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# MORPHOMETRIC ORGAN WEIGHT AND HISTOPATHOLOGICAL STUDIES OF BROILER CHICKENS FED DIET CONTAINING GRADED LEVEL OF PRO-VITAMIN-A CASSAVA (*Manihot esculenta)* LEAF MEAL AS REPLACEMENT FOR GROUNDNUT CAKE

**Urom, S.M.O.C., Nsing, F.E., Onunkwo, D. N., Zachary, B.N., Onabanjo, R.S. and Oko, E. C.**
Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
Corresponding Author's email: donunkwo1@gmail.com

**Abstract**
The study was conducted to investigate the morphometric organ weight and histopathology of broiler chickens fed diet containing Pro-vitamin-A Cassava Leaf Meal (PVACLM) as a replacement for groundnut cake (GNC) protein. A total of 120-day old Ross-308 broiler chicks were randomly allotted into 4 treatment groups of 30 birds per group, each group was further divided into 3 replicates of 10 chicks per replicate in a Completely Randomized Design (CRD). The group were tagged as treatment 1($T_1$), treatment 2($T_2$), treatment 3($T_3$) and treatment 4($T_4$) at the ratio of 0%, 5%, 10% and 15%. PVACLM $T_1$ had no PVACLM and served as the control. The results of the organ weight of broiler chickens showed that the heart, kidney, bile and spleen differed significantly ($p>0.05$) among the treatment groups. $T_4$ had the highest value (0.7567 g) for the heart, kidney (1.0367 g), bile (0.2300g), and spleen (0.2300g). The result of 0% inclusion of Pro Vitamin A-Cassava leaf meal on broiler chickens showed normal hepatic histo-architecture, while 5% and 10% inclusion of Pro Vitamin A-Cassava leaf meal showed sections of the liver with multifocal areas of hepatocellular necrosis with marked infiltration of inflammatory leukocytes (Plate 1-4). The result of 15% inclusion of Pro Vitamin A-Cassava leaf meal on broiler chickens showed mild periportal infiltration of inflammatory leukocytes Therefore for efficient productivity and reduction in cost of production, 15% inclusion of graded PVACLM is recommended.

***Keywords: PVACLM, GNC, heart, kidney, bile, sple***en

## Introduction

Provision of feed is the most important consideration in poultry enterprise. Feed alone has been reported to account for 60-80% of the total cost of poultry production in developing countries (Onunkwo *et al*., 2021). Limitations imposed by scarcity and high cost of conventional feedstuff due to its consumption by humans has forced many farmers into employing other readily available alternative source of protein/energy such as cassava. Cassava is one of the alternative feed ingredients that can replace a considerably proportion of conventional feed resources in livestock feed industry (Bokanga, 1995). Its products have been in use for a long time in place of cereal grains for livestock (Ervubetine *et al*., 2003), but its use as animal feed is being constrained by the present of toxic cyanogens linamarin and lotaustrulin in its leaves and tubers (Ogundu *et al*., 2014). Dried cassava leaves processed for food or feed (Cassava Leaf Meal or CLM; also called cassava leaf powder) has been analysed in detail as a potential source of dietary protein and other nutrients. Average leaves contain about 70% water, whereas, the dried meal is approximately 9–10% moisture (Wobeto *et al*., 2007). Energy content in leaves is high for both ruminants and swine, with digestibility ranging from 62–73%, and Digestible Energy (DE) (MJ/kg DM) values of 12.3–13.2, slightly higher (15.2) measured in wilted forage fed to growing pigs. Energy values are considerably lower for poultry [apparent Metabolizable Energy (ME) 7.8 MJ/kg DM for broilers), due to high fiber levels in leaves. Fiber content increases with maturity; both Nitrogen Detergent Fiber (20–30% of DM, up to 60% in some reports) and Crude fiber (8–20%) fractions are not insignificant. Crude protein, with highest levels in leaves approximately 12 months of age, is reported to vary from ~17 to 40% of Dry matter (Wobeto *et al*., 2007), averaging ~21%; current summary data average slightly higher (25–28%). Almost 85% of the crude protein fraction is true protein according to Onunkwo *et al.* (2021). Cassava leaf meal could be included up to 20% in broiler diets, whereas, the inclusion levels of cassava foliage meal were slightly lower (Khieu, 2005). To close the gap between high cost of feed ingredients and non-availability of non-conventional feedstuffs, there is need to use more of un- conventional feedstuff. Consequently, the optimum

level of inclusion PVCLM in poultry diets has not been fully studied. This has become essential in animal feeding to minimize the competition of livestock with human for conventional fed and for economic reasons.

## Materials and Methods

### *Experimental Site*

The study was carried out at the Poultry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria (MOUAU). The area falls within the Tropical rain forest zone, it is located at latitude 05° 21°N and longitude 07° 33°E, its elevation is about 112m above sea level. It has an average Rainfall of about 2177mm/annum, Relative Humidity of about 50-90% and a monthly temperature range of 17°-36°C. (Meteorological Station, NRCRI, Umudike, 2020).

### *Experimental Animals and Management*

A total of 120-day-old chicks (broilers) used for the research was purchased from a reputable hatchery in Ibadan, Oyo State, Nigeria. The chicks were brooded in a deep litter for 4 weeks. They were provided with heat using kerosene lampshade, hot coal pot and a 400watt electric bulb. The broilers were well vaccinated and medicated for the period. The birds were randomly divided into 4 treatment groups of 30 birds per group and each group was divided into 3 replicates of 10 birds per replicate. Feed and water were provided *ad libitum*. The broiler chickens were reared on the deep litter house using wood shaving. This study lasted for 8 weeks.

### *Experimental design*

### *Statistical model*

### *Experimental Diet and Preparation*

Pro vitamin A cassava leaf (*Manihot esculenta)* gotten from National Root Crop Research Institute (NRCRI), Umudike was air dried (drying under shade) for about 2-3 days, then chopped into pieces and milled (using hammer miller). The pro-vitamin A cassava leaf meal (PVACLM) was used to substitute groundnut cake (GNC) protein at levels of 0%, 5%, 10% and 15% for $T_1$ (control), $T_2$, $T_3$, and $T_4$ inclusion levels for both starter mash and finisher mash as shown in Tables 1 and 2 respectively.

### *Data Collection*

### *Organ weight evaluation and parameters measured*

At the end of the 8 weeks research, 2 birds were taken from each replicate for organ weight evaluation**.** The birds slaughtered were fasted for 24 hours before slaughtering to reduce the contents of the gastro-intestinal tract, but water was supplied to them *ad libitum.* Slaughtering was done by making a clean cut across the jugular vein and the birds allowed to bleed for at least two minutes. The weights of the birds before slaughtering and after slaughtering were taken. Each bird was dipped in hot water of about 60°C for about a minute and then defeathered. The defeathered weights of the birds were also taken.

### *Histopathology of the liver of the birds*

Sections of the liver were collected for histopathological examination. The samples were fixed in 10% phosphate buffered formalin for a minimum of 48 hours. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in 3 grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5µm thick with a rotary microtome, floated in water bathe and incubated at 60°C for 30 minutes. The 5µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90%, 80% and 70%). The sections were then stained with Hematoxylin for 15 minutes. Blueofing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX. The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were randomly taken using a Motic™ 5.0 megapixels microscope camera at x160 magnifications.

### *Statistical Analysis*

Data collected were subjected to analysis of variance (ANOVA). The mean separation was carried out using Duncan's Multiple Range Test as described by Duncan (1955).

## Results and Discussion

The result of the morphometric organ weight of broiler chickens fed pro-vitamin A cassava leaf meal (PVACLM) (% of live weight) is shown in Table 3. The result showed no significant difference (P> 0.05) among the treatment groups except the heart, kidney, bile and the spleen. All these parameters measured followed the same trend which increased with high levels of the test ingredient. The highest value of 0.7567g was recorded in the $T_4$ in the heart, followed by $T_3$0.6200g, $T_2$ 0.6100g, while the least value of 0.5767g was recorded in $T_1$. The kidney followed the same trend as the heart with the highest value recorded in $T_4$ (1.031g) and the least value of 0.7076g recorded in $T_1$. In Bile, the highest value of 0.2300g was recorded in $T_4$, while the lowest value of 0.1867 g was recorded in $T_1$. Also, the highest value (0.2300g) in spleen was recorded in $T_4$ and the least value of (0.1867g) recorded in $T_1$.

### *Histopathology of broiler birds fed pro vitamin A cassava leaf meal*

The effect of 0%($T_1$), 5%($T_2$), 10%($T_3$) and 15%($T_4$) inclusion of Pro-Vit-A Cassava on broiler chickens is presented in Plate 1,2,3 and 4 respectively. The $T_1$ without the test ingredient was normal and showed the normal hepatic histo-architecture arranged in two-layer thick interconnecting cords separated by normal hepatic Sinusoids. Hepatic Sinusoids (arrow); Portal area (P). H&Ex160. $T_2$ and $T_3$ showed section of the liver with multifocal areas of hepatocellular necrosis with marked infiltration of inflammatory leukocytes (arrow). Portal area (P). H&Ex160. T4 showed mild periportal

infiltration of inflammatory leukocytes (arrow). Portal area (P). H&Ex160. Though this did not affect the health of the birds. This result corroborates with the finding of Ebenebe *et al.* (2018) and Esiegwu *et al.* (2013) who reported that that Garcinia kola seed meal caused some histological alteration in the liver of rats and laying hens respectively.

**Conclusion**

Pro vitamin A Cassava leaves meal are a good source of protein, high in lysine but deficient in methionine and tryptophane, and are rich in vitamins and minerals. Pro-Vit-A Cassava leaf meal can replace levels of GNC protein at inclusion of 15% inclusion level. The findings of this study suggest that broilers could be placed at 15% level of inclusion of PVACLM for better performance. Recommendation of pro vitamin A cassava leaf meal vary within wide ranges according to several research carried out by other authors. Protein quality can be improved by further processing cassava leaves into leaf protein concentrate. The price for cassava leaves is generally low when compared to the price of protein sources used in feed formulation. Therefore, could be easily affordable and accessible as protein source for broiler birds, which will aid improvement in birds' physiological performance.

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**Table 1: Composition of broiler starter diet containing Pro-vitamin-A Cassava Leaf Meal (PVACLM)**

| Ingredients | $T_1$ 0%PVACLM | $T_2$ 5%PVACLM | $T_3$ 10%PVACLM | $T_4$ 15%PVACLM |
|---|---|---|---|---|
| White maize | 51.30 | 51.30 | 51.30 | 51.30 |
| Groundnut cake | 18.00 | 16.80 | 15.60 | 14.40 |
| Soya bean meal | 17.00 | 17.00 | 17.00 | 17.00 |
| Pro-vitamin. A cassava leaf meal | 0.00 | 1.20 | 2.40 | 3.60 |
| Wheat offal | 5.00 | 5.00 | 5.00 | 5.00 |
| Bone meal | 3.00 | 3.00 | 3.00 | 3.00 |
| L – lysine | 0.10 | 0.10 | 0.10 | 0.10 |
| Fish meal | 5.00 | 5.00 | 5.00 | 5.00 |
| DL – Methionine | 0.10 | 0.10 | 0.10 | 0.10 |
| Common salt | 0.25 | 0.25 | 0.25 | 0.25 |
| Premix | 0.25 | 0.25 | 0.25 | 0.25 |
| **Total** | **100.00** | **100.00** | **100.00** | **100.00** |
| **Calculated analysis** | | | | |
| Crude protein | 23.23 | 22.98 | 22.74 | 22.50 |
| ME (Kcal/Kg) | 2770.3 | 2744.11 | 2717.92 | 2691.73 |

**Table 2: Composition of broiler finisher diet containing Pro-vitamin-A Cassava Leaf Meal (PVACLM)**

| Ingredients | $T_1$ 0%PVACLM | $T_2$ 5%PVACLM | $T_3$ 10%PVACLM | $T_4$ 15%PVACLM |
|---|---|---|---|---|
| White maize | 59.30 | 59.30 | 59.30 | 59.30 |
| Groundnut cake | 13.00 | 12.35 | 11.70 | 11.05 |
| Pro-vitamin A cassava leaf meal | 0.00 | 0.65 | 1.30 | 1.95 |
| Soya bean meal | 16.00 | 16.00 | 16.00 | 16.00 |
| Fish meal | 4.00 | 4.00 | 4.00 | 4.00 |
| Wheat offal | 4.00 | 4.00 | 4.00 | 4.00 |
| Bone meal | 3.00 | 3.00 | 3.00 | 3.00 |
| L – lysine | 0.10 | 0.10 | 0.10 | 0.10 |
| DL – Methionine | 0.10 | 0.10 | 0.10 | 0.10 |
| Common salt | 0.25 | 0.25 | 0.25 | 0.25 |
| Premix | 0.25 | 0.25 | 0.25 | 0.25 |
| **Total** | 100.00 | 100.00 | 100.00 | 100.00 |
| **Calculated analysis** | | | | |
| Crude protein | 20.06 | 19.95 | 19.83 | 19.71 |
| ME (Kcal/Kg) | 2831.61 | 2817.43 | 2803.23 | 2789.04 |

**Table 3: Morphometric organ weight of broiler fed pro-vitamin A cassava leaf meal (PVACLM) (% of live weight)**

| Parameter (g) | $T_1$ 0%PVACLM | $T_2$ 5%PVACLM | $T_3$ 10%PVACLM | $T_3$ 10%PVACLM | |
|---|---|---|---|---|---|
| Heart | $0.57^{b}$ | $0.61^{b}$ | $0.62^{b}$ | $0.75^{a}$ | 0.02 |
| Liver | 2.82 | 2.98 | 2.83 | 3.13 | 0.08 |
| Kidney | $0.70^{b}$ | $1.00^{a}$ | $0.92^{ab}$ | $1.03^{a}$ | 0.49 |
| Gizzard | 2.47 | 2.77 | 2.72 | 2.58 | 0.09 |
| Proventriculus | 0.54 | 0.61 | 0.56 | 0.58 | 0.02 |
| Lungs | 0.85 | 0.94 | 1.06 | 0.96 | 0.04 |
| Bile | $0.18^{b}$ | $0.22^{a}$ | $0.21^{a}$ | $0.23^{a}$ | 0.01 |
| Crop | 0.78 | 0.91 | 0.95 | 1.03 | 0.05 |
| Abdominal fat | 0.37 | 0.34 | 0.44 | 0.17 | 0.05 |
| Spleen | $0.18^{b}$ | $0.22^{ab}$ | $0.23^{a}$ | $0.23^{a}$ | 0.01 |
| Small intestine | 5.98 | 5.83 | 7.11 | 7.09 | 0.27 |
| Large intestine | 1.02 | 0.99 | 0.71 | 0.76 | 0.08 |
| Pancreas | 0.43 | 0.39 | 0.54 | 0.45 | 0.03 |

**$^{ab}$ Means within the rows with different superscripts differ significantly($p<0.05$); SEM-Standard error of mean**

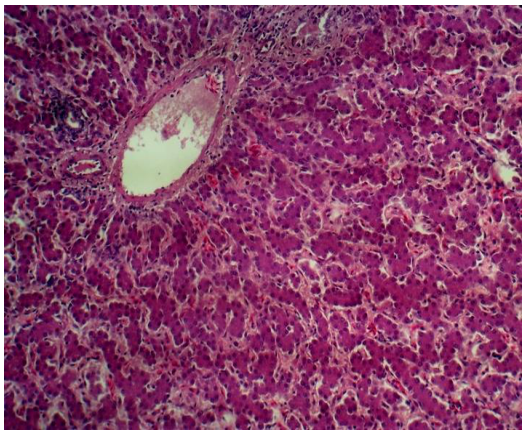
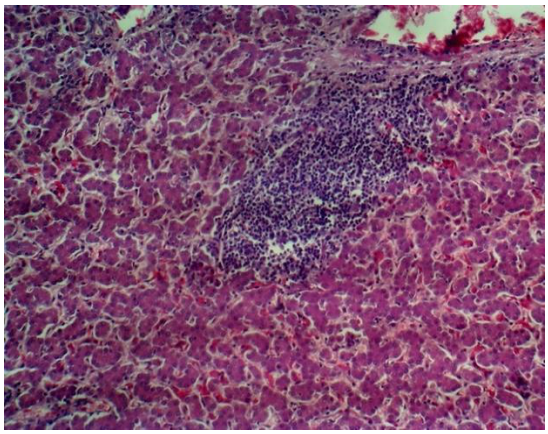


**Plate 1:** H&Ex160



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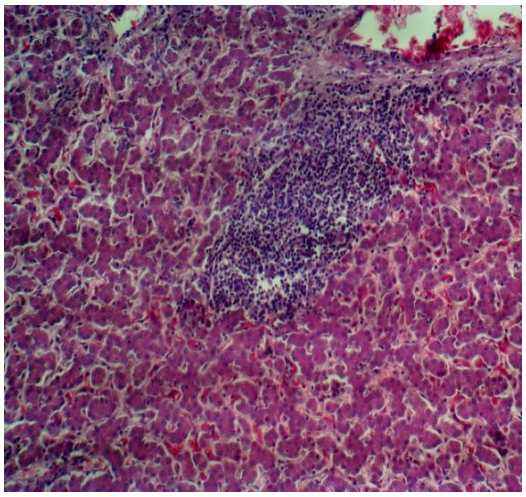

**Plate 3:** H&Ex160

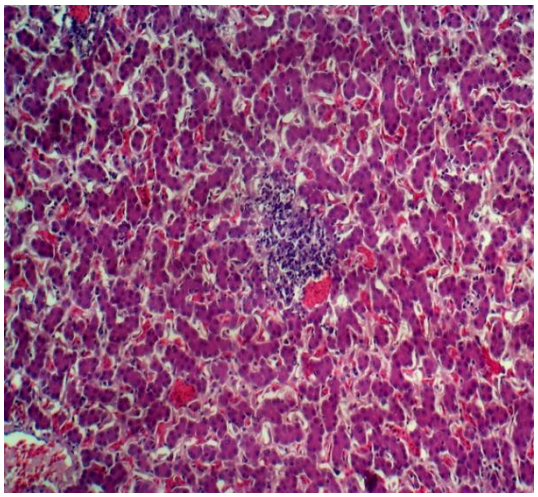

**Plate 4:** H&Ex160