Starter Broiler, Wet Season.

Introduction

Poultry industry has been considered one of the most dynamic and ever-expanding sectors in the world. It helps to fill the gap between requirement and availability of high-quality protein for human consumption (Pervez and Abdul 2011). There is an increasing demand for quality in animal products, as well as concerns about the effects of these products on human health. Therefore, animal production systems will have to focus not only on obtaining high production, but also on their

impact on the environment as well as on human and animal health (Mountzouris *et al.*, 2010). The abuse of antibiotic growth promoters has become a global issue in animals and human's well-being (Nawaz *et al.*, 2016). This situation has driven much research on the search for alternatives that are able to maintain high productivity and to be economically feasible, as well as not being harmful to human and animal health, thereby complying with the requirements of consumers. Among these alternatives, the use

of probiotics in animal feeds stands out. These products do not leave residues in animal products and promote animal performance and health (Nawaz *et al.*, 2016; Patterson and Burkholder, 2003; Zulkifli *et al.*, 2000), because they improve diet digestibility (Apata, 2008), resulting in better nutrient utilization and consequently, higher productivity (Kabir *et al.*, 2004, Mountzouris *et al.*, 2007; 2010). The gastrointestinal tract (GIT) of the chicken and its gut state and condition are accountable for nutrient digestion, metabolism and absorption (Mabelebele *et al.*, 2014).

The addition of pure lactobacillus cultures and other bacteria to broiler diets has produced variable results. Probiotics display numerous health benefits beyond providing basic nutritional advantages (Quinteiro-Filho *et al.*, 2010). Probiotic products consisting of beneficial micro flora can help to establish and maintain the balance of the intestinal micro flora in commercial broilers. A consistent improvement in body weight gain of chickens fed a culture of lactobacillus has also been reported (Awad *et al.*, 2009). The gastrointestinal tracts (GITs) of chickens harbor microbial communities, or microbiomes, that play important roles in growth and development, including the production of energy-rich short chain fatty acids (SCFA), promotion of GIT villus and crypt morphology, nutrient utilization, including reduction in luminal viscosity, the deconstruction of dietary polysaccharides, nutrient absorption and well-being of their chicken hosts, including detoxification (Yeoman *et al.*, 2012; Hai *et al.*, 2010). The composition of the GIT microbiome reflects co-evolution among the inhabiting microbes, genetic, immune, and metabolic interactions with the host, and environmental influences (Yeoman *et al.*, 2011). The objective of this study is to evaluate the performance of starter

broiler chicken fed levels of probiotic products in the feed from 1 to 49 days of age.

Materials and Methods

Experimental site

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Science, Ahmadu Bello University, Zaria. The experimental site lies between latitude 11° 09' 06" N and longitude 7° 38' 35" E and at an altitude of 640m above sea level (Ovimaps, 2019). The temperature ranges between 14.5 – 39.5°C with humidity range of 21 – 77%. The climate is characterized by well-defined dry and wet seasons which are divided into cold-dry season (November - February), hot-dry season (March - May) and wet season (June - October) with annual rainfall ranging from 700-1400mm (IAR, 2019).

Experimental materials

The experimental materials used for this study were *Bacillus subtilis* (Enviva® PRO 202 GT) from Danisco Animal Nutrition with recommended level by manufacturer of 6g/100kg. *Lactobacillus acidophilus* probiotic strain (*LACTO Acidophilus*) manufactured by MIAVIT® in Germany with recommended level by manufacturer of 200g/100kg were used for the study.

Management of experimental animals

A total of 360 unsexed Cobb day-old broiler chicks were used for this study. The chicks were randomly allocated to 6 dietary treatments with three replicates consisting of 20 birds each in a completely randomized design. Birds were raised in a deep litter poultry house with feed and water provided *ad libitum*. Routine vaccines were given as recommended and mortality records kept as it occurred.

Table 1: Composition of Broiler Starter Diets in the Wet Season

Ingredients (%)	Treatment					
	Control	<i>B. subtilis</i>		<i>L. acidophilus</i>		Oxytetracycline
		6g	9g	200g	300g	
Maize	56.00	56.00	56.00	56.00	56.00	56.00
GNC	13.70	13.70	13.70	13.70	13.70	13.70
SBC	26.00	26.00	26.00	26.00	26.00	26.00
Bone Meal	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50	0.50
Common Salt	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.05	0.05	0.05	0.05	0.05	0.05
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Vit/min Premix ^A	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analyses						
ME Kcal/kg	2,904	2,904	2,904	2,904	2,904	2,904
Crude Protein (%)	23.21	23.21	23.21	23.21	23.21	23.21
Crude Fibre (%)	3.89	3.89	3.89	3.89	3.89	3.89
Ether Extract (%)	3.39	3.39	3.39	3.39	3.39	3.39
Calcium (%)	1.32	1.32	1.32	1.32	1.32	1.32
Phosphorus (%)	0.87	0.87	0.87	0.87	0.87	0.87
Lysine (%)	1.35	1.35	1.35	1.35	1.35	1.35
Methionine (%)	0.50	0.50	0.50	0.50	0.50	0.50
Feed cost (₦/Kg)	129.10	133.57	135.80	134.70	137.50	135.10

^A Vitamin- mineral premix provide per kg of diet: vit. A, 13,340 i.u; vit. D₃, 2680 i.u; vit. E, 10 i.u; vit. K, 2.68 mg calcium pantothenate, 10.68mg; vit. B₁₂, 0.022mg; folic acid, 0.668mg; choline chloride, 400mg; chlorotetracycline, 26.68mg; manganese, 13mg; iron, 66.68mg; zinc, 53.34mg; copper, 3.2mg; iodine, 1.86mg; cobalt, 0.268mg; selenium, 0.108mg. Oxytet = Oxytetracycline; GNC = Groundnut cake; SBM = Soya beans meal, *B. subtilis*= *Bacillus subtilis*, *L. acidophilus*= *Lactobacillus acidophilus*.

Table 2: Proximate Composition of Formulated Broiler Finisher Diet

Components	Control	<i>B.</i> 6g	<i>B.</i> 9g	<i>L.</i> 200g	<i>L.</i> 300g	Oxyte.
Dry matter	92.34	92.34	92.34	92.34	92.34	92.34
Crude protein (%)	25.75	25.75	25.75	25.75	25.75	25.75
Crude fibre (%)	4.72	4.72	4.72	4.72	4.72	4.72
Ether Extract	3.25	3.25	3.25	3.25	3.25	3.25
Ash (%)	6.62	6.62	6.62	6.62	6.62	6.62
NFE (%)	59.66	59.66	59.66	59.66	59.66	59.66

B. = *Bacillus subtilis*, *L.* = *Lactobacillus acidophilus*, Oxyte. = Oxytetracycline

Data collection and statistical analysis

Data was collected on growth performance (initial and final body weights, total weight gain and total feed consumed, feed conversion ratio feed cost/weight gain), haematological

indices (Packed Cell Volume (PCV), White Blood Cell (WBC), Red Blood Cell (RBC), Haemoglobin (Hb), Lymphocyte, Heterophil, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean

Corpuscular Haemoglobin Count (MCHC)), serum biochemical (Glucose (GLC), Total protein (TPR), Albumin (ALB), Globulin (GLB), Aspartate amino transferase (AST), Alanine amino transferase (ALT), Alkaline phosphatase (ALP), Uric acid, Creatinine), lipid profile (total cholesterol, triglyceride, high and low density lipoprotein and triglyceride: high density lipoprotein) and villus morphometric (area, perimeter, villus height, villus width, crypt depth and VH/CD). Blood samples were collected from the birds at the end of the experiment and used to determine haematological indices, serum biochemical indices and lipid profile. The data obtained were subjected to a one-way ANOVA test of the SAS (2002) version package while significant difference between means were separated using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussions

The result on growth performance of broiler starter chicks fed levels of probiotic based diet in wet season is presented in Table 3. The results showed that there were significant (P<0.05) differences in final weight and total

weight gain, feed intake and feed/gain ratio. In this experiment, final weight and total weight gain were enhanced and FCR was significantly similar for control, 200g *L. acidophilus* and oxytetracycline treatments. In agreement with our results, Zhang and Kim (2014) reported that dietary supplementation with probiotic significantly improved body weight gain and FCR in broiler chickens compared with chickens from the control treatment. Likewise, Sinol *et al.* (2012) reported that inclusion of *Bacillus subtilis* in broiler chicken diets resulted in improved FCR and weight gain. The result from this study is not in agreement with (Al-Khalaifah *et al.*, 2019) whose results showed that there was no significant effect of the different probiotics on the productive performance of broilers. Irrespective of the insignificant result reported by Al-Khalaifah *et al.* (2019), broiler bird's gastrointestinal tract consists of a diverse and dynamic population of microorganisms living in symbiotic relationship with their host. This mutualistic relationship is important for host nutrition, metabolism, and immunity. Therefore, the complex ecosystem works as a virtual organ system that aids in host homeostasis.

Table 3: Growth Performance of Broiler Starter Chicks Fed Levels of Probiotic Base Diet (0 – 4 weeks)

Parameters	Control	<i>B. subtilis</i>		<i>L. acidophilus</i>		Oxtet	SEM
		6g	9g	200g	300g		
Initial Weight (g/bird)	45.93	45.93	45.93	45.93	45.93	45.93	0.0 ^{ns}
Final Weight (g/bird)	1073.33 ^b	1074.20 ^b	1061.67 ^b	1116.97 ^a	1061.67 ^b	1132.97 ^a	20.31*
Total weight gain	1027.53 ^b	1028.40 ^b	1015.87 ^b	1071.17 ^a	1015.87 ^b	1087.17 ^a	20.31*
Total Feed Consumed (g/bird)	1966.50 ^c	2107.93 ^a	2081.67 ^a	2073.40 ^{ab}	2055.00 ^b	2145.17 ^a	37.44*
Feed Conversion Ratio	1.92 ^a	2.05 ^b	2.05 ^b	1.93 ^a	2.02 ^b	1.97 ^a	0.05*
Feed cost/gain (₦/Kg gain)	132.63 ^c	137.36 ^c	137.93 ^{bc}	144.30 ^{ab}	139.70 ^b	146.90 ^a	2.68*
Mortality	0.00	0.00	0.00	0.00	0.00	0.00	0.00

^{abc}= Means with different superscript on the same row are significantly different (P<0.05), SEM= Standard Error of Mean, Oxytet= Oxytetracycline, ns= not significant.

With regard to the influence of probiotic on haematological parameters investigated in this study, there were statistical changes observed

among treatment groups at all times of this trial as illustrated in Table 4. The findings agree with that of Cetin *et al.* (2005), who observed

that the probiotic supplementation caused statistical increase in the PCV, haemoglobin concentration and haematocrit values of Turkeys. The differences may be attributed to type and number of species of bacteria present in probiotics. In contrast, the findings disagree with the study done by Alkhalf *et al.* (2010) who found that the probiotic supplementation did not affect the blood constituents especially, haemoglobin concentrations. However, all parameters fall within the normal range for healthy chickens as reported by Mitruka and Rawnsley (1997) and Jain, (1986). The blood is used as an indicator that birds are adequately nourished and fed diets of good quality. Isaac *et al.* (2013) reported that good blood

composition results in good performance of animals. This indicates that probiotics may improve health condition by enhancing concentration of haemoglobin and red blood cells count in broiler chicks. Owasibo *et al.*, (2013) reported that probiotics did not show any harmful changes on haematological parameters and it could improve health condition by enhancing concentration of haemoglobin, hematocrit and red blood cell count in broilers. Increased PCV, and in broilers would enhance blood formation, improve oxygen, CO₂ and sugar carrying capacity of the blood, prevent oxidative damage and improve health status (Wakenell, 2010).

Table 4: Effect of Probiotic Levels on Haematological Profile of Broiler Chickens at 0 – 4 weeks

Parameters	Control	<i>B. subtilis</i>		<i>L. acidophilus</i>		Oxytet	SEM
		6g	9g	200g	300g		
PCV (%)	41.00 ^a	43.73 ^a	42.26 ^a	35.00 ^b	41.43 ^a	38.86 ^{ab}	2.93*
Hb (g/dL)	12.30 ^a	12.60 ^a	12.70 ^a	10.16 ^b	12.40 ^a	11.90 ^a	0.82*
WBC (x10 ⁹ /uL)	112.17 ^a	115.07 ^a	111.03 ^a	92.47 ^b	109.80 ^a	109.47 ^a	8.16*
RBC (x10 ¹² /uL)	2.88	2.99	2.97	3.42	2.94	2.77	0.35 ^{ns}
Lymphocytes (%)	82.53 ^{ab}	84.06 ^a	84.80 ^a	84.03 ^a	82.76 ^{ab}	81.23 ^b	1.38*
Heterophil	6.66 ^a	6.80 ^a	6.66 ^a	7.06 ^a	3.56 ^b	7.76 ^a	1.40*
MCV (fL)	142.23 ^a	146.13 ^a	142.00 ^a	113.57 ^b	143.67 ^a	140.30 ^a	11.58*
MCH (Pg)	42.80 ^a	42.16 ^a	42.86 ^a	33.23 ^b	43.00 ^a	42.86 ^a	3.60*
MCHC (g/dL)	30.06 ^a	28.80 ^b	30.16 ^a	28.90 ^b	29.96 ^{ab}	30.60 ^a	0.68*

^{abc}= Means with different superscript on the same row are significantly different (P<0.05), SEM= Standard Error of Mean, PCV= packed cell volume, Hb= haemoglobin, WBC= white blood cell, TP= total protein, Heat= heterophil, Lymph= lymphocyte, RBC= red blood cell, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin count, ns= not significant.

The results on serum biochemical indices of broiler chickens fed levels of probiotics-based diets in wet season is shown in Table 5. The results show significant (P<0.05) differences among treatment groups in most parameters measured except GLB, AST, ALT and uric acid. Serum glucose increased in probiotics supplemented groups compared to the control. The present results agree with Shareef and Al-

Dabbagh, (2009) who recorded that addition of probiotics significantly increase the level of serum glucose, whereas it disagrees with Azza *et al.*, (2012) who reported no significant differences in the groups fed probiotics.

The results of this study concurred with that of Siadati *et al.* (2017) who observed that probiotic supplementation increased the total protein and improved the growth performance

in Japanese quail. Yazhini *et al.* (2018) also reported that probiotics significantly increased mean total serum protein and albumin as compared to the birds that were not supplemented. However, the results of this study did not agree with findings of Li *et al.* (2014) (*Lactobacillus sporogenes*) and Abdel-Hafeez *et al.* (2017) who observed that the serum total protein concentration of birds supplemented with probiotic was significantly ($p<0.05$) lower than the control birds. The lactic acid bacteria competitively exclude the pathogenic bacteria which reduce the breakdown of proteins to nitrogen and reduce the efficiency of dietary protein (Yazhini *et al.*,

2018). Thus, the utilization of amino acids and proteins is improved. The liver is another important organ in regulating immunity due to its abundance of innate cells responsible for defending foreign antigens (Holz *et al.*, 2008). Creatinine is a chemical waste molecule that is generated from muscle metabolism when creatinine phosphate is catabolized. Higher creatinine level in broilers could suggest depletion of muscle protein and impairment of protein utilization which could be attributed to poor growth performance in such birds (Fafiolu *et al.*, 2014). The creatinine levels in this study did not pose any harmful threat to the bird.

Table 5: Effect of Probiotic Levels on Serum Biochemical Parameters of Broiler Chicks in Wet Season (0 – 4 weeks)

Parameters	Control	<i>B. subtilis</i>		<i>L. acidophilus</i>		Oxytet	SEM
		6g	9g	200g	300g		
GLC (mmol/L)	107.60 ^c	114.60 ^{bc}	112.50 ^{bc}	114.56 ^{bc}	115.40 ^b	131.33 ^a	3.64*
TPR (g/L)	4.64 ^b	5.33 ^a	4.76 ^{ab}	4.65 ^b	4.51 ^b	4.64 ^b	0.29*
ALB (g/dL)	1.62 ^b	2.20 ^a	1.97 ^{ab}	1.81 ^{ab}	2.12 ^a	1.56 ^b	0.24*
GLB (g/L)	3.08	2.82	3.00	2.79	2.53	2.53	0.31 ^{ns}
AST (μL)	45.30	45.16	47.33	47.43	47.50	48.13	3.03 ^{ns}
ALT (μL)	27.60	30.20	25.40	27.93	28.46	28.60	2.50 ^{ns}
ALP (μL)	71.20 ^b	73.33 ^b	78.56 ^{ab}	85.40 ^a	75.63 ^{ab}	81.50 ^{ab}	5.31*
Uric acid (mg/dL)	4.66	5.33	5.00	5.66	4.33	4.66	0.69 ^{ns}
Creatine (mg/dL)	1.16 ^b	1.30 ^{ab}	1.46 ^{ab}	1.40 ^{ab}	1.40 ^{ab}	1.53 ^a	0.16*

^{abc}= Means with different superscript on the same row are significantly different ($P<0.05$), GLC= Glucose, TRP= Total protein, ALB=Albumin, GLB=Globulin, AST=Aspartate amino transferase, ALT=Alanine amino transferase, ALP=Alkaline phosphatase, SEM= Standard Error of Mean, Oxytet= Oxytetracycline, ns= not significant

Table 6: Effect of Probiotic Levels on Lipid Profile of Broiler Chickens in Wet Season (0 – 4 weeks)

Parameters	Control	<i>B. subtilis</i>		<i>L. acidophilus</i>		Oxytet	SEM
		6g	9g	200g	300g		
TCHOL (mg/dL)	86.65 ^a	86.38 ^a	79.74 ^{ab}	73.18 ^b	79.50 ^a	82.17 ^{ab}	5.20*
TRYGL (mg/dL)	40.56 ^{ab}	37.40 ^b	41.08 ^{ab}	36.39 ^b	46.91 ^a	38.72 ^b	4.08*
HDL (mg/dL)	23.96	24.95	20.37	22.01	19.56	28.11	5.12 ^{ns}
LDL (mg/dL)	15.85 ^{bc}	24.30 ^a	18.48 ^b	14.77 ^c	16.62 ^{bc}	15.33 ^{bc}	1.68*
Tg/HDL (mg/dL)	2.01 ^{ab}	1.71 ^b	2.35 ^{ab}	1.78 ^{ab}	3.02 ^a	1.38 ^b	0.64*

^{abc}= Means with different superscript on the same row are significantly different ($P<0.05$), TCHOL., = Total Cholesterol, TRYGL., = Tryglyceride, HDL., = High Density Lipoprotein, LDL = Low Density Lipoprotein, Oxytet = Oxytetracycline SEM= Standard Error of Mean ns= not significant.

The result on the effects of probiotics-based diet on lipid profile of broiler chickens in wet season is shown in Table 6. There were significant ($P < 0.05$) differences in all parameters measured except for HDL. Results of lipid profile showed significant decrease in the values of cholesterol, triglycerides, HDL and LDL in all probiotics groups compared with the control during the whole period of the experiment. This could be attributed to reduced absorption and/or synthesis of cholesterol in the gastrointestinal tract by probiotic supplementation. Mohan *et al.*, (1996) and Surono, (2003) speculated that *Lactobacillus acidophilus* reduces cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis. Also, Uzochukwu *et al.* (2015) reported that probiotics may possess the property of reducing cholesterol in the blood by inhibition of its hepatic synthesis, or by deconjugating the biliary salts. However, probiotics reduces abdominal fat in broilers due to microbiota present in gastro-intestinal tract of birds which impairs the absorption of cholesterol and bile acid (Mohammadreza *et al.*, 2016). Patterson and Burkholder, (2003) reported that probiotics decrease triglycerides and attributed the reason that probiotics secrete inulin which have an inhibitory effect on the process of building triglycerides.

Conclusion

It is be concluded that supplementation of the probiotics significantly increased serum total protein, and albumin and significantly lowered mean total serum cholesterol, LDL cholesterol, and triglycerides as compared to the birds that were not supplemented with any probiotic. Dietary supplementation of probiotic *Lactobacillus acidophilus* 200g at the early days of the rearing periods of broiler chickens had a positive effect on intestinal microbial population, leading to more nutrients being assimilated by the

chickens and consequently greater performance in broiler chickens.

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