SERUM BIOCHEMICAL INDICES AND PROTEIN EXPRESSION IN YANKASA RAMS FED CASSAVA PEELS AND *GMELINA ARBOREA* SILAGE

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ABSTRACT
A 21-day feeding trial was conducted to evaluate the effects of ensiled Gmelina arborea leaves and cassava peel mixtures on serum biochemistry and protein expression in Yankasa rams before and after feeding. Ten (10) rams were randomly assigned to two (2) experimental diets: 100% Gmelina arborea leaves (Diet A) and a 50:50 cassava peel-Gmelina arborea mixture (Diet B), both ensiled for 75 days using randomized two-group experiment design. Before the feeding trial, all measured biochemical parameters were not significantly different (P > 0.05), except for cholesterol and triglycerides. However, after the feeding trial (21-days), significant (P < 0.05) increase was observed in all parameters, except for cholesterol and low-density lipoprotein (LDL), which decreased when compared to pre-trial values. Protein expression analysis revealed increased expression and intensity of myosin (245 kDa) and transferrin (80 kDa) in rams fed Diet B compared to those fed Diet A. These findings suggest that Diet B is beneficial as it regulates and improves the biochemical profile, with reduction in cholesterol and LDL levels. Diet B, is therefore, recommended for enhanced biochemical health and protein expression in rams.
Keywords: Lipid profile, Protein expression, Sheep, Silage

INTRODUCTION

The use of agro-industrial by-products and underutilized tropical plants offers a promising solution to feed scarcity in ruminant production especially during the dry season (Oso and Ibhaze, 2023). *Gmelina arborea* is a fast-growing leguminous tree, whose leaves contain good portion of protein (12.59%), fiber (19.09%), and carbohydrates (51.80%) and low antinutritional factors, making it suitable for livestock feed (Abdullahi *et al*., 2020). Similarly, cassava peels, a by-product of cassava processing, are a valuable energy source containing 70.52% fermentable carbohydrates (Aro *et al*., 2010). However, raw cassava peels are high in antinutrients like hydrogen cyanide and oxalates, which can negatively affect metabolic processes and overall health of animals. Processing methods like fermentation or ensiling reduce these antinutrients, enhance feed quality, digestibility, and nutrient availability (Ibhaze *et al*., 2020).

Evaluating feed quality, serum biochemical indices such as enzymes, lipid profiles, and metabolites are reliable biomarkers, giving valuable information about organ functionality and the physiological state of animals (Ibhaze *et al*., 2021). Similarly, proteomic studies provide insights into diet-induced molecular changes, revealing mechanisms behind metabolism, expression, function and overall cellular responses (Rodrigues *et al*., 2012). This study, therefore, aimed at investigating the biochemical indices and protein expression of Yankasa rams fed cassava peel and *Gmelina arborea* silage-based diets.

MATERIALS AND METHODS

Experimental site

The study was conducted at the Teaching and Research Farm of the Federal University of Technology Akure, Ondo State, Nigeria.

Experimental diets

Cassava (*Manihot esculenta*) peels were sourced from cassava processing industries while *Gmelina arborea* leaves were harvested within the University community. Prior to ensiling, the materials were chopped to enhance compaction. Two experimental diets were prepared: 100% *Gmelina arborea* leaves (Diet A) and a 1:1 mixture of cassava peels and *Gmelina arborea* leaves (Diet B) both ensiled for 75 days.

Feeding and management of experimental animals

Ten (10) Yankasa rams were randomly selected from the flock available on the farm which were already vaccinated and treated against endemic diseases and ectoparasites. The rams were weighed and assigned to the experimental diets using a randomized two-group experiment design. During the 21-days feeding trial, rams were fed *ad libitum* and had access to clean water.

Collection of blood

A 10 ml of blood samples were collected from each ram via the jugular vein, before (baseline) and after the feeding trial. Then, 5mL were placed in Ethylene-diaminetetra acetic acid (EDTA) bottles for protein expression analysis, while the remaining 5 mL were placed in plain test tube for serum biochemical analysis.

Gel electrophoresis analysis

Agarose gel was prepared by dissolving 0.5 g of agarose powder into 50 mL of 1X TBE buffer. The mixture was heated until completely dissolved, cooled to room temperature, and poured uniformly onto a gel casting tray to solidify. For protein identification, the gel was placed in an electrophoresis chamber and 6 µL of a protein ladder was loaded into the first well as a standard, while purified serum proteins (3 µL each) from the experimental rams were pre-mixed with 3 µL of loading buffer (1:1 ratio) and loaded into the remaining wells, following the Inqaba Biotec protocol. Electrophoresis was conducted at 100 V for 1 hour using 1X TBE SDS running buffer. The gel was subsequently stained with Coomassie Brilliant Blue R-250 solution (40% methanol, 10% glacial acetic acid, 0.25% Coomassie Brilliant Blue R-250) for 2–3 hours at room temperature with constant rocking. De-staining was performed using a solution of 20% methanol and 5% acetic acid for another 2–3 hours, until distinct protein bands were revealed.

Identification and Measurement of protein intensity

Proteins were identified by comparing the observed bands to the standard chart. The molecular processes, cellular functions, and biological roles of the proteins were further investigated using the Ensembl protein database (https://www.ensembl.org).

The intensity of the protein bands was quantified and plotted using the ImageJ Gel Analyzer software.

Serum biochemical analysis

Serum biochemical analyses were conducted using Randox test kits in accordance with the manufacturer's protocols. Parameters evaluated included Alanine Aminotransferase, Aspartate Aminotransferase, glucose, cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) with SPSS software (version 26). Least Significant Difference (LSD) of the same software was used to separate the means.

RESULTS AND DISCUSSION

Lipid profile and serum biochemical indices of rams before and after feeding trial

Serum biochemical indices are valuable for assessing liver functionality, metabolic and physiological status in animals (Gong *et al*., 2020). Table 1 presents the lipid profile and serum biochemical indices of Yankasa rams before and after the feeding trial. Baseline parameters were not significant ($P>0.05$), except for cholesterol and triglyceride levels. Glucose levels increased in both groups, with rams fed Diet B showing a significantly ($P<0.05$) higher value (68.17 ± 17.78 mg/dL) when compared to rams fed Diet A (60.00 ± 9.53 mg/dL). Glucose, the primary metabolic fuel for cells, remained within the reference range of 50–80 mg/dL (Jackson and Cockcroft, 2002). This suggests that the rams did not experience negative energy balance, helping to prevent the incidence of ketosis or hypoglycemia. The higher glucose levels in rams fed Diet B could likely result from the high portion of easily digestible fermentable carbohydrates in cassava peel silage, which are rapidly converted into glucose during metabolism (Aro *et al*., 2010).

Cholesterol levels decreased significantly ($P<0.05$) after the trial. Rams placed on Diet B showed lower values (59.02 ± 19.19 mg/dL) while those on Diet A had higher value (66.44 ± 10.71 mg/dL). These levels remained within the normal physiological range of 43–103 mg/dL as reported by Jackson and Cockcroft, (2002). Reduced serum cholesterol which is primarily from exogenous dietary source may be linked to a lower fat content in the test diets and low ruminal acetate supply which is the primary precursor for cholesterol synthesis (Kapourchali *et al*., 2016). This reduction is beneficial, contributing to improved overall health and reduced risks associated with high cholesterol build up.

After feeding, triglycerides and HDL increased, particularly in Diet B (23.95 ± 2.03 mg/dL, 22.99 ± 2.94 mg/dL) compared to Diet A (22.82 ± 13.96 mg/dL, 21.72 ± 8.61 mg/dL) respectively. Conversely, LDL levels decreased significantly in Diet B (20.25 ± 16.93 mg/dL) compared to Diet A (22.15 ± 16.51 mg/dL). However, these parameters were within the respective reference ranges of 10–30 mg/dL, 18–40 mg/dL and 10-30 mg/dL for triglyceride, HDL and LDL respectively (Jackson and Cockcroft, 2002). Reduction in LDL which is considered bad cholesterol, is beneficial as it will help reduce the risk of atherosclerosis, which causes arterial hypertension or vascular complications.

Serum enzyme, specifically AST and ALT, increased after the feeding trial but remained within the normal ranges of 60–280 U/L and 22–38 U/L as reported by Jackson and Cockcroft, (2002) respectively. These results suggest proper functioning of the liver, kidney, heart, and muscles, as deviations in these enzymes often indicate poor detoxification or liver dysfunction. Since the enzymes do not increase beyond the safe threshold, it implies no signs of liver disease, inflammation, or injury, confirming the safety and nutritional adequacy of the test diets. Thus, feeding cassava peel and *Gmelina arborea* silage-based diets can help improve myosin and transferrin expression to optimize their biochemical and cellular functions within the cell..

Table 1: Lipid profile and serum biochemical indices of rams before and after feeding trial

	Before feeding trial		After feeding trial	
Parameters	**A**	**B**	**A**	**B**
Glucose (mg/dL)	57.00 ± 2.94	57.83 ± 2.68	60.00[b] ± 9.53	68.17[a] ± 17.78
Cholesterol (mg/dL)	67.00[a] ± 8.71	63.61[b] ± 4.11	66.44[a] ± 10.71	59.02[b] ± 19.19
Triglyceride (mg/dL)	13.53[a] ± 3.84	10.57[b] ± 2.12	22.82[b] ± 13.96	23.95[a] ± 2.03
High density lipoprotein (mg/dL)	20.08 ± 1.98	19.15 ± 1.36	21.72[b] ± 8.61	22.99[a] ± 2.94
Low density lipoprotein (mg/dL)	23.88 ± 3.11	22.34 ± 5.45	22.15[a] ± 16.51	20.25[b] ± 16.93
Aspartate aminotransferase (U/L)	86.20 ± 2.51	89.50 ± 2.80	102.63[b] ± 3.13	109.17[a] ± 22.01
Alanine aminotransferase (U/L)	17.90 ± 0.24	18.87 ± 0.50	20.50[b] ± 0.96	18.33[a] ± 13.44

Mean ± SD, A= *Gmelina aborea* (100%), B= *Gmelina aborea* + Cassava peel (50:50)
Means in row with different superscripts are significantly (P<0.05) different

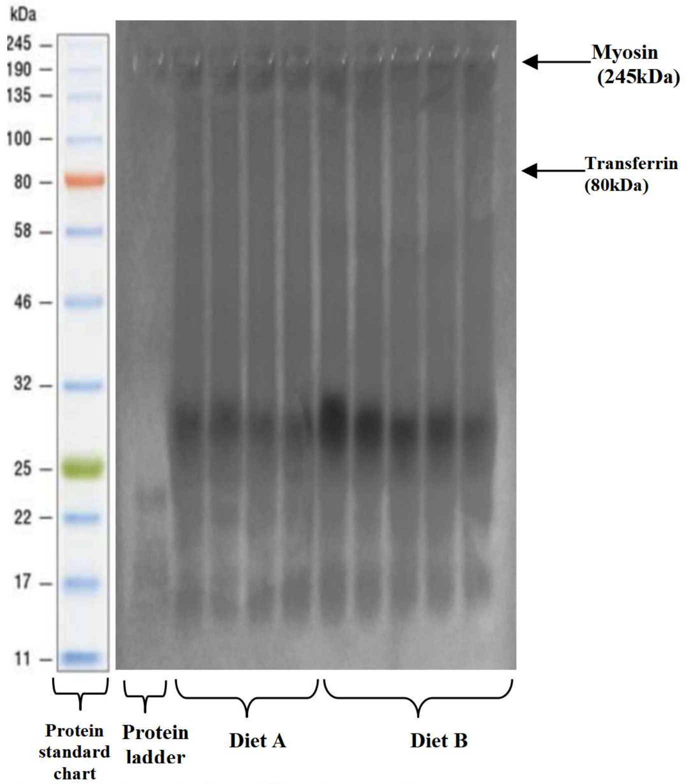

Figure 1: Protein identification on Agarose gel

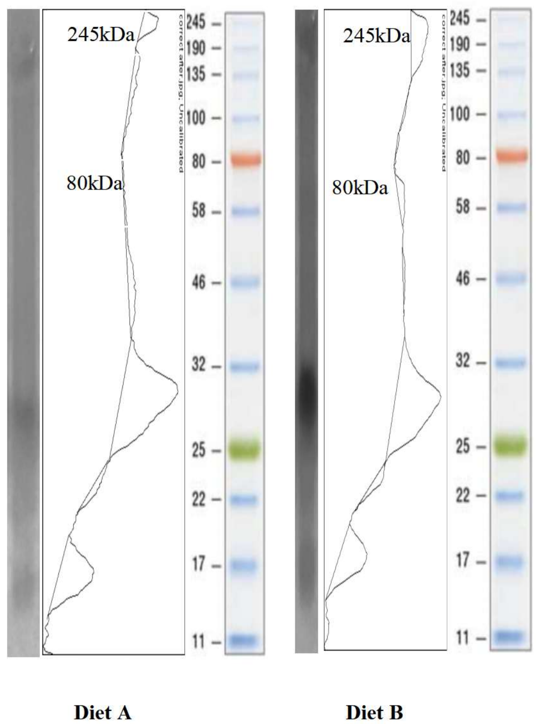

Figure 2: Protein intensity on ImageJ gel alyzer

Protein identification and intensity of rams after feeding trial

Figures 1 and 2 present the identification and intensity of serum proteins in rams fed Diet A and B. Analysis using ImageJ gel analyzer revealed an increased expression of myosin and transferrin protein.

Serum myosin (245 kDa) was detected in both groups of rams but was predominantly expressed in rams fed Diet B. The increased myosin expression enhances the conversion of chemical energy (ATP) into mechanical energy, which is crucial for metabolic activities (Trivedi *et al*., 2020). Elevated myosin levels in blood and muscle contribute to healthy growth and provide sufficient energy for physiological functions.

The iron-binding protein transferrin (80 kDa), which plays a critical role in iron transport and blood formation, also showed higher expression in rams fed Diet B. This increase can be attributed to the iron richness of cassava peel, which supports erythropoiesis. The availability of iron in the body likely enhanced transferrin expression, improving its function in maintaining adequate iron levels for physiological and metabolic processes (Ajayi *et al*., 2024).

CONCLUSION AND RECOMMENDATIONS

The diets effectively supported stable glucose levels, reduced LDL deposition, and maintained normal enzyme activities, highlighting its potential to enhance and sustain good metabolic and physiological health in ruminants.

Additionally, the increased expression of myosin and transferrin, proteins required for energy metabolism and erythrocyte formation, underscores the nutritional adequacy of the diets.
It is therefore recommended that ruminant animals be fed 50% cassava peel + 50% *Gmelina arborea* ensiled diet, particularly during the dry season as the diet contained adequate nutrients with no adverse effect on biochemical indices.

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