

Effects of Feeding Cassava Root Meal and Activated Charcoal Supplementation on the Liver and Intestinal Histology of Grower Pigs

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Abstract: Feeding value of cassava root meal supplemented with and without activated charcoal was investigated using sixty-four (64) grower pigs (Large White x Landrace). The pigs were allotted to 8 treatments with 8 pigs per treatment and each treatment replicated twice. Four diets were compounded such that cassava root meal (CRM) replaced maize at 0, 25, 50 and 75% levels. Another four diets containing the same levels of CRM were compounded with activated charcoal supplementation at 40g/kg diet. Results showed that increasing level of CRM resulted in varying degrees of inflammatory infiltration and wide lumen in architectural background with attendant degenerative changes in the lobules and some measure of fibrotic portal tracts in the liver. However, activated charcoal was able to make correction up to 50% CRM level. Histology of small intestine revealed that as CRM level increased there was attendant increase in the damage in terms of erosion of the villi, desquamation of epithelial cells and numerous lymphoid aggregates. However, activated charcoal supplementation was able to make correction reasonably up to 50% CRM level. It was concluded that feeding cassava root meal based diets at 50% replacement level for maize with activated charcoal supplementation at 40g/kg tended to give corrective measure on the liver and intestinal integrities.

Keywords: Cassava root meal, activated charcoal, histology, grower pigs

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I. Introduction

Cassava (*Manihot esculenta* also known as manioc, or yuca) is one of the leading food and feed plants in the world. It ranks fourth among staple crops with a global production of about 160 million tons per year (Lawrence and Moore, 2005). A great number of recent studies have reported many biotechnological approaches to improve the safety and quality of cassava products (Onitilo *et al.*, 2007) and the effect of different processing modalities of the tuberous roots on the level of these toxic substances and functional properties has been assessed (Nambisan and Sundaresan, 1985; Udensi, *et al.*, 2005).

Cassava is a staple food that provides carbohydrates or energy for more than 2 billion people in the tropics (Ukwuru and Egbonu, 2013). Cassava plays a dominant role in the rural economy in the agro-ecological zone (Adenijiet *et al.*, 2001).

The bulky roots contain much moisture (60-65%) making their transportation from rural areas difficult and expensive. Processing the tuber into a dry form reduces the moisture content and converts into a more durable and stable product with less volume which makes it more transportable (IITA 1990, Ugwu, 1996). Cassava in the fresh form contains cyanide which is extremely toxic to humans and animals and therefore needs to be processed to reduce the cyanide content to safe level. (Eggelston, *et al.*, 1992).

Traditional cassava processing methods involve several steps including peeling, soaking, grinding, steeping in water and leaving in the air to allow fermentation to occur, and drying. Cassava processing by traditional methods is labour-intensive but the application of improved processing technology has reduced processing time, labour and encourage further production (Adenijiet *et al.*, 2001).

Cassava is fed to livestock in the fresh or processed form. While different methods reduce the cyanide level in cassava, the reported loss in cyanide content differs considerably due to analytical methods, the combination of methods and extent to which the process is carried out. Drying is one of the methods of reducing the cyanide level. An improvement in sun drying of cassava roots using inclined tray-drying was reported by Gomez *et al.*, (1984). The residual total cyanide content was 10-30% of the fresh sample, with about 60-80% of the total cyanide in the dried chips occurring as free cyanide. Gomez *et al.*, (1984) indicated that more than 86% HCN is lost during sun drying.

Excess consumption of unprocessed cassava led to enzymatic breakdown of the glycoside releasing HCN and thereby causing poisoning. Cassava toxicity may be acute and / or chronic. Acute toxicity results from ingestion of lethal dose and death is caused by the inhibition of cytochrome oxidase of the respiratory chain by cyanide. This has been reported in goats ingesting cassava leaves (Obioha, 1972) and also in non-ruminants like pigs, when fed fresh uncooked tubers. Chronic cyanide toxicity in animals can affect both the growth and reproductive phases of development. Supplemental values of the nutrients in cassava had been evaluated using protein and amino acids (Job, 1975), palm oil (Omole and Onwudike, 1982), iodine and other dietary minerals. The use of activated charcoal to reduce the cyanide in gut of pigs will add to existing external assessment of reducing the HCN content of cassava product. Activated charcoal is non-nutritive and an inert adsorbent. It has ability to bind chemicals, aflatoxin etc. and reduce their absorption from the gastro intestinal tract. Activated charcoal is not absorbed from the gastro intestinal tract and has ability to bind physically with the chemical substances and precludes their absorption (Heshamet *et al.*, 2004). The major advantages include expense, safety and easy administration through addition to the animal feed (Ledoux *et al.*, 1999).

The objective of this study was to evaluate the effects of feeding cassava root meal diets supplemented with activated charcoal on the liver and intestinal histology of grower pigs.

II. Materials And Methods

Site of the experiment

The experiment was carried out at the Piggery Unit of the Teaching and Research Farm, Ladok Akintola University of Technology, Ogbomosho; Oyo-State Nigeria. The area is in the derived savannah zone of Nigeria. It is located between latitudes 8° 07'N and 8° 12'N and longitudes 4°04'E and 4°15'E. The mean annual rainfall is 1247mm with a relative humidity of between 75 and 95%. It is situated at about 500m above sea level with a mean annual temperature of 26.2°C (Oguntoyinbo, 1978).

Collection of test ingredients

Fresh cassava of sweet variety (TMS 3052) was obtained from Aarada central market, located in the Ogbomosho South Local Government Area of Oyo State while activated charcoal was purchased from a chemical store around Oke-Ado Sabo Area, Ogbomosho.

Processing of the test ingredient

Fresh cassava roots obtained were cleaned of accompanying dirt, chopped and thinly spread on concrete slab for sun drying and left to dry until constant weight was attained with frequent turning to prevent fermentation. The dried chips were then milled using hammer mill to obtain cassava root meal (CRM). This was then stored in sacks kept from dampness until needed.

Preparation of experimental diets

Four diets were compounded to contain cassava root meal (CRM) at 0, 25, 50 and 75% replacement levels for maize and supplemented with activated charcoal at 40g/kg diet. Another four (4) diets containing the same levels of CRM were constituted without activated charcoal supplementation to make eight diets in all. The composition of the experimental diets is shown in Table 1

Table 1: Gross Composition of experimental diets for grower pigs

Ingredients	1(0%)	2(25%)	3(50%)	4(75%)	1(0%)	2(25%)	3(50%)	3(75%)
Maize	55.0	41.25	27.50	13.75	55.0	41.25	27.50	13.75
Cassava root meal	-	13.75	27.50	41.25	-	13.25	27.50	41.25
Soyabeanmeal	10	10	10	10	10	10	10	10
Fish meal	2	2	2	2	2	2	2	2
Blood meal	3	5	6	7	3	5	6	7
Wheat offal	25	23	22	21	25	23	22	21
Bone meal	2	2	2	2	2	2	2	2
Oyster shell	2	2	2	2	2	2	2	2
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Activated charcoal	+	+	+	+	-	-	-	-
Calculated Analysis								
Crude protein%	18.04	18.22	17.79	17.34	18.04	18.22	17.79	17.34
Metabolizable								

Energy (ME)	2747	2719	2690	2661	2747	2719	2690	2661
(kcal/kgdiet)								
Crude;fibre(%)	3.94	4.09	4.12	4.24	3.94	4.09	4.12	4.24

* Each kg feed contained: Vit. A, 1500IU; Vit. B₁, 1500IU; Vit. B₂, 2,500IU; Vit. B₆, 20mg; Vit. B₁₂, 0.02mg; Chlorine chloride, 400mg; Mn 120mg; Fe 70mg; Cu 100mg; I 1;2.2m; Se 0.2mg; Zn 45mg; Co; 0.02mg.

Experimental animals and management

Sixty four (64) grower (Large White x Landrace) pigs of 16 weeks of age were balanced for weight and allotted to eight treatments with eight (8) pigs per treatment, and each treatment replicated twice. The animals were intensively managed in individual pen each measuring 0.46 x 0.9m with concrete floor and equipped with feeding and drinking troughs. They were allowed one week adjustment period during which they were fed the control diet and water supplied *ad-libitum*. The animals were dewormed against both internal and external parasites using Ivomec®. Other routine management practices were strictly followed. To reduce feed wastage the animals were fed twice daily (Morning – 8.00 hours and afternoon – 16.00 hours).

Experimental design

The experiment was arranged using 2 x 4 factorial arrangement in a completely randomized design such that there were two factors: Charcoal supplementation (factor 1) which had 2 levels (supplementation and no supplementation) while factor 2 (% CRM) had 4 levels (0, 25, 50 and 75%).

Histological Analysis

After slaughtering and dissection, segments of the intestine and liver samples were harvested. The intestinal segments were flushed with physiological saline and fixed in Bouin's fluid. The samples were later transferred to 70% alcohol and cleared in chloroform before being embedded in two changes of molten paraffin wax for 20 minutes each in an oven at 57°C. They were placed vertically in molten paraffin wax inside a metal mould and left overnight to cool and solidify. They were later trimmed and mounted on wooden blocks. Serial sections were cut using rotary microtome at 5 microns. Sections were floated on water bath to spread out and later picked into albumenized slides and dried on a hot plate at 52°C. Staining was done with haematoxylin-eosin, and slides were prepared from the tissues. Mountant (a drop) was placed on the surface of slide and covered with a 22 by 22mm cover slide (Bustos – Obregon and Gonzalez – Hormazabal (2003)). The histological examinations were done using a microscope connected to a computer system. A photomicrographic software – Phoenix Micro Image Analysis (2003) version 1.33 was used to project the slides on the computer for clear assessment.

III. Results

Histological changes in the liver of grower pigs fed cassava root meal based diets with and without activated charcoal supplementation.

The micrographs of the liver of grower pigs on the control diet (0% CRM without activated charcoal) is presented in Plate 1a while that of the control supplemented with activated charcoal is presented in Plate 1b. Liver architecture was intact. Dense inflammatory cells within the fibrous tissues, separating the lobules are distinct which characterize the histological presentation of pigs. No degenerative change and necrosis were noticed. In Plate 1b, the presentation was similar.

The micrograph of the liver of grower pigs fed 25% CRM without activated charcoal supplementation is shown in Plate 2a. Few of the portal tracts showed inflammatory infiltrations. Other presentations are normal. Plate 2b shows the micrograph of the liver of grower pigs fed 25% CRM with activated charcoal supplementation. The histological presentation here showed that the lobules are well partitioned, with no histological alteration. The infiltration caused by CRM diets was corrected by activated charcoal supplementation.

Liver micrographs of grower pigs fed diets containing 50% CRM without activated charcoal supplementation is shown in Plate 3a. Few of the portal tracts showed inflammatory infiltrations with attendant wide lumen in the architectural background of the lumen. Plate 3b showed the micrograph of the liver of grower pigs fed 50% CRM with activated charcoal supplementation. Although the tissues separating the lobules appeared fibrotic, yet the lobules are well partitioned with the architectural background remaining intact. This shows the tendency of activated charcoal in reducing the adverse effects of CRM diet on the liver of grower pigs.

The micrograph of the liver of grower pigs fed 75% CRM without activated charcoal supplementation is shown in Plate 4a. Portal tracts were inflamed with degenerative changes in the lobules as a result of higher level of infiltrations. Very visible fibrous tissues show some measure of infiltrations into the lobules as a result of ulceration in the liver of pigs fed 75% CRM based diets supplemented with activated charcoal (Plate 4b).

Histological changes in the intestinal morphology of grower pigs fed cassava root meal based diets with and without activated charcoal supplementation.

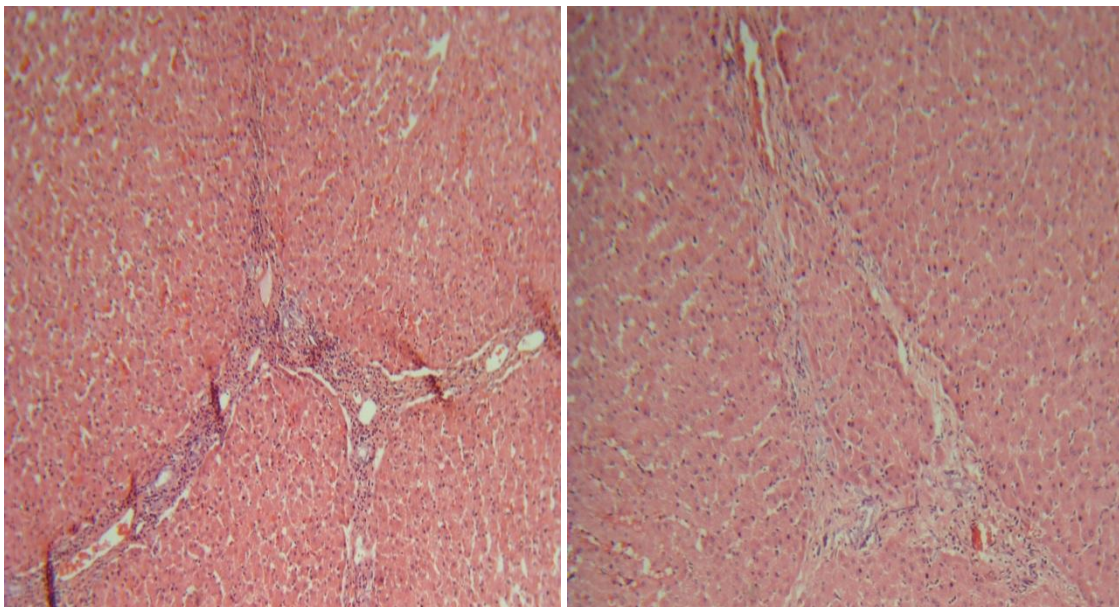
The micrograph of the intestinal morphology of grower pigs fed control (0%CRM) without activated charcoal supplementation is presented in Plate 5a. No desquamation of the epithelium and integrity of the intestinal mucosa are maintained. This shows that there is no erosion and inflammation of the crypt and villus. Plate 5b shows the micrograph of the intestinal morphology of grower pigs fed control diet (0% CRM) supplemented with activated charcoal. The presentation is similar with Plate 5a where no erosion and inflammation of the epithelium occurred.

Intestinal micrographs of grower pigs fed diets containing 25% CRM without activated charcoal supplementation is shown in Plate 6a. Histological presentation showed that there are focal areas of mild erosion on the villi with inflammatory cells infiltration. However, both the villi and crypts integrities are maintained in pigs fed 25% CRM with activated charcoal supplementation (Plate 6b).

The micrograph of the intestinal morphology of grower pigs fed 50% CRM without activated charcoal supplementation is presented in Plate 7a. There were extensive desquamations of the epithelium in terms of areas of erosion and complete loss of mucosa. Plate 7b shows the micrograph of the intestinal morphology of grower pigs fed 50% CRM with activated charcoal supplementation. The presentation is such that there was no erosion but numerous lymphoid aggregates were observed.

Extensive areas of erosion and complete loss of villi were observed in the intestine of grower pigs fed 75% CRM diets without activated charcoal supplementation (Plate 8a). This presentation reduced the height of the villi and desquamate the mucosal layer of the crypt. The micrograph of the intestinal architecture of the grower pigs fed 75% CRM diets with activated charcoal supplementation is presented in Plate 8b. Focal areas of erosion were observed although not extensive but with numerous lymphoid aggregates and partial desquamation of the epithelium of intestinal mucosa.

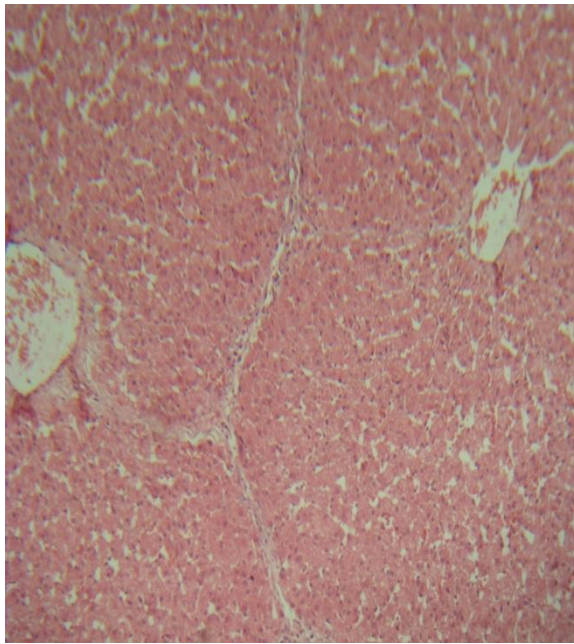
PLATE 1: LIVER OF EXPERIMENTAL GROWER PIG AS AFFECTED BY CONTROL DIET AND CONTROL + ACTIVATED CHARCOAL



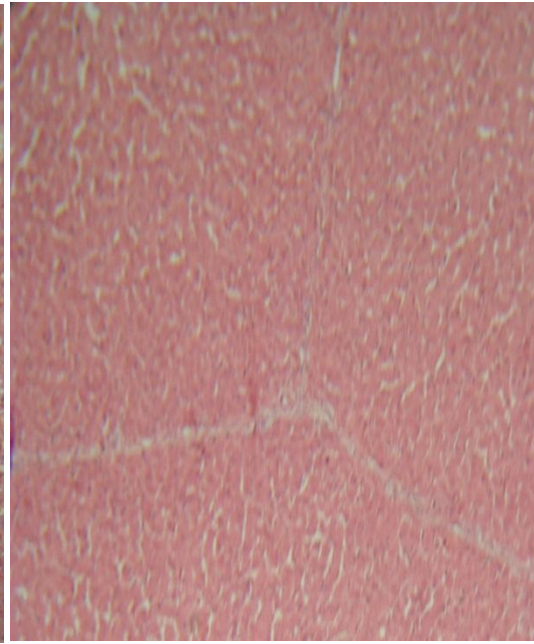
A: Liver architecture of experimental grower pig fed the control diet.

B: Liver architecture of experimental grower pig fed the control diet + activated charcoal

PLATE 2: LIVER OF EXPERIMENTAL GROWER PIG AS AFFECTED BY 25% CRM AND 25% CRM + ACTIVATED CHARCOAL

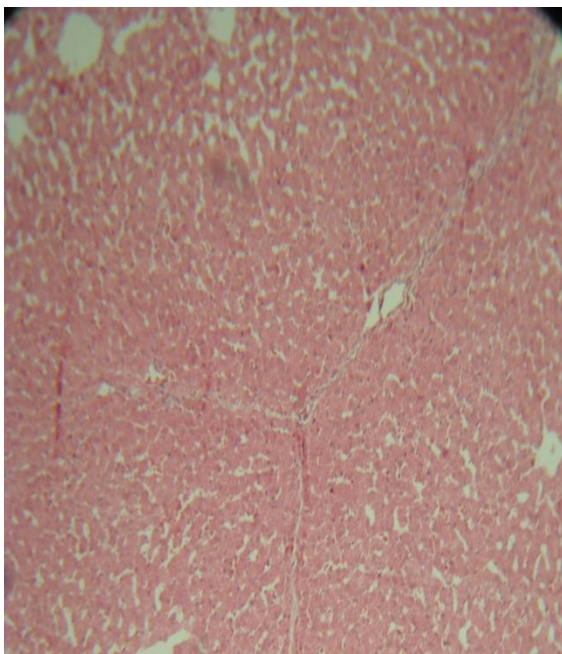


A: Liver of experimental grower pig fed 25% CRM

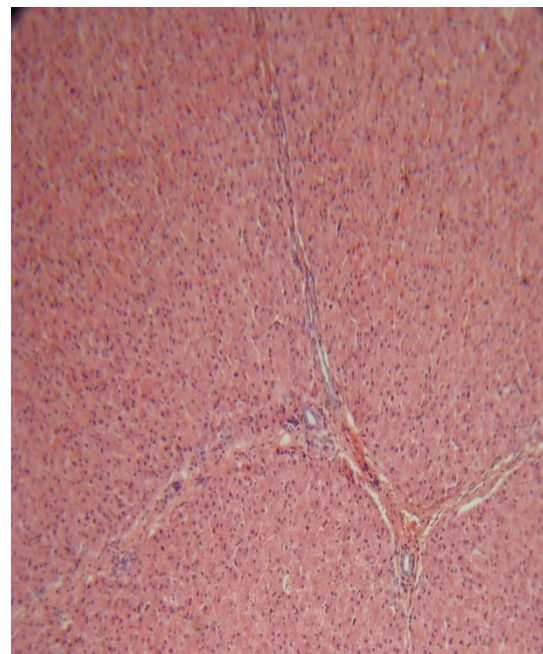


B: Liver of experimental grower pig fed 25% CRM + activated charcoal

PLATE 3: LIVER OF EXPERIMENTAL GROWER PIG AS AFFECTED BY 50% CRM AND 50% CRM + ACTIVATED CHARCOAL

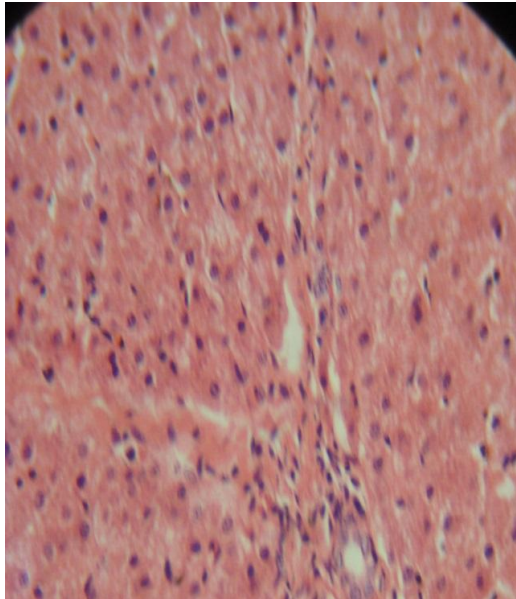


A : Liver of experimental grower pig fed 50% CRM

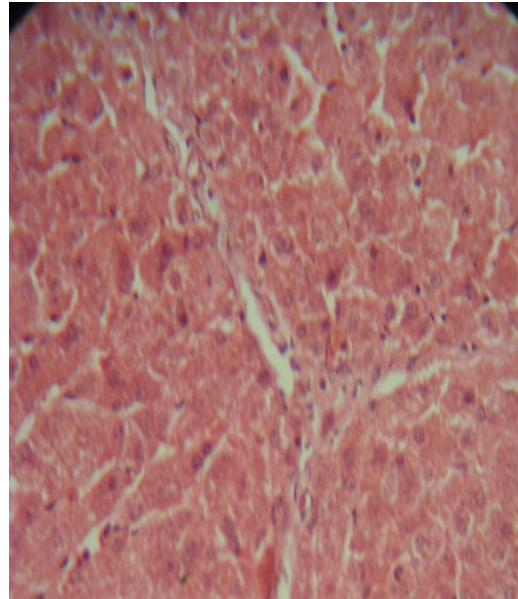


B: Liver of experimental grower pig fed 50% CRM + activated charcoal

PLATE 4: LIVER OF EXPERIMENTAL GROWER PIG AS AFFECTED BY 75% CRM AND 75% CRM + ACTIVATED CHARCOAL

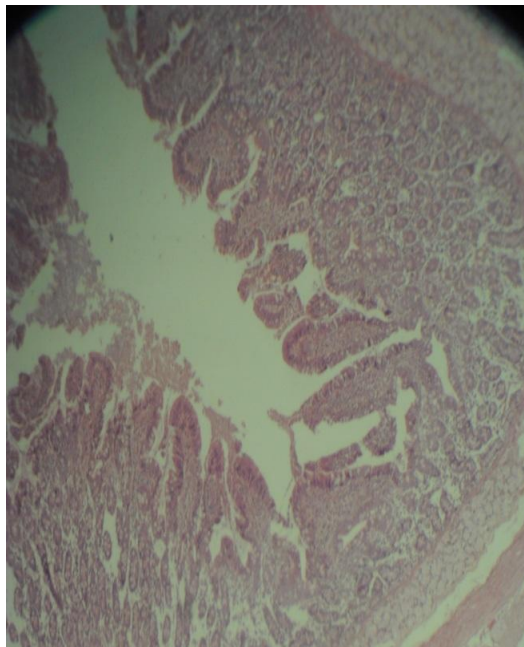


A : Liver of experimental grower pig fed 75% CRM

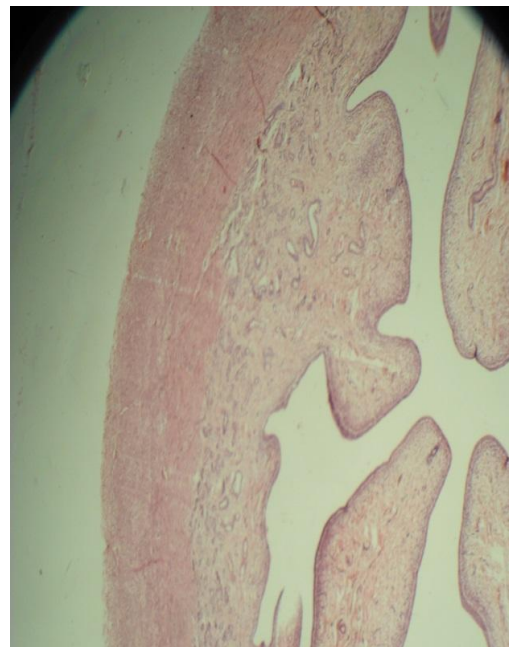


B: Liver of experimental grower pig fed 75% CRM + activated charcoal

PLATE 5: INTESTINAL VILLI OF EXPERIMENTAL GROWER PIG AS AFFECTED BY CONTROL DIET AND CONTROL + ACTIVATED CHARCOAL

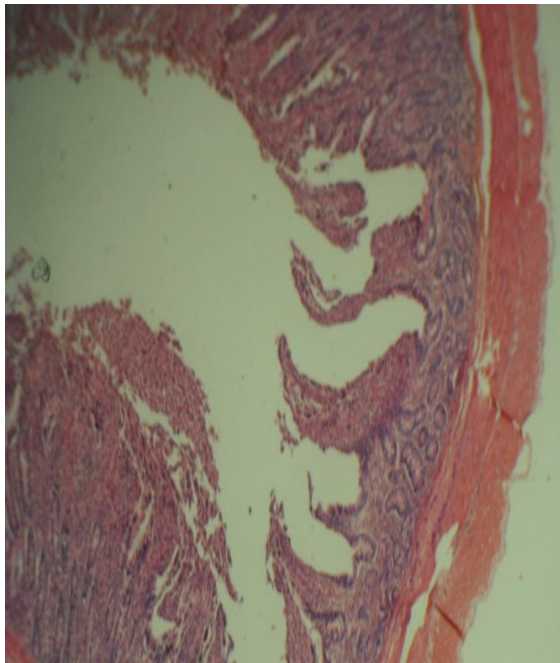


A : Intestinal villi of experimental grower pig fed the control diet

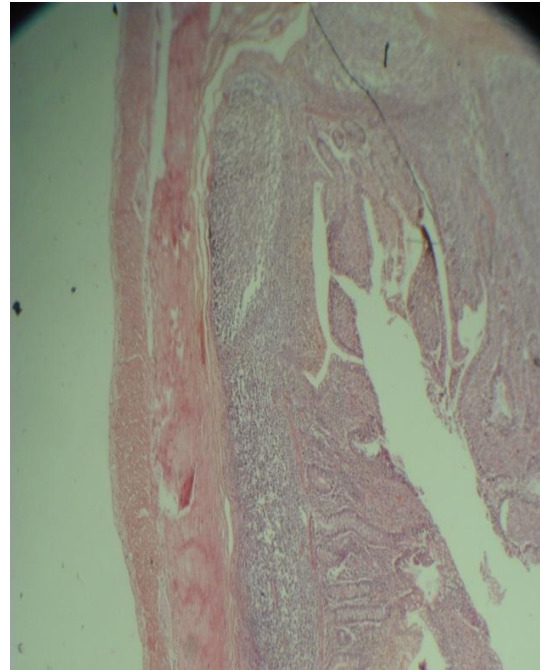


B : Intestinal villi of experimental grower pig fed the control diet + activated charcoal

PLATE 2: INTESTINAL VILLI OF EXPERIMENTAL GROWER PIG AS AFFECTED BY 25% CRM AND 25% CRM + ACTIVATED CHARCOAL

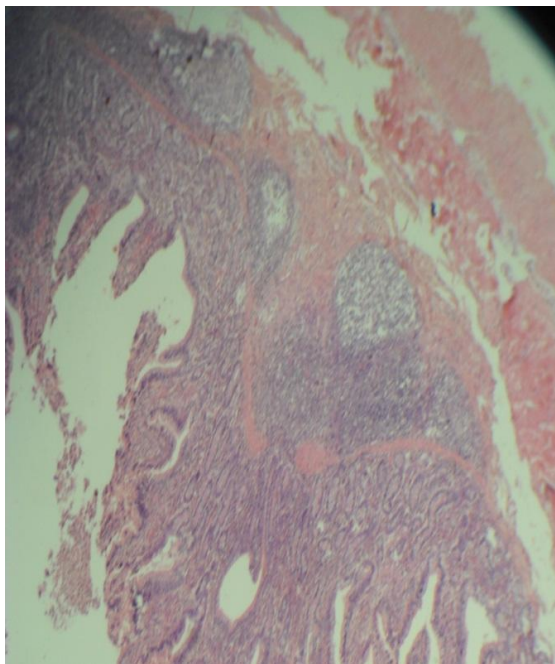


A: Intestinal villi of experimental grower pig fed 25% CRM

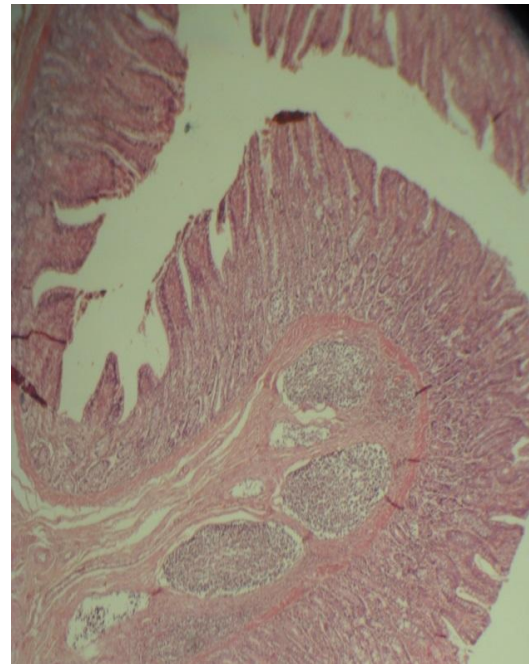


B: Intestinal villi of experimental grower pig fed 25% CRM + activated charcoal

PLATE 3: INTESTINAL VILLI OF EXPERIMENTAL GROWER PIG AS AFFECTED BY 50% CRM AND 50% CRM + ACTIVATED CHARCOAL

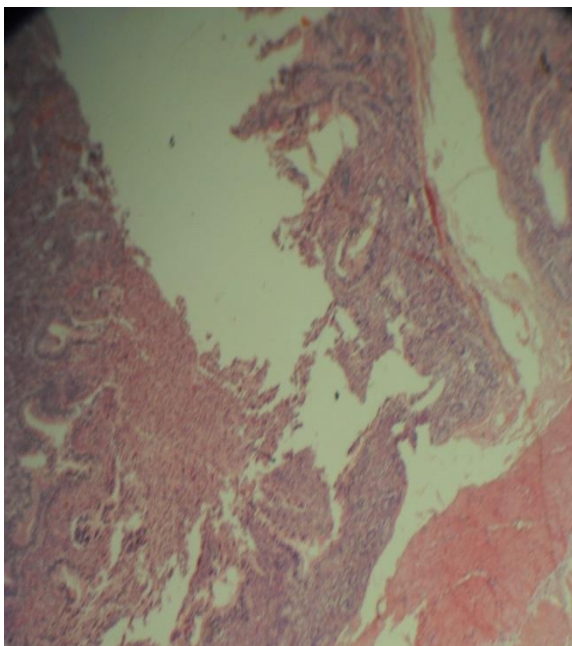


A: Intestinal villi of experimental grower pig fed 50% CRM

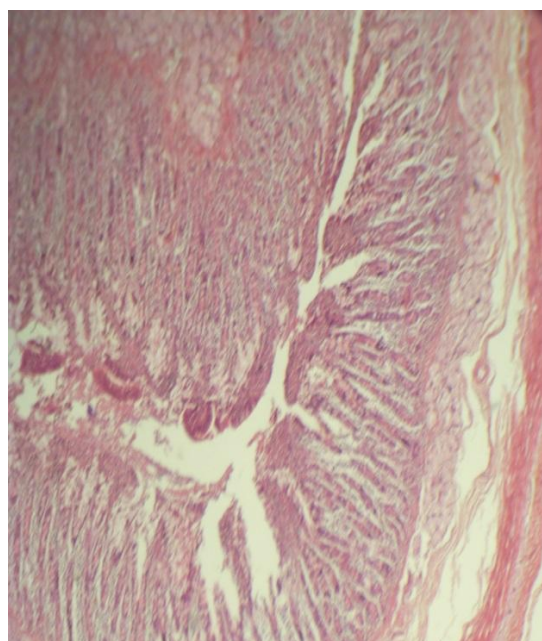


B: Intestinal villi of experimental grower pig fed 50% CRM + activated charcoal

PLATE 4: INTESTINAL VILLI OF EXPERIMENTAL GROWER PIG AS AFFECTED BY 75% CRM AND 75% CRM + ACTIVATED CHARCOAL



A: Intestinal villi of experimental grower pig fed 75% CRM



B: Intestinal villi of experimental grower pig fed 75% CRM + activated charcoal

IV. Discussion

Histological changes in the liver

The observation, from this study was that the liver of grower pigs on the control diet and control diets with activated charcoal were not affected. The few hepatic inflammations in the liver of pigs fed 25% CRM could be attributed to mild action of HCN. With charcoal supplementation, this mild anomaly was corrected. Severity of lesions in the livers of broilers fed low level of aflatoxin was reduced after addition of kaolin and activated charcoal. Intoxications of the HCN became more severe as level of CRM increased but supplementation with activated charcoal caused reduction in the severity. The relative ability of activated charcoal to reverse to some extent the damage to the liver as a result of ingesting CRM based diets could be related to ability reduce uptake and subsequent distribution of HCN to the blood and target organs. It is also reported that effectiveness of activated charcoal is dose dependent (Ayanwale *et al.*, 2006). However, Wang *et al.*, (2006) suggested that activated charcoal at 2% dosage could be harmful for birds even in normal diets.

Histological changes in intestinal morphology

It was observed that intestinal integrities of grower pigs fed control diet and control diet with activated charcoal were maintained. Absence of anti-nutritional factor could justify this presentation. Morphologically, it has been suggested that long villi result in an increased surface area that is capable of greater absorption of available nutrients (Casparly, 1992) and that greater villus height and more numerous cell mitosis in the intestine are indicators that the function of the intestinal villi is activated. (Langhout *et al.*, 1999; Yassar and Forbed, 1999). Increased villi size has been associated with activation of cell proliferation (Lauronen *et al.*, 1998). Residual effect of HCN is being manifested in the intestinal morphology of pigs fed 25% CRM, although, it was corrected by activated charcoal inclusion. Short villi and erosion on the villi are known to be accompanied by reductions in the villus surface area (Park *et al.*, 1998) resulting in reduced absorptive functions. As the level of HCN increased in the diets, corresponding loss of intestinal integrity was observed, although corrections of observed histological alterations were being made by activated charcoal up to 50% CRM level. Numerous histological studies have revealed that the intestinal villi and cells in piglets (Mekbungwan *et al.*, 2003) are well recognized to be affected by dietary components. Fat has been identified to exert a strong stimulatory effect for intestinal mucosal regeneration (Buts *et al.*, 1990). Although the mechanism is not explained, but ability of activated charcoal to reverse the anomaly in intestinal integrity is similar to that of fat. At 75% CRM level the extent of damage was enormous which could be mildly corrected by activated charcoal. Quantity of HCN consumed and absorbed, as well as prolonged exposure to the diets could be responsible. Residual anti nutrient

factor (ANF) had been identified to interfere with digestion, absorption and utilization of nutrients in the diets (Jianoet al., 2000; Fasinaet al., 2004).

Trypsin inhibitor (TI) is well known to interfere with the proper function of trypsin and chymotrypsin leading to abnormal intestinal morphology (Liener and Kakade, 1993). It could be inferred in this study that since HCN is one of the ANFs, it might interfere with certain enzymes (especially brush border enzymes) leading to loss of intestinal integrity. Zarkadas and Wiseman (2000) demonstrated a negative correlation between TI level in soybean meal and villus height in weaned piglets. Improvement of intestinal morphology may be associated with degradation of antigenic materials in feed stuffs.

- a. Increasing level of CRM resulted in varying degrees of inflammatory infiltration and wide lumen in architectural background with attendant degenerative changes in the lobules and some measure of fibrotic portal tracts in the liver. However, activated charcoal was able to make correction up to 50% CRM level.
- b. Histology of small intestine revealed that as CRM level increased there was attendant increase in the damage in terms of erosion of the villi, desquamation of epithelial cells and numerous lymphoid aggregates. However, activated charcoal supplementation was able to make correction reasonably up to 50% CRM level.
- c. Long term feeding of CRM caused histological alterations to the liver and small intestine which were corrected up to 50% CRM level by activated charcoal supplementation.

It is therefore recommended that hydrogen cyanide content of cassava root meal that imposed limitation on the use of cassava root meal in diets of pigs above certain level could be overcome by supplementing with activated charcoal at 40g/kg

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