Effect of dietary supplementation with probiotics and prebiotics on haematological indices, serum chemistry and gut salmonella count of broilers sourced from Salmonella-infected hatcheries in south-west zone of Nigeria.

Abstract

This study investigated the effect of dietary inclusion of probiotics and prebiotics against gut salmonella, haematological indices and serum biochemistry of broilers sourced from Salmonella infected hatcheries within south-west zone of Nigeria**.**

In this experiment, a total of 480 male, day-old broilers (Anak 2000 Strain) (160 birds from each state) were collected from hatcheries located in 3 different areas of south west Nigeria that were positive to *Salmonella* organisms and used for performance testing for 8 weeks. The birds were laid out in a 3x5 factorial arrangement comprising of 3 hatchery location fed diet supplemented with 5 different feed additives (no additive, antibiotics, mannose oligosaccharides, arabinoxylan oligosaccharides, Sim Lac®). Data were collected on blood, and gut salmonella count. Main effect of location showed that total serum protein, white blood cell count, haemoglobin, uric acid and serum creatinine were highest (P<0.05) for broiler starter sourced from Location 3. All broiler starter fed diet supplemented with different additives had reduced (P<0.05) white blood cell, uric acid concentration, increased (P<0.05) serum glucose and albumin. Broiler starter sourced from Location 1 and 3 fed diet supplemented with varying additives had reduced serum uric acid concentration when compared to birds fed control diet. At the finisher phase, haemoglobin, red blood cell, serum uric acid and creatinine concentration were highest for birds sourced from Location 3. Finisher broilers fed diet supplemented with varying additive had increased (P<0.05) serum albumin and reduced (P<0.05) serum uric acid concentration. Finisher broilers sourced from location 3 and fed diet supplemented with additive had reduced (P<0.05) white blood cell count, serum uric acid and increased (P<0.05) serum albumin when compared with group on control diet. In all the phases of the study, gut salmonella reduced drastically with inclusion of various additives. Birds fed diet supplemented with MOS showed the least gut salmonella count. Mannose oligosaccharides and arabinoxylan oligosaccharides could be used as replacement for antibiotics to improve performance and control the prevalence of *Salmonella* organism in broiler chickens.

Keywords: Haematological indices, oxytetracycline, prebiotics, probiotics, serum chemistry

INTRODUCTION: Poultry production is an attractive enterprise because it yields high economic value within a very short generation interval. Poultry production is an important source of animal protein, income, employment, industrial raw materials, and manure (Rosen, 1995). Therefore, importance of broiler production cannot be over emphasized in the face of the rising demand for animal protein within developing countries like Nigeria. To maximize the genetic potential of broiler for production, these birds must be free from diseases as well as fed with appropriate diets that will meet their requirement for optimal production

(Tannock, 1998). Enteric diseases are an important concern to the poultry industry because it reduced productivity, increased mortality and could cause contamination of poultry products leading to food poisoning in humans (Tannock, 1998). In the healthy animal, balance in floral ecology in the gastrointestinal tract helps in efficient digestion, optimal absorption of nutrients, and increases the body's resistance to infectious diseases (Rosen, 1995).

Incidence of Salmonellosis has been traced to the presence of pathogenic Salmonella organism in human food chain as a result of infected droppings material, poor hygiene or from infected eggs. Literatures have confirmed that major aetiological source for wide prevalence of typhoid and paratyphoid cases in approximately 13 million cases of paratyphoid infections worldwide (Murugkar *et al*., 2005). Unfortunately, poultry meat is the major source of food borne Salmonella paratyphoid infection (Mayrhofer *et al.,* 2003) which had caused severe economic losses, high mortality and medication cost but with attendant reduction in egg production in breeder flocks, poor chicks quality and high cost for eradication and control measures.

Salmonellosis has been reported as a major cause of high mortality of poultry birds, affecting many bird species worldwide (Hafez, 2001). Several strains of Salmonella bacterium are more closely associated with poultry morbidity than their ubiquitous distribution of various pathogenic serotypes (serovars) like pullorum, gallinarum, enteritidis and typhimurium (Hafez and Jodas, 2000). These pathogenic serotypes constantly passed down the food chain through poultry products to infect numerous consumers. In the poultry industry, antibiotics are used worldwide to prevent poultry pathogens and disease so as to improve meat and egg production. However, the use of dietary antibiotics or feed additives has resulted in high level antimicrobial resistance (Sorum and Sunde, 2001), while its residues in poultry products significantly enhance imbalance of normal intestinal microflora and mucosa fluid and electrolyte. Therefore, alternative and safe intervention to overcome these important problems is the use of probiotics and prebiotics bacteria which have a wide range of nutritional benefits, support physiological absorption activity of intestinal mucosa and effectively combat diarrheal syndromes (Huyghebaert *et al.,* 2011). Competition for nutrients, production of antimicrobial compounds (such as low pH volatile fatty acids and bacteriocins), binding sites on the intestinal epithelium and stimulation of immune system (Rolfe, 2000) are proposed mechanisms of pathogen inhibition by probiotics organisms and prebiotics (Koenen *et al*., 2004).

The use of alternative in-feed growth stimulant like probiotics and prebiotics beneficially affect the host microbial flora (Huyghebaert *et al.,* 2011). Probiotics are direct-fed microbial feed supplements which modulate the gut microflora by successfully competing with pathogens through a competitive exclusion process (Mountzouris *et al.,* 2007). Probiotics are usually live or attenuated organisms and spores (Patterson and Burkholder 2003), non-traditional chemicals (Moore *et al.,* 2006), bacteriophages (Higgins *et al*., 2005), organic acids (Jarquin *et al*., 2007; Wolfenden *et al.,* 2007) and several others that have emerged in the last decades which are potentially useful (Revolledo *et al.,* 2009). Beneficial effects of probiotics were observed in toxin neutralization, inhibition of microbial metabolism and immunity stimulation (Sleator, 2008, Sherman *et al.,* 2009) conferring healthy gastrointestinal microbiota on host (Gibson *et al.,* 2004).

Prebiotics are defined as ''non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth or activity or both of limited number of bacteria in the colon, which can improve host health. One of such prebiotic is mannose oligosaccharide (MOS) which is derived from the cell wall of the yeast Saccharomyces cerevisiae and effectively influences intestinal activities and animal performance. MOS has the capacity to modulate the immune system and the gut microflora, agglutinate a wide range of pathogenic bacteria and as a result preserve the integrity of the intestinal absorptive surface (Sevage *et al;* 1996). Reducing intestinal colonization of pathogenic microbes using competitive exclusion compounds

(probiotics) during the grow-out period is crucial to improve the microbiological quality of poultry products as well as for consumer health (Vugia 1993).

This study therefore seeks to investigate the effect of dietary supplementation with various feed additives (antibiotics, mannose oligosaccharides, arabinoxylan oligosaccharides, Sim Lac®) on haematological indices and serum biochemistry of broilers sourced from Salmonella-infected hatcheries within south-west zone of Nigeria, West Africa.

MATERIALS AND METHODS

Experimental Location: The *in vivo* studies were carried out at the Teaching and Research Farm Development (TREFAD) of the University of Agriculture, Abeokuta, Nigeria, West Africa (7^0 10^1N and 3^0 2^1E) area which is 76m above sea level, humid and located in the tropical rain forest vegetation zone with an average temperature of 34.7^{o}C.

Salmonella screening of hatcheries

Preliminary screening of all hatcheries located within south-west Nigeria were done by collecting hatching eggs (50 eggs/hatchery) on weekly basis over a period of 8 weeks from all the hatcheries existing within this area. The findings of this preliminary screening of hatcheries which has been published by the author (Olorunsola *et al*., 2012) identified three potentially infected hatcheries in 3 different locations (Location 1, 2 and 3). These identified hatcheries were used in this study as the source of day-old chicks used for feeding trial.

Experimental birds and management: A total number of four hundred and eighty (480) male, day-old broilers (Anak 2000) collected from the three infected hatcheries [located in Oyo state (Location 1), Ogun state (Location 2) and Lagos state (Location 3), southwest Nigeria] were used in this study. One hundred and sixty (160) day-old broiler chicks were randomly sourced from each hatchery making a total of four hundred and eighty (480) broilers used for the study. Birds from each location were allotted into 5 treatment groups of 32 birds each. Each treatment was replicated four times with 8 birds each. Birds contained in each replicate were housed in individual pen each measuring 2.7m × 0.9m with a total floor area of 2.43m^2. Birds were brooded for 21 days on a deep litter system littered with wood shavings. Water and feed were supplied *ad libitum*. No medication was administered throughout the experimental period which lasted for 56 days.

Dietary Treatments: A standard basal diet containing no additive (control diet) was formulated for the starter and finisher phase of the broilers while four additional experimental diets were formulated such that basal diets were supplemented each with antibiotics (oxyteraxycline at 1g/kg), MOS (1 g/kg), arabinoxylose oligosaccharide (1g/kg) and Sim®-Lac (1g/kg). SimLac (a product containing 1×10^{10}cfu viable strain of *Prediococcus acidilactis per gram*) was supplied by a commercial company (Simbiyotec Biological Product Inc. Tuzaistanbul). Mannose oligosaccharides (MOS) and arabinoxylans oligosaccharides (AXOS) were obtained commercially (Alltech Inc. Kentucky, USA) and used as prebiotics. The inclusion level of Sim®-Lac used in this study was according to manufacturer's specifications. Inclusion level of prebiotics (AXOS and MOS) used in the current study was based on previous studies (Bovera *et al.*, 2010) while the therapeutic dosage of oxytetracycline was used as a positive control. The diets were formulated to meet the NRC (1994) nutrient requirement of broilers.

Table 1 Percentage Composition of experimental diets fed to starter broilers (0 -4 weeks)

Ingredients %	Experimental		Diets		
	1	**2**	**3**	**4**	5
Maize	46.00	46.00	46.00	46.00	**46.00**
SBM	19.00	19.00	19.00	19.00	**19.00**
GNC	15.00	15.00	15.00	15.00	**15.00**
Fish meal (72%)	2.00	2.00	2.00	2.00	**2.00**
Wheat offal	11.95	11.95	11.95	11.95	**11.95**
Bone meal	2.00	2.00	2.00	2.00	**2.00**
Oyster shell	3.00	3.00	3.00	3.00	**3.00**
Salt	0.25	0.25	0.25	0.25	**0.25**
∗Premix	0.25	0.25	0.25	0.25	**0.25**
Methionine	0.30	0.30	0.30	0.30	**0.30**
Lysine	0.25	0.25	0.25	0.25	**0.25**
[1].oxytetracycline	-	+	-	-	-
[2].MOS	-	-	+	-	-
[3].AXOS	-	-	-	+	-
[4]. Sim® lac	-	-	-	-	+
Total	**100.00**	**100.00**	**100.00**	**100.00**	100.00
	DeterminedAnalysis				
Metabolizable energy	11.83	11.83	11.83	11.83	**11.83**
Crude protein %	23.22	23.22	23.22	23.22	**23.22**
Crude fibre %	4.88	4.88	4.88	4.88	**4.88**
Ether extract %	5.63	5.63	5.63	5.63	**5.63**
Available Ca %	1.97	1.97	1.97	1.97	**1.97**
Available P %	0.44	0.44	0.44	0.44	**0.44**
Lysine %	1.34	1.34	1.34	1.34	**1.34**
Methionine %	0.65	0.65	0.65	0.65	**0.65**

∗Vitamin and mineral premix based on 2.5kg/ ton [Vit A; 4000000 Iu, Vit D:800000, Vit B12: 25mg, Niacin:60000mg, Vit E 40000, Viut k3 800mg, Vit B3 1000mg, Vit B26000mg, Vit B6 5000mg, Panthotenic Acid: 20000, Folic Acid: 200mg, Biotine 8mg, Maganese:300000mg, Iron 80000mg, Zinc: 20000mg, Copper: nill, Cobalt: 80mg, Iodine: 400mg, Selenium:40mg,Choline:800000mg**]**

Table 2 Percentage Composition of experimental diets fed to finisher broilers (4 -8 weeks)

Ingredients %	Experimental		Diets		
	1	**2**	**3**	**4**	5
Maize	50.00	50.00	50.00	50.00	**50.00**

SBM	12.00	12.00	12.00	12.00	**12.00**
GNC	11.00	11.00	11.00	11.00	**11.00**
Fish meal (72%)	2.00	2.00	2.00	2.00	**2.00**
Wheat offal	19.00	19.00	19.00	19.00	**19.00**
Bone meal	2.00	2.00	2.00	2.00	**2.00**
Oyster shell	3.00	3.00	3.00	3.00	**3.00**
Methionine	0.25	0.25	0.25	0.25	**0.25**
Lysine	0.25	0.25	0.25	0.25	**0.25**
∗Premix	0.25	0.25	0.25	0.25	**0.25**
Salt	0.25	0.25	0.25	0.25	**0.25**
[1].Oxytetracycline	-	+	-	-	-
[2].MOS	-	-	+	-	-
[3].AXOS	-	-	-	+	-
[4].Sim® lac	-	-	-	-	+
Total	**100.00**	**100.00**	**100.00**	**100.00**	100.00
Determined					
Metabolizable	12.43	12.43	12.43	12.43	**12.43**
Crude protein %	19.90	19.90	19.90	19.90	**19.90**
Crude fibre %	5.66	5.66	5.66	5.66	**5.66**
Ether extract %	4.81	4.81	4.81	4.81	**4.81**
Available Ca %	1.97	1.97	1.97	1.97	**1.97**
Available P %	0.44	0.44	0.44	0.44	**0.44**
Lysine %	1.13	1.13	1.13	1.13	**1.13**
Methionine %	**0.56**	**0.56**	**0.56**	**0.56**	**0.56**

- Vitamin and mineral premix based on 2.5kg/ ton

Vit A; 4000000 Iu, Vit D:800000, Vit B12: 25mg, Niacin:60000mg, Vit E 40000, Viut k3 800mg, Vit B3 1000mg, Vit B2 6000mg, Vit B6 5000mg, Panthotenic Acid: 20000, Folic Acid: 200mg, Biotine 8mg, Maganese:300000mg, Iron 80000mg, Zinc: 20000mg, Copper: nill, Cobalt: 80mg, Iodine: 400mg, Selenium: 40mg, Choline: 800000mg

Blood sample collection

At 28th and 56th day of the study, blood samples (2.5 ml each) were collected from the brachial wing vein of four birds per replicate (*n = 16 per treatment*) into vials containing Ethylene diamine tetra-acetate (EDTA) for the determination of haematological indices while another set of blood was collected into plain bottles (without EDTA), centrifuged (2,500 × *g* for 15 min) and used for serum chemistry.

Determination of haematological indices: Haemoglobin concentration (Hb) was estimated using the cyanmethaemoglobin method (Cannan 1958). Packed cell volume (PCV) of blood samples were determined in a Wintrobe haematocrit tube according to the method of Schalm et al. (1975) while total red blood cell and white blood cell count (WBC) were determined using standard method. Differential leucocyte counts (heterophils, lymphocytes, eosinophils and monocytes) were carried out on blood smears stained with May-Grunwald-Giemsa stain and further calculated.

Estimation of serum chemistry: Total serum protein, serum albumin and globulin were determined using bromocresol purple method (Varley *et al.*, 1980). Serum creatinine and uric acid concentration was determined according to standard procedures. Serum enzymes (alanine transaminase (ALT) and aspartate transaminase (AST) were analysed spectrophotometrically using Randox^{R} diagnostic kit. The serum cholesterol was estimated using standard kit (Qualigens India. Pvt. Ltd., Catalogue number 72201-04).

Gut Salmonella count and isolation

A bird per replicate at day 1, 28 and 56 of the study were selected, slaughtered and dissected to separate the gastro-intestinal tract. Intestinal content of the birds gut was aseptically collected into sterile plain bottle and total salmonella load was estimated with the aid of appropriate agar (Xylose Lysine Deoxycholate agar) using pour plate method as described by Hedges (2012). Salmonella colonies were counted and the results were expressed according to plate count proceedings established in Normative Instruction (Brazil, 2003). Bacterial count was carried out according to modified method of Desmidt *et al., (1998*).

Statistical Analysis: Data obtained from this study was laid out in a 3 × 5 factorial arrangements consisting of broilers sourced from 3 different hatcheries (Location 1, 2 and 3) and fed diet supplemented with 5 different additives (no additive, antibiotics, mannose oligosaccharides, arabinoxylan oligosaccharides, Sim Lac®). The design of the experiment was a completely randomized design (CRD). Bacterial colony counts formerly in colony forming unit per gram (CFU/g) were transformed into log base 10. Data obtained were subjected to analysis of variance using SAS (1998) while significant means among variables were separated using the Tukeys Test.

RESULTS

The result of the main effect of hatchery source and inclusion of various feed additives on the haematological indices and serum biochemistry of starting broiler is presented in Table 3. Result revealed that total serum protein, white blood cell count, haemoglobin, uric acid and serum creatinine were highest ($P<0.05$) for broiler starter sourced from Location 3. Birds from Location 1 and 2 recorded reduced ($P<0.05$) total serum protein, white blood cell count, haemoglobin, uric acid and serum creatinine values. Birds from Location 1 and 2 had the highest serum albumin and serum glucose concentration, respectively. Birds fed with no additive (control diet) had the least ($P<0.05$) serum glucose, serum albumin and the highest ($P<0.05$) serum uric acid concentration. Birds fed diet supplemented with SimLac had the least ($P<0.05$) white blood cell count while those fed diet supplemented with arabinoxylans oligosaccharides recorded the highest ($P<0.05$) serum glucose. All birds fed diet supplemented with additive had reduced ($P<0.05$) white blood cell, uric acid concentration, increased ($P<0.05$) serum glucose and albumin.

The interaction effect of hatchery source and feed additives on the haematological indices and serum biochemistry of starting broiler is shown in Table 4. The results revealed a significant ($P<0.05$) interaction effect on PCV, Hb, WBC count, red blood cells, total serum protein, serum albumin and uric acid. Birds souced from Location 3 and fed diet supplemented with arabinoxylans oligosaccharides had the highest ($P<0.05$) haemoglobin concentration. Highest ($P<0.05$) white blood cell count was obtained with birds sourced from Location 1 and fed control diet. Highest ($P<0.05$) total serum protein was obtained with birds sourced from Location 2 and fed diet supplemented with SimLac. All birds sourced from Location 1 and 3 supplemented with varying additives had reduced serum uric acid concentration when compared to birds fed control diet. Glucose, serum globulin, cholesterol and creatinine showed no significant ($P>0.05$) interaction effect.

The result of the main effect of hatchery source and inclusion of various feed additives on the haematological indices and serum biochemistry of finisher broiler is presented in Table 5. Result revealed that haemoglobin, red blood cell, serum uric acid concentration and creatinine were highest for birds sourced from Location 3. Birds sourced from Location 2 and 3 recorded the highest ($P<0.05$) serum globulin. Birds sourced from Location 2 had the highest serum glucose while birds sourced from Location 1 has the least ($P<0.05$) haemoglobin concentration. Birds fed diet supplemented with SimLac recorded the least ($P<0.05$) white blood cell count. Birds fed control diet having no additive recorded the least ($P<0.05$) serum glucose while birds fed diet supplemented with mannan oligosaccharides had the least ($P<0.05$) serum globulin. All birds fed diet supplemented with varying additive had increased ($P<0.05$) serum albumin and reduced ($P<0.05$) serum uric acid concentration.

The interaction effect of hatchery source and feed additives on the haematological indices and serum biochemistry of starting broiler is shown in Table 6. The results revealed a significant ($P<0.05$) interaction effect on all parameters with the exception of serum globulin, glucose, cholesterol and creatinine. All birds sourced from location 3 and fed diet supplemented with additive had reduced ($P<0.05$) white blood cell count, serum uric acid and increased serum albumin when compared with group on control diet. Highest ($P<0.05$) serum albumin was obtained with birds sourced from Location 1 fed diet supplemented with MOS and birds sourced from Location 3 supplemented with mannan oligosaccharides.

Table 7 showed that the main effect of location showed no effect on gut salmonella count of the birds. There was a significant reduction ($P<0.05$) in Salmonella count at day 1 of the study when various additives were added. At day 28 and 56, inclusion of MOS showed the least salmonella count. Similar salmonella counts were obtained at day 28 for birds fed diet supplemented with antibiotics and AXOS. In all the phases of the study, gut salmonella reduced drastically with inclusion of various additives. Birds fed diet supplemented with MOS showed the least gut salmonella count.

Table 3 Main effect of location of hatchery and feed additives on blood parameters of broiler chickens (0-4weeks)

	Location of Hatchery				Additives					
Parameters	1	2	3	SEM	Control	AB	MOS	AXOS	SIML	SEM
PCV (%)	31.9	32.7	35.9	1.55	34.8	31.1	31.1	33.0	33.8	1.99
Hb(g/dl)	10.6^{b}	11.0^{b}	14.6^{a}	0.50	12.6	12.2	11.3	12.4	12.0	0.65
WBC ($10^3/mm^3$)	30.69	30.27	30.69	27.01	31.46^{a}	30.53^{bc}	31.48^{ab}	30.25^{c}	29.37^{d}	3.49
RBC (x 10^{12}/L)	2.7^{b}	2.7^{b}	2.8^{a}	0.11	4.1	4.3	3.8	4.1	3.9	0.15
Glucose (mg /dl)	137.1^{c}	154.4^{a}	141.1^{b}	3.84	123.5^{d}	151.2^{b}	141.2^{bc}	168.6^{a}	136.4^{c}	4.96
T. Protein(g/l)	41.7^{b}	39.2^{c}	43.7^{a}	1.18	38.4	43.0	41.4	43.5	41.5	1.5
Serum Globulin(g/l)	14.3^{b}	17.6^{a}	18.3^{a}	0.56	18.3	18.6	14.1	16.9	15.8	0.73
Serum Albumin (g/l)	27.4^{a}	21.6^{c}	25.4^{b}	1.02	20.1^{c}	24.4^{b}	27.3^{a}	26.6^{a}	25.7^{ab}	1.31
Uric Acid (mg/dl)	9.9^{b}	7.9^{c}	13.9^{a}	0.21	11.8^{a}	10.5^{b}	10.5^{b}	9.6^{c}	10.1^{bc}	0.27
Cholesterol (mg/dl)	240.7	232.0	244.9	11.61	252.8^{b}	287.2^{a}	212.7^{c}	228.8^{bc}	214.2^{c}	14.99
Creatinine(mg/dl)	0.9^{c}	1.5^{b}	4.9^{a}	0.30	2.2	2.1	2.3	2.1	3.2	0.4

^{abc} means on the same row having different superscript are significantly different ($P<0.05$); Control=No Additives; AB= Antibiotics (oxytetraxycline); MOS=Mannose oligosaccharide; AXOS=Arabinoxylans Oligosaccharide; Sim lac=*Pedioccocus acidilactici*

Table 4 : Interaction effect of location of hatchery and additives on blood parameter of starting broiler

	Location 1					Location 2					Location 3					
Parameters	Control	AB	MOS	AXOS	SIML	Control	AB	MOS	AXOS	SIML	Control	AB	MOS	AXOS	SIML	SEM
PCV (%)	27.5^{g}	36.5^{c}	28.5^{fg}	36.0^{c}	31.0^{ef}	45.5^{a}	27.5^{g}	32.3^{de}	23.0^{h}	35.3^{c}	31.5^{d}	40.5^{b}	32.5^{be}	40.0^{b}	35.0^{cd}	1.46
Hb(g/dl)	9.1^{l}	11.8^{e}	9.5^{l}	12.7^{cd}	10.1^{ef}	15.5^{b}	9.0^{l}	11.0^{ef}	7.7^{j}	12.0^{d}	13.1^{c}	15.8^{b}	13.5^{c}	16.7^{a}	14.1^{bc}	0.68
WBC (10^{3}/mm^{3})	33.733^{a}	30.200^{e}	30.867^{c}	28.578^{g}	30.075^{f}	26.900^{h}	31.175^{d}	31.708^{bc}	33.60^{b}	27.98^{gh}	33.737^{a}	30.204^{e}	30.870^{c}	28.582^{g}	30.079^{f}	5.1649
RBC (x 10^{12}/L)	2.4^{f}	3.2^{d}	2.3^{l}	3.1^{d}	2.6^{ef}	3.6^{c}	2.4^{f}	2.8^{e}	2.2^{i}	2.7^{e}	4.4^{b}	5.3^{a}	4.3^{b}	5.1^{a}	4.6^{ab}	0.27
Glucose (mg /dl)	115.3	140.3	127.1	171.7	130.9	136.0	169.1	165.3	158.3	143.3	119.3	144.3	131.1	175.7	134.9	4.85
T. Protein(g/l)	38.7^{l}	45^{cd}.0	42.5^{e}	46^{c}.o	36.5^{j}	35.8^{k}	37.0^{is}	37.1^{is}	36.4^{j}	49.4^{a}	40.7^{f}	47.0^{b}	44.5^{d}	48.0^{ab}	38.5^{l}	1.19
Globulin(g/l)	17.4	17.9	9.2	15.5	11.5	16.0	16.1	19.8	15.7	20.3	21.4	21.9	13.2	19.5	15.5	0.90
Albumin (g/l)	21.3^{gh}	27.1^{e}	33.3^{a}	30.5^{b}	24.9^{f}	19.8^{l}	20.9^{h}	17.3^{j}	20.6^{h}	29.2^{cd}	19.3^{l}	25.1^{f}	31.3^{c}	28.5^{d}	22.9^{g}	1.24
Uric Acid (mg/dl)	12.0^{d}	9.8^{e}	9.4^{e}	8.7^{f}	9.4^{e}	7.2^{j}	8.3^{fi}	8.7^{f}	7.6^{l}	7.5^{i}	16.0^{a}	13.4^{b}	13.4^{b}	12.7^{c}	13.4^{b}	5.68
Cholesterol (mg/dl)	267.8	319.9	203.0	218.5	194.4	218.9	217.7	228.1	245.5	249.8	271.8	323.9	207.0	222.5	198.4	10.22
Creatinine(mg/dl	0.9	0.8	1.0	0.8	0.9	1.0	0.9	1.0	0.8	1.7	2.8	2.8	3.0	2.8	2.9	0.24

^{abc} means on the same row having different superscript are significantly different (P<0.05); Control=No Additives; AB= Antibiotics (oxytetraxycline); MOS=Mannose oligosaccharide; AXOS=Arabinoxylans Oligosccharide; Sim lac=*Pedioccocus acidilactici*

Table 5 Main effect of Location and feed additives in blood parameters of broiler (4-8weeks)

	Location of hatchery				Additives					
Parameters	**1**	**2**	**3**	**SEM**	**Control**	**AB**	**MOS**	**AXOS**	**SIML**	**SEM**
PCV (%)	36.9	37.7	40.9	1.00	39.8	36.1	36.1	38.0	38.8	0.66
Hb(g/dl)	12.6^{c}	14.0^{b}	16.6^{a}	1.04	14.6	14.2	13.3	14.4	14.0	0.20
WBC ($10^3/mm^3$)	30.700	30.281	30.704	2.70	31.466^{a}	30.5367^{ab}	31.159^{ab}	30.264^{bc}	29.386^{c}	3.25
RBC (x 10^{12}/L)	3.7^{b}	3.7^{b}	5.7^{a}	0.54	5.1	5.3	4.8	5.1	4.9	0.08
Glucose (mg /dl)	140.1^{c}	154.4^{a}	141.1^{b}	3.77	123.5^{d}	161.2^{b}	141.2^{bc}	168.6_{a}	136.4^{c}	7.37
T. Protein(g/l)	36.7^{ab}	34.2^{b}	38.7^{a}	1.06	33.4	38.0	36.4	38.5	36.5	0.79
Serum Globulin(g/l)	10.3^{b}	13.6^{a}	14.3^{a}	1.01	14.3^{a}	14.6^{a}	10.1^{c}	12.9^{ab}	11.8^{bc}	0.74
Serum Albumin (g/l)	26.4^{a}	20.6^{b}	24.4^{a}	1.39	19.1^{b}	23.4^{a}	26.3^{a}	25.6^{a}	24.7^{a}	1.14
Uric Acid (mg/dl)	11.9^{b}	9.9^{c}	12.9^{a}	0.72	13.8^{a}	12.6^{b}	12.5^{b}	11.6^{c}	12.1^{bc}	0.33
Cholesterol (mg/dl)	230.7	222.0	234.7	3.06	242.8^{ab}	277.2^{a}	202.7^{b}	218.8^{b}	204.2^{b}	12.51
Creatinine(mg/dl)	1.7^{b}	1.3^{b}	2.7^{a}	0.34	2.0	2.9	2.1	1.9	2.9	0.19

^{abc} means on the same row having different superscript are significantly different (P<0.05);
Control=No Additives; AB= Antibiotics (oxytetraxycline); MOS=Mannose oligosaccharide; AXOS=Arabinoxylans Oligosaccharide; Sim lac=*Pedioccocus acidilactici*

Table 6: Interaction effect of Location of hatchery and additive on blood parameter of finishing broiler.

	Location 1					**Location 2**					**Location 3**					
Parameter	Control	AB	MOS	AXOS	SIML	Control	AB	MOS	AXOS	SIML	Control	AB	MOS	AXOS	SIML	SEM
PCV (%)	32.5^{l}	41.5^{c}	33.5^{f}	41.0^{c}	36.0^{e}	50.5^{a}	32.5^{l}	37.3^{d}	28.0^{j}	40.3^{c}	36.5^{e}	45.5^{b}	37.5^{d}	45.0^{b}	40.0^{c}	1.46
Hb(g/dl)	11.1^{i}	13.8^{e}	11.5^{i}	14.7^{cd}	12.1^{ef}	17.5^{b}	11.0^{i}	13.0^{ef}	9.7^{j}	14.0^{d}	15.1^{c}	17.8^{b}	15.5^{c}	18.7^{a}	16.1^{bc}	0.68
WBC (103/mm3)	33.743^{a}	30.210^{e}	30.876^{e}	28.588^{g}	30.085^{f}	26.910^{h}	31.185^{d}	31.718^{bc}	33.610^{b}	27.985^{gh}	33.747^{a}	30.214^{e}	3.0880^{c}	28.592^{g}	30.089^{f}	5.17.
RBC (x 1012/L)	3.4^{f}	4.3^{d}	3.3^{l}	4.1^{d}	3.6^{ef}	4.6^{c}	3.4^{f}	3.8^{e}	3.2^{i}	3.7^{e}	7.4^{b}	8.3^{a}	7.3^{b}	8.1^{a}	$7.6a^{b}$	2.49
Glucose (mg /dl)	115.3	140.3	127.1	171.7	630.9	136.	169.1	165.3	158.3	143.3	119.3	144.3	131.1	175.7	134.9	31.67
T. Protein(g/l)	33.7^{f}	40^{bc}	37.5^{d}	41.0^{b}	31.5^{j}	30.8^{ij}	32.0^{i}	32.1^{i}	31.4^{j}	44.4^{a}	35.7^{e}	42.0^{ab}	40.5^{e}	43.0^{a}	33.5^{f}	1.20
Globulin(g/l)	13.4	13.9	52	11.5	9.5	12.0	12.1	15.8	11.7	16.2	17.4	17.9	10.2	15.5	11.6	8.17
Albumin (g/l)	20.3^{e}	26.1^{c}	32.3^{a}	29.5^{b}	22.0^{d}	18.8^{i}	19.9^{f}	16.3^{j}	19.6^{f}	28.2^{bc}	18.3^{i}	24.1^{d}	30.3^{ab}	27.5^{bc}	21.9^{de}	1.39
Uric Acid (mg/dl)	14.0^{d}	11.8^{e}	11.4^{e}	10.7^{f}	11.4^{e}	9.2^{i}	10.3^{fi}	10.7^{f}	9.6^{i}	9.5^{i}	18.0^{a}	15.8^{b}	15.4^{b}	14.7^{c}	15.4^{b}	0.69
Cholesterol (mg/dl)	257.8	309.9	193.0	208.5	184.4	208.9	207.7	218.1	235.5	239	261	313.9	197.0	212.5	188.4	10.20
Creatinine(mg/dl)	0.6	0.6	0.8	0.6	0.7	0.8	0.7	0.8	0.6	0.5	0.6	0.6	0.8	0.6	0.7	0.02

^{abc} means on the same row having different superscript are significantly different (P<0.05); **Control=No Additives; AB= Antibiotics (oxytetraxycline); MOS=Mannose oligosaccharide; AXOS=Arabinoxylans Oligosaccharide; Sim lac=*Pedioccocus acidilactici***

Table 7 Main effect of feed additives on Salmonella count in the gut of broiler chicken CFUX10^{6}

	Main effect of Location				Main effect of additives						Location × Additive
Days	1	2	3	SEM	Control	AB	MOS	AXOS	SIM L	SEM	P-Values
Day 1	0.24	0.31	0.28	0.02	0.43^{a}	0.31^{b}	0.30^{b}	0.29^{b}	0.31^{b}	0.10	NS
Day 28	0.21	0.20	0.12	0.04	0.59^{a}	0.11^{c}	0.04^{d}	0.13^{c}	0.44^{b}	0.15	NS
Day 56	0.15	0.17	0.07	0.02	2.81^{a}	0.18^{b}	0.02^{d}	0.11^{c}	0.16^{b}	0.09	NS

^{abc} means on the same row having different superscript are significantly different (P<0.05); **Control=No Additives; AB= Antibiotics (oxytetraxycline); MOS=Mannose oligosaccharide; AXOS=Arabinoxylans Oligosaccharide; Sim lac=*Pedioccocus acidilactici***

NS= Not significant

Discussion

Several species of *Salmonellae* infections are now recognized as important food borne diseases most especially from Salmonella contaminated or pre-infected poultry product. In many cases, salmonella infection in broilers or layers sometimes remain sub-clinical, but poultry products are considered as important sources for human food borne outbreaks (*Bielke et al., 2003*).

In the present study, the packed cell volume (PCV) value obtained lied within the normal range and similar to other findings for avians (Oyewole and Ajibade, 1990; Banerjee *et al.*, 2002). The red blood cell counts (RBC) and PCV also lied within the normal range reported for chickens while highest heamoglobin level (Hb), RBC, total serum protein and serum globulin obtained for broiler chicks sourced from hatcheries in location 3 showed improved health status of the birds. Low Hb concentrations values have been reported to be indications of poor protein intake and severe parasitic infection and damage to liver (Lindsay, 1997, Corcionivoschi *et al* 2010). Dietary treatment have been reported to influence the concentration of both red blood cell and packed cell volume (Banerjee *et al.*, 2002). This implied that birds sourced from hatcheries in location 3 efficiently utilize the feed offered.

The high serum glucose obtained for birds fed with various additives showed improved energy utilization and increased serum glucose which implies high or increased energy availability. All birds at the starter phase fed diet supplemented with various additives showed reduced serum uric acid concentration which usually indicate efficient protein utilization and reduced deamination (Oduguwa and Ogunmodede, 1995). High high serum uric concentration above normal is typical of animals fed with nutritionally imbalanced amino

acids in their diets. This agreed with the findings of Szabo *et al*. (2005) who reported high levels of serum uric acid due to poor protein utilization. There is significant reduction in serum cholesterol in broiler chicks fed diet supplemented with different additives. Prebiotic and probiotic supplement used in this study has been shown to reduce serum cholesterol concentration in chicken (Kanna *et al*., 2005). Reduction in serum cholesterol observed in this study for broiler fed with probiotic well corroborate the findings of Pereira and Gibson (2002) who reported that oral administration of probiotic significantly reduced serum cholesterol level by as much as 22 to 33%. The ability of probiotic to lower serum cholesterol is usually due to inhibition of intestinal cholesterol absorption and suppression of bile acid re—absorption mechanism (Guo *et al*., 2012)

At the finisher phase, birds sourced from hatchery 3 recorded the highest Hb, RBC and total serum protein. This followed similar trend with what was observed at the starter phase. This is suggestive of the fact that birds sourced from hatchery 3 showed a better healthy status than other birds sourced from the other hatcheries. Low total protein levels could suggest a liver or kidney disorder which could be caused by poor protein metabolism due to poor digestion or absorption. It was observed in the present study that dietary feed additives supplementation influenced serum protein and albumin concentrations, with highest serum protein and albumin concentrations in the additives compare with the control. Several authors have identified that probiotic supplementation in diets increased serum protein and albumin concentrations, in broilers (Agawane and Lonkar, 2004, Capcarova *et al.*, 2011), piglets (Harding *et al.*, 2008) and fish (Zhou *et al.*, 2010). Harding *et al.*, (2008) reported that probiotics did not stimulate gastrointestinal protein synthesis or reduce the severity of intestinal colitis, but reported an unidentified signaling mechanism between the gut and liver which could be responsible for the increase in liver protein and plasma protein synthesis induced by probiotics. Furthermore, dietary probiotics and prebiotics supplementation may increase the serum protein and albumin concentrations by increasing iron absorption in the lower intestine (Eizaguirre *et al.*, 2002). The increased serum protein concentration in broilers sourced from hatchery 3 could be due to improvement in protein synthesis and carbohydrate and lipid metabolism (Rosa *et al.*, 2001). Reduced WBC noticed with finishing broilers fed diet supplemented with AXOS and SIML is suggestive of absence/low population of foreign bodies or pathogens introduced in the system of the birds resulting in reduced production of antibodies to fight against such. White blood cells (WBC) played prominent role in disease resistance especially with respect to the generations of antibodies and the process of phagocytosis. An elevated value of WBC is an indication that the birds were reacting to one or more factors in the feed (Oduguwa, 2006).

Dietary inclusion of various additives supplemented in the diets showed reduced serum uric acid at both the starting and finishing phases of the birds. This is implicative of good health status. There exist an indirect relationship between serum uric acid concentration and efficiency of utilization of ingested protein (Szabo *et al*. 2005). Although birds fed with various additives showed

reduced gut salmonella counts, dietary supplementation of MOS recorded the least gut salmonella count at both the starter and grower phases of the study. This could be attributed to binding effect of mannose oligosaccharides on the intestinal epithelium of the birds. Most pathogenic microbes including salmonella attach to mannose –containing cells in the intestinal tract. Studies on MOS pointed out that the oligosaccharides is able to bind to mannose-specific lectin of gram-negative pathogens that express Type 1- fimbriae such as Salmonella, preventing salmonella organisms from proliferation hence resulting in their excretion from the intestine (Baurhoo *et al.*, 2007). This resulted in the reduction noticed in the gut salmonella count of broilers fed with MOS in this study.

Conclusion

Although broiler chicks obtained from source 3 showed prospects for improved haematological indices, dietary inclusion of MOS and AXOS in ration for broilers sourced from salmonella infected hatcheries showed improved haematological indices, reduced serum cholesterol and gut salmonella count. Therefore, AXOS and MOS are beneficial for improved healthy status of broiler and prevention of Salmonella morbidity and mortality in poultry.

Acknowledgment

We thank Professor Mrs DaisyEruvbetine for her financial support, who also helped in the sourcing of the Additives used in the cause of the research work. Furthermore, we thank Dr Oso Abimbola in the Department of Animal Nutrition, University of Agriculture Abeokuta Ogun State for critical reading and helpful comments on the manuscript.

Conflict of interest

All authors have no conflict of interest

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