

**EFFECT OF VARYING LEVELS OF IRON FORTIFIED
LOCALLY PRODUCED NATURAL VITAMIN PREMIXES ON
THE HISTOLOGY AND SPECIFIC ENZYME ACTIVITIES
OF BROILERS.**

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Target Audience: Researchers, feed manufacturers, teachers, consumers
and extension workers.

ABSTRACT

Enzyme activities and histology of some organs were investigated in broilers fed diets containing graded levels of iron-fortified locally produced natural vitamin premix (LPNVP) in a completely randomized design.

Histological alterations were observed in organs such as liver, kidney and villi for birds fed diets containing iron-fortified (LPNVP). These alterations were more pronounced in broilers fed lower levels of LPNVP. Histology of the red blood cell confirmed the necessity for iron fortification of LPNVP.

Plasma alkaline phosphatase (PAP) and serum aspartate amino transferase (AST) values were significantly different ($P < 0.05$) among the different treatments. However, normal values were observed for broilers fed Commercial vitamin-mineral premix (CVMP)- based diet. The values reported for serum alanine amino transferase (ALT) was not different ($P > 0.05$) among the various treatments.

In summary, the results of enzyme activities tended to corroborate with the observed alterations in the histological studies. Lower inclusion levels of the iron-fortified (LPNVP) did not adequately supply the needed vitamins for use in the broilers. However, as the levels of inclusion of the LPNVP increased, there was consistent improvement in the histological competence and enzyme profile of broilers.

Keywords: Iron-fortified locally produced natural vitamin premix, commercial vitamin-mineral premix, broiler.

DESCRIPTION OF PROBLEM

Formulation of herbal alternatives of the various essential synthetic biological substrates is a current trend in animal nutrition. This is informed by the possibility of synthetic products to precipitate carcinogenic conditions in biological systems (12). In an earlier study, (3) reported that inclusion of alternative natural vitamin premix at 0.5% and 1.0% were optimum for layers; 0.5% was also reported optimum for broilers. It was also recommended that reduction of inclusion

levels might be possible to enable the natural vitamin premix compete (in the provision of vitamins) adequately with the commercial vitamin/mineral premix (CVMP).

Commercially sold synthetic analogues differ in inclusion levels depending on the manufacturer. Most of the Vitamin /mineral premixes are included at 2.5kg/ton of feed e.g. Agricare, (Pfizer); Anupco and Delavit premixes; 3kg/ton for Toyola premix and 5.0kg/ton of feed for Peterhand premix. (4) reported specific mineral deficiencies (especially iron) in the performance of broilers fed LPNVP-based diets. It was also observed that haematological parameters especially haemoglobin concentration, packed cell volume and erythrocyte counts were affected. Most of the experiments conducted so far, have not taken into consideration the effects of the LPNVP on the histology and enzyme activities of broilers. It has been reported however that these parameters are indirect assessments of vitamin status in birds (8,9).

The current experiment was conducted to investigate the response of broilers to inclusion levels and mineral fortification (iron from blood meal) of LPNVP.

MATERIALS AND METHODS

One hundred and twenty (120) day-old mixed sex Hubbard broilers chicks (34-40g) were housed in electrically heated battery cages and fed experimental diet as shown in (Table 1). They were allocated to a previously completely randomized design comprising of six groups of twenty (20) birds each. The birds within the same group had equal mean weights. Each treatment was

Table 1 Composition of Experimental Diets (%).

Item	Diets					
	1	2	3	4	5	6
Basal ingredients**	99.75	99.90	99.85	99.80	99.75	99.70
ANUPCO Premix*	0.25	-	-	-	-	-
LPNVP***	-	0.10	0.15	0.20	0.25	0.30
Total	100	100	100	100	100	100

** Contained:

Maize; soybean; groundnut cake; fishmeal; oyster shell; bone meal; blood meal; wheat bran; methionine and salt Analyzed nutrient content: crude protein (%), 24; crude fat (%), 2.5; crude fibre (%) 3.6; total ash (%), 13. Minerals (as calculated) ca (%), 0.9; K (%), 0.2; Na (%) 0.15, cl (mg), 800; cu (Mg), 4; Se (mg), 0.1; Zn (mg), 40.

* Contain (Kg):-

Retinol, 4×10^5 i.u; cholecalciferol, 1.2×10^6 i.u; atocopherol, 3200 i.u. Menadione, 800 mg; riboflavin, 2200 mg; thiamin, 3200mg; niacin, 400 mg; pyridoxine, 480 mg; Calcium pantothenate, 2800 mg; folate 240 mg; choline choride, 2×10^5 mg; biotin, 12 mg; Se. 40 mg; Mn, 32000 Cu, 3200 mg; Zn, 2×10^5 mg; Co, 180 mg; I, 800 mg; Mg, 400 mg.

*** Formulated to meet NRC(1994) requirement. Blood meal to supply 200mg/kg

Table 2 Effect of varying levels of Iron-fortified LPNVP on enzyme activities of broilers

Enzymes	Diets						SEM
	1	2	3	4	5	6	
Plasma Alkaline Phosphatase (IU/l)	35 ^a	45 ^a	52 ^{ab}	57 ^{ab}	76 ^c	42 ^a	3.44
Serum Alanine amino Transferase ALT (IU/l)	12	12	14	15	15	12	1.25
Serum Aspartate amino Transferase AST (IU/l)	52 ^a	104 ^b	62 ^a	104 ^b	120 ^b	59 ^a	3.85

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

replicated in four (4) units. LPNVP-based diets were formulated with LPNVP varied at five levels (treatments) viz. 0.10, 0.15, 0.20, 0.25 and 0.30%. The control diet contained ANUPCO broiler premix at the dietary recommended level of 0.25%.

Sourcing and Preparation of Vitamin Ingredients.

Fish discards were collected within Ilorin, Kwara state, Nigeria. These discards consist of abdominal fat and liver. Daily collections were kept in sealed polythene bags in refrigerator (4°C) for about 16 hrs. Fish oil was extracted from these discards under mild heat. The extracted oil was stored at 4°C in a refrigerator. The extracted oil was premixed with other powdery ingredients prior to its incorporation into the locally produced natural vitamin premix (LPNVP).

Following oil extraction of the fish discards, the remaining residues were dried at 60°C in the oven for 24 hrs. Colostrum (milk produced between day 1 – 3 following parturition) was collected from local Fulanis (nomadic cattle rearers). Daily collections were dried to a semi-solid state. Palm oil was bought from Ipata market, Ilorin, Kwara state, Nigeria and was premixed in this form with other powdery premix ingredients prior to its use in the LPNVP. Bakers' yeast (Fermipan) was bought from Ipata market, Ilorin, Kwara state, Nigeria. Rice bran was collected from rice millers within Ilorin, Kwara state, Nigeria. The husk components were removed from the mixture by physical separation. Rice bran was added in this form with other LPNVP ingredients. Leaf vegetables such as *Telfaria occidentalis*, *Celosia spp*, *Capsicum frutescens* (Red pepper) and *Amaranthus spp* and tree leaves such as *Adansonia digitata* were bought fresh from Ipata market, Ilorin, Kwara state, Nigeria. Ethiopian pepper (*Xylopi aetiopica*) was bought in the dried form from Ipata market, Ilorin, Kwara state, Nigeria. The dried ingredients were ground to a convenient particle size (1mm) prior to their use in the LPNVP.

Histopathological Samples.

Following carcass analysis, the organs required for histology were quickly dissected out, freed from any adhering fat, blotted free of blood and preserved in 10% formalin solution. The tissues were trimmed, fixed in Bouins fixative for 24hrs, embedded in wax, sectioned at 5-6m with a microton (Leitz Wetzair) and stained with hematoxylin and eosin (H&E). A histological study was carried out according to methods described by (8).

Samples for Enzyme Studies.

Serological samples were taken from clotted bloods. The clotted blood samples were centrifuged at 400rpm for 3mins and the supernatant sera were harvested in Bijou bottles for specific serological parameter determination. Serum aspartate amino transferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) activities were determined according to the colorimetric method of (9). Fresh blood was centrifuged at 4000rpm and the supernatant plasma harvested. Plasma Alkaline phosphatase (APEC.3.1.3.1) activity was determined by the kinetic method of (7)

Chemical Analysis

Vitamin analysis was carried out at National Agency fo Food, Drug Administratiion and Control (NAFDAC), Kaduna Regional office using standard HPLC analytical procedures outlined for vitamin determination in Pharma and food premixes by (10).

Statistical Analysis

Data collected were subjected to analysis of variance using the model for completely randomized design (13). Differencesamong treatment means were subjected to Duncan's multiple range test (6).

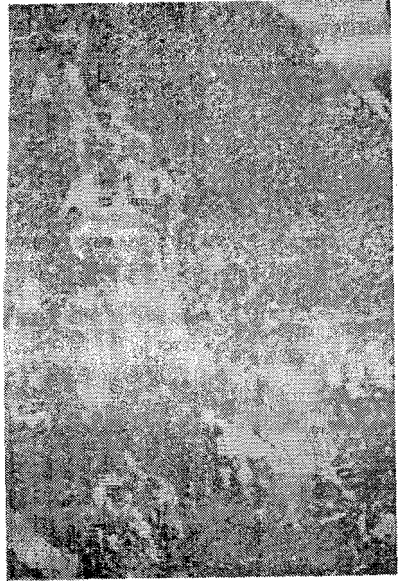
RESULTS AND DISCUSSION

Major histological lesions were observed especially in birds fed various inclusion levels of LPNVP. The organs mostly affected were the liver, villi, and kidney (F1g 1 – 3). In these organs, major changes in the cellular integrity and organ architecture were noticed. Other anomalies observed were cellular vacuolation, heavy cellular inflammation and depositions in the liver; slight muscular necrosis of the striated muscles and major intracellular infiltration of the kidney. These observations were at variance with those of broilers fed CVMP-based diet, which tended to suggest serious vitamin deficiencies at the lower levels of LPNVP inclusion. Histology of the blood however was not affected by varying dietary treatments as shown in (Fig 4).

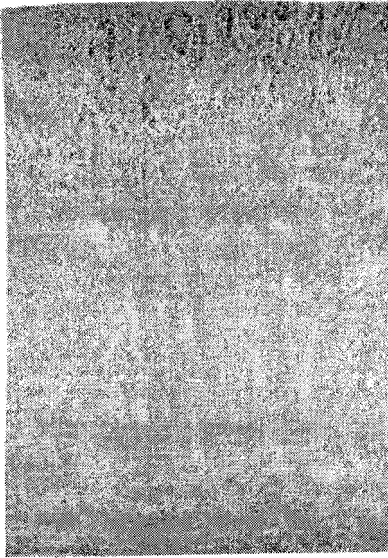
Histological observations as revealed in this study tended to suggest a



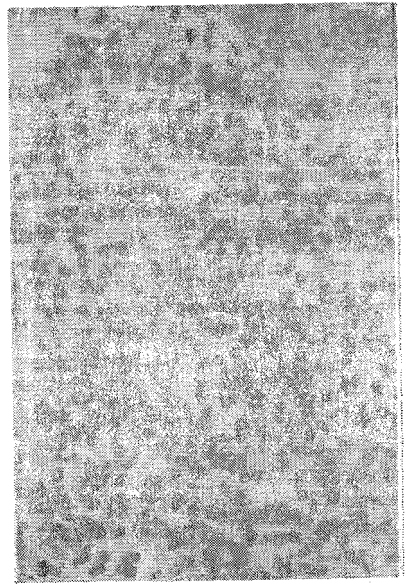
**Micrograph 1. Hepatic Cellular Infiltration at
0.25% Iron fortified LPNVP. x 60**
A = Cellular Detachment
B = Definite Deposit
C = Vacuolation



**Micrograph 2. Kidney Cells (Renalocytes)
Derangement at Various Levels of
LPNVP Inclusion. x 60**



**Micrograph 3. Denuded Villi at 0.15% of
Iron Fortified LPNVP. x 60**



Micrograph 4. Normal Haematology
A = Leukocytes
B = Erythrocytes

hypovitaminotic condition. Vitamin nutrition has been reported to alter the normal cellular integrity leading to eroded tissue architecture. Other histological revelations attributable to vitamin inadequacies are denuded epithelium coverings, cellular infiltration and vacuolations (14). The major organs affected during histological studies of birds fed LPNVP-based diets were liver, kidney and villi. That the liver architecture and integrity was altered is an indication of specific vitamin absence or deficiency. This observation agrees with the findings of (1). Kidney function is measured by uric acid and creatinine concentration in the serum. These parameters are however modified by specific vitamin nutrition (5). Villus denudation has been reported to be modified by vitamin A provision *in vivo* (14). That villus height and integrity was affected may tend to confirm vitamin A inadequacy. It is noteworthy that birds fed diets containing LPNVP recorded these histological aberrations at the various levels of inclusion. Although, these lesions tended to reduce with increasing levels of LPNVP, the differences were sometimes not noticeable. Histology of the blood reported in this study has proved that iron fortification of the LPNVP was necessary for the optimum haematological competence of broilers fed LPNVP-based diets.

The specific enzymed activities (Table 2) were different ($P < 0.05$) for broilers on the diets except for ALT. Plasma Alkaline phosphate was significantly high for higher inclusion levels of LPNVP. However, at 0.3 and 0.1% inclusion levels, comparable PLP activities were noticed. Serum Aspartate amino transferase (AST) was also $P < 0.05$ by the various diets. Birds fed LPNVP-based diets demonstrated higher serum activity for this enzyme.

Enzymological manifestations may proffer vitamin status of animals (2,9). Plasma ALP has been used to measure or reflect liver and heart damages, and muscle necrosis (11). The observation from histology thus corroborates the ALP values for the birds fed LPNVP based diets. Serum AST has been implicated as a membrane-associated enzyme involved in the inter-conversion of glutamate to its α -keto analogue in the cells. During conditions of liver damage, AST and ALT values have been observed to rise. This observation has also been implicated in cellular metabolism and energy processes (1). During conditions of hypovitaminosis occasioned by decreasing LPNVP levels, inconsistent but high serum values were observed for these parameters suggesting physiological defects microscopically confirmed at histology.

CONCLUSIONS AND APPLICATION

- (1) Lower levels of inclusion of the LPNVP had adverse effects on the histology and enzyme activities of broilers and should not be applied in broiler diets.
- (2) As the level of inclusion increases (signifying increasing level of vitamins), histological and enzyme activities of broilers fed LPNVP-based diets were

relatively comparable with the birds fed CVMP-based diets. Inclusion levels of LPNVP in broiler diets should be 0.30% and above.

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