UNDER PEER REVIEW

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

Abstract

This study investigated ~~theeffect~~ 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, 480 male Anak 2000 day-old broiler chickens (160 birds from each state) were collected from hatcheries 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 5 dietary treatments including a control and 4 different feed additives. Data were collected on blood, and gut salmonella count. Birds sourced from location 3 showed highest haemoglobin (Hb), white blood cell, serum total protein, uric acid and serum albumin. Among the broiler included in the study, haemoglobin, RBC, total serum protein, serum albumin, uric acid and creatinine were significantly influenced (P<0.05) with highest values recorded for source 3. The WBC, Glucose, serum globulin, uric acid and cholesterol were significantly (P<0.05) reduced by the various additives included. 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. MOS and AXOS could be used as replacement for antibiotics to improve performance and control the prevalence of *Salmonella* organism in broiler chickens.

Keywords: Oxytetracycline, Mannose oligosaccharide, Arabiloxylans oligosaccharide and Pediccocusacidilactici.

INTRODUCTION: Poultry production is an attractive enterprise because the production of poultry birds yields high economic value within a very short generation interval. Poultry 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 be linked with 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

UNDER PEER REVIEW

digestion, optimal absorption of nutrients, and increases the body's resistance to infectious diseases (Rosen, 1995).

Increase in Salmonella contamination of poultry product which enter the human food chain as a result of infected droppings material, poor hygiene or from infected eggs were reported as major aetiological source for wide prevalence of Salmonella 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.

Salmonella infection or salmonellosis, is major cause of high mortality of wild birds, affecting many bird species worldwide (Hafez, 2001). Several strains of Salmonella bacteria are more closely associated with poultry morbidity than their ubiquitous distributionof various pathogenic serotypes (serovars) like Salmonella pullorum and S. gallinarum to zoonotic serotypes such as S. enteritidis and S. typhimurium (Hafez and Jodas, 2000).These pathogenic serovars 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 microfloraand 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 benefit, support physiological absorption activity of intestinal mucosa and effectively combat diarrheal syndromes (Huyghebaert*et al.,* 2011). Competition for nutrients, production of antimicrobial compounds (such aslow pH volatile fatty acids andbacteriocins), 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), while pobioticswhich are direct-fed microbial feed supplements modulate the gut microflora by successfully competing with pathogens through a competitive exclusion process (Mountzouris*et al.,* 2007). 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 microbiotaon host(Gibson *et al.,* 2004).

One of such prebiotic is mannose oligosaccharide (MOS) which is derived from the cell wall of the yeast Saccharomyces cerevisiaeand 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 with S. Enteritidis using competitive exclusion compounds (probiotics)

UNDER PEER REVIEW

duringthe grow-out period is crucial to improve the microbiological quality of poultry products as well as for consumerhealth (Vugia 1993). 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*etal*., 2007; Wolfenden*et al.,* 2007) and several others that have emerged in the last decades which are potentially useful as prebiotic for pathogen control and poultry performance especially with S. Enteritidis in newly hatched chicks with successful results(Revolledo et al., 2009).

This study therefore seeks to investigate the effect of dietary inclusion of MOS probiotics on health status, 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. Abeokuta (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^{0}C (ICF8)

Experimental birds and management: 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. Three potentially salmonella infected hatcheries identified in previous findings by the author (Olorunsola*et al*., 2012) were used in this study. A total number of four hundred and eighty (480) male, Anak 2000 day-old broiler birds sourced from the three major hatcheries located in Oyo state (Location 1), Ogun state (Location 2) and Lagos state (Location 3), all in southwest Nigeria with incidence of salmonellosis were selected for 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 2.7mx 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.

Experimental Diets: A basal diet containing no additive (control diet) was formulated while additional four diets were formulated to include 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 *Prediococcusacidilactis per gram*) was supplied by a commercial company (Simbiyotec Biological Product Inc. Tuzaistanbul). Mannose oligosaccharides (MOS) and arabinoxylans oligosaccharides (AXOS) (Alltech Inc. Kentucky, USA) were used as prebiotics. The inclusion level of Sim®-Lac used 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 therapeutic dosage of

UNDER PEER REVIEW

oxytetracycline was used as a positive control. The additives were added to the basal diet and thoroughly mixed to formulate the experimental diets.

Table 1 Percentage Composition of Broiler Starter diets and essential proximate in Broiler Starter diets (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 Broiler Finisher Diets (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**

UNDER PEER REVIEW

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

Determination of haematological Parameters: At 28 days, blood samples (2.5 ml each) were collected from the brachial wing vein of four birds per pen (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 heparinised tubes for serum biochemical determination. Haemoglobin concentration (Hb) was estimated using the cyanmethaemoglobin method (Cannan 1958). Packed cell volume (PCV) of blood samples were determined in a Wintrobehaematocrit tube according to the method of Schalm et al. (1975) whiletotal 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 biochemistry parameters: Total serum protein, albumin and globulin were determined using bromocresol purple method (Varleyet *al*.,1980). Serum creatinine and uric acid concentration was determined according to standard procedures. Serum enzymes (alanine transaminase (ALT) and aspartate transaminase (AST) were analysedspectrophotometrically using Randox^{R} diagnostic kit. The serum cholesterol was estimated (Qualigens India. Pvt. Ltd., Catalogue number 72201-04)

Salmonella bacteriaestimation and identification: Intestinal content of the birds gut was aseptically collected into sterile plain bottle and total salmonella load was estimated using pour plate method described by Hedges (2012). Salmonella colonies were counted and the

UNDER PEER REVIEW

results were expressed according to plate count proceedings established in Normative Instruction (Brazil, 2003). Salmonella bacteria isolation was carried out according to modified method of from Desmidt*et al (1998*). The intestinal sample, 1 gramme of different probiotic, prebiotic fed bird and control was pre-enriched at 37°c for 1 hour in 2 ml BPW (Buffer Peptone Water) and later directly inoculated onto XLD agar (Xylose Lysine Deoxycholate agar). Bacteria colonies found were examined for their respective colonial morphology and biochemically characterized for Salmonella identification while antigenic serological assay was also performed.

Statistical Analysis: The experimental layout was 3 x 5 factorial arrangements within a randomized complete design. Data obtained were subjected to analysis of variance using SAS (1998). Significant means among variables were separated using Duncan Multiple Range Test as outlined by Steel and Torrie (1990). The colony count data were transformed into log base 10 and were subjected to one-way analysis of variance and those with statistical differences were submitted to the Tukey's test at the 0.05% level using Stat view for windows 5.0 (SAS Inc, Cary, NC, USA)

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 Hb, RBC, total serum protein, serum albumin, uric acid and creatinine was significantly influenced (P<0.05) with highest values recorded for hatchery in Location 3. The WBC, Glucose, serum globulin, uric acid and cholesterol were significantly (P<0.05) reduced by the various additives included. However, dietary inclusion of various additives showed no significant effect (P>0.05) on the PCV, Hb, RBC, total serum protein, serum albumin and creatinine, respectively. The WBC also reduced significantly (P<0.05) across the treatments all the hatcheries with the control diet having higher (P<0.05) value of 31,456x10^{3}/mm^{3}. Serum cholesterol reduced (P<0.05) with birds fed diet supplemented with MOS and Sim® Lac having the least (P<0.05) values of 212.7 and 214.2 mg/dl respectively.

The interaction effect of hatchery source and feed additives on the haematological indices and serum biochemistry of starting broiler is compared and 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. Total serum protein showed higher (P<0.05) values for birds sourced from location 1 hatchery and fed diet supplemented with Sim® Lac. However, there was a significant (P<0.05) increase in uric acid of birds sourced from hatcheries in location 3 and fed with the control diet. Glucose, serum globulin, cholesterol and creatinine showed no significant (P>0.05) interaction effect.

The result of the main effect of hatchery sources and dietary inclusion of feed additives on haematological indices and serum biochemistry of finisher broilers is presented in Table 5. Hb and RBC count were significantly (P<0.05) increased with birds sourced from hatcheries in location 1 and 2 while birds from hatcheries in location 3 had the highest values. The

UNDER PEER REVIEW

serum glucose, serum total protein, serum albumin, serum globulin uric acid and creatinine levels of the broilers significantly (P<0.05) affected by the location of the hatchery. Birds fed with the control showed higher (P<0.05) values of serum albumin and uric acid. The control diet and antibiotics had statistically similar values for white blood cell, serum albumin and serum cholesterol. Other parameters measured, PCV, Hb, Red blood Cell, total protein and creatinine across the treatment were not significantly (P>0.05) affected. This result followed the same trend with the result of starting broilers.

The interaction effect of hatchery source and additives on the haematological indices and serum biochemistry of finishing broiler is presented in table 5. The result showed that packed cell volume, haemoglobin and white blood cells were significantly (P<0.05) increased in the control diet across the locations compared to other dietary treatments applied. The control diets had 50.5% PCV and 33,743 of white blood cells. Higher significant (P<0.05) value for red blood cells was recorded for antibiotic and AXOS at hatchery location 3. Total serum protein recorded a significant (P<0.05) increase with increasing AXOS, Sim lac® which were statistically similar to antibiotic. The uric acid recorded significant (P<0.05) reduction across the location having the control diet with higher values of 18.0 and 14 mg/dl respectively. The serum globulin, cholesterol and creatinine were not significantly (P>0.05) affected by interaction of location of hatchery and feed additives.

Table 7 showed a significant (P<0.05) reduction in Salmonella count at day 1 of the study when various additives were added. Main effect of location showed no effect on gut salmonella count of the birds. 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

UNDER PEER REVIEW

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=*Pedioccocusacidilactici*

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=ArabinoxylansOligosccharide; Sim lac=*Pedioccocusacidilactici*

UNDER PEER REVIEW

UNDER PEER REVIEW

228 **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

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

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UNDER PEER REVIEW

235 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

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

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UNDER PEER REVIEW

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					
Days	1	2	3	SEM	Control	AB	MOS	AXOS	SIM L	SEM
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
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
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

^{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=*Pedioccocusacidilactici***

Discussion

several species of *Salmonellae* infections are now recognized as important food borne diseases contracyed majorly 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 packedcell 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 indicateinefficient protein utilization, resulting in wastages and excess protein in form of uric acid. 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 disorderwhich 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

UNDER PEER REVIEW

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.

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UNDER PEER REVIEW