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Effects of Improved (Addition of Antimicrobials and Antioxidants) Locally Produced Natural Vitamin Premix on Performance, Hematology and Some Serum Constituents of Broiler Chickens

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Abstract

An experiment was conducted to compare the haematology and some serum constituents and performance of broilers fed diets containing improved (addition of anti-microbial and anti-oxidant) locally produced natural vitamin premix (LPNVP), LPNVP without improvement and commercial vitamin premix (CVMP). Haematology of the birds was not affected by the dietary treatments. However, red blood cell (RBC) count was significantly high ($P<0.05$) for the birds fed diet containing improved LPNVP. The haemoglobin, PCV, RBC and WBC values observed for birds fed improved LPNVP were 13 gm%, 32%, 246 x 10^{12}/L and 3.1 x 10^{10}/L respectively. Serological parameters observed in the study were not significantly different ($P>0.05$) for all the dietary conditions studied. Birds fed diet containing improved LPNVP also recorded comparable serum components to control diet containing the commercial vitamin/mineral premix. Serum calcium, phosporus, glucose, ureate, creatinine and total protein observed for birds fed diet containing improved LPNVP were 2.32%, 0.70%, 3.60Mmol/L, 2.9Mmol/L, 0.25Mmol/L, 58Mmol/L and 66g/100ml respectively. Feed intake, weight gain and feed utilization were similar ($P>0.05$) for the various dietary treatments. Birds' fed improved LPNVP based diets tended to record better feed intake than did the control birds on CVMP based diets and unimproved LPNVP. Generally, improvement of LPNVP tended to give better performance of broilers than did other dietary treatments.

Key words: locally produced Natural vitamin premix, commercial vitamin/mineral premix, haematology, serum

Introduction

Locally produced natural vitamin premix (LPNVP) had been developed and used in broiler diets (Bolu and Balogun 1998). Latest improvements to this developed natural alternative involve iron fortification using blood meal as the natural source (Bolu and Balogun, 2000). Commercial vitamin premixes (CVP) sold in Nigeria have non-nutritive additives such as antioxidants, antibiotics, yolk colourants to ensure efficiency

of production. Antioxidants and antimicrobials have been used in diets of chickens as non-nutritive feed additives to prevent lipoxidation and improve feed utilization respectively.

Factors implicated as catalyst to lipid peroxidation are pro-oxidants such as oxygen, metals and peroxidative enzymes (Ashgar *et al.*, 1990); polyunsaturated fatty acid (PUFA) (Gaal *et al.*, 1995); aging and carcinogenesis (Miyake *et al.*, 1997).

Antimicrobials have been reported to improved growth and efficiency of feed utilization (Morrison *et al.*, 1974), improve egg production (Andrews *et al.*, 1966) and improve reproductive performance (Janos, 1974). Antimicorbials are usually included at low levels (about 1 - 50mg/kg diet). Some commercial vitamin/mineral premixes have considerable levels of antimicrobials added as growth promoters. Herbal formulation of substrates for use in animal nutrition as a replacement for their synthetic analogues, is a current view in animal production (Venkatarraman *et al*, 1994).

The objective of this study was to determine the effects of the addition of antioxidant and antimicrobial to the LPNVP fed to broiler chickens on their haematology, serum biochemical constituents and performance. These additions were expected to improve the commercial value of LPNVP and also bring its quality close to the marketed analogues.

Materials and Methods

A total of one hundred and twenty (120) day-old chicks were used in this study. The birds, which were of mixed sex, had initial body weights that ranged between 37 - 45g. The birds were weighed and allocated to each treatment in a completely randomized design. The three vitamin formulations formed the treatments (Table 1). There were four replicates per treatment and forty birds per treatment. The average initial group weight was almost identical per group. The starter phase, which lasted for a period of four (4) weeks (0 - 4 weeks), was conducted in a heated tier brooder. The birds used for the starter phase were transferred to deep litter house for the finishing phase, which lasted for another four (4) weeks (4 - 8 weeks). Birds were fed *ad libitum* on the diet formulated (Table 1). They were also vaccinated against Infections bronchitis (IB), New castle disease (NCD) and Infectious Bursal disease (IBD). They were also medicated against coccidiosis. Weight gain, feed intake was determined weekly. From these values, feed conversion efficiency and cost: benefit ratios were calculated.

Blood collection for Heamtological Examination.

Blood samples were taken from the wing vein of each group into a collecting tube containing ethylene diamine tetra acetate (EDTA). Haematological assay was carried out as described by Dacie and Lewis, (1977) to determine packed cell volumen (PCV), haemoglobin concentration (Hb), total red blood cell count (RBC), total leukocyte count (WBC) and differential leukocyte count for each sample.

TABLE 1 Composition of Experimental Diets (%).

Ingredients	Treatments		
	CVMP	Improved LPNVP	Unimproved LPNVP
Basal ingredients *	99.75	99.50	99.50
ANUPCO Premix **	0.25	-	-
***LPNVP^{+}		0.50	-
***LPNVP^{++}	-	-	0.50
Total	100.00	100.00	100.00

* Contained: -
Maize: soybean: groundnut cake; fishmeal; oyster shell; blood meal; wheat bran; methionine and salt. Analyzed nutrient content: crude protein (%), 24; crude fat (%), 2.5; crude fibre (%) 3.6; total ash (%), 3.2. Minerals (as calculated) Ca (%), 0.9; K (%), 0.2; Na (%) 0.15, Cl (mg), 800; Cu (Mg), 4; Sc (mg), 0.1; Zn (mg), 40.

** Contained (/Kg):-
Retinal, $4x10^6$ IU; cholecalciferol, $1.2x10^6$ IU; α-tocopherol, 3200 IU; Menadione, 800 mg; riboflavin, 2200 mg; thiamin, 3200mg; niacin, 400 mg; pyridoxine, 480 mg; Calcium pantothenaet, 2800 mg; folate 240 mg; choline choride, $2x10^3$ mg; biotin, 12mg; se, 40mg; Min, 32000 Cu, 3200 mg; Zn, $2x10^5$ mg; Co, 180 mg; Ir 800mg; Mg, 400mg.

*** Contained: (1 Kg).
Retinal $2.8x10^6$ IU; cholecalciferol, 3200 IU; α- tocopherol, 4000 IU; menadione, 800mg; riboflavin, 800 mg; thiamin, 400mg; pyridoxine, 500mg; cyanocobalamin, 2.4mg; folate, 440 mg; Biotin, 20 mg; Choline, $8x10^4$ mg; Ir, 100mg; Zn, $1.5x10^5$ mg; Cu 1600mg; Ir, 800 mg; Co, 100mg; Sc. 40 mg.

\+ Formulated according to NRC requirement (NRC 1994). Iron fortified at 200mg/Kg diet. With anti-microbials and anti-oxidant.

++ Formulated according to NRC requirement (NRC 1994). Iron fortified at 200mg/Kg diet. Without anti-microbials and anti-oxidant.

Clotting time was determined using glass slide method as described by Oji *et al.*, (1986).

Serum Collection

Serological samples were taken from clotted bloods. The clotted blood samples were centrifuged at 400rpm for 3 minutes and the supernatant sera were harvested in bijou bottles for specific serological parameter determination. Serum total protein was determined by the method of Wooton (1964), uric acid and glucose were determined according to the procedure outlined by Searcy (1969), while plasma cholosterol and creatinine were determined according to Scott (1965); calcium and available phosphorus were determined according to AOAC (1990).

Proximate analysis of the basal diet was carried out according to the method described in the AOAC (1980). Vitamin and mineral analyses were carried out according to (ROCHE, 1990). Data collected were subjected to Analysis of Variance using the model for completely randomized design (Steel and Torrie, 1980).

Results and Discussion

Feed intake, weight gain and feed utilization efficiency (Table 2) were not effected ($P>0.05$) by the different dietary treatments. Birds fed diets containing improved LPNVP tended to favour these parameters and in some cases were observed to record superior performances to the values reported for the birds fed diet containing the commercial vitamin/mineral premix and

TABLE 2 Effect of Improved LPNVP on the Performance of Broiler chickens.

CHARACTERISTICS	Treatments		
	CVMP	Improved LPNVP	Unimproved LPNVP
Total feed intake (kgb^{-1})	4.52	4.57	3.58
Initial body weight (gb^{-1})	42	45	45
Final body weigh (kgb^{-1})	2.33	2.34	1.49
Total body weight gain (kgb^{-1})	2.29	2.29	1.45
Feed utilization efficiency	0.50	0.50	0.42
Cost: benefit ratio (₦)	28.00[b]	12.10[a]	12.06[a]
Mortality (%)	0.11	0	0.15

a.b. Treatment means within rows followed by different superscripts are significantly different ($P<0.05$).

unimproved LPNVP. Average feed intake, weight gain and feed utilization efficiency recorded for birds fed improved LPNVP based diet were 4.56, 2.29 kg/bird and 0.50 respectively. Although non-significant, improved feed intake observed in this study agrees with earlier studies where antibiotic was incorporated into the feeds of livestock (Patterson, 1965). Homeostatic regulation of feed intake has been established for vitamin deficiencies. Hughes and Wood Gush (1971) reported that birds demonstrated acuity for thiamin deficient diets. Vitamins have also improved feed intake upon supplementation (Bains 1998). The holistic effect of the improvement of LPNVP was the enhancement of feed intake for broiler as recorded in this study and it agreed with earlier findings. Weight gain and feed utilization efficiency were found to have improved considerably with the addition of antioxidants and antimicrobials *vis-a-viz* unimproved LPNVP. These observations are in consonance with the findings of Morrison *et al.*, (1974) and Gaal *et al.*, (1995) that antimicrobials improved growth and efficiency of feed utilization. This report also corroborates the findings of Janos (1974) that antimicrobials improved the efficiency of feed utilization by as much as 18%. Antioxidants have been reported to prevent vitamin loses (Grundboeck *et al.*, 1977). Growth depression resulting from low cumulative weight gains has been established for specific vitamins (Kazemi and Kratzer 1980). Apparently, the improvements incorporated into the LPNVP synergized positively to reduce stresses and improve gains and efficiency of feed utilization and translated to the relative cost: benefit ratio, since it was cheaper to produce LPNVP *vis-à-viz* CVMP. Coupled with the concomitant comparable performances of broilers on both types of vitamins, it is logical to expect better economic returns for LPNVP-based diets than for synthetic analogue CVMP. Result observed for mortality may tend to tip improved LPNVP as a good alternative to CVMP. Alternative feedstuffs have been reported to affect the returns on investment positively when adequately harnessed (Longe, 1986). The cost of producing 1kg of broiler flesh was significantly higher ($P<0.05$) for CVMP-based diet (₦28.00) *vis-à-viz* LPNVP-based diet (₦12.01). For every 1kg of weight gained, birds' fed LPNVP-based diet tended to save ₦16 compared to birds on CVMP-based diet. Thus, the cost of production suggests better economic advantage for improved LPNVP.

All haematological parameters measured were not significantly influenced by the dietary treatments (Table 3) ($P>0.05$) except for the RBC, which was significantly increased ($P<0.05$) by the improved LPNVP. The RBC count observed for birds' fed LNPVP based diet was 246×10^{12}/L. Haematology of broilers fed LPNVP based diets was observed to be adequate and compare with normal ranges earlier reported by Orji *et al.*, (1986). Blood clotting time recorded in this study was acceptable. Griminger *et al.*, (1970) reported that prothrombin-clothing time of decalcified plasma reflects vitamin K status. The percentage haemoglobin, PCV and RBC count were normal suggesting that vitamin was adequate for

TABLE 3 Effect of Improved LPNVP on the Haematology of Broiler Chickens.

Blood Parameters	Treatments			SEM
	CVMP	CVMP	CVMP	
Haemoglobin (gm%)	12	13	11	1.14
Haematocrit (PCV) (%)	29	32	30	5.30
RBC ($x10^{12}$/L)	196^{b}	246^{a}	210^{b}	2.96
WBC ($x10^{9}$/L)	3.2	3.1	2.9	0.98
Lymphocytes (%)	63	68	66	4.97
Nutrophils (%)	29	30	28	1.32
Eosinophils (%)	6	2	4	5.44
Monocytes (%)	-	-	-	-
Basophils (%)	2	-	2	2.80
Blood clotting time (min)	5	5	5	1.23

a.b. Treatment means within rows followed by different superscripts are significantly different ($P<0.05$).

haemopoietic processes. Goeff *et al.*, (1953) reported that riboflavin deficiency increased PCV, MCV and decreased MCH and heterophil leukocytosis prior to neurologic signs. Vitamin E has also been implicated in maintaining RBC integrity thus preventing haemolysis, (Dobinska *et al.*, 1982). NRC (1994) established that vitamin B_{12} and folate deficiencies precipitate bodies, numerous mitosis and hyper-segmented granulocytes.

Table 4 showed the treatment means of the various serological parameters for broilers on the CVMP and LPNVP-based diets. The values were close for both treatments and were not significantly different ($P>0.05$). They were also comparable to the reported values. Serum biochemistry was favourable to the dietary conditions; the values observed were also within normal ranges. Annongu (1997) observed that high serum ureate is correlated with liver damage. This observation is also in line with NRC (1994) that high serum NPN (a result of pyridoxine deficiency) precipitates liver damage. It has been reported that cellular glucose is affected by vitamin A in poultry via indirect phosphorylase activities (Nockel and Philips, 1971); as a result, high muscle glycogen ten to suggest vitamin A deficiency and production of glucose from

TABLE 4 **Effect of Improved LPNVP on some Serum Parameters of Broiler Chickens.**

Serum Parameters	Treatments			SEM
	CVMP	CVMP	CVMP	
Ca (%)	2.34	2,34	2.33	2.94
P (%)	0.75	0.70	0.72	0.25
Glucose (Mmol/L)	3.4	3.6	3.2	1.23
Cholesterol (Mmol/L)	2.6	2.9	2.7	0.96
Uric acid (Mmol/L)	0.20	0.25	0.25	1.45
Creatinine (Mmol/L)	62	58	59	4.62
Total protein (g/100ml)	68	66	66	5.68

a.b. Treatment means within rows followed by different superscripts are significantly different ($P<0.05$).

protein (Brubacher *et al.*, 1985). The end product of this process is the deposition of uric acid in kidney and ureter, which may precipitate kidney dysfunction (Chandra *et al.*, 1984). This observation (i.e. normal serum glucose) may therefore suggest vitamin adequacy especially vitamin A in the LPNVP-diet. Reduced serum cholesterol concentration implies impared transport of and metabolism of lipids (Shukla and Pachaurii, 1995). This reduction has been reported to be a result of riboflavin and has also been reported during afflatoxicosis with a concomitant calcium absorption inhibition.

Serum phosporus and calcium have been reported to be a function of vitamin D nutrition since vitamin D is thought to perform a major role in bone mineralization by homeostastically regulating their blood economies. McDowell (1985) reported that vitamin D performs this role by including the synthesis of calcium binding prtein *Cabp* in the intestine, kidney and uterus; enhancing calcium absorption from GIT, and recovery from urine and shell deposits. Vitamin D deficiency has also been reported to depress Ca^{2+} absorption from the GIT resulting in rickets and hypocalcaemia thereafter (McCarthy *et al.*, 1984). Serum protein has been implicated as a pointer to strong amino acid metabolism (Shukla and Pachaurii 1995). Decreased serum protein concentration suggests alteration of normal

protein metabolism due to interference of protein utilization. This observation is also thought to affect liver function of protein metabolism.

Increase serum uric acid and creatinine are also due to the metabolism of muscle proteins and indicative of increased protein degradation enzymes e.g. arginase, ornithine trans-carbamilase responsible for regulating creatinine and ureate levels. This observation agrees with the findings of Balogun (1995) that increased uric acid points to liver damage. This is thought to result from changes in dietary protein components of total urinary nitrogen (Ward *et al.*, 1975). During conditions of decreased dietary protein, less of uric acid and ammonia are excreted while amino acid excretion remains constant. High serum non-protein nitrogen has been linked with pyridoxine deficiency (Attar *et al.*, 1967). That these parameters were normal in the study conducted is a pointer to positive synergistic effect of the innate vitamins and the improved LPNVP on the biochemical processes within the body.

Generally, improvement of LPNVP via the addition of BHT and antimicrobial resulted in positive synergism for the performance of broilers. It was relatively cheaper to use LPNVP as vitamin source *vis-à-viz* CVMP as a source of vitamins for broilers.

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