



INFLUENCE OF DIETARY PROTEIN LEVELS ON THE BLOOD PROFILE OF YANKASA RAMS RAISED IN THE SEMI-ARID ZONE OF NIGERIA

Bashir, F. A.^{1*}, Getso, M. M.², Abdullahi, S.³, Usman, K.¹, Muhammad, I. R.⁴ and Muhammad, B.⁴

¹Department of Animal Production, Adamawa State University, Mubi, Nigeria

²Department of Animal Health and Production, Federal University of Science and Technology Kabo (FUSTK) Kano State, Nigeria.

³Department of Animal Science, Federal University of Agriculture, Mubi Adamawa State. Nigeria.

⁴Department of Animal Science, Bayero University, Kano, Nigeria

***Corresponding author:** zahrabash77@gmail.com Phone Number: +2348036458527

Abstract

This study evaluated the influence of dietary protein levels on the blood profile of Yankasa rams. Twelve rams (average weight 16 kg; approximately 12 months old) were assigned to four dietary treatments in a completely randomized design, with three animals per treatment. The control diet contained 14.26 % Crude Protein (CP), while the other diets contained 14.94, 15.45, and 16.15 % CP presented as 20 %, 40 %, and 60 % increment of CP of the control diet. The basal diet comprised fresh grass and soybean hay, and the trial lasted 90 days. Blood samples collected at the end of the experiment were analyzed for haematological and serum biochemical parameters. Data were subjected to analysis of variance, and means were separated using Duncan's Multiple Range Test. Results indicated that the 60 % CP (16.15%) diet had the highest crude protein content as expected. No significant differences ($P>0.05$) were observed in the haematological parameters, whereas significant differences ($P<0.05$) were observed in some selected serum biochemical parameters among treatments. The majority of the parameters were within normal recommended physiological ranges. It was concluded that increasing dietary protein improved the physiological and metabolic status of Yankasa rams, with 16.15% CP supplementation producing favourable responses without adverse effects on the haematological and serum biochemical parameters studied. Further studies are recommended to establish optimal protein requirements under semi-arid conditions.

Keywords: Blood, Dietary protein, Rams, Semi-arid zone, Yankasa

1.0

INTRODUCTION

Nutrition remains the foundation of sustainable livestock production, particularly in efforts to meet the growing demand for animal protein. In the seasonally dry tropics, ruminant productivity is largely constrained by fluctuations in feed availability and quality between the wet and dry seasons (Muhammad, 2008). During the dry season, forages are typically characterized by low crude protein and energy contents, resulting in inadequate nutrient intake to meet maintenance and production requirements. Consequently, ruminants experience weight loss, reduced growth rate, poor digestibility, and overall low productivity (Alemayehu, 2006; Nigale *et al.*, 2010; Adewumi and Ajayi, 2010).



The low nutritive value of dry-season forages imposes significant nutritional stress on animals grazing without supplementation, leading to depressed voluntary intake and inefficient nutrient utilization (Jiwuba *et al.*, 2021). To address this challenge, supplementation of forage-based diets with energy and protein-rich concentrate feeds has been widely recommended to bridge nutrient deficits and improve pasture utilisation.

Concentrate supplementation enhances rumen fermentation by improving rumen microbial activity, fibre digestibility, and forage intake (Peyraud, 2001; Leng, 2011). Adequate protein and energy supplementation also optimize rumen fermentation by maintaining appropriate pH and ammonia-nitrogen ($\text{NH}_3\text{-N}$) concentrations, thereby promoting microbial protein synthesis and volatile fatty acid production, with potential reductions in methane emission (Khampa and Wanapat, 2007). Such improvements in rumen function are critical for increasing ruminant productivity under tropical production systems.

Blood biochemical and haematological parameters serve as reliable indicators of the physiological and health status of farm animals, reflecting the interaction between nutrition and metabolic processes (Jiwuba *et al.*, 2016). These indices are also useful in assessing immune competence and stress response in ruminants (Ambore *et al.*, 2009; Al-Seaf and Al-Harbi, 2012). The Yankasa sheep, an important indigenous breed in northern Nigeria, contributes significantly to meat production but is highly affected by seasonal feed scarcity. Therefore, this study evaluated the influence of dietary protein levels on the blood profile of Yankasa rams raised in semi-arid Nigeria.

2.0 MATERIALS AND METHODS

2.1 Study Area

The experiment was conducted at the Bayero University, Kano Livestock Teaching and Research Farm, Department of Animal Science, Faculty of Agriculture, located in Kano, Nigeria.

2.2 Experimental Animals and Management

Twelve (12) Yankasa growing rams, approximately 12 months old with an average body weight of 16 kg, were used for the study. Prior to the commencement of the experiment, pens were thoroughly cleaned, disinfected, and allowed to dry for two weeks. The animals were dewormed orally with albendazole to control internal parasites, and an acaricide (amitraz solution) was applied externally to control ectoparasites. Long-acting oxytetracycline (LA) was administered intramuscularly at a dosage of 1 mL

per 10 kg body weight as a prophylactic measure against bacterial infections. The rams were individually housed in well-ventilated pens and managed under standard husbandry practices throughout the experimental period. Clean drinking water was provided *ad libitum*.

2.3 Experimental Diets and Treatments

Feed supplements were collected from private ranches and analyzed for crude protein content. The mean CP value obtained was used to formulate the control diet. Subsequently, concentrate rations were formulated to increase crude protein levels in increments of 20, 40 and 60 %. The ingredient composition and calculated nutrient profile of the experimental diets are presented in Table 1.

Table 1: Composition (%) of Ingredients in the supplementary concentrate

Ingredients	Treatments			
	1	2	3	4
Wheat bran	50	48	38	30
Maize bran	35	26	24	24
Soy Bean Meal	4	12	20	28
Cotton Seed Cake	5	8	12	12
Salt	2	2	2	2
Bone meal	3	3	3	3
Toxin binder	1	1	1	1
Total	100	100	100	100

1= control (crude protein of supplements from farmers' farms; 13.4% CP), 2 = Low Protein (16.15% CP), 3 = Medium Protein (18.1% CP), 4 = High Protein (21.3% CP).

2.4 Experimental Procedure and Design

A 90-day feeding trial was conducted using a Completely Randomized Design (CRD). The experimental rams were randomly allotted to the four dietary treatments with three animals per treatment. Experimental diets were offered at 5 % of body weight on a dry matter basis. The dietary regimen consisted of 40 % concentrate supplement and 60 % basal diet (fresh grass and hay). The daily feed allowance was weighed and divided into two equal portions. One portion was offered at 09:00 h, and the second at 16:00 h.



2.5 Blood Collection

A new hypodermic needle and syringe for each animal to avoid cross-contamination was used to collect 10 mL of the blood sample via the jugular vein from two randomly selected rams from each treatment. The jugular area was disinfected with cotton wool soaked in methylated spirit prior to blood collection. Half of the blood sample was released into a sample bottle containing anticoagulant Ethylene Diamine Tetra Acetic acid (EDTA) and was rocked gently to ensure easy mixing of the blood with the anticoagulant for estimation of haematology parameters. The remaining 5 mL of blood samples was discharged into a sterile plain bottle to allow blood to clot at room temperature within two hours of collection, and serum obtained for chemistry analysis. The bottles containing the blood samples were kept in an ice- pack and taken to the laboratory for analysis.

2.6 Laboratory Analyses

Blood samples were collected and centrifuged at 3,500 rpm for 15 minutes to separate the plasma. The serum was subsequently separated and stored at -20°C until analysis. Serum biochemical parameters, including total protein, albumin, cholesterol, glucose and urea were determined using commercial ELISA/multiplex assay kits (Pfizer Animal Health, New York, NY, USA) according to the manufacturer's instructions. Haematological parameters, including packed cell volume (PCV), white blood cell (WBC) count, red blood cell (RBC) count, and haemoglobin (Hgb) concentration, were determined using an automated blood analyser. The red blood cell indices namely mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated according to the formulae described in the Merck Veterinary Manual (2016):

$$\text{MCV (fL)} = \text{PCV (\%)} \times 10 \div \text{RBC} (\times 10^6/\mu\text{L})$$

$$\text{MCH (pg)} = \text{Hb (g/dL)} \times 10 \div \text{RBC} (\times 10^6/\mu\text{L})$$

$$\text{MCHC (g/dL)} = \text{Hb (g/dL)} \times 100 \div \text{PCV}$$

2.7 Data Analysis

All data generated were subjected to Analysis of Variance (ANOVA) using the completely randomized design model in SAS (2003). Treatment means were separated using Duncan's Multiple Range at 5 % probability level ($P < 0.05$). The statistical model used was: $Y_{ij} = \mu + T_i + e_{ij}$ Where: Y_{ij} = observation, μ = overall mean, T_i = treatment effects, and e_{ij} = experimental error.

3.0 RESULTS AND DISCUSSION

3.1 Chemical Composition of Experimental Diets

The chemical composition of the experimental diets is presented in Table 2. Significant ($P < 0.05$) differences were observed for all parameters except Dry Matter (DM). Treatment 4 (21.3CP %) diet recorded the highest values for Crude Protein (CP), Ether Extract (EE), Hemicellulose (HEM), and Metabolizable Energy (ME). In contrast, the control diet had the highest values for Crude Fibre (CF), ash, Nitrogen-Free Extract (NFE), Neutral Detergent Fibre (NDF), and Acid Detergent Fibre (ADF), respectively. These results indicate progressive improvement in nutrient density with increasing concentrate inclusion.

Table 2: Chemical composition of experimental diets (%)

Parameters	Treatments					
	1	2	3	4	SEM	P-Value
Dry matter	75.96	76.08	75.38	75.59	0.16	0.05
Crude protein	14.26 ^d	14.94 ^c	15.45 ^b	16.15 ^a	0.40	0.01
Crude fibre	29.10 ^c	27.84 ^b	27.15 ^a	26.79 ^a	0.51	0.03
Ether extract	6.27 ^b	7.65 ^a	7.55 ^a	7.75 ^a	0.35	0.01
Ash	6.95 ^a	6.49 ^b	6.13 ^c	6.41 ^d	0.17	0.02
Nitrogen-Free-Extract	44.11 ^a	42.15 ^{bc}	41.41 ^c	39.97 ^d	0.86	0.04
Neutral-Detergent-Fibre	52.64 ^a	51.40 ^b	47.95 ^c	45.64 ^d	1.63	0.04
Acid-Detergent-Fibre	35.39 ^a	34.72 ^b	32.73 ^c	27.93 ^d	1.69	0.01
Hemicellulose	17.25 ^a	16.68 ^b	15.22 ^c	17.71 ^a	0.54	0.01
Metabolizable energy (kcal/kg)	1736.6 ^d	1876.3 ^{bc}	1886.9 ^b	1929.2 ^a	41.83	0.02

abc: Values with the same superscript within a row are not significantly different ($P > 0.05$). 1= control (crude protein of supplements from farmers' farms; 13.4% CP), 2 = Low Protein (16.1% CP), 3 = Medium Protein (18.1% CP), 4 = High Protein (21.3% CP).

The chemical composition of the experimental diets indicate their suitability as supplements for growing ruminants. The adequate DM content observed supports optimal voluntary intake and proper rumen function, as diets with appropriate DM enhance microbial fermentation efficiency (McDonald *et al.*, 2011). Crude protein increased progressively from 14.26 % CP in the control diet to 16.15 % CP, meeting



the protein requirements for growing sheep. This agreed with NRC (2007), Abu *et al.* (2023) who stated that adequate CP enhances nitrogen availability for rumen microorganisms, promoting fibre degradation and nutrient utilization. The fibre fractions (CF, NDF, and ADF) declined with increasing protein levels which reduced the rumen fill. High NDF and ADF are known to limit voluntary intake and energy availability in sheep (McDonald *et al.*, 2011). Therefore, their reduction implies improved feed efficiency and greater energy utilization. Ether extract increased to 7–8 %, enhancing dietary energy density without exceeding recommended levels. Although ash content slightly declined, values remained within acceptable ranges, indicating adequate mineral supply necessary for metabolic and skeletal functions (McDowell, 2003).

Nitrogen-free extract decreased with higher protein inclusion, reflecting lower rapidly fermentable carbohydrates. Excess fermentable carbohydrates may predispose animals to rumen acidosis (Plaizier *et al.*, 2017); thus, the observed reduction may support rumen stability while maintaining energy supply through increased EE (Beauchemin *et al.*, 2020). Metabolizable energy increased significantly from 1,736.6 to 1,929.2 kcal/kg, demonstrating improved dietary energy density. Energy is often the primary limiting nutrient in sheep production systems (Solaiman *et al.*, 2018). Higher ME supports anabolic processes and improves feed efficiency (Mekonnen *et al.*, 2021; González-Rivas *et al.*, 2022).

3.2 Haematological and Serum Biochemical Parameters

The haematological and serum biochemical parameters of Yankasa rams fed diets containing different levels of dietary protein are presented in Table 3. The results showed no significant differences ($P > 0.05$) in WBC, RBC, HGB, PCV, MCH, and MCHC, although numerical variations were observed. Majority of the haematological values obtained in this study fell within or close to the normal physiological ranges reported for sheep (Merck Veterinary Manual, 2022; Aiello & Moses, 2016), indicating that the animals remained physiologically stable throughout the experimental period.

However, the serum biochemical parameters (Total protein, albumin, globulin, cholesterol, globulin, glucose and urea) showed significant differences ($P < 0.05$) among treatment groups. Total protein, albumin, globulin, glucose, and urea increased progressively with increasing dietary protein levels, with the highest values recorded in rams fed diet 4 (16.15% crude protein). Cholesterol values showed a different trend, with the highest concentration observed in diet 2 (14.95% crude protein), followed by a decline in higher protein diets. These findings suggest that increasing dietary protein levels enhanced protein metabolism, improved nutrient utilization, and supported metabolic efficiency in Yankasa rams.

Table 3. Haematological and Serum Biochemical parameters of Yankasa rams fed different levels of protein

Parameters	*Ref.values	Treatments				SEM	P-Value
		1	2	3	4		
WBC ($10^9/L$)	4 – 15	10.27	14.91	19.12	13.76	3.02	0.3197
RBC ($\times 10^{12}/L$)	5– 18	6.22	6.16	6.29	6.72	0.59	0.0560
HGB (g/dl)	8 – 16	8.60	9.55	9.35	8.50	0.99	0.1666
PCV (%)	22 – 45	26.65	26.55	26.60	28.80	3.17	0.0983
MCV (fI)	16 – 60	42.75	42.80	42.25	42.90	1.54	0.5312
MCH (pg)	8 – 40	13.75	15.60	14.75	12.65	0.80	0.2021
MCHC (g/L)	30 – 38	32.15	36.75	34.90	29.55	2.14	0.1195
Total protein (g/dl)	5.9 – 7.8	5.65 ^b	5.75 ^b	7.45 ^a	7.55 ^a	0.25	0.0013
Albumin (g/dl)	2.2 – 3.7	2.74 ^b	2.83 ^b	3.20 ^a	3.22 ^a	0.00	<0.0001
Globulin (g/dl)	2.7 – 4.4	2.85 ^c	2.97 ^c	3.24 ^b	3.50 ^a	0.00	<0.0001
Cholesterol (mmol/l)	1.05 – 7.5	3.52 ^b	3.70 ^a	2.72 ^d	2.84 ^c	0.00	<0.0001
Glucose (mmol/l)	1.7 – 4.4	2.66 ^d	3.02 ^c	3.23 ^b	3.45 ^a	0.00	<0.0001
Urea (mmol/l)	0.4 – 9.3	3.60 ^c	5.85 ^b	7.15 ^a	7.50 ^a	1.51	0.0478

^{abc}: Means within the same row with different subscripts are significantly different ($P < 0.05$). SEM=Standard error of means; WBC=White Blood Cell; RBC=Red Blood Cell; Hgb= Haemoglobin concentration; PCV= Packed Cell Volume; MCV=Mean corpuscular volume; MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular haemoglobin concentration. *Merck Veterinary Manual (2022), Research Animal Resources, (2009) and Veterinary Medicine (2006). 1= control (13.4% CP), 2 = Low Protein (16.1% CP), 3 = Medium Protein (18.1% CP), 4 = High Protein (21.3% CP).

Haematological parameters are important indicators of the physiological and health status of animals, reflecting their nutritional condition, metabolic activity, and response to environmental or dietary changes (Afolabi *et al.*, 2010; Ashiru, 2014). In the present study, the absence of significant differences in most haematological indices suggests that dietary protein levels did not negatively affect the blood profile of Yankasa rams. This indicates that all dietary treatments supported adequate physiological function without inducing anaemia or immunological stress.

The WBC values recorded ($10.27\text{--}19.12 \times 10^9/L$) were above the normal physiological range for sheep ($4\text{--}15 \times 10^9/L$; Merck Veterinary Manual, 2022). The relatively higher WBC observed in some treatments may reflect a normal immune surveillance response rather than pathological stress, as similar variations have been reported in sheep exposed to dietary modifications (Olorunnisomo *et al.*, 2012;



Jackson & Cockcroft, 2002). The RBC and PCV values obtained ($6.16\text{--}6.72 \times 10^{12}/\text{L}$ and 26.55–28.80%, respectively) were within established reference ranges (Radostits *et al.*, 2006; RAR, 2009), indicating adequate erythropoietic activity and oxygen-carrying capacity, and suggesting that the animals were not anaemic.

Haemoglobin concentrations (8.50–9.55 g/dL) were also within normal physiological limits (8–15 g/dL), confirming adequate oxygen transport capacity and normal haematopoietic function across all treatments. Although numerical differences were observed, the lack of statistical significance suggests that dietary protein inclusion did not exert a strong influence on red blood cell indices under the conditions of this study.

Serum biochemical indices provided clearer evidence of dietary effects on metabolism. The significant increase in total protein, albumin, and globulin with increasing dietary protein levels indicates improved protein intake, absorption, and hepatic synthesis. Albumin and globulin are important indicators of nutritional status and immune competence; therefore, their elevation suggests enhanced protein metabolism and improved physiological resilience in rams fed higher protein diets (Kaneko *et al.*, 2008; Ikyume *et al.*, 2018).

Similarly, the progressive increase in serum glucose with increasing dietary protein levels suggests improved energy availability and gluconeogenic activity, which may support growth and metabolic demands. Blood urea levels also increased with higher protein intake, reflecting increased protein catabolism and nitrogen metabolism, which is expected when dietary protein supply is elevated (Prvulovic *et al.*, 2012; Saleh & Sanusi, 2019). Importantly, all urea values remained within normal physiological ranges, indicating efficient nitrogen utilization without evidence of renal dysfunction.

Cholesterol concentrations varied significantly among treatments, with the highest value recorded in the moderate protein diet and lower values in higher protein treatments. This trend suggests that dietary protein level may influence lipid metabolism, possibly through altered energy partitioning and reduced reliance on lipid synthesis pathways at higher protein intake levels (Banerjee, 2009; Olorunnisomo *et al.*, 2012). The serum biochemical responses demonstrate that increasing dietary protein levels positively influenced metabolic processes in Yankasa rams without causing physiological imbalance.

4.0 Conclusion

Based on the findings of this study, it was concluded that higher dietary protein improved selected serum biochemical and haematological indices without adverse physiological effects.



LIMITATION OF THE STUDY

The study was designed primarily to evaluate the blood parameters as complementary indicators of animal health and physiological status. Resource limitations prevented blood analysis from being conducted on all experimental animals. A future study with larger sample sizes is recommended.

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