

Histopathology, haematology and serum chemistry of growing pigs fed varying levels of wild sunflower (*Tithonia diversifolia*) leaf meal as protein supplements

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Abstract: *Tithonia diversifolia* (wild sunflower) leaf meal (TDLM) was prepared and fed to 24 Large White growing pigs in 3 varying inclusion levels (10%, 20% and 30%) as protein supplements in a completely randomized designed experiment. Haematological and histopathological examinations were carried out. White blood cells (WBC) count of pigs on 30% TDLM diet had the highest ($p < 0.05$) WBC value of 18083mm^3 . The urea and creatinine values increased across the increasing inclusion of TDLM may be suggestive of nephrotoxic bioactivity of the TDLM. Total cholesterol content was highest at $5.94\mu\text{mol/l}$ for pigs on 30% TDLM inclusion and decreased as the TDLM inclusion declined. There were noticeable degeneration, bleeding and inflammation of the stomach inner linings and external appearance of the large intestine. It can be concluded that safe nutritional regimes existed below and at levels not exceeding 20% TDLM inclusion in diets of growing pigs when used as protein supplement. *Tithonia diversifolia* leaf meal (TDLM) could be a suitable health diet to combat some fatty acid metabolic diseases at 10% inclusion in the diet of pigs. The histopathology revealing severe epithelial erosion and haemorrhages could be instructive of the toxicological effects of TDLM at high inclusion levels above 20%.

Keywords: Histopathology, haematology, serum chemistry, *Tithonia diversifolia*

I. Introduction

Blood parameters have been shown to be major indices of physiological, pathological and nutritional status of an organism and changes in the constituent compounds of blood when compared to normal values, could be used to interpret the metabolic state of an animal as well as quality of feed [1]. Administration of a chemical compound may bring about significant changes in the structure, function, metabolic transformation and concentration of biomolecules, enzymes and even metabolic pathways. These alterations, which may be rapid or slow may lead to different biochemical mechanisms producing similar pathological, clinical and laboratory findings [2].

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compound including plant extracts on the blood. Such laboratory investigations have been reported to be highly sensitive, accurate and reliable, and it remains the bedrock of ethical and rational research, disease diagnosis, prevention and treatment [3, 4].

Despite the predictive possibility of green forages as livestock (monogastric) feed supplement, the presence of antinutritional factors and their high fibre levels have constantly been the major obstacles in the ample use of these green forages [5].

However, pigs have been fingered as particular monogastric specie with remarkable capacity to consume and digest fibre, and leaves from trees, shrubs and crop plants that are relatively high in fibre [6].

Tithonia diversifolia (wild sunflower) is a green forage plant that can be utilized as feed stuff for animals due to its protein content [7, 8]. There have been various information that exist on the effects of wild sunflower (*Tithonia diversifolia*) leaf meal on the growth performances, carcass traits and haematological indices of poultry birds, rabbits, ruminants and pigs [9, 10, 11].

Therefore, the objective of the present study is to assess the health status of growing pigs fed varying levels of TDLM as protein supplements through the determination of their haematological parameters and histopathological assessment in cases of mortality.

II. Materials And Methods

2.1 Preparation of test ingredients

The test ingredients *Tithonia diversifolia* leaf meal (TDLM) was prepared by harvesting daily, the fresh and matured leaves of *Tithonia diversifolia* plants of different ages before flowering. The whole leaves were chopped manually using kitchen knives and then sun-dried. Sun-drying was done for 4 days, and the chopped leaves were manually turned using a rake so as to guarantee even-drying to 12-13% moisture content. Fresh and

dried samples of *Tithonia diversifolia* leaves were taken to the laboratory for proximate and chemical analyses even before the inclusion of the dried samples into the diets.

2.2 Experimental animals

A total of 24 male growing pigs (about 2¹/₂ months old) of commercially available Large White breed with a mean body weight of 13.3±0.5kg were used for this study. The experimental pigs were given adequate medication to prevent piglet anaemia and worms at the first week of their arrival.

2.3 Feeding trial study

The feeding trials were carried out at the piggery unit of the Teaching and Research Farm (TRF) of the Ekiti State University, Ado-Ekiti. The 24 male growing pigs were all randomized into separate pens for this pre-feeding trial. They were initially served a standard growing diet for pigs compounded with conventional feedstuffs for acclimatization for a period of 10 days. Daily feeding rate was 3.30% of the pigs' live weight [12].

Water was given to the pigs *ad libitum* throughout the period of the standardization trial. The daily feed consumption in g/day was calculated and divided by the average daily weight gain to obtain the feed conversion ratio means for all the pigs on the standard pig diet.

The feeding trial proper was carried out for 102 days with four diets (D1, D2, D3 and D4) formulated to contain about 19.0% crude protein and a digestible energy value of about 12.55MJ kg⁻¹. The control diet was a standard growing diet for pigs compounded with conventional feed stuffs e.g. maize, soybeans, palm kernel cake (PKC), oyster shell, bone meal, brewer's dry grain (BDG), wheat offals, fish meal, salt and grower premixes. The other three diets were compounded such that *Tithonia diversifolia* leaf meal (TDLM) progressively replaced soybeans at 10%, 20% and 30% inclusion levels in diets 2, 3 and 4, respectively. Daily feeding rate was 3.30% of the pigs' live weight [12]. Water was given to the pigs *ad libitum* throughout the period of the experiment. The daily feed consumption in g/day was calculated and was divided by the average daily weight gain, to get the feed conversion ratio means for all the pigs on the four experimental diets.

2.4 Haematological assessment

Blood samples collection was done on day 102 of the feeding trial. It was done in the morning after the pigs were starved overnight in order to attain a stable haematological evaluation and also for easy handling. Four pigs were randomly selected from each experimental diet, and blood samples were collected with the aid of 10-gauge needle inserted into the anterior vena cava. Two sets of blood samples were taken from each pig to determine the haematological indices and serum metabolites.

Blood samples meant for haematological parameters were emptied into vacutainer tubes containing ethylene diamine tetra-acetic acid (EDTA) as anti-coagulant. The tubes were immediately capped and the contents mixed gently for about 1 minute by repeated inversion. The samples were then taken immediately to the laboratory to determine packed cell volume (PCV), white blood cells (WBC), neutrophils (N), lymphocytes (L), monocytes (M) and eosinophils (E). PCV was determined by the micro-haematocrit method [13]. WBC was estimated using the improved Neubauer haemocytometer method as described by [14]. Neutrophils (N), eosinophils (E), lymphocyte (L) and monocyte (M) were determined [15].

Blood samples meant for serum metabolites were collected in vacutainer tubes without anticoagulants and sent to the laboratory. The tubes were kept in a slanting wooden rack, and the blood samples were allowed to clot overnight. The serum (supernatant) was separated clearly by decanting after the blood samples were spun in a centrifuge at 3000 rpm for 10 minutes. The serum samples were kept in sterile vacutainer tubes and kept deep frozen at -10°C prior to analysis to determine urea [16], total cholesterol [17], aspartate aminotransferase (AST) [18] and alanine aminotransferase (ALT) [19] activities using spectrophotometric method. Total protein (TP) was determined [20], while albumin was determined using the BCG (bromocresol green) method [20].

2.5 Histopathology

Histopathology was conducted for some organs obtained from the only dead pig in the diet containing the highest TDLM inclusion level of 30%. Typical organ samples of the large and small intestine, liver, kidney and lungs of the dead pig placed on the highest inclusion level of TDLM at 30% was taken to the laboratory for histopathology and cells examination. Typical treatments of these organs were carried out before they were examined with a light microscope as described [21] as follows:

1.5.1 Fixation was done with some agents, such as formalin, that will coagulate the protein and prevent further changes in the tissues of the organ such as autolysis and bacterial action.

- 1.5.2 Embedding of the tissue of this organ was done by immersing in paraffin or nitrocellulose to permit the cutting of very thin sections. Since most embedding media are not water-soluble, the fixed tissue was dehydrated and then infiltrated with xylene, which is miscible with the embedded medium.
- 1.5.3 Sectioning of the tissue was carried out to obtain very thin slices (between 2 and 20 μ in thickness), so that the sections could be placed on a glass slide using a microtome which consisted of a sharp knife blade and a mechanism for moving the tissue past the blade and then advancing it a definite distance after each cutting.
- 1.5.4 Staining of the cut sections was done with *hematoxyline* together with *eosine* (H & E stain) so that different cells or different parts of cells can be differentiated according to colour. The *hematoxylin* would stain the acid portions of a cell dark blue or purple (called basophilic areas) and the *eosin* would stain the basic portions of a cell pink to red (called acidophilic areas).
- 1.5.5 Examination of the stained sections of tissues on the slide by means of a microscope and light transmitted through the section was the last step. Magnified photographs were taken of the examined sections.
- 1.6 Statistical analysis
- The data collected in the completely randomized experimental design (CRD) were subjected to statistical analysis using the Minitab Computer Software package [22]. A significant difference was considered at level of $p > 0.05$

Table 1. Haematological indices of pigs fed varying levels of TDLM- based diets

Parameters	Diets			
	1	2	3	4
	% inclusion levels of TDLM			
	0	10	20	30
Packed cell volume (PCV), %	30.20 \pm 0.43 ^a	40.20 \pm 0.39 ^b	40.10 \pm 0.41 ^b	41.06 \pm 0.45 ^b
White blood cells (WBC), mm ³	6298 \pm 0.56 ^a	3931 \pm 0.49 ^b	16105 \pm 0.43 ^c	18093 \pm 0.51 ^d
Neutrophils (N), %	30.25 \pm 3.21 ^a	36.10 \pm 2.43 ^b	35.20 \pm 3.43 ^b	35.15 \pm 4.10 ^b
Lymphocytes (L), %	58.10 \pm 1.58 ^a	50.15 \pm 2.53 ^b	50.10 \pm 3.15 ^b	51.15 \pm 2.97 ^c
Monocytes (M), %	8.10 \pm 3.56 ^a	10.15 \pm 3.03 ^b	10.05 \pm 4.31 ^b	10.15 \pm 3.34 ^b
Eosinophils (E), %	4.05 \pm 5.46 ^a	4.10 \pm 3.42 ^a	5.05 \pm 3.21 ^b	4.05 \pm 2.45 ^a

^{a,b,c} Mean values within rows with different superscript letters are significantly different ($P < 0.05$)

Table 2. Serum metabolites of experimental pigs fed varying levels of TDLM based diets

Parameters	Diets			
	1	2	3	4
	% inclusion levels of TDLM			
	0	10	20	30
Urea, mmol/l	5.89 \pm 2.25 ^a	3.72 \pm 2.45 ^b	6.04 \pm 2.32 ^c	4.19 \pm 2.42 ^d
Creatinine, μ mol/l	63.69 \pm 3.49 ^a	136.39 \pm 4.42 ^b	150.06 \pm 5.01 ^c	150.12 \pm 4.21 ^c
Total cholesterol, μ mol/l	4.42 \pm 3.39 ^a	4.15 \pm 4.42 ^b	4.90 \pm 5.32 ^c	5.94 \pm 4.50 ^d
Triglyceride, mmol/l	1.06 \pm 2.37 ^a	0.74 \pm 3.32 ^b	0.36 \pm 3.45 ^c	0.37 \pm 3.67 ^c
Aspartate aminotransferase, mmol/l	67.10 \pm 3.65 ^a	125.10 \pm 3.23 ^b	52.05 \pm 2.97 ^c	47.15 \pm 2.21 ^d
Alanine aminotransferase, mmol/l	34.05 \pm 4.52 ^a	29.20 \pm 3.65 ^b	39.05 \pm 2.96 ^c	25.15 \pm 2.78 ^d
γ -glutamyl transferase, mmol/l	42.44 \pm 2.98 ^a	10.58 \pm 3.94 ^b	31.36 \pm 4.45 ^c	10.65 \pm 3.98 ^b
Alkaline phosphatase, mmol/l	107.70 \pm 4.21 ^a	88.43 \pm 3.87 ^b	73.10 \pm 4.01 ^c	84.65 \pm 3.96 ^d
Total protein, ng/l	0.46 \pm 2.34 ^a	0.47 \pm 2.78 ^a	0.45 \pm 2.54 ^a	0.4 \pm 3.21 ^a
Albumin, ng/l	26.28 \pm 1.43 ^a	28.18 \pm 1.75 ^b	24.42 \pm 1.67 ^c	29.10 \pm 2.11 ^d

^{a,b,c} Mean values within rows with different superscript letters are significantly different ($P < 0.05$)

III. Results And Discussion

3.1 Haematological and histopathological implications of TDLM as protein supplements in pig feed

Haematological indices are presented in Table 1. Packed cell volume (PCV), neutrophils and monocytes were not significantly ($p>0.05$) affected as the levels of TDLM increased in dietary treatments. The white blood cells (WBC) count values from blood samples of pigs were significantly affected ($p<0.05$) by the varying levels of TDLM inclusion at 0%, 10%, 20% and 30% in diets 1, 2, 3 and 4 respectively. Pigs on diet 4 had the highest ($p<0.05$) value of 18093mm^3 , followed by those on diets 3 and 1 at 16105mm^3 and 6298mm^3 , respectively. WBC value for pigs on diet 2 had the lowest ($p<0.05$) value of 3932mm^3 .

Apart from diet 1 whose pigs had the significantly lowest ($p<0.05$) average value of neutrophils (N) at 30.25%, diets 2, 3 and 4 had similar values ($p>0.05$) of 36.10%, 35.20% and 35.15%, respectively.

A significantly highest ($p<0.05$) lymphocytes (L) value was recorded for pigs on the control diet 1 at 58.10%. Pigs on diets 2 and 3 had similar ($p>0.05$) lymphocytes (L) values at 50.15% and 50.10%, respectively. The lymphocytes (L) value of 51.15% obtained for pigs on diet 4 was also significantly different ($p<0.05$) from the other lymphocytes (L) values.

Apart from the significantly lowest monocytes (M) value of 8.10% obtained for pigs on diet 1, other monocytes values were similar ($p>0.05$) at 0.15%, 10.05% and 10.15% for pigs on diets 2, 3 and 4, respectively.

Apart from pigs on diet 3 which had a significantly highest ($p<0.05$) eosinophils (E) value of 5.05%, other values were similar ($p>0.05$) at 4.05%, 4.10% and 4.05% for pigs on diets 1, 2 and 4, respectively.

Packed cell volume (PCV) for TDLM inclusion diets were superior to the control diet (0% TDLM). This report was similar to that where broiler chicks were served fluted pumpkin (*Telfaria Occidentalis*) leaves extract supplement [23]. It could also be related to the ability of *Tithonia diversifolia* leaf meal to provide and maintain the essential amino acids and minerals in the diets which are necessary for the normal functioning of the blood cells producing tissues and organs [9]. The range of PCV in this study (30.20 to 41.06%) was different and higher than that reported as 29.0 to 38.0% [24], but lower than the reported value of 41.50% [23] when broiler chicken were fed fluted pumpkin leaves extract supplement.

The supplementation of the diets at 0, 10, 20 and 30% TDLM inclusion levels was followed by a significant increase in packed cell volume (PCV). The phytochemical profile of TDLM revealed high levels of flavonoids and phytin [25]. Moreover, sesquiterpene lactones in TDLM were reported to be free radical scavengers [26]. It is therefore conceivable that these components may have competed with haemoglobin (Hb) in red blood cell (RBC) for oxygen, resulting in hypoxea which stimulated synthesis and RBC production. It is also possible that the end product of TDLM metabolism in the body stimulated the kidney directly to cause formation of and secretion of erythropoietin, and also production of cortisol (stress) which are humoral regulators of RBC production [27, 28].

TDLM produced a significant increase in white blood cell (WBC) of the animals in a dose dependent pattern where WBC value increased as the level of TDLM in diets increased, which was different from that reported that gradual decrease in the value of WBC with increasing levels of TDLM in poultry diets [29], but similar to report where broiler chicks were served fluted pumpkin (*Telfaria Occidentalis*) leaves extract supplement [23].

The significant increase level of WBC, could be attributed to the presence of sesquiterpene lactones which are feeding deterrents that were found in the leaves of *Tithonia diversifolia* [15]. It could also be related to the ability of TDLM leaf meal to provide and maintain the essential amino acids and minerals in the diets which are necessary for the normal functioning of the blood cells producing tissues and organs [9]. It has been reported that granulocyte-macrophage colony stimulating factor, interleukins IL-2, IL-4 and IL-5 regulate the proliferation, differentiation maturation of committed stem cells responsible for the production of white blood cells [30, 31]. It is suggestive therefore, that *Tithonia diversifolia* has immunostimulatory effects.

With the exception of the value of lymphocyte (L) which was the highest for pigs placed on the control diet (0%), other parameters like PCV, WBC (20% and 30%), neutrophils (N), monocytes (M) and eosinophils (E) (10% and 20%), for TDLM inclusion diets were superior to the control diet (0% TDLM).

Increase in WBC differentials (in 20% and 30% inclusion) cut across all the cell types including the neutrophils, lymphocytes, monocytes and eosinophils. At 10% TDLM inclusion level, there was reduction in the WBC value. This suggests that the 20% and 30% TDLM inclusion levels could be prescribed for medicinal (immunostimulation) purposes and as such, period of exposure should not be protracted, otherwise toxicosis could set in. The 10% inclusion had no negative effect on the WBC.



Photo 1: Inflammation and necrosis of the wall of the stomach of pig fed TDLM highest level of 30%.



Photo 2: External degeneration and inflammation of large intestine of pig fed highest TDLM dietary inclusion of 30%.

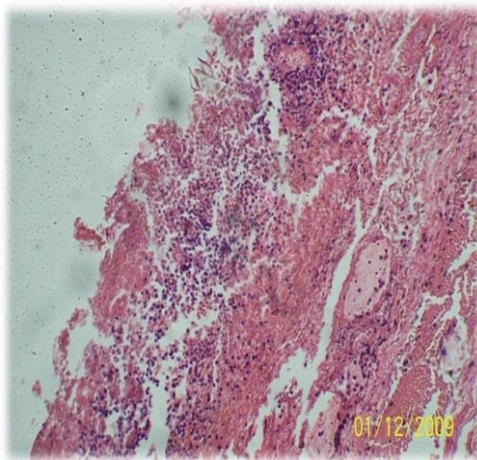


Photo 3: Histopathology of the large intestine of pig fed TDLM at 30% inclusion level showing severe epithelial erosion and necrosis, with neutrophilic cellular infiltration (Mag. X40).

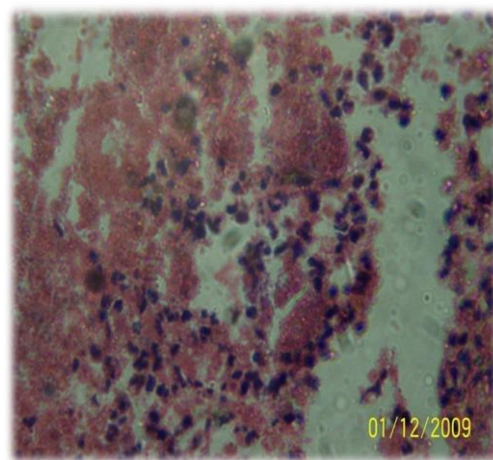


Photo 4: Histopathology of the large intestine of pig fed TDLM at 30% inclusion level showing severe epithelial necrosis, with neutrophilic cellular

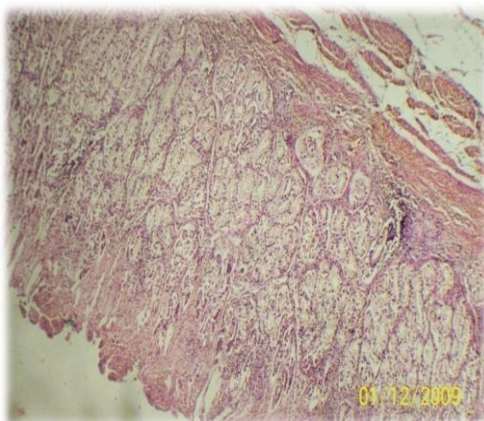


Photo 5: Histopathological appearance of the small intestine of pig fed highest level of TDLM at 30% inclusion level showing the stunted structure of the villi with no visible lesions on other parts (Mag. X40).

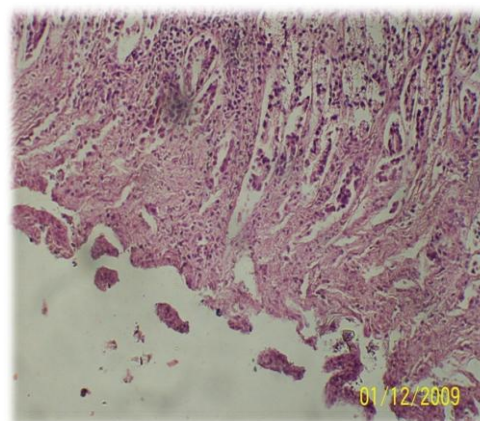


Photo 6: Histopathological appearance of the small intestine of pig fed highest level of TDLM at 30% inclusion level showing the stunted structure of the villi with no visible lesions on other parts (Mag. X100).

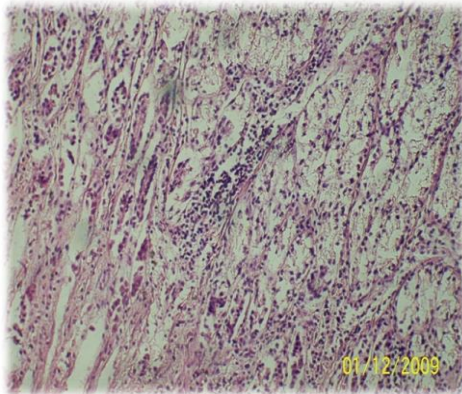


Photo 7: Histopathological appearance of the small intestine of pig fed highest level of TDLM at 30% inclusion level showing the stunted structure villi with no visible lesions on other parts (Mag. X400).

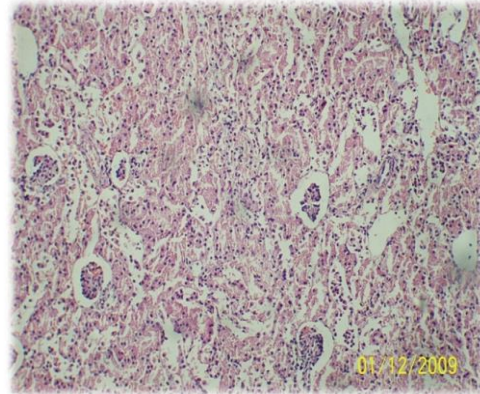


Photo 8: Histopathology of kidney of pig fed TDLM at 30% inclusion level, showing all the ultra structures without any visible lesion of the (Mag. X100).

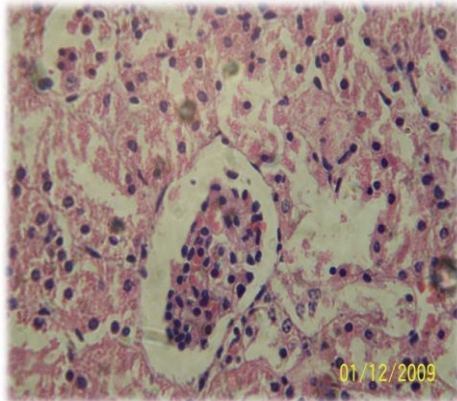


Photo 9: Histopathology of kidney of pig fed TDLM at 30% inclusion level, showing all the ultra structures without any visible lesion (Mag. X400).

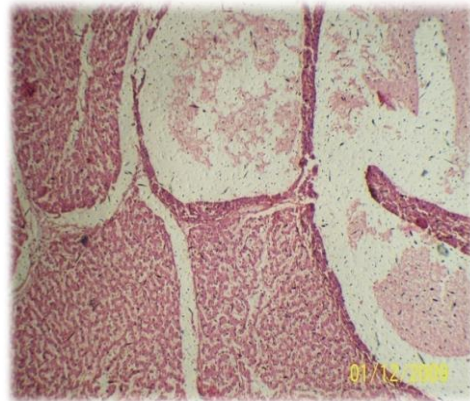


Photo 10: Histopathology of the liver obtained for pigs fed highest level of TDLM at 30% suggesting hepatic degeneration and necrosis, with the presence of inflammatory cells in the tissues (Mag. X40).

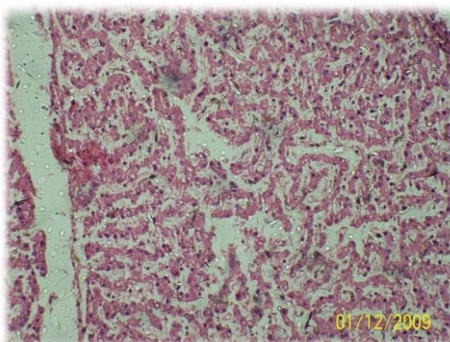


Photo 11: Histopathology of the liver obtained from pigs fed highest level of TDLM at 30% suggesting hepatic degeneration and necrosis, with the presence of inflammatory cells in the tissues (Mag. X100).

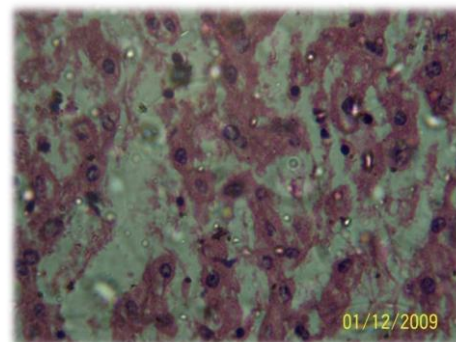


Photo 12: Histopathology of the liver obtained for pigs fed highest level of TDLM at 30% suggesting hepatic degeneration and necrosis, with the presence of inflammatory cells in the tissues (Mag. X400).

3.2 Effect of TDLM as protein supplement on serum metabolites of experimental pigs

Serum metabolites of experimental pigs fed varying levels of TDLM based diets are presented in Table 2. The values of urea in the blood of pigs on diet 1 (control), 2 (10% TDLM inclusion), 3 (20% TDLM inclusion) and 4 (30% TDLM inclusion) at 5.89 mmol/l, 3.72mmol/l, 6.04mmol/l and 4.19mmol/l were significantly different ($p<0.05$). Pigs on diet 3 had the highest significant ($p<0.05$) urea level, while pigs on diet 2 had the significantly lowest urea level. Creatinine levels of pigs on diets 3 and 4 were similar ($p>0.05$) at 150.06 $\mu\text{mol/l}$ and 150.12 $\mu\text{mol/l}$ respectively. The highest significantly different ($p<0.05$) value of 163.69 $\mu\text{mol/l}$ was obtained for pigs on diet 1, while pigs on diet 2 had the lowest significantly different ($p<0.05$) value of creatinine at 136.39 $\mu\text{mol/l}$. There were significant differences ($p<0.05$) in the total cholesterol contents in the blood samples of pigs on diets 4, 3, 1 and 2 in a decreasing order of 5.94 $\mu\text{mol/l}$, 4.90 $\mu\text{mol/l}$, 4.42 $\mu\text{mol/l}$ and 4.15 $\mu\text{mol/l}$, respectively. Serum triglyceride content of 1.06 mmol/l obtained for pigs on the control diet 1 was the highest and significantly different ($p<0.05$) from other values. The triglyceride contents of pigs on diets 3 and 4 had similar ($p<0.05$) values of 0.36 mmol/l and 0.37 mmol/l, respectively. There seemed to be a decreasing trend in the triglyceride contents from diet 1 to diet 3.

There were significant differences ($p<0.05$) in the values of aspartate aminotransferase (AST) obtained for all experimental pigs on diets 1, 2, 3 and 4. Experimental pigs on diet 2 had the highest value of 125.10 mmol/l, while pigs on diet 4 had the lowest value of 49.15 mmol/l. Alanine aminotransferase (ALT) value obtained for pigs on diet 3 (20% TDLM inclusion) was significantly different ($p<0.05$) and was the highest at 39.05 mmol/l, while pigs on diet 4 (30% TDLM inclusion) had the significantly lowest ($p<0.05$) value of 25.15 mmol/l. Pigs on control diet 1 had the significantly highest ($p<0.05$) gamma-glutamyltransferase (GGT) level of 42.44 mmol/l, followed by 31.86 mmol/l obtained for pigs on diet 3. Pigs on diets 2 and 4 had similar ($p>0.05$) gamma-glutamyltransferase (GGT) values of 10.56 mmol/l and 10.65 mmol/l, respectively.

Pigs on diet 1 had the highest alkaline phosphatase (ALP) value of 107.70mmol/l and pigs on diet 3 had the lowest alkaline phosphatase value (ALP) of 73.10mmol/l. There was no significant differences ($p>0.05$) among total protein (TP) mean values obtained for all experimental pigs on varying TDLM dietary treatments.

The TP values of 0.46ng/l, 0.47ng/l, 0.45ng/l and 0.48ng/l obtained for pigs on diets 1, 2, 3 and 4 were similar ($p>0.05$).

There were significant differences ($p<0.05$) in the values of albumin contents in the blood samples of pigs on experimental diets. The values of those on diet 4 was significantly superior ($p<0.05$) to other diets at 29.10 ng/l. This value was closely followed by those on diet 2, 1 and 3 at 28.18ng/l, 26.28 ng/l and 24.42 ng/l, respectively.

Of all the serum metabolite parameters examined, only the total protein was not affected significantly by the inclusion levels of TDLM for all the experimental diets, including the control. This further ascertained the fact that TDLM is fortified with protein and all other essential amino acids [32]. However, this report differs from the one where significant difference occurred in the total protein value of birds served fluted pumpkin leaves extract supplement [23], but similar to study where total protein did not vary significantly in all diets when birds were served *Telfairia occidentalis* leaf meal as protein supplement [5].

Total protein is an indirect index for measuring the nutritional protein adequacy [33, 34]. Similarity between the control and the other diets with TDLM inclusions observed in this study suggests that the nutritional quality of TDLM as a protein source may be acceptable, as it compared favourably with other known conventional sources [35, 36].

The values of urea, triglyceride, alanine aminotransferase (ALT) and gamma-glutamyl transferase (YGT) which were significantly different for all the experimental diets, also followed a pattern of initial increase and gradual decrease as the level of inclusion of TDLM increased. This increase took place mostly at 20% TDLM inclusion level.

Values of urea were higher on pigs served TDLM compared to the control diets. The value of urea which ranged from 3.72 to 6.04 mmol/l in this study is considerably lower than 21.01 to 24.00mmol/l when broilers were served mimosa leaf meal [37] and 10.01 to 19.00mmol/l when broilers were served fluted pumpkin leaves extract [23]. Previous reports [34, 38] emphasised that urea depends on both the quality and quantity of the protein supplied in the diet of pigs. Higher levels of urea in blood could be attributed to the presence of some anti-nutritional factors which might have lowered the quality of the protein, indicating imbalance of amino acids in the diet, which caused elevated blood urea concentration.

The urea and creatinine values which reduced across the increasing inclusion with little consistency indicates that *Tithonia diversifolia* has no nephrotoxic bioactive principles.

Increase in urea and creatinine values are cardinal in diagnosis of kidney toxicosis and kidney malformation may raise the level of blood urea [39]. This is supported by the histopathological pictures (Photos 8 and 9) of the kidney of pig fed highest TDLM level of 30%, revealing all the ultrastructures without any visible lesion.

Fatalities in the high TDLM inclusions could be majorly attributed to the degeneration, bleeding and inflammation of the stomach inner linings and external appearance of the large intestine (Photos 1 and 2).

Moreover, severe epithelia erosion and necrosis, with neutrophilic cellular infiltration (Photo 3 and 4) were observed in the histopathological appearance of the large intestine, and stunted structure of the villi of the small intestine with no visible lesions on other parts (Photos 5, 6 and 7). This was evidently captured by the pictorial images of the large intestine with clear haemorrhages and congested mesenteries (Photo 2).

At the highest TDLM inclusion of 30%, a collaborative and additive irritating effects of the bioactive components such as tagitinins A, B, C and F with diversifol, tirotundin, tithonine and sulphurein were suspects [40].

The liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) had reduced values at 30% inclusion of TDLM. Value of ALT was higher on pigs served 20% TDLM compared to the control diet and ALT value in this study, at 25.15 to 34.5mmol/l was higher than previous report at 23.50 to 24.84mmol/l [23]. This shows that TDLM is not hepatotoxic though histopathological pictures (Photos 10, 11 and 12) of the liver obtained for pigs fed highest level of 30% TDLM suggest hepatic degeneration and necrosis, with the presence of inflammatory cells in the tissues. These effects may be associated with haematological effects of increased WBC infiltration at highest inclusion level of TDLM at 30%.

Alkaline phosphate (ALP) value was observed to decrease at 20% TDLM inclusion, but later increased at 30% TDLM inclusion. Previous report [41] stated that ALP activity can be utilized to assess the health of the liver as it owes its origin to the osteoblasts and some of it are normally excreted in the bile. So, if there is interference of bile flow, the ALP activity is said to be increased. In some abnormal cases when there is biliary stasis, excessive ALP may be formed by the liver.

Albumin value was observed to decrease at 20% TDLM inclusion, but later increased at 30% TDLM inclusion. The significant albumin variation for all diets in this study contradicted the report when *Telfaira occidentalis* leaf meal was served as protein supplement to broilers [5], but similar to the significant difference reported when fluted pumpkin leaves extract supplement was served to broilers [23]. Previous reports stated that the most sensitive biochemical indices of mild or impending protein deficiency is a drop in serum albumin into the marginal range [42, 43].

It has been reported that a reading of total albumin than the normal physiological value usually indicates hypo-albuminemia which may result from deficient intake of protein, deficient synthesis of albumin, excess protein breakdown, chronic liver diseases, starvation and chronic gastro-intestinal disease with their interference with protein digestion and absorption [4]. However, hyper-albuminemia are rarely seen except in cases of dehydration and shock. Generally speaking, increase in albumin are masked by increase in total plasma volume [45].

Total cholesterol in the control diet was higher than the 10% TDLM inclusion, but gradual increase was observed as the inclusion level of TDLM increased from 20% to 30%. Values of cholesterol ranged from 4.15 to 5.94 mmol/l which was different from other reported values at 143.10 to 163.00 mmol/l [23].

Cholesterol is an essential structural component of cell membrane and lipo proteins and serve as the precursor for steroid hormones and bile acids [46]. There is an association between blood levels of cholesterol and the risk of coronary heart disease in humans [47] and premature development of atherosclerosis [48].

The high cholesterol recorded for diet 4 (30% TDLM inclusion) could be attributed to the presence of high saponin content which has been shown to bind to serum lipids, especially cholesterol, thereby, easing their excretion from circulation [49]. It has further been enlightened that increased blood cholesterol values are found in the following conditions: chronic and acute nephritis, chronic nephrosis, diabetes mellitus, high liquid hypothyroidism and biliary obstruction [41]. These conditions were lacking in this present.

Anti-lipidemic properties have been well defined [50] and the increased cholesterol and reduced triglyceride across the increasing inclusion levels of TDLM underscores TDLM as an anti-lipidemic agent. This suggests that TDLM could be used to treat some metabolic conditions involving fatty acids and glucose in animals.

IV. Conclusions

All haematological parameters and serum metabolites examined indicated safe nutritional regimes up to 20% TDLM inclusion. The abnormal excessive activity of ALP in the liver seemed reduced or normalised at 20% TDLM inclusion in pigs' diets.

Tithonia diversifolia leaf meal (TDLM) could be a suitable health diet to combat some fatty acid metabolic diseases at 10% inclusion in the diet of pigs. The histopathology revealing severe epithelial erosion and haemorrhages could be instructive of the toxicological effects of TDLM at high inclusion levels above 20%.

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