Blood parameters of wistar albino rats fed processed tropical sickle pod (Senna obtusifolia) leaf meal-based diets

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ABSTRACT: A feeding trial was conducted for 28 d to evaluate the effects of feeding albino rats with processed Senna obtusifolia leaf meal (SOLM) based diets. Five experimental diets were compounded to contain 0% and 20% each of the sun-dried, boiled, fresh fermented, and boiled and fermented SOLM-based diets designated as T1, T2, T3, T4, and T5, respectively. A total of 90 young albino rats with initial weight of 13.52–14.48 g were randomly allocated to the dietary treatments in a completely randomized design with three replicates of six rats each. The hematological parameters revealed nonsignificant (P > 0.05) difference except for the rats fed the sun-dried SOLM-based diet, which had the lowest packed cell volume (34%), red blood cell (5.32 × 10⁶/µL), and hemoglobin (13.67 g/dL). The hematological values recorded were, however, within the normal reference ranges. The biochemical indices were also not significantly (P > 0.05) different. The total protein, creatinine, alanine aminotransferase, and total bilirubin recorded were within the ranges of 71.50–77.73 g/L, 1.03–1.23 mg/dL, 20.67–24.37 µ/L, and 10.13–11.67 µ/L, respectively. It was concluded that the different processed SOLM-based diets had no adverse effects on the blood parameters of the albino rats and are, therefore, recommended for feeding albino rats.

Key words: biochemical indices, hematological parameters, processed Senna obtusifolia

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INTRODUCTION

The scarcity and high cost of conventional feed resources have continued to pose serious limitation on the growth and development of the Nigerian livestock industry. Kwari et al. (2019) also reported that Nigeria is presently faced with the problem of inadequate supply and high cost of conventional feed ingredients, which consequently led to a setback in sustainable livestock production in Nigeria. These problems, therefore, necessitate the need to look inward and exploit the nutritional potentials of underutilized wild legumes. Over the years, much effort has been directed toward the exploitation and use of nonconventional ingredients in feed production for livestock (Nduaka, 2006). One of such alternative feed resource that can be exploited in Nigeria is the leaf of Senna obtusifolia plants. The chemical properties of the leaves as reported by Yakubu et al. (2017) revealed

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that it has good nutritional value but also contains antinutritional factors, such as tannins, oxalates, and phytates. These antinutritional factors have adverse effects on the blood constituents and overall performance of an animal. Therefore, it is necessary to detoxify the leaves by subjecting them to appropriate processing treatments before incorporation in livestock diets. It is important to note that hematological and biochemical investigations are indicators of the effects of nutrition in terms of antinutrients on the health status of an animal (Akinmutimi, 2004). At the moment, there appears to be paucity of information on the effects of processed S. obtusifolia leaf meal (SOLM) based diet on the blood constituents of albino rats, hence the need to conduct more studies in order to bridge this information gap. It is in view of the above that this study was conducted to evaluate the effects of processed SOLM-based diet on the hematological and biochemical parameters of wistar albino rats.

**MATERIALS AND METHODS**

**Institutional Animal Care and Ethic Committee Approval**

The use of albino rats for the experiment was approved by the Animal Care and Ethic Committee of the Adamawa State University, Mubi. The ethical considerations of the use of the rats were in line with the specifications of Nuffield Council on Bioethics.

**Location of the Study Area**

The study was conducted at the Animal House of the Department of Biological Sciences, Adamawa State University, Mubi. The area is located between 9°30’N and 11°N and 13°E and 9°45’E (Adebayo, 2004).

**Collection and Processing of S. obtusifolia Leaves**

The leaves of S. obtusifolia were harvested in bushes around Mubi area of Adamawa State, Nigeria. The leaves were divided into four batches: the first batch was properly sun-dried for 5 d until the leaves became crispy; the second batch was boiled for 30 min and the boiling time was taken immediately after the water started to boil; the third batch was wilted placed in polythene bags and were well compressed to expel air. Thereafter, the leaves were placed in an air-tight container and allowed to naturally ferment for 9 d; the fifth batch was boiled for 30 min, cooled, drained, and placed in an air-tight container and allowed to ferment for 7 d.

**Experimental Diets and Treatments**

Five experimental diets were compounded to contain 0% and 20% each of the sun-dried, boiled, fresh fermented, and boiled and fermented SOLM designated as T1, T2, T3, T4, and T5, respectively, as presented in Table 1.

**Table 1. Ingredient composition and calculated analysis of the experimental diets**

| Ingredient, g | T1 (0% SOLM) | T2 (20% SDSOLM) | T3 (20% BSOLM) | T4 (20% FFSOLM) | T5 (20% BFSOLM) |
|---------------|--------------|----------------|----------------|----------------|----------------|
| Maize         | 49.00        | 49.00          | 49.00          | 49.00          | 49.00          |
| Maize offal   | 15.00        | 7.15           | 7.15           | 7.15           | 7.18           |
| Roasted soya bean meal | 25.65 | 13.50          | 13.50          | 13.50          | 13.50          |
| Fish meal     | 7.00         | 7.00           | 7.00           | 7.00           | 7.00           |
| PSOLM         | 0.00         | 20.00          | 20.00          | 20.00          | 20.00          |
| Salt (NaCl)   | 0.30         | 0.30           | 0.30           | 0.30           | 0.30           |
| Bone meal     | 2.50         | 2.50           | 2.50           | 2.50           | 2.50           |
| Methionine    | 0.20         | 0.20           | 0.20           | 0.20           | 0.20           |
| Lysine        | 0.15         | 0.15           | 0.15           | 0.15           | 0.15           |
| Premix        | 0.20         | 0.20           | 0.20           | 0.20           | 0.20           |
| Total         | 100.00       | 100.00         | 100.00         | 100.00         | 100.00         |
| CP, %         | 20.61        | 19.13          | 19.06          | 19.39          | 19.48          |
| Crude fiber, %| 4.70         | 5.64           | 4.80           | 3.75           | 3.25           |
| Calcium, %    | 2.43         | 3.13           | 3.08           | 3.41           | 3.47           |
| Phosphorus, % | 2.75         | 3.21           | 3.03           | 3.22           | 3.25           |
| Energy*, kcal/kg | 2,877.61 | 2,877.58       | 2,849.83       | 2,851.75       | 2,861.50       |

BFSOLM, boiled and fermented Senna obtusifolia leaf meal; BSOLM, boiled Senna obtusifolia leaf meal; CP, crude protein; EE, ether extract; FFSOLM, fresh fermented Senna obtusifolia leaf meal; FSOLM, fermented Senna obtusifolia leaf meal; ME, metabolizable energy; NFE, nitrogen-free extract; PSOLM, processed Senna obtusifolia leaf meal; SDSOLM, sun-dried Senna obtusifolia leaf meal.

*Energy calculated according to the formula of Pauzenga (1985): ME (kcal/kg) = 37 × %CP + 81 × %EE + 35.5 × %NFE.
Chemical Analysis

The processed SOLM and the experimental diets were analyzed using standard laboratory procedure of AOAC (2010).

Experimental Animals and Their Management

A total of 90 young albino rats with average initial weight of 13.52–14.48 g were managed inside constructed metal cages. The rats were acclimatized to the cages and the experimental diets for 1 wk. Thereafter, they were fed with measured quantity of feed, which was supplied at ad libitum. Clean drinking water was regularly supplied. The rats were managed in accordance with the procedure of handling experimental animals as described by Ochei and Kolhatkar (2007) and the ethics of the Adamawa State University, Mubi Animal Welfare and Ethical Committee. The experiment lasted for 28 d.

Experimental Design

A total of 90 young albino rats with initial weight of 13.52–14.48 g were randomly allocated to the five dietary treatments in a completely randomized design with three replicates of six rats each.

Parameters Measured

Blood collection and analysis. Exactly 3.5 mL of blood samples were collected from each treatment in triplicates. Blood samples for full blood count analysis were collected into ethylenediamine-tetraacetate (EDTA) treated tubes, while blood samples for the biochemical analysis were collected into EDTA-free test tubes. Blood samples for the biochemical analysis were centrifuged for 15 min and the serum separated using a Vanguard 5000 model centrifuging machine. Blood samples for hematological and biochemical parameters were analyzed using spin cell 3 automated hematology and biochemistry analyzer Urit 3000 plus version 07/2015 model.

Statistical Analysis

Data obtained were subjected to analysis of variance using Statistix 9.0, and Duncan’s Multiple Range Test was used to separate the means where significant difference occurred.

RESULTS AND DISCUSSION

The analyzed chemical composition of the experimental diets is presented in Table 2. The results indicated that the different processing methods were observed to be effective in reducing the levels of the antinutritional factors. The proximate composition of the diets is adequate to meet the nutritional requirements of albino rats.

Hematological and Biochemical Indices

The hematological and biochemical indices of the albino rats fed processed SOLM-based diets are presented in Tables 3 and 4. The packed cell volume (PCV), red blood cell (RBC), and the hemoglobin (Hb) were significantly (*P* < 0.05) different. The albino rats fed the sun-dried SOLM-based diet indicated the lowest parameters mentioned above, which might be attributed to the effect of antinutritional factors present in the sun-dried SOLM. This suggests that sun drying alone might not give the desired detoxification. This finding is consistent with the report of Ardo et al. (2019) who reported the same for albino rats.

Table 2. Analyzed chemical composition of the experimental diets

| Proximate components, % | T1 (SDSOLM) | T2 (BSOLM) | T3 (FFSOLM) | T4 (BFSOLM) |
|-------------------------|-------------|------------|------------|------------|
| Dry matter              | 93.55       | 92.75      | 93.75      | 93.55      |
| CP                      | 19.77       | 18.75      | 20.45      | 19.87      |
| Crude fiber             | 5.21        | 5.07       | 4.77       | 4.25       |
| Ash                     | 4.55        | 4.18       | 5.25       | 5.20       |
| EE                      | 2.65        | 2.33       | 2.25       | 2.25       |
| NFE                     | 41.07       | 40.75      | 39.09      | 40.65      |
| Energy*, kcal/kg         | 2,404.13    | 2,275.10   | 2,326.60   | 2,368.62   |
| Tannins, mg/100g        | 3.90        | 2.03       | 1.85       | 1.75       |
| Phenols, mg/100g        | 22.76       | 13.13      | 9.18       | 8.25       |

BFSOLM, boiled and fermented *Senna obtusifolia* leaf meal; BSOLM, boiled *Senna obtusifolia* leaf meal; CP, crude protein; EE, ether extract; FFSOLM, fresh fermented *Senna obtusifolia* leaf meal; ME, metabolizable energy; NFE, nitrogen-free extract; SDSOLM, sun-dried *Senna obtusi-folia* leaf meal.

*Energy calculated according to the formula of Pauzenga (1985): ME (kcal/kg) = 37 × %CP + 81 × %EE + 35.5 × %NFE.*
rats fed sun-dried SOLM-based diets. However, the PCV, RBC, and Hb of the albino rats were within the normal ranges of 36–54%, 6–8 g/dL, and 11–19.2 g/dL as reported by Wikivet (2012). It, therefore, implies that feeding albino rats with SOLM-based diets had no adverse effects on their hematological parameters. The white blood cells (WBCs) revealed nonsignificant (P > 0.05) difference and the values are also within normal ranges of 4,400–14,800 and 3,600–14,500 per/mm³ for male and female rats reported by Shehani et al. (2018). This suggests that the inclusion of 20% processed SOLM had no adverse effects on the immune systems of the rats, implying that all the processing methods were effective in detoxifying the toxic components of *S. obtusifolia* leaves. Eric (2015) reported that body systems of an animal produce more WBCs when there is any abnormality, which is not the case with the findings of this study. The mean corpuscular volume (MCV), mean Hb concentration, and mean corpuscular Hb concentration (MCHC) were not significantly (P > 0.05) different and are within the normal ranges of 48–70 fl, 17.1–20.4 pg, and 32.9–37.5 g/dL reported by Mary and Charles (2008) and Wikivet (2012).

The total protein and albumin values of the albino rats indicated no significant (P > 0.05) difference and are within the normal ranges of 5.6–7.6 g/dL and 3.8–4.8 g/dL reported by Wikivet (2012) and 5.2–7.1 g/dL and 3.4–4.8 g/dL reported by Mary and Charles (2008). Healthline (2017) explained that low or high total protein is an indication of liver disorders and malnutrition. Based on the total protein and globulin values obtained in this study, it means that the experimental diets had no adverse effects on the liver of the albino rats. This is a clear indication that the processing methods were effective in detoxifying the *S. obtusifolia* leaves. The values for serum electrolytes were not significantly (P > 0.05) different. The values for sodium, potassium, chloride, and bicarbonate

### Table 3. Hematogical indices of albino rats fed processed *Senna obtusifolia* leaf meal-based diet

| Parameters       | T₁ (0%SOLM) | T₂ (20%SDSOLM) | T₃ (20%BSOLM) | T₄ (20%FFSOLM) | T₅ (20%BFSOLM) | SEM     |
|------------------|-------------|----------------|---------------|---------------|---------------|---------|
| Packed cell volume, % | 45.00a      | 34.00b         | 41.67a        | 37.00b        | 39.00b        | 0.50*   |
| RBC, ×10⁶/mm³ | 7.37a       | 5.32b          | 7.15a         | 6.32b         | 6.75b         | 0.18*   |
| Hb, g/dL       | 15.00a      | 11.27b         | 13.67b        | 12.27b        | 13.00b        | 0.34*   |
| WBCs, ×10⁶/mm³ | 6,256.67    | 6,200.00       | 6,320.00      | 6,400.00      | 6,200.00      | 3.81**  |
| MCV, fl        | 61.21       | 61.04          | 60.30         | 59.63         | 59.17         | 1.77**  |
| MCH, pg        | 20.42       | 21.20          | 19.12         | 19.46         | 19.44         | 0.86**  |
| MCHC, g/dL     | 33.31       | 33.11          | 32.80         | 33.18         | 33.30         | 0.50**  |

BFSOLM, boiled and fermented *Senna obtusifolia* leaf meal; BSOLM, boiled *Senna obtusifolia* leaf meal; FFSOLM, fresh fermented *Senna obtusifolia* leaf meal; MCH, mean corpuscular Hb; SDSOLM, sun-dried *Senna obtusifolia* leaf meal.

*Means in the same row with different superscript are significantly different at 5% level of probability.

### Table 4. Biochemical indices of albino rats fed processed *Senna obtusifolia* leaf meal-based diet

| Parameters       | T₁ (0%SOLM) | T₂ (20%SDSOLM) | T₃ (20%BSOLM) | T₄ (20%FFSOLM) | T₅ (20%BFSOLM) | SEM     |
|------------------|-------------|----------------|---------------|---------------|---------------|---------|
| Total protein, g/L | 77.73       | 74.63          | 71.50         | 72.70         | 75.27         | 0.57**  |
| Albumin, g/L      | 36.10       | 32.43          | 32.10         | 31.07         | 32.33         | 0.33**  |
| Globulin, g/L     | 41.63       | 42.27          | 41.30         | 39.67         | 42.93         | 0.64**  |
| Creatinine, g/dL  | 1.07        | 1.03           | 1.23          | 1.05          | 1.07          | 0.08**  |
| Urea, mmol/L      | 5.5         | 5.0            | 4.97          | 5.32          | 5.03          | 1.02**  |
| Sodium, mmol/L    | 145.40      | 142.57         | 142.40        | 141.53        | 145.40        | 0.60**  |
| Potassium, mmol/L | 5.13        | 5.07           | 4.80          | 4.53          | 5.50          | 0.08**  |
| Chloride, mmol/L  | 118.67      | 103.00         | 118.00        | 104.67        | 112.23        | 1.00**  |
| Bicarbonate, mmol/L | 28.17      | 27.38          | 28.30         | 30.43         | 30.93         | 0.35**  |
| ALP, μ/L          | 32.37       | 34.10          | 30.60         | 29.27         | 34.90         | 0.51**  |
| ALT, μ/L          | 21.20       | 20.67          | 22.93         | 23.30         | 24.37         | 0.55**  |
| AST, μ/L          | 15.43       | 15.80          | 14.98         | 15.53         | 15.76         | 0.39**  |
| TB, mg/dL         | 11.17       | 10.50          | 11.43         | 10.13         | 11.67         | 0.16**  |
| DB, mg/dL         | 4.70        | 4.67           | 4.20          | 5.73          | 4.80          | 0.08**  |

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BFSOLM, boiled and fermented *Senna obtusifolia* leaf meal; BSOLM, boiled *Senna obtusifolia* leaf meal; DB, direct bilirubin; FFSOLM, fresh fermented *Senna obtusifolia* leaf meal; FSOLM, fermented *Senna obtusifolia* leaf meal; SDSOLM, sun-dried *Senna obtusifolia* leaf meal; TB = Total bilirubin.
fells within the normal ranges of 140–150, 4.3–5.6, and 95–115 mmol/L as reported by Wikivet (2012). Similarly, the creatinine and urea values showed no significant \( P > 0.05 \) variation and are close to the normal ranges of 0.5–1 mg/dL and 10–2 mg/dL reported by Wikivet (2012). Normal ranges for serum electrolytes, creatinine, and urea are evidences that the kidneys of the albino rats are without any forms of disorders. Increase and abnormal creatinine and blood urea nitrogen levels have been reported as evidence of kidney disorders and adrenal dysfunction (Ada Health GmbH, 2009).

The alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the bilirubin levels indicated no significant \( P > 0.05 \) variation. The alkaline phosphatase and ALT values obtained are within the normal ranges of 16–50 and 35–80 µL, reported by Wikivet (2012). Thapa and Anu (2007) reported that AST and ALT are increased in all liver disorder, disease, or damage. Based on the levels of the serum enzymes obtained in this study, it is very clear that the liver of the albino rats were not compromised by the experimental diets. Similarly, the bilirubin levels indicated non-significant \( P > 0.05 \) difference and are within the normal ranges of 0.2–0.5 mg/dL as reported by Wikivet (2012). Thus, the bilirubin levels are indicators of normal liver function and condition.

CONCLUSION

It was concluded that the inclusion of 20% of the sun-dried, boiled, fresh fermented, and boiled and fermented SOLM had no adverse effects on blood constituents of albino rats and are, therefore, recommended for feeding albino rats.

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Conflict of interest statement. None declared

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