

Haematological and serum biochemical parameters of noiler chickens fed with different biotic additives at chicks phase

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Abstract

*The rampant use of antibiotics in animal production and the use of low doses of nontherapeutic antibiotic as led to animal products contamination. This has resulted to consumer outcry and preference for antibiotic free animal products. Therefore, alternatives to the use of growth promoter/antibiotics must be found to enhance the health of farm animal. The focus of growth promoters is to enhance the health status of birds for optimum production performance and to increase the farmers' income. The study was conducted to evaluate the haematological and serum biochemical parameters of noiler chicken. Parameters measured were full blood count, serum total protein, cholesterol, glucose, liver function test and heterophil lymphocyte ratio was calculated. Three hundred and sixty day-old noiler chicks were randomly distributed into 12 treatments with three replicates of 10 birds each. The birds were fed with different growth promoters additives (prebiotic, probiotic symbiotic and antibiotic. At three dosage inclusion rates (0, 0.5 and 1g) into the feed for a period of 42 days. The experiment was laid out in a 4 x 3 factorial arrangement. The result of the haematological parameters of noiler chicken fed with different growth promoters additive significantly ($P < 0.05$) affected the heterophil lymphocyte ratio which was lower for birds fed with probiotic. The cholesterol was significantly ($P < 0.05$) higher for treatment without biotic supplement across the treatment level. Based on the result of heterophil lymphocyte ratio, probiotic primilac[®] which contained (*Enterococci faecium*, *Bacillus species*, *Bifidobacterium bifidum*, and yeast *Saccharomyces cerevisiae*) can be used as a growth promoters anti-stress and at 0.5g/kg of feed level of inclusion anti-cholesterolemic property can be achieved in noiler chickens.*

Keywords: Growth promoters, prebiotic, probiotic, synbiotic, Noiler



Paramètres hématologiques et biochimiques sériques de poulets noilers nourris avec différents additifs biotiques au stade poussin

Résumé

L'utilisation généralisée d'antibiotiques en production animale et l'utilisation de faibles doses d'antibiotiques non thérapeutiques ont conduit à la contamination des produits d'origine animale. Cela a entraîné un tollé des consommateurs et une préférence pour les produits d'origine animale sans antibiotiques. Par conséquent, des alternatives à l'utilisation de stimulateurs de croissance/antibiotiques doivent être trouvées pour améliorer la santé des animaux d'élevage. L'objectif des promoteurs de croissance est d'améliorer l'état de santé des oiseaux pour des performances de production optimales et d'augmenter les revenus des agriculteurs. L'étude a été menée pour évaluer les paramètres biochimiques hématologiques et sériques du poulet Noiler. Les paramètres mesurés étaient la formule sanguine complète, les protéines sériques totales, le cholestérol, le glucose, le test de la

*fonction hépatique et le rapport des lymphocytes hétérophiles a été calculé. Trois cent soixante poussins de noiler âgés d'un jour ont été répartis au hasard en 12 traitements avec trois répétitions de 10 oiseaux chacune. Les oiseaux ont été nourris avec différents additifs activateurs de croissance (prébiotique, probiotique symbiotique et antibiotique. À trois taux d'inclusion de dose (0, 0,5 et 1 g) dans l'aliment pendant une période de 42 jours. L'expérience a été présentée dans un factoriel 4 x 3 Le résultat des paramètres hématologiques du poulet noiler nourri avec différents additifs promoteurs de croissance a significativement ($P<0,05$) affecté le ratio de lymphocytes hétérophiles qui était plus faible pour les oiseaux nourris avec des probiotiques. Le cholestérol était significativement ($P<0,05$) plus élevé pour le traitement sans Sur la base du résultat du ratio de lymphocytes hétérophiles, le probiotique (*Enterococcifaecium*, espèce *Bacillus*, *Bifidobacteriumbifidum* et levure *Saccharomyces cerevisiae*) peut être utilisé comme stimulateur de croissance anti-stress et à raison de 0,5 g/kg d'aliment le niveau de propriété anticholestérolémique d'inclusion peut être atteint chez les poulets plus nuls.*

Mots clés : Promoteurs de croissance, prébiotique, probiotique, symbiotique, Noiler

Introduction

Probiotics can be characterized as selected life counts of beneficial bacteria and yeasts that are administered orally in order to establish a favourable intestinal microflora. Probiotic/symbiotic in livestock stimulates the direct uptake of dissolved organic material mediated by the bacteria, and enhances the immune response against pathogenic microorganisms (Balcázar *et al.*, 2007). It has been discovered that it can inhibit pathogens by competition for colonization sites or nutritional sources and production of toxic compounds, or stimulation of the immune system (Musa *et al.*, 2009). On the other hand, prebiotics are defined as non-digestible food ingredients that affect the host beneficially by selectively stimulating the growth and/or activity of bacteria in the colon (Gibson and Roberfroid, 1995). However, the combinations of prebiotic and probiotic are referred to as synbiotic. Growth promoters have been shown to confer many health benefits to livestock and human. The arbitrary use of antibiotics in industrial food animal production has sequel to alteration of normal microbial floral and interaction of an antibiotics and bacteria. Lopez (2000),

reported that the use of routine prophylactic antibiotics in animal agriculture “contributes to the emergence of antibiotic-resistant bacteria in Livestock. The bacteria can contaminate the animal products and make the products unwholesome because of antibiotic residue. Alternative to antibiotic growth promoter should be sought for in Livestock production to serve as growth enhancer and immunomodulator in farm Animal. There is an increasing interest in finding alternate growth promoters that will enhance a healthy microbial flora and/or prevent the uncontrolled growth of unwanted microorganisms. Thus, there is a growing interest in developing alternatives, such as probiotic, prebiotic and synbiotic which offers alternate to antibiotics usage in animal production. Noiler chicken is a hybrid of broiler and cockerel. It has the resistance traits of cockerels and some meat characteristics of Broilers. It is a two in one purpose bird that grows quickly like the broiler but not as much as broiler and the female lay many eggs like the conventional layers. It was originated an initiated by Amo Farm Sieberer Hatchery Limited. Little information is available on this new breed.

Materials and methods

Study location

The experiment was carried out at the

poultry unit of Directorate of University Farm, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The area is located on Latitude 7° 10' N and Longitude 3° 2' E. It receives a mean precipitation of 1037mm per annum an average temperature of 34.7°C and an average annual relative humidity of 82%. (Google Earth, 2021).

Experimental birds and management

Test ingredient profile

The probiotics used for this experiment was “Primilac® which contained *Enterococci faecium*, *Bacillus* species, *Bifidobacterium bifidum*, and yeast *Saccharomyces cerevisiae* while the prebiotic used was manna oligosaccharides (MOS) and the Synbiotic “Biotin that contained *Enterococci faecium*, *Bacillus* species, *Bifidobacterium bifidum*), and the yeast (*Saccharomyces cerevisiae*), sea weed, and amylase as the active ingredients while the antibiotic was oxytetracycline hydrochloride.

Animal management

Three hundred and sixty day old Noiler chicks were acquired from Amo farm Sieberer hatchery limited in Awe, Oyo State for this experiment. The birds were randomly distributed into twelve treatments with three replicates of ten birds each.

Experimental treatments consisted of four different growth promoters (probiotic, prebiotic, synbiotic and antibiotic) at three inclusion rates (0, 0.5 and 1g) into kg of feed respectively. The experiment commenced at the bird's arrival and the chicks were placed on these for a period of six weeks representing the chick phase. Prior to the arrival of the birds, the poultry house was thoroughly cleaned and disinfected. The feeders and drinkers were kept clean. Feed and water were made available to the birds *ad libitum*. On arrival, the birds were given feed containing probiotic, prebiotic, symbiotic and antibiotic and these continued for a period of forty two days representing the starter phase.

Experimental layout

The experiment was laid out in a 4x3 factorial arrangement. There were two factors: growth promoters (prebiotic, probiotic, symbiotic and antibiotic and level of inclusion (0, 0.5, 1g/kg) in feed.

Data collection

The following data were collected in the course of the experiment: Hematological indices; packed cell volume was determined by blood samples that were collected into EDTA bottles and placed in micro haematocrit centrifuge and subjected to spinning for 5 minutes at a revolution of 11000rpm. The PCV values were subsequently determined by measuring the height of the red cell column and expressing this as a ratio of the height of the total blood column while white blood cells were determined by standard methyl alcohol and giemsa stain that were prepared into a dilute buffer solution consisting of disodium hydrogen orthophosphate, potassium dihydrogen orthophosphate and distilled water. Thereafter an air dry film of blood fixed in the methyl alcohol giemsa stain and the buffer solution were prepared. One volume of giemsa stain was diluted with nine volume of buffer solution which was used to flood the film of blood and stain for 15 minutes. This was washed and differentiated with buffer solution until the cells could be identified and air dried and then observed under low power and high power oil immersion for cell counting and hemoglobin. A plain capillary tube was filled to about three quarter full, with the bottom end sealed. It was placed in microhaematocrit centrifuge with sealed end facing out and resting on the rubber run cushion. The other end was covered with a plastic material. The counter of capillary tubes was centrifuged for five minutes at 11000rpm; after which the tube was placed in a microhaematocrit reader. This has a linear scale; the bottom of the tube content is at 100. From the scale, the level of the top

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of RBC was determined and the haemoglobin value was determined.

Experimental design and data analysis

The experiment was laid out in a 4 x 3 factorial arrangement. There were two factors: Growth promoters additives

(prebiotic, probiotic, synbiotic and antibiotic) and levels of inclusion (0, 0.5, 1g/kg) in feed. Data generated were subjected to analysis of variance in a completely randomised design as described by Steel and Torrie (1980). Significant difference among treatment means were separated using the Duncan multiple range test in the SAS package.

Table 1: Percentage composition of noilerchicks diet

Ingredients	Composition (%)
Maize	50.00
Soybean meal	21.00
Wheat offal	12.00
Groundnut cake	10.00
Fish meal (72% CP)	2.00
Bone meal	2.50
Oyster shell	1.50
*Vitamin and Mineral premix	0.25
Lysine	0.25
Methionine	0.25
Salt (NaCl)	0.25
TOTAL	100.00
Calculated Analysis	
Crude protein (%)	23.35
Crude fiber (%)	3.40
Ether extracts (%)	3.32
Cal (%)	1.32
P (%)	0.71
ME (Kcal/kg)	2822.38
Determined Analysis (%)	
Dry matter	89.63
Crude protein	22.94
Crude fiber	3.84
Ether extract	3.56
Ash	5.20

*Premix composition per kg diet: Vit A: 400000IU, Vit D: 80000IU, Vit E: 40000ng, Vit K₃: 800mg, Vit B₁: 1000MG, Vit B₂: 6000mg, Vit B₆: 500mg, Vit B₁₂: 12.25mg, Niacin: 6000mg, Panthothenic acid: 2000mg, Folic acid: 200mg, Biotin: 8mg, Manganese:300000mg, Iron: 8000mg, Zinc: 20000mg, Cobalt: 80mg, Iodine: 400mg, Selenium: 40mg, Choline:800000mg

Table 2: Vaccination regime for noiler at starter phase

Age	Vaccination
Day 1	Mareks
Day 8	Infectious bursal disease vaccine (IBDV) 1 st dose
Day 14	Newcastle disease vaccine (LASOTA) 1 st dose
Day 17	Infectious bursal disease vaccine (IBDV) booster dose
Day 32	Newcastle disease vaccine (LASOTA) 2nd dose

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Table 3: Main effect of different biotic additive on heamatological parameters of noiler chicken at chicks phase

Parameters	Growth promoter additives				SEM	Dosage Inclusion			(g/kg)
	Prebiotic	Probiotic	Synbiotic	Antibiotic		0	0.5	1	SEM
Pack cell volume %	33.88	33.22	32.77	31.77	5.79	33.41	33.08	33.75	7.50
Haemoglobin g/dl	10.88 ^b	11.33 ^a	10.76 ^b	10.30 ^b	3.10	10.76	10.74	10.89	5.50
White blood cells x10 ³ /ul	10.99	11.52	12.30	12.79	3.90	11.98	11.58	12.06	4.10
Heterophil %	30.33 ^a	23.66 ^b	31.22 ^a	29.56 ^a	5.50	30.25 ^a	21.75 ^c	27.33 ^b	7.40
Lymphocyte %	66.11 ^a	56.11 ^b	64.77 ^a	66.89 ^a	8.60	66.00 ^a	57.75 ^b	66.66 ^a	9.90
Monocyte %	1.11	2.10	1.33	1.00	0.19	1.58	1.62	1.10	1.40
Eosinophil %	1.55	1.33	1.77	1.88	0.97	2.16	1.50	1.25	0.98
Basophil %	0.88	1.33	0.88	0.66	0.30	0.00	1.25	1.58	0.55
Heterophil: Lymphocyte	0.46 ^a	0.38 ^c	0.48 ^a	0.44 ^b	0.01	0.45	0.41	0.43	0.01

^{a,b,c}: Means in the same row not sharing common superscript are significantly different (p<0.05) SEM:

Standard Error mean

Results and discussion

Haematology is the analysis of blood, which show the health status of the animals. The results of the haematological parameters of noiler chicken fed with different biotic additive for a period of forty two days had no significant (P<0.05) effect on the packed cell volume, white blood cells, monocyte, eosinophil and basophil, although there are variation in the values across biotic additives nevertheless the values are all within the recommended values by Mercks Veterinary Manual with exception of haemoglobin which is a function of availability of oxygen for tissue maintenance. Haemoglobin is the concentration of the haeme group and the iron atom is the site of oxygen binding. However, heterophil: lymphocyte ratio was significantly improved for treatment with probiotic that contain *Enterococci faecium*, *Bacillus* species, *Bifidobacterium bifidum*, and yeast *Saccharomyces cerevisiae* (P<0.05), this can serve as an antistress in livestock animal. The number of lymphocytes per unit of blood increases and the number of heterophils decreases in birds fed with probiotic as compared to other biotic diets. Although, in birds under stress, the number of heterophils per unit of blood increases and the number of lymphocytes

decreases, a normal ratio is about 0.4 (Gross and Siegel, 1983) and treatment with probiotic recorded a lower value as compared to other treatments. Probiotic in livestock stimulates the direct uptake of dissolved organic material mediated by the bacteria, and enhances the immune response against pathogenic microorganisms (Balcázar *et al.*, 2007). The use of antibiotic growth promoters has played a significant role in poultry industry and stoppage of it can have a negative effect on production performance most especially during the heat period. Lin *et al.* (2006) reported that heat stress can be detrimental to poultry production in tropical area and causes a severe economic loss. Persistent heat stress can be detrimental to poultry performance ranging from decrease in feed intake, decline in body weight gain, mortality and poor meat quality characteristics (Frag and Alagawany, 2018; Saeed, Abbas, *et al.*, 2019). The result of the serum biochemical parameters observed a significant higher value for total protein of treatment fed with probiotic as compared to other additives followed by prebiotic, this is related by work done by Mohan *et al.* (1996), which shows that use of probiotics improved nitrogen utilization in broilers while antibiotic biotic treatment

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recorded the lowest value. Moreover, inclusion of probiotic at 0.5g into a kilogram of feed improved the blood cholesterol as compared with birds from other growth promoters and this was related to work done by (Ashayerizadeh, 2009). The report of the experiment conducted for a period of thirtyfive days from (Pandal *et al.*, 2001 and Kannan *et al.*, 2005) further established reduction in serum *cholesterol* in a probiotic fed birds as compared with the control birds treatment ($P<0.05$). Further studies from work (Pelicano, 2014) and (Owosibo *et al.*, 2013) corroborated this finding. Preliminary studies in human and animal have shown that lactic acid bacteria are effective in reduction of serum cholesterol, presumably by breaking down bile in the gut, thus inhibiting its re-absorption back into the blood Kumar *et al.* (2012).

However, the value of glucose was significantly improved for treatment that contained synbiotic and antibiotic across the biotic additives. While aspartate transaminase and alkaline transaminase were all within the normal range which is an indication that there are no damages done to the liver and tissue cells.

Moreover, the interaction of different additive on dosage inclusion on serum biochemical parameters significantly ($P<0.05$) affected the alkaline phosphatase at 1g/kg of feed inclusion which recorded a higher significant value and a caution to prolong usage of oxytetracycline hydrochloride in food animal. ALP is an enzyme mostly found in the liver, bone kidney and digestive system it becomes available in the blood when there are liver damage.

Table 4: Main effect of levels of biotic additives on serum biochemical parameters of noiler chicken at chicks phase

Parameters	Biotic additives					Dosage Inclusion (g/kg)			
	Prebiotic	Probiotic	Synbiotic	Antibiotic	SEM	0	0.5	1	SEM
Serum total protein	3.96 ^b	4.15 ^a	3.43 ^b	3.27 ^c	0.43	3.33 ^b	3.79 ^b	3.99 ^a	0.23
Cholesterol (mg/dL)	72.08 ^a	70.38 ^b	69.68 ^c	70.33 ^b	23.00	79.64 ^a	65.06 ^b	67.00 ^b	23.81
Glucose (mg/dL)	116.02 ^b	119.07 ^b	129.45 ^a	123.32 ^a	45.00	108.52 ^c	125.54 ^b	131.35 ^a	19.49
Aspartate transaminase u/L	62.44	64.44	65.22	64.11	9.34	64.08	64.33	63.75	8.30
Alkaline transaminase u/L	31.00	27.77	31.11	29.11	24.69	28.75	28.25	32.25	4.96
Alkaline phosphate u/L	28.00 ^a	24.55 ^b	26.88 ^b	28.88 ^a	7.94	26.75 ^b	24.91 ^b	29.58 ^a	7.90

^{a,b,c}: Means in the same row not sharing common superscript are significantly different ($p<0.05$) SEM: Standard Error mean

Table 5: Interactive effect of levels of additives on haematological parameters of noiler chicken at chicks phase

Parameters	Prebiotic			Probiotic			Synbiotic			Antibiotic			SEM
Dosage Inclusion	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	
Park cell volume %	32.66 ^b	32.00 ^b	37.00 ^a	35.66 ^a	29.60 ^c	35.00 ^a	32.67 ^b	29.33 ^c	29.67 ^c	32.67 ^a	29.33 ^c	33.00 ^a	2.50
Haemoglobin g/dl	10.60	10.17	11.90	11.26	10.50	11.23	10.60	11.70	9.90	10.60	9.83	10.48	0.70
White blood cell x10 ³ /uL	11.66	10.37	10.66	12.93	11.28	10.37	11.66	12.57	12.67	11.67	12.13	14.57	0.77
Heterophil(x10 ⁹ /L)	30.33 ^b	32.00 ^b	28.67 ^c	30.00 ^b	21.10 ^b	20.00 ^c	30.33	31.33 ^b	32.00 ^b	30.33 ^b	30.33 ^b	26.33 ^c	4.25
Lymphocyte (x10 ⁹ /L)	65.66 ^c	68.33 ^a	64.33 ^c	67.00 ^a	62.67	68.66 ^a	65.66 ^c	65.33 ^c	63.33 ^a	65.66 ^b	64.66 ^b	70.33 ^a	
Heterophil: Lymphocyte ratio	0.46 ^a	0.47 ^a	0.44 ^b	0.44 ^b	0.47 ^a	0.39 ^c	0.46 ^a	0.47 ^a	0.50 ^a	0.46 ^a	0.46 ^a	0.37 ^c	0.03
Eosinophils (x10 ⁹ /L)	2.33	1.00	1.33	1.66	1.00	1.33	2.33	1.66	1.33	2.33	2.33	1.00	1.06
Basophils (x10 ⁹ /L)	0.00 ^d	1.00 ^b	1.6 ^b	0.00 ^d	2.33 ^a	1.66 ^b	0.00 ^d	1.00 ^b	1.60 ^b	0.00 ^d	0.66 ^c	1.33 ^b	0.43

^{a,b,c}; Means in the same row not sharing common superscript are significantly different ($p < 0.05$) SEM: Standard Error mean.

Table 6: Interactive effect of levels of additives on serum biochemical parameters of noiler chicken at chicks phase

Parameters	Prebiotic			Probiotic			Synbiotic			Antibiotics			SEM
Levels	0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1	
Serum total protein (g/L)	4.50	4.16	4.23	3.50	3.90	5.00	3.16	3.30	3.86	3.17	3.86	2.80	0.14
Cholesterol (mg/dL)	79.00 ^a	70.00 ^b	66.57 ^c	80.00 ^a	62.33 ^c	68.88 ^c	80.00 ^a	64.00 ^c	64.50 ^c	79.23 ^a	63.07 ^c	68.70 ^c	4.80
Glucose (mg/dL)	109.43	120.80	117.83	108.26	122.50	126.46	108.43	138.78	141.20	107.93	120.13	141.90	9.72
Aspartate transaminase u/L	65.00	65.66	56.66	62.33	62.67	66.33	64.00	66.66	65.00	65.00	62.33	65.00	7.16
Alkaline transaminase u/L	29.00	30.66	33.33	29.03	24.00	30.33	28.66	32.33	32.33	28.33	26.00	33.00	4.96
Alkaline phosphatase u/L	27.67 ^c	27.66 ^c	28.66 ^c	27.00 ^c	21.66 ^c	25.00	25.66 ^d	24.66 ^d	30.33 ^b	26.66 ^d	25.66 ^d	34.33 ^a	2.81

^{a,b,c}; Means in the same row not sharing common superscript are significantly different ($p < 0.05$) SEM: Standard Error mean

Conclusion

The results of the haematological parameters of noiler chicken improved the haemoglobin concentration and reduced the heterophil lymphocyte for treatment with probiotic additive. The implication of Heterophil lymphocyte ratio correlation is that higher number of heterophil to decrease in lymphocyte is an indication of birds in distress. Probiotic recorded the least heterophil: lymphocyte ratio which shows that inclusion of probiotic at 1g/kg of feed level of inclusion can serve as antistress in noiler chicken. However, the serum cholesterol observed indicated that probiotic have an anticholesterolemic property.

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