

Changes in Some Haematological Parameters of Pubertal Yankasa Rams Fed Graded Levels of Dietary Protein Using Cotton Seed and Palm Kernel Cake

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Abstract

The effects of graded levels of dietary protein on some haematological parameters in pubertal Yankasa rams were studied. They were randomly divided into three treatment groups (A, B and C). Groups A, B and C received 10%, 15% and 20% crude protein respectively. All rams were fed a basal diet of hay (*Digitaria* spp) *ad-libitum* and given a supplement ration of concentrate mixture at 2% body weight/day. Blood was collected once weekly via jugular venepuncture into heparinised test-tubes, and transported to the laboratory for determination of red blood cell counts, packed cell volume, white blood cell counts and total protein for twelve weeks. The overall mean red blood cell values obtained were $4.2 \pm 0.1 \times 10^6 \mu\text{l}$, $5.2 \pm 0.1 \times 10^6 \mu\text{l}$ and $5.06 \pm 0.1 \times 10^6 \mu\text{l}$, for groups A, B and C respectively. The difference between values obtained for groups B and C were not significantly different ($P > 0.05$). Mean packed cell volumes obtained in this study revealed that rams fed 15% crude protein had significantly ($P < 0.05$) higher values ($31.3 \pm 0.9 \%$) than those fed 10% ($25.9 \pm 0.7 \%$) but similar to those fed 20% ($30.33 \pm 0.56 \%$) crude protein. There were no significant ($P > 0.05$) differences in mean white blood cell counts and total protein concentrations between all groups of animals fed 10% CP. Feeding rams diets containing 15 % CP (cotton seed cake and palm kernel cake) gave better values in terms of packed cell volume and red blood cell counts, than feeding diets containing 10% and 20% C.P. While graded levels of dietary protein was shown to affect packed cell volume and total protein concentration, it had no significant effect on red blood cell and white blood cell counts.

Keywords: Haematological Parameters; Protein diet; Pubertal; Yankasa rams.

Introduction

Proteins are also involved in growth, repair of body tissues, synthesis of nucleoproteins, hormones, enzymes and antibodies. The various sources of proteins in livestock diet include; animal by-products, cereal, legumes and forages (Boland et al., 2013). Groundnut cake and soybean meal are the major conventional sources of protein ingredients in livestock production in Nigeria (Boland et al., 2013). However, the utilisation of these protein sources would largely depend on their type and or animal species to be fed. The synthesis of plasma blood protein is negatively affected when the supply of amino acid is inadequate (Ahmed and Abdelatif, 1992; Kheir and Ahmed, 2008). This protein helps

to transport calcium and phosphorous and other substances in the blood (Njidda et al., 2014). The Red blood cell value for sheep is ($8.9 - 9.3 \times 10^6/\mu\text{l}$) as reported by (Etim et al., 2014), this range could be distorted by nutrition, age or sex difference as stated by (Etim et al., 2014). Several authors have stated the importance of dietary protein to blood parameters in rams, hence this study to investigate the effects of graded levels of dietary protein using cotton seed and palm kernel cakes on haematological values in pubertal rams.

Materials and Methods

The study was carried out at the National Animal Production Research Institute (NAPRI) Shika, Ahmadu Bello University

Zaria, situated in the Northern Guinea Savannah, between latitudes 11° 8' 19.5"N and between longitude 7° 45' 51.22" E at an elevation of 646 m above sea level. The study was conducted between March and June.

Experimental Animals

Fifteen yankasa rams with mean age of 19.06 ± 2.4 months and mean weight of 19.4 ± 1.6 kg with good body condition scores of 3.5 were randomly divided into three treatment groups (A, B and C) according to the dietary protein inclusion level fed, of 5 rams each. Group A received 10% crude protein level feed, group B received 15% crude protein level feed, while group C received 20% crude protein level feed respectively. The rams were screened for blood and helminth parasites, and

appropriate treatment carried out before the commencement of the research.

Experimental diets

The three levels of protein were formulated to contain 10% crude protein, 15% crude protein and 20% crude protein (Table 1).

The rams were managed under intensive system, kept in separate pens, fed individually and allowed two weeks adjustment period. All rams under study were fed a basal diet of hay (*Digitaria* spp) *ad-libitum* and given a supplement ration of concentrate mixture at 2% body weight/head/day, with water provided *ad libitum*. All test diets (Table 2) were subjected to proximate analysis using the method of AOAC, (1990). The animals were fed for a period of three months.

Table 1: Ingredients and nutrient composition of experimental diets

Groups/Ingredients (%)	A (10% C.P)	B (15% C.P)	C (20% C.P)
Maize Bran	23.5	10	2
Bagasse	20	8.5	2.5
Palm Kernel Cake	10	30	45
Cotton Seed Cake	15	30	48
Rice Bran	30	20	1
Bone meal	1	1	1
Common Salt	0.5	0.5	0.5
Total	100.00	100.00	100.00

Table 2: Proximate analysis of feeds (10%, 15% and 20%) Crude Protein levels

Groups	Percentage Content (%)					
	Dry Matter	Ash	Crude Fibre	Nitrogen	Crude Protein	Energy
A (10%)	96.21	11.94	35.69	1.67	10.44	2.060
B (15%)	95.57	9.26	31.15	2.43	15.19	2.120
C (20%)	96.05	6.70	30.50	3.30	20.63	2.210

*Energy (MJ/Kg DM ME)

Haematological Evaluation

Blood was collected once weekly from individual animal via jugular venepuncture into heparinised test-tubes and transported to the laboratory in a container with icepacks for analyses. This was done in the morning.

The Packed Cell Volume (PCV) was determined using the microhaematocrit method as described by Benjamin, (1978). Red and white blood cell counts were done using haemocytometer method as described by Schalm et al. (1975). Total protein was determined according to the method described by Benjamin, (1978), known as Biuret reaction which is based on the principle that protein forms coloured complex with copper ions (Cu^{2+}) in alkaline solution as a result of nitrogen reaction complex.

Data Analyses

Data collected were expressed as means and standard error of the mean (\pm SEM). Significance of differences between

treatments means were estimated at $P \leq 0.05$ with Tukey-Kramer multiple comparison test of repeated measure analysis of variance (ANOVA). Statistical analysis was conducted using the Graphpad InStat computer programme (GRAPHPAD for Windows, Inc., version 3.05 of 2000).

Results

Total Red Blood Cell Count

The mean RBC values obtained for the rams fed 10%, 15% and 20% crude protein ranged from $3.8 \pm 0.1 \times 10^6 \mu\text{l}$ to $4.9 \pm 0.5 \times 10^6 \mu\text{l}$, $4.5 \pm 0.5 \times 10^6 \mu\text{l}$ to $5.8 \pm 0.2 \times 10^6 \mu\text{l}$ and 4.7 ± 0.2 to $5.7 \pm 0.2 \times 10^6 \mu\text{l}$ respectively (Table 3). There was significant ($P \leq 0.05$) difference between values for groups A and B and groups A and C. The overall mean RBC values obtained were $4.2 \pm 0.1 \times 10^6 \mu\text{l}$, $5.2 \pm 0.1 \times 10^6 \mu\text{l}$ and $5.06 \pm 0.1 \times 10^6 \mu\text{l}$, for groups A, B and C respectively (Table 3). The difference between values obtained for group B and C were not significantly different ($P > 0.05$), although group C had lower values than group B.

Packed Cell Volume

There was significant difference ($P < 0.05$) between PCV values obtained for groups A and B. and for groups B and C, but not ($P > 0.05$) between group A and C. The packed cell volume level of rams fed 20% CP ranged from $28.4 \pm 1.2\%$ to $34.4 \pm 1.4\%$ (Table 4). That of those fed 15% and 10% CP ranged from $26.8 \pm 2.8\%$ to $35.0 \pm 1.6\%$ and $22.6 \pm 0.8\%$ to $29.6 \pm 2.2\%$ respectively.

At the end of the study, the overall mean PCV of rams fed 10% CP was $25.9 \pm 0.7\%$ (Table 4) which was significantly lower ($P < 0.05$) than values obtained for rams fed 15% and 20% CP. Rams fed 15% CP had an overall mean value of 31.3 ± 0.9 which was higher than what was obtained for group C ($30.3 \pm 0.6\%$), though the difference was not statistically significant.

Total White Blood Cell Count

Rams fed 10% CP had mean white blood cell counts ranging from $7.5 \pm 0.7 \times 10^3/\mu\text{l}$ to $10.0 \pm 2.1 \times 10^3/\mu\text{l}$ (Table 5), while those rams fed 15% and 20% CP had values that ranged from $7.4 \pm 0.8 \times 10^3/\mu\text{l}$ to $9.5 \pm 1.0 \times 10^3/\mu\text{l}$ and $6.6 \pm 0.7 \times 10^3/\mu\text{l}$ to 9.2 ± 1.5 , respectively. The overall mean WBC obtained for rams fed 10%, 15% and 20% were $8.43 \pm 0.7 \times 10^3/\mu\text{l}$, $8.37 \pm 0.2 \times 10^3/\mu\text{l}$ and $8.40 \pm 0.2 \times 10^3/\mu\text{l}$, respectively.

Table 3: Mean \pm SEM weekly Red Blood Cell Counts ($\times 10^6/\mu\text{l}$) of Yankasa rams fed graded levels of dietary crude protein (CP) from cotton seed and palm kernel cakes

Weeks	10% CP	15% CP	20% CP
1	4.50 \pm 0.33	4.76 \pm 0.37	4.73 \pm 0.19
2	4.50 \pm 0.33	4.60 \pm 0.37	4.83 \pm 0.22
3	4.76 \pm 0.29	5.00 \pm 0.54	4.80 \pm 0.17
4	3.76 \pm 0.11	5.40 \pm 0.32	4.76 \pm 0.12
5	3.90 \pm 0.40	5.40 \pm 0.27	5.00 \pm 0.19
6	4.40 \pm 0.34	4.46 \pm 0.47	5.43 \pm 0.31
7	3.90 \pm 0.30	4.56 \pm 0.32	5.06 \pm 0.24
8	4.93 \pm 0.46	5.63 \pm 0.35	5.73 \pm 0.21
9	4.40 \pm 0.36	5.83 \pm 0.25	5.20 \pm 0.09
10	4.40 \pm 0.36	5.83 \pm 0.27	5.13 \pm 0.12
11	3.96 \pm 0.21	5.56 \pm 0.12	4.90 \pm 0.15
12	3.90 \pm 0.30	5.40 \pm 0.32	5.13 \pm 0.12
	4.23 \pm 0.11 ^a	5.21 \pm 0.14 ^b	5.06 \pm 0.08 ^{bc}

Thus values obtained for rams fed 10% CP were highest, while those fed 20% and 15% CP gave lesser values (Table 5) but these differences were not statistically ($P \leq 0.05$) significant.

Total Protein

Those rams fed 10% had total protein values, ranging from 5.1 ± 0.3 g/l to 7.00 ± 0.71 g/l, rams fed 15% CP had values ranging from 6.4 ± 0.1 g/l to 7.3 ± 0.3 g/l, while those fed 20% CP had values ranging from 5.8 ± 0.2 g/l to 7.8 ± 0.5 g/l, (Table 6). The overall mean total protein values obtained for the groups were 5.9 ± 0.2 g/l, 6.8 ± 0.0 g/l and 7.0 ± 0.2 g/l for group A, B and C respectively. There were statistically ($P \leq$

0.05) significant differences between groups A and B, and groups A and C, but not between groups B and C.

Table 4: Mean \pm SEM weekly packed cell volumes (%) of Yankasa rams fed graded levels of dietary crude protein (CP) from cotton seed and palm kernel cakes

Weeks	10% CP	15% CP	20% CP
1	27.00 \pm 2.00	28.60 \pm 2.20	28.40 \pm 1.16
2	27.00 \pm 2.00	27.60 \pm 2.23	29.00 \pm 1.30
3	28.60 \pm 1.72	30.00 \pm 1.24	28.80 \pm 1.02
4	22.60 \pm 0.78	32.40 \pm 1.91	28.60 \pm 0.75
5	23.40 \pm 2.40	32.40 \pm 1.63	30.00 \pm 1.14
6	26.40 \pm 2.04	26.80 \pm 2.81	32.60 \pm 1.89
7	23.40 \pm 1.81	27.40 \pm 1.94	30.40 \pm 1.43
8	29.60 \pm 2.23	33.80 \pm 2.11	34.40 \pm 1.29
9	26.40 \pm 2.13	35.00 \pm 1.55	31.20 \pm 0.58
10	26.40 \pm 2.13	35.00 \pm 1.64	29.40 \pm 0.93
11	23.80 \pm 1.24	33.40 \pm 1.08	31.20 \pm 0.58
12	23.40 \pm 2.40	33.80 \pm 2.11	29.40 \pm 0.93
	25.87 \pm 0.69 ^a	31.33 \pm 0.95 ^b	30.33 \pm 0.56 ^{bc}

Discussion

The Packed Cell Volume (PCV) recorded for rams fed 10% CP and 15% CP in this study were lower than what was reported by Njidda et al. (2014), who reported a normal value of 28.90% for Yankasa rams. Rams fed 15% CP had higher PCV values than what was reported by Njidda et al. (2014). Rams fed 10% C.P had the lowest PCV which was significantly lower than values obtained for rams fed 15% and 20% C.P. This is similar to report by (Babeker and Abdalbagi, 2015), who reported a significant difference in goats fed medium levels of nutrition compared to those fed high level of nutrition. This difference could be as a result of age difference in animals used and also difference in species.

Table 5: Mean \pm SEM weekly White blood cell counts ($\times 10^3/\mu\text{l}$) of Yankasa rams fed graded levels of dietary crude protein (CP) from cotton seed and palm kernel cakes

Weeks	10% CP	15% CP	20% CP
1	7.48 \pm 0.71	8.04 \pm 1.45	6.60 \pm 0.74
2	9.04 \pm 1.03	7.44 \pm 0.78	8.00 \pm 0.44
3	9.80 \pm 0.73	8.60 \pm 0.59	8.44 \pm 0.17
4	9.96 \pm 2.09	8.72 \pm 1.05	8.56 \pm 0.41
5	6.98 \pm 0.79	9.40 \pm 1.16	9.16 \pm 1.48
6	8.32 \pm 0.85	8.24 \pm 1.22	8.88 \pm 0.34
7	8.08 \pm 0.57	9.52 \pm 0.99	8.28 \pm 0.61
8	9.00 \pm 0.71	7.92 \pm 0.42	8.80 \pm 0.37
9	8.16 \pm 0.89	7.88 \pm 0.39	7.92 \pm 0.26
10	6.98 \pm 0.78	7.88 \pm 0.39	9.16 \pm 1.48
11	8.28 \pm 0.61	8.20 \pm 0.58	8.08 \pm 0.57
12	9.08 \pm 0.81	8.60 \pm 0.24	8.96 \pm 0.87
	8.43 \pm 0.28	8.37 \pm 0.18	8.40 \pm 0.21

Table 6: Mean weekly total protein concentration (g/l) of Yankasa rams fed graded levels of dietary crude protein (CP) from cotton seed and palm kernel cakes

Weeks	10% CP	15% CP	20% CP
1	6.00 ± 0.15	6.52 ± 0.14	6.72 ± 0.54
2	5.64 ± 0.13	6.36 ± 0.11	6.80 ± 0.58
3	6.02 ± 0.57	6.72 ± 0.10	7.72 ± 0.59
4	5.52 ± 0.14	7.08 ± 0.14	6.72 ± 0.46
5	5.08 ± 0.33	6.72 ± 0.36	6.48 ± 0.48
6	5.60 ± 0.35	6.64 ± 0.19	5.76 ± 0.24
7	7.00 ± 0.71	6.60 ± 0.35	7.84 ± 0.49
8	6.00 ± 0.34	7.20 ± 0.23	6.72 ± 0.46
9	6.04 ± 0.53	7.32 ± 0.27	7.48 ± 0.41
10	5.80 ± 0.37	7.24 ± 0.27	7.40 ± 0.49
11	6.00 ± 0.37	6.76 ± 0.27	7.36 ± 0.73
12	7.00 ± 0.71	6.72 ± 0.36	6.72 ± 0.46
	5.92 ± 0.18 ^a	6.82 ± 0.01 ^b	6.98 ± 0.17 ^{bc}

The result from this study could be due to decrease in by-pass protein in this species of animals fed above required amount of C.P. This process would not improve the quantity of amino acids available for the animal to use. The low level of PCV recorded in rams fed 10% is reflection of low amino acid concentration to drive haematopoiesis, resulting in low levels of PCV. This agrees with report of Babeker and Abdalbagi, (2015), who suggested that reduction in PCV and red blood cells values are indicative of low protein intake. Such high PCV values obtained in rams fed 15% is similar to the report Addas et al. (2010) who reported high PCV as signs of healthy and high productivity in animals. Rams fed 10% CP had the lowest value for total protein. This is in contrast to reports of Benjamin, (1978), who reported higher values in goats, fed medium level nutrition, while goats fed high levels of nutrition had lower values. Total blood protein is an indication of the quantity or quality of protein in the diet (Mohamed et al., 2012). The synthesis of plasma protein is markedly reduced when the supply of amino acid for digestive process is not adequate. This could be the reason for the results observed in this study, as rams fed 10% had the least total protein. This is because supply of amino acids which is usually from protein isn't adequate. This finding is similar to what was recorded in animals fed low level of Lucene hay (Ahmed and Abdelatif, 1992; Kheir and Ahmed, 2008) which had low levels of total protein. Plasma protein helps to transport calcium and phosphorous and other substances in the blood (Njidda et al., 2014). Result in this study is similar to reports by (Mohamed et al., 2012) who stated that rams maintained on low level of feeding showed lower total protein. In a study by Bello and Tsado, (2013), total protein was high in rams fed 14% C.P. This corroborates the findings in this present study as the required protein levels for rams is between 12-16%. The range of white blood cell count observed in this study is similar to what was reported for rams by Etim et al. (2014), who gave a normal range of 4 – 12 x10³/µl. This could be due to a low level of plasma corticosterone levels, which are known to decrease the immunity in higher concentrations (Keser et al.,

2008). Although this differed from reports by Babeker and Abdalbagi, (2015) who reported higher WBC in goats fed medium level of nutrition. White blood cells/ within normal values is an indication that there were no microbial infections or presence of foreign bodies or parasites (Bello and Tsado,2013). Animals fed 15% CP had the lowest WBC; this could mean they were in a healthy state with resultant production of lower WBC (Etim et al., 2014). This result differs from reports by Bello and Tsado (2013), who fed 15% C.P of protein diet to rams and had a higher WBC. Those fed 10% C.P. had the highest WBC, which may be as a result of a disease condition (Etim et al., 2014). This might be because of higher plasma corticosterone levels associated with nutritional restrictions which are known to negatively influence the immune system, making the animal susceptible to infectious diseases. (Keser et al., 2008). The Red blood cell values that were recorded in this study ranged from 3.76 to 5.83 x10⁶/µl and these values are lower than what was reported for sheep by (Etim et al., 2014). The difference might be due to nutrition, age or sex difference as stated by (Etim et al., 2014). Rams fed 15 and 20% C.P had the higher values for RBC than those fed 10% CP which indicated that the feed with 15 and 20% CP improved the erythropoetic process in the animals (Togun et al., 2007). Rams fed 10% C.P had the lowest values of RBC which could be as a result of decreased protein to drive erythropoiesis. This study revealed that animals on 15 and 20% CP had higher RBC which in turn would lead to an increased transport of oxygen and absorbed nutrients. It was observed that, there was no significant difference in values of RBC obtained from animals fed 15% CP and 20% CP.

Conclusion

Feeding rams diets containing 15 % CP (cotton seed cake and palm kernel cake) gave better results as regards Packed Cell Volume, Red Blood Cell Counts, than feeding rams diets containing 10% and 20% C.P. Graded levels of dietary protein had no significant effect on total protein, white blood cell counts.

Conflict of interest

The authors declare that there is no conflict of interest.

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