Nigerian Journal of Scientific Research (NJSR)
ISSN-0794-0378

EFFECT OF DIFFERENT PROCESSING METHODS OF *HIBISCUS SABDARIFFA* L. SEEDS ON GROWTH PERFORMANCE AND BLOOD PROFILE OF YANKASA RAM LAMBS

IBRAHIM, T.A.[1]*, ABDU, S.B.[1], ADAMU, H.Y.[1], LAWAL M.[2] AND BABAYO, J.[1]
[1]Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria, [2]Agricultural Education Department, Federal College of Education, Katsina, Nigeria

ABSTRACT

The need to evaluate the nutritional value of different processing methods and higher inclusion levels of *Hibiscus sabdariffa L.* seeds and sprouts in diets of small ruminants necessitated this study to investigate the performance of Yankasa ram lambs fed raw and differently processed *H. sabdariffa* seeds in maize offal-based diets. Three processing methods; soaking in water (WS), sprouting (SP) and grinding (GR) at 25% dietary inclusions and 15% raw *H. sabdariiffa* seeds (RHS) and a control diet, i.e. no hibiscus seeds (NHS) were adopted in a 5×5 Latin square arrangement, with five replicates per treatment using Yankasa ram lambs of 17-24 kg. The data collected were subjected to analysis of variance using general linear model procedure of SAS and treatment means were compared using Duncan Multiple Range test. The 25% dietary inclusion of water soak (WSHS), 25% ground (GRHS), and 15% RHS and the NHS diets were statistically similar and significantly higher ($P<0.05$) for weight gain, lower feed conversion ratio (FCR) and feed cost/kg gain than 25% inclusion of SPHS while the 25% dietary inclusion of WSHS had the least feed cost/kg gain among the treatment groups. The dietary inclusion of WSHS had higher ($P<0.05$) digestibility coefficients for dry matter, crude protein, crude fibre and nitrogen free extract among treatment group while the 25% inclusion of SPHS recorded least digestibility values except for crude ash content. The blood indices obtained in this study were all within the normal range values, though inclusions of RHS and all processed *H. sabdarriffa* seeds increased ($P<0.05$) the haemoglobin, white blood cells and neutrophil of Yankasa ram lambs compare to the control NHS diet. Also, the dietary inclusion of 25% WSHS had higher ($P<0.05$) total protein, globulin and cholesterol than the control diet. It could be concluded that dietary inclusions of WSHS and GRHS were more economical and could potentially be more adoptable compared to the SPHS, although inclusion of WS seeds gave a better feed cost/kg gain and nutrient digestibility. The inclusion of SPHS imposed a negative effect on the performance of Yankasa ram lambs. Therefore, there is need for further studies to evaluate the performance of small ruminants with higher dietary inclusions of WSHS and GRHS.

Keywords: Roselle seed, processing, performance, small ruminant
***Correspondence:** taibrahim@abu.edu.ng, 08065313133

INTRODUCTION

The problem associated with animal protein shortages are a perennial one which may be partly due to the excess demand over supplies in Nigeria [1, 2]. The population of sheep in Nigeria was reported to be 50.3 million [3]. This population if well managed and fed will help in providing animal protein to meet up with the increasing demand [4] and [5]. Sheep are generally known to have high production efficiency and during periods of unpredictable food shortage, they have proven to be very useful to human beings in the supply of meat and milk products [6, 7]. In addition to its nutritional importance to the teeming population, sheep and goats constitute a good source of family income and livelihood, assets and agricultural resources for smallholder farmers [7, 8, 9].

Feed is one of the major factors that influence effective sheep production in livestock industries and according to Ibrahim *et al.* [10], feed intake is a factor that greatly influences animal's growth, nutrient utilization and health. Therefore, shortage of feed for the production of the increasing sheep population has warranted research into alternative feed resource. Efforts to reduce the high cost of feeds and by extension the cost of small ruminant production in Nigeria have been concentrated on using feed formulated from available and cheap alternative feedstuffs of no or less nutritional value in human diets [11, 12, 2] especially those of high protein content. Some of the alternative feed ingredients that could easily be obtained are various

plant seeds with different crude protein content such as *Amaranthus spp* (14.5%CP), *Pisum sativum* (20.8%CP), *Acacia colei* (23.4%) and *Hibiscus sabdariffa* L. (33.5%CP) as reported by Ismail *et al*. [13].

The *H. sabdariffa* seeds known as "roselle seeds" in English are cheap alternative protein feedstuff readily grown in the north eastern part of Nigeria. The seeds had been used in feed formulation at different inclusion levels of different processing methods to enhance the performance of growing Red Sokoto bucks [2, 14]. The seeds have a moderate to high content of crude protein ranging from 21-39%, NFE from 21.4-40%, fat from 6-19% and crude fibre from 12-22% [15, 16].

The use of *H. sabdariffa* seeds as protein source in livestock diet is hampered by its anti-nutritional content [14] which has negative effects on nutrient utilization and enzyme catalyzed reactions [17] and hence must be processed for effective anti-nutrient destruction. Different processing methods have been reported to reduce the toxic factors in feeds. Earlier reports observed that processing methods such as soaking, cooking or sprouting significantly improved the nutritional and functional properties of plant seeds such as bambara groundnut [18] and *H. sabdariffa* seeds [15, 16].

The use of inclusion levels of crushed, water soaked and lime-soaked *H. sabdariffa* seeds up to 20% in the diets of small ruminant with improved growth performance [19, 20, 2], nutrient utilization and good hematopoietic effects on blood [14] had been documented. There were also suggestions that higher inclusion levels and other processing methods could be further investigated to validate the potential of *H. sabdariffa* seed meal as ruminant's feed ingredient. However, from our previous study [2], while the 10% inclusion level of raw *H. sabdariffa* seeds in rice offal-based diets was comparable in performance to the 20% processed seeds, the 20% inclusion level of raw seeds meal recorded a negative effect on performance of Red Sokoto bucks. Therefore, there is need to further investigate higher inclusion levels and other processing methods of *H. sabdariffa* seed meals on the growth performance, nutrient digestibility and blood profile of Yankasa ram lambs.

MATERIALS AND METHODS

Description of study site

The study was carried out at the small ruminant unit of the Department of Animal Science, Teaching and Research Farm, Ahmadu Bello University, Samaru, Zaria. Zaria is within the northern Guinea savannah zone of Nigeria, on latitude 11^0 16.5759' N and longitude 7^0 63.2304' E at an altitude of 610m above sea level. The climate is relatively dry with a mean annual rainfall of 1100-1200mm per annum, occurring from the months of April and mid-October. The mean temperature fluctuates from 40.2^{o}C at dry season to 9.2^{o}C at wet season [21].

Collection and sorting of *H. sabdariffa* seeds

The seeds of red variety of Hibiscus *H. sabdariffa* were purchased from an open market in Lere Local Government of Kaduna state, Nigeria. The seeds were carefully sorted out and impurities were removed before further processing.

Methods of processing *H. sabdariffa* seeds

The sprouting and soaking of *H. sabdariffa* seeds were carried out following laid down procedures [14, 16]. For the sprouting method, the *H. sabdariffa* seeds were first soaked in clean water (1 kg seed to 5 L of water) inside plastic buckets for 8hrs. Sprouting was carried out by spreading each of the soaked seeds on porous metallic trays for 6 days of germination. The seeds were kept wet throughout sprouting period by spraying with clean water (at 1 L to 2 kg seeds) twice daily at 8:00am and 4:00pm. The sprouts were sun-dried for two days after which it was stored in jute bags until required for laboratory analysis and diet formulation. For the soaking method, every 1 kg of *H. sabdariffa* seeds was soaked in 3 L of clean water for four days into clean plastic buckets so as to completely submerge the seeds. A water changing pattern of 24hrs interval was carried out for four days. The seeds were then removed, rinsed and sun-dried on metallic trays for three days and then stored in jute bags until required for laboratory analysis and feed formulation. The raw seeds of *H. sabdariffa* were milled into smaller particles using a sieve size of 1.0mm, after which they were packaged into a jute bag.

Chemical analysis

The experimental diet, feacal samples, raw and processed samples of *H. sabdariffa* seeds were analysed at the Biochemical laboratory, Department of Animal Science, Ahmadu Bello University, Zaria Nigeria, using the methods described by Association of Official Analytical Chemist [22]. The dry matter samples of the diet, faeces and *H. sabdariffa* seeds were analysed by oven-drying for 48 h at 60^{o}C. The nitrogen was evaluated using the micro Kjeldahl method with Tecator Product apparatus (Kjeltec TM2100) and the crude protein was further determined by multiplying estimated nitrogen by 6.25. The crude ash content for each sample was determined by combustion of the dry material in a muffle furnace at 600^{o}C for 8 h. The ether extract was measured using Soxhlet extraction procedure with electromantle ME. The crude fibre determination was done using Tecator

Line (FT 122 FibertecTM) as described by Van Soest and Wine [23]. The nitrogen free extract was obtained by subtracting the crude protein, oil, ash and crude fibre from 100. The concentration of total tannins was determined calorimetrically according to AOAC [22], in which tannic acid was used as a reference standard. The saponin concentration was determined using the modified method of Yoko *et al*. [24] while phytic acid was determined using spectrophotometer after addition of sulphuric acid, molybdate reagent into a micro Kjeldhal flask. Further digestion took place until the solution was clear or coluorless, the reading at this point was used to determine the phytic acid concentration of the sample. The content of metabolisable energy (ME) in each diet was estimated using the equation of Pauzenga [25]. ME (Kcal/kg DM) = 37 × %CP + 81.8 × % EE + 35 × % NFE.

Experimental diets

The experimental diets were formulated to consist of maize offal, rice offal, cotton seed cake, cowpea shell, bone meal, salt and the differently processed and raw seeds of *H. sabdariffa*. The diets are control diet with no *H. sabdariffa* seeds (NOHS), raw *H. sabdariffa* seeds (RHSS) included at 15% dietary inclusion, water soaked *H. sabdariffa* seeds (WSHS) included at 25% dietary inclusion, sprouted *H. sabdariffa* seeds (SPHS) included at 25% dietary inclusion, ground *H. sabdariffa* seeds (GRHS) included at 25% dietary inclusion.

Experimental animals, design and management

The experimental animals were purchased from an open market in Lamba, Sabon Gari Local Government, Kaduna state, Nigeria. Five growing Yankasa ram lambs of 10 – 14 months old weighing 17 – 24kg were used for the study. At their arrival, the experimental animals were housed in a pen and were treated against endo and ecto-parasites using Amitix, oxytetracyclin and Albendazole® according to the manufacturer's recommendation. The rams were given experimental diets for a fourteen-day adjustment period before the commencement of the experiment. At the commencement of each phase of the growth trial, the animal's body weights were taken using a foldable hangable weighing scale (B03E). The animals were housed in the experimental pens in a 5 by 5 Latin square arrangement. The animals were fed 3% of their body weight at 7:00am in a single dose daily. The five dietary treatments were rotated to the five animals as designed in the experiment, constituting five replicates per treatment group. Left over of the feeds from the previous day were recorded and subtracted from the total feed offered to each animal to obtain the total feed intake used in calculating growth and digestibility indices. Faecal weight was also recorded on daily bases for the period of the experiment, subsampled and later bulked for analysis. Faecal samples were collected using metabolic faecal bags. The digestibility coefficient (*d*) of nutrients was calculated as follows: *d* = Nutrient intake (g/d) – Faecal nutrient excretion (g/d)/ Nutrient intake (g/d) [14]. A 3 L of clean drinking water was also provided on daily basis in a 5 L plastic container for each animal. The final weight gain of the animals was also taken using a foldable hangable weighing scale (B03E) at the end of each phase (14 days) of the trial and their rations were adjusted according to the new live weight changes for the subsequent phases.

Blood sampling for hematological and serum biochemistry analyses

5mls of blood samples was taken from jugular vein using hypodermic needle by 8:00 am at every end of 14 days collection period into two types of test tubes: plain test tubes for determination of serum biochemical parameters and EDTA test tubes for determination of heamatological parameters. The samples were taken to the haematology laboratory at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria Nigeria, with immediate effect for preservation and further analysis. All the haematological parameters obtained in this study were determined using the haematocrit auto analyzer (MSLXC03). The biochemical parameters in this study were analysed using 23AUV-Spectrophotometer.

Statistical analysis

All data collected were subjected to one-way analyses of variance (ANOVA) using general linear models (GLM) procedure of SAS version 9.13 [26]. $Y_{ij(k)} = \mu + T_i + R_i + C_j + e_{ij(k)}$; where $Y_{ij(k)}$ is the observation of i^{th} row and j^{th} column receiving the k^{th} treatment, μ is the overall mean, T_i is the effect of the i^{th} treatment, R is the effect of i^{th} row, C is the effect of the j^{th} column and $e_{ij(k)}$ is the residual error. The effect of treatment was tested and significant difference between treatment means was compared using Duncan multiple range test [34].

RESULTS AND DISCUSSION

Chemical composition of raw and processed *H. sabdariffa* seeds

The result of Table 1 shows the chemical composition of raw and processed *H. sabdariffa* seeds. The dry matter content in processed *H. sabdariffa* seeds differ numerically but within the range of 99.24 – 99.99%. The crude protein content obtained in this study is within a range of 19.38-21.5%, crude fibres range from 29.05 – 35.13%. The ether extract is within the range of 9.57 - 11.15% while ash and nitrogen free extract

were at the ranges of 3.59 – 4.42% and 27.73 - 34.87%, respectively. The crude fibre and ether extract were lower for SPHS while the ash content was lower for water soaked compared to the GRHS. On a contrary, there was an increase in the nitrogen free extract in the WSHS and SPHS compared to the ground seeds. The saponin, phytate and tannin contents of raw and processed *H. sabdariffa* seeds range from 1.82% - 2.93%, 0.16% - 0.19%, and 2.45% - 3.20%, respectively with the WSHS having the least value.

The crude protein value of 19.38 – 21.50% in this study was slightly lower than the value of 23.79% reported by Abdu *et al.* [15], 21.79% - 23.20% obtained by Ari *et al.* [27], 25.18% obtained by Ibrahim *et al.* [14]. These variations may be due to the varietal differences, soil and agronomic practices. The ether extract obtained in this study (9.57 - 11.15%) falls within the range (6 – 19%) reported by Abdu *et al*. [15], but higher than the range 4.1 – 4.6% reported by Ibrahim *et al*. [14]. The phytochemical analysis of *H. sabdariffa* seeds revealed that both water soaking and sprouting method of processing reduced saponin, tannin, and phytate content compared to ground seeds. Leaching out effects and changes in chemical activity could be responsible for tannin reduction in WSHS as reported by Ibrahim *et al.* [2]. The reduction in tannins accompanied by decrease in crude fibre and ash of both WSHS and SPHS in this study agrees with the report of Abu El Gasim *et al*. [28] who reported that the reduction of phenols like tannins by soaking, cooking or sprouting were accompanied by changes in nutritional profile of processed seeds. Yagoub and Abdalla [18] revealed in their study that processing methods such as soaking, cooking or sprouting significantly improved the nutritional and functional properties of plant seeds which was in tandem with other works on *H. sabdariffa* seeds [15,14, 16].

Table 1: Chemical composition of raw and processed *H. sabdariffa* seeds

Parameters (%)	WSHS	SPHS	GRHS
Dry matter	99.24	99.99	99.56
Crude protein	19.38	21.50	20.13
Crude fibre	32.20	29.05	35.13
Ether extract	11.01	9.57	11.15
Ash	3.59	4.00	5.42
Nitrogen free extract	33.06	34.87	27.73
Saponin	1.82	2.68	2.93
Phytate	0.16	0.16	0.19
Tannin	2.45	2.60	3.20

WSHS: Water soaked hibiscus seeds, SPHS: Sprout hibiscus seeds, GRHS: Ground hibiscus seeds

Percentage ingredient and proximate compositions of experimental diets

Table 2 shows the percent ingredient and proximate compositions of experimental diets. Cowpea shell, rice offal, bone meal, and common salt constitute the fixed ingredients and the cotton seed cake and maize offal were varied in the dietary formulations to ensure iso-nitrogenous and iso-caloric diets. From the proximate compositions, the crude protein content ranged from 14.18% in GRHS meal diet to 16.02% in SPHS. On a contrary, the nitrogen free extract values ranged from 58.54% in SPHS diets to 61.52% in GRHS meal diet.

Table 2: Percentage ingredient and proximate compositions of experimental diets

Ingredients (%)	NHS	15%RHS	25%GRHS	25%WSHS	25%SPHS
***H. sabdariffa* seeds**	0.00	15.00	25.00	25.00	25.00
Cotton seed cake	29.00	20.00	13.00	14.00	12.00
Cowpea shell	17.00	17.00	17.00	17.00	17.00
Maize offal	36.00	30.00	27.00	26.00	28.00
Rice offal	15.00	15.00	15.00	15.00	15.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Common salt	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition (%)					
Dry matter	86.42	87.36	88.32	88.30	88.34
Crude protein	15.65	15.04	14.18	14.59	16.02
Crude fibre	17.51	17.09	15.96	16.06	16.41
Ether extract	1.01	1.20	0.98	0.87	1.06

Ash	6.17	6.52	7.36	5.97	7.97
NFE	60.27	60.15	61.52	62.51	58.54
ME (kcal/kg DM)	2771	2759	2758	2798	2725

NHS: Control, RHS: Raw hibiscus seeds, GRHS: Ground hibiscus seeds, WSHS: Water soaked hibiscus seeds, SPHS: Sprout hibiscus seeds, ME: Metabolizable energy.

Growth performance and feed intake of Yankasa ram lambs fed dietary inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet

Table 3 shows the growth performance of Yankasa ram lambs fed inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet. There were significant differences (P<0.05) in total weight gain, average daily weight gain, feed conversion ratio (FCR) and feed cost in ₦/kg gain. The diets containing 25% SPHS had lower (P<0.05) weight gain but higher feed conversion ratio (FCR) and feed cost per weight gain than the control (NHS) diet, 25% WSHS, 25% GRHS, and 15% RHSS dietary inclusions of *H. sabdariffa* seeds. However, there was no significant difference (P>0.05) in feed intake among the dietary treatments.

The dietary inclusions of 25% WSHS, 25% GRHS, and 15% RHSS compete favourably with the NHS diet for weight gain of Yankasa ram lambs at reduced feed costs per kg gains, while the diet containing SPHS decreased the weight of Yankasa ram lambs with poor FCR and at higher feed cost per kg gain. The poor weight gain and feed utilization observed in the dietary inclusion of SPHS may not be unconnected with the poor digestibility coefficient (Table 4) associated with the SPHS dietary treatment. The non-difference in the feed intake noticed in this study shows that the processing methods adopted for *H. sabdariffa* seed meals probably had no influence on the palatability of the diets. The lamb fed diet containing WSHS had the least FCR which could be responsible for better feed cost ₦/Kg gain of the diet. This implies that diets WSHS was more economical for production purposes. The diet containing SPHS is uneconomical due to poor FCR (10.57) and this may be attributed to the poor quality of the feed in which the animal had to consume more to gain live body weight similar to other treatments. The economic justification of diet containing WSHS could probably be attributed to increased nutritional values and better utilization as a result of decrease in antinutrients of the WSHS, especially with the lower saponin content of the processed seeds (Table 1). Although, Ibrahim *et al.* [14] reported improved weight gain, FCR and nutrients utilization for Red Sokoto bucks fed up to 20% dietary inclusion levels of WSHS, the present study recorded similar successes with higher inclusion level. The performance of the diet containing GRHS which was comparable to the diet containing WSHS could be attributed to increase in surface area for enzymatic reaction which enhanced feed utilization and carcass characteristics as earlier reported by Elamin *et al.* [20] and Ari *et al*. [27].

Table 3: Growth performance and feed intake of Yankasa ram lambs fed inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet

	Dietary treatments of raw and processed HS (%)						
Parameters	**NHS**	**15%RHSS**	**25%GRHS**	**25%WSHS**	**25%SPHS**	**SEM**	**P value**
Initial weight (kg)	21.40	21.34	21.80	22.04	22.04	1.63	0.96
Final weight (kg)	22.54	22.68	23.18	23.54	22.72	1.71	0.09
Total weight gain (kg)	1.14^{a}	1.34^{a}	1.40^{a}	1.48^{a}	0.78^{b}	0.21	0.02
ADWG (g/day)	81.00^{a}	95.60^{a}	101.20^{a}	105.40^{a}	48.20^{b}	16.00	0.04
Tot feed intake (kg)	8.60	8.60	8.64	8.91	8.28	0.89	0.99
ADFI (kg/head/day)	0.61	0.61	0.61	0.63	0.59	0.063	0.94
FCR	8.01^{b}	7.14^{b}	6.50^{b}	6.38^{b}	10.57^{a}	1.19	0.03
Feed cost (₦/Kg gain)	700.10^{b}	547.20^{b}	495.80^{b}	484.20^{b}	953.91^{a}	108.82	0.02

ab = Means with different superscript shows significant difference (P<0.05), SEM = Standard Error Mean, NHS: Control, RHS: Raw hibiscus seeds, GRHS: Ground hibiscus seeds, WSHS: Water soaked hibiscus seeds, SPHS: Sprout hibiscus seeds, ADWG: Average daily weight gain, ADFI: Average daily feed intake. FCR: Feed conversion ratio

Nutrient digestibility of Yankasa ram lambs fed inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet

The nutrient digestibility of Yankasa ram lambs fed with raw and processed *H. sabdariffa* seeds in maize offal-based diet are presented in Table 4. The dietary

inclusion of 25% WSHS had higher (P<0.05) digestibility coefficient for dry matter, crude protein, crude fibre and nitrogen free extract than the NHS diet, 25% SPHS, and 15% RHS, while the dietary treatment of the SPHS recorded the least values for digestibility coefficient. There was no difference (P>0.05) in ether extract digestibility among the lambs fed dietary *H. sabdariffa* seed meals. The ash digestibility of the dietary inclusion of 25% WSHS and 25% GRHS were higher (P<0.05) than the diet containing 25% *H. sabdariffa* sprouts and the control diet, but similar (P>0.05) to the dietary inclusion of 15% RHSS.

In this study, the better digestibility coefficient observed in the diet containing WSHS could be partly attributed to the reduction in antinutrients like saponin in the WSHS (Table 1). The improved nutrient digestibility observed in this study is also in consonant with the previous report of Ibrahim *et al*. [14] who fed water soak *H. sabdariffa* seeds up to 20% dietary inclusion to Red Sokoto bucks. The low coefficient of digestibility observed in the diet containing 25% *H. sabdariffa* sprouts could be attributed to the poor utilisation of the feed.

Table 4: Nutrient digestibility of Yankasa ram lambs fed inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet

Parameters (%)	Dietary treatments of raw and processed HS (%)					SEM	P value
	NHS	15%RHS	25%GRHS	25%WSHS	25%SPHS		
Dry matter	66.64^{b}	66.31^{b}	71.46^{b}	78.77^{a}	59.03^{c}	3.20	0.0001
Crude protein	77.08^{b}	76.28^{b}	80.16^{ab}	84.52^{a}	64.43^{c}	2.43	0.0003
Crude fibre	62.02^{b}	60.38^{b}	63.21^{b}	72.45^{a}	51.89^{c}	4.52	0.0023
Ether extract	78.94^{a}	78.19^{a}	78.42^{a}	79.10^{a}	70.23^{b}	3.72	0.0036
Ash	54.80^{b}	62.90^{ab}	75.71^{a}	78.85^{a}	56.93^{b}	7.54	0.0340
Nitrogen free extract	68.33^{b}	68.28^{b}	72.92^{b}	79.30^{a}	51.95^{c}	3.11	0.0001

abc = Means with different superscript shows significant difference (P<0.05), SEM = Standard Error Mean, NHS: Control, RHS: Raw hibiscus seeds, GRHS: Ground hibiscus seeds, WSHS: Water soaked hibiscus seeds, SPHS: Sprout hibiscus seeds.

Haematology of Yankasa ram lambs fed inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet

Table 5 shows haematology of Yankasa ram lambs fed dietary inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet. The dietary inclusion of 25% WSHS had significantly higher (P<0.05) pack cell volume (PCV) and lymphocyte than the 25% SPHS, 25% GRHS, 15% RHS and the control NHS diet. The dietary inclusion of 25% WSHS and 25% GRHS were statistically similar for haemoglobin and significantly higher (P<0.05) than diet containing 25% SPHS and 15% RHSS, while the control diet recorded the least significant value for haemoglobin. There was no significance difference (P>0.05) observed in red blood cells, monocyte and eosinophil among the treatment lambs. The white blood cells (RBC) and neutrophil were significantly lower (P<0.05) for the control diet compared to the dietary inclusions of both RHSS and processed *H. sabdariifa* seeds.

All the haematological parameters obtained in this study are within the range of the hematological values for sheep as reported by Research Animal Resources [29]. The increase in hemoglobin and white blood cell could be attributed to the haematopoiesis in *H. sabdariffa* seeds, an effect which was earlier noted by Ibrahim *et al*. [14] who reported increase in PCV, RBC, Haemoglobin and white blood cells of Red Sokoto bucks when fed *H. sabdariffa* seeds diets up to 20% inclusion levels. The haematopoietic effect of *H. sabdariffa* may be attributable to the presence of appreciable amount of iron minerals [14] and also iron minerals and vitamins [30] in the plant materials. In this study, Haemoglobin level of the Yankasa ram lambs fed the control diet containing 29% inclusion of cotton seed cake fell below the reference value for sheep, although the lambs were physically healthy. From literature, feeding cotton seed products beyond 30% has causes haematological alteration where the gossypol in cotton seeds possibly bind the iron thereby leading to aneamia in ruminants [35] while 10% dietary inclusion of cotton seed cake affected the reproductive performance in rabbits [36].

Table 5: Haematology of Yankasa ram lambs fed inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet

Parameters	NHS	15%RHS	25%GRHS	25%WSHS	25%SPHS	SEM	P value	RV
Pack cell vol. (%)	31.40^{b}	30.20^{b}	31.60^{b}	34.40^{a}	31.60^{b}	1.32	0.023	24 – 45

Haemoglobin (g/dL)	5.92^{c}	7.12^{b}	8.74^{a}	8.74^{a}	7.26^{b}	0.55	0.008	8 – 16
Red blood cell (10^6/uL)	6.16	6.35	6.16	6.83	6.06	0.42	0.710	6 – 15
WBC (x10^9/l)	5.06^{b}	9.34^{a}	7.54^{a}	8.06^{a}	7.84^{a}	0.96	0.003	4 – 12
Neutrophil (%)	52.60^{b}	67.80^{a}	67.80^{a}	66.0^{a}	60.6^{a}	3.39	0.001	30 – 70
Lymphocyte (%)	45.60^{bc}	48.40^{bc}	50.80^{b}	62.40^{a}	41.40^{c}	4.09	0.001	40 – 70
Monocyte (%)	0.40	0.80	0.60	0.70	0.60	0.31	0.311	0– 6
Eosinophil (%)	1.00	1.00	1.40	1.60	1.00	0.38	0.697	0 – 10

abc = Means with different superscript shows significant difference (P<0.05), SEM = Standard Error Mean, NHS: Control, RHS: Raw hibiscus seeds, GRHS: Ground hibiscus seeds, WSHS: Water soaked hibiscus seeds, SPHS: Sprout hibiscus seeds, WBC: White Blood Cells. RV: Reference values**.**

Serum biochemistry of Yankasa ram lambs fed inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet

Table 6 shows the Serum biochemistry of Yankasa ram lambs fed inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet. The total protein of the diet containing 25% WSHS was higher (P<0.05) than the control diet but statistically similar (P>0.05) to the diet containing 15% RHS, 25% SPHS, and 25% GRHS. The albumin in the diet containing 25% SPHS was significantly higher (P<0.05) than the control diet and the diet containing 15% raw *H. sabdariffa* seeds but similar (P>0.05) to the diet containing 25% GRHS and 25% WSHS. The globulin and cholesterol contents for dietary inclusion of 25% WSHS were higher (P<0.05) than the diet containing 15% RHS and the control diet but similar (P>0.05) to the diet containing 25% GRHS and 25% SPHS. The blood urea in the diet containing 25% GRHS was significantly higher (P<0.05) than the control diet and the diet containing 25% SPHS but similar (P>0.05) to the diet containing 25% WSHS and 15% RHS.

The dietary inclusion of *H. sabdariffa* seeds in this study is indicative that the seeds have the potential to increase the serum biochemical indices of animals, in addition to their haematopoietic effect. The higher albumin content in the blood of the lambs fed diet containing sprout, water soak and ground *H. sabdariffa* seeds could be attributed to the response of the animal to the antioxidative potency of *H. sabdariffa* as reported by Olushola *et al*. [31] who found the antioxidative potency of *H. sabdariffa* calyx in rabbit resulted to gradual increase in the concentration of albumin and globulin. The range values for total protein, albumin, globulin, cholesterol and blood urea obtained in this study were similar to the ones reported by Garba and Abubakar [32] and within the normal serum biochemistry values of rams reported by Al-Hadithy and Badawi [33] on Awassi sheep. It was also noted that total protein and globulin levels of all the Yankasa ram lambs examined in this study fell below the reference values for sheep while the cholesterol exceeded the reference values. The alterations in these biochemical indices could partly be attributed to the diets. Prolong feeding with cotton seed products has been reported to impose toxicity on ruminants and causing alteration in blood parameters [35] while in another report, dietary inclusion of cotton seed meals below 20% increased serum biochemical indices, improve growth and reproductive performance in ruminants especially cotton seed meals of higher protein [37].

Table 6: Serum biochemistry of Yankasa ram lambs fed inclusions of raw and processed *H. sabdariffa* seeds in maize offal-based diet

Parameters	**Dietary treatments of raw and processed HS (%)**					**SEM**	**P value**	**RV**
	NHS	**15%RHS**	**25%GRHS**	**25%WSHS**	**25%SPHS**			
Total protein (g/dL)	5.24^{b}	5.42^{ab}	5.60^{ab}	5.84^{a}	5.53^{ab}	0.15	0.04	6-8
Albumin (g/dL)	2.66^{b}	2.68^{b}	2.80^{ab}	2.80^{ab}	2.92^{a}	0.08	0.03	2.4-3
Globulin (g/dL)	2.65^{b}	2.64^{b}	2.81^{ab}	2.91^{a}	2.83^{ab}	0.08	0.03	3.5-5.7
Cholesterol (mg/dL)	79.55^{b}	81.53^{b}	85.94^{ab}	90.97^{a}	86.78^{ab}	3.41	0.01	52-79
Blood urea (mg/dL)	5.75^{b}	5.99^{ab}	6.21^{a}	5.95^{ab}	5.76^{b}	0.19	0.04	3.4-12

abc = Means with different superscript shows significant difference (P<0.05), SEM = Standard Error Mean, NHS: Control, RHS: Raw hibiscus seeds, GRHS: Ground hibiscus seeds, WSHS: Water soaked hibiscus seeds, SPHS: Sprout hibiscus seeds, RV: Reference values**.**

CONCLUSIONS

1. The WSHS and SPHS methods of processing in this study increased nitrogen free extracts but reduced the ash, saponin, phytate, and tannin contents of *H. sabdariffa* seeds compared to the GRHS.
2. The dietary inclusions of 15% RHS, 25% GRHS, and 25% WSHS had similar growth performance, feed utilization but at cheaper feed cost per kg gain when compared to the control diet in Yankasa ram lambs, with dietary inclusion of 25% WSHS being most cost effective in terms feed cost in ₦/Kg gain. On a contrary, dietary inclusion of 25% SPHS decreased growth performance, feed utilization and increased the feed cost per kg gain of the Yankasa ram lambs fed maize offal-based diet compared to the control diet.
3. The Yankasa ram lambs on dietary inclusions of 25% WSHS had higher dry matter, crude fibre, and nitrogen free extract digestibility than the 15% RHS, 25% GRHS, and the control diet while the inclusion of 25% SPHS had the poorest coefficient of digestibility among the treatment lambs.
4. Although, most of the blood indices were within the range of the reference values, the Although, most of the blood indices were within the range of the reference values, the inclusions of 25% WSHS in the diet of Yankasa ram lambs fed maize offal based diet increased PCV, total protein, globulin and cholesterol when compared to the control diet.

RECOMMENDATIONS

1. The water soak method of processing *H. sabdariffa* seeds could be recommended as better alternative method of processing for improved nutritional quality, lower processing cost and ease of practice by the farmer.
2. For better performance of Yankasa ram lambs, dietary inclusion of 25% WSHS in maize offal-based diet is therefore recommended for improved digestibility, feed utilization, and lower feed cost per kg gain.
3. Further studies should be carried out on higher inclusion levels of WSHS, so as to determine its optimum inclusion level for small ruminants and also to evaluate its haematopoietic effect.

ACKNOLEDGMENTS

Although, this research was self-funded, the authors sincerely acknowledge the efforts and contributions of the farm technicians who participated actively during the data collections.

REFERENCES

1. AKINMUTIMI, A.H. & ONWUKWE, C.C. (2002). Effect of cooking with various concentrated potash on nutrient composition of lima beans. *Journal of Agricultural Biotechnology and Environment*, **1**: 1-3.
2. IBRAHIM, T.A, ABDU, S.B., HASSAN, M.R., YASHIM, S.M. & ADAMU, H.Y (2016). Growth performance of Red Sokoto bucks fed inclusion level of raw and soaked roselle (*Hibiscus sabdariffa.* L) seeds in rice offal-based diet. *Nigerian Journal of Animal Science*, **18**(2): 434-443.
3. DORIS, D.S. (2024). Number of sheep in Nigeria from 2010 to 2022. https://www.statista.com/1297940/stock-of-sheep-in-nigeria/ Retrieved August 8, 2024.
4. MANDAL, A., PRASAD, H., KUMAR, A., ROY, R. & SHARMA, N. (2007). Factors associated with lamb mortalities in Muzaffarnagari sheep. *Small Ruminant Research*, **7**(1-3): 273-279.
5. MUHAMMAD, N., MAIGANDI, S.A., HASSAN, W.A. & DANEJI, A.I. (2008). Growth performance and economics of sheep production with varying levels of rice milling waste. *Sokoto Journal of Veterinary Sciences*, **7**(1): 59 – 64.
6. GATENBY, R.M. (2002). *Sheep. Trop. Agricultural series*. (Rene` Coste, ed). CTA, Macmillan, 33-34 Alfred Place, London WC1E 7DP. Pp 178.
7. IYAYI, E.A. & TONA, G.O. (2004). Management practices among small holders of sheep, goats and pigs in derived savannah zone in Oyo state, Nigeria. *Nigerian Journal of Animal Production*, **31**(1): 86-93.
8. SHITTU, A., CHAFE, U.M., BUHARI, S., JUNAIDU, A.U., MAGAJI, A.A., SALIHU, M.D. & JIBRIL, A. (2008). An overview of mastitis in Sokoto red goat, Nigeria. *Sokoto Journal of Veterinary Sciences*, **7**(1): 65-70.
9. SALEM-BEN, S. & SMITH, T. (2008). Feeding strategies to increase small ruminant production in dry environments. *Small Ruminant Research*, **77**(2-3): 174-194.
10. IBRAHIM, T.A., TURANG, S., JAMILU, M., ABDU, S.B., HASSAN, M.R., MUSA, A. & ALHABIB, I.K. (2022). Performance and Blood Profile of Red Sokoto Bucks Fed Different Replacement levels of Maize offal with Sun dried Mango (*Mangifera indica*) fruit waste meals. *Nigeria Journal of Animal Production*, **34**(1): 84-95.

11. ALIYU, I.D., MAIGANDI, S.A., MUHAMMAD, I.R. & GARBA, Y. (2012). Haematological Indices and Blood Urea Nitrogen of Yankasa Ram Lambs Fed Urea, Poultry Droppings and or Urea Treated *Pennisetum pedicellatum* (Kyasuwa Grass). *Nigerian Journal of Basic and Applied Science*, **20**(1): 39-43.
12. IBRAHIM, T.A. & YASHIM, S.M. (2014). Growth Response, Nutrient Digestibility and Haematological Parameters of Red Sokoto Bucks Fed Lime Treated Maize Cob Supplemented with Concentrate Diet. *Nigeria Journal of Animal Science*, **16**(2): 264-271.
13. ISMAIL, A., IKRAM, E.H.K & NAZRI, H.S.M. (2008). Roselle (*Hibiscus sabdariffa.* L) Seeds – Nutrition composition, protein quality and health benefits. *Food Global Science Book*.
14. IBRAHIM, T.A., ABDU, S.B., HASSAN, M.R., YASHIM, S.M., ADAMU, H.Y. & LAMIDI, O.S. (2018). Nutrient Utilization and Blood Chemistry of Red Sokoto Bucks Fed Inclusion levels of Raw and Soaked Roselle (*Hibiscus sabdariffa* L.) Seeds in Rice Offal Based Diets. *Journal of Agriculture and Rural Development in the Tropics and Sub-tropics*, **119**(1): 45-54.
15. ABDU, S.B., ADEGOKE, T.F., ABDULRASHID, M., DURU S., YASHIM S. M. & JOKTHAN G.E. (2008). Effect of cooking duration on chemical composition of Roselle (*Hibiscus sabdariffa*) seeds In: Bawa, G.S., Akpa G.N. Jokthan, G.E., Kabir M. & Abdu S.B. (Eds) 13th *Proc, of the Animal Science Association of Nigeria (ASAN)*, at A.B.U. Zaria 15th September 2008 Pg 521 – 523.
16. IBRAHIM, T.A., JAMILU, M., TURANG, S., ABDU, S. B., HASSAN, M.R., JINADU, L.A. & ISHIYAKU, Y. M. (2019). Effects of Cooking and Sprouting Duration on the Chemical Compositions and Dry Matter Losses of *Hibiscus sabdariffa L*. (Roselle) Seeds. *Nigeria Journal of Animal Science*, **21**(1): 135-142.
17. ALETOR, V. (2009). Cyanide in gari 1: Distribution of total, bound and free hydrocyanide acid in commercial gari, and the effect of fermentation time on residual cyanide content. *International Journal of Food Science and Nutrition*, **44**(4): 281-287. DOI:10.3109/09637489309017447.
18. YAGOUB, A.A. & ABDALLA, A.A. (2007). Effect of domestic processing methods on chemical, in vitro digestibility of protein and starch and functional properties of bambara groundnut (*Voandzeia subterranea*) seed. *Research Journal of Agriculture and Biological Sciences*, **3**: 24-34.
19. BESHIR, A.A. & BABIKER, S.A. (2009). Performance of Sudanese Desert Lambs Fed Graded Levels of *Hibiscuss sabdariffa* (*Hibiscus subdariffa*) Seeds Instead of Groundnut Cake. *Pakistan Journal of Nutrition,* **8**(9): 1442-1445.
20. ELAMIN, K.M., HASSAN, H.E., ABDALLA, H.O., ARABI, O.H. & TAMEEM ELDAR, A.A. (2012). Effect of Feeding Crushed *Hibiscuss sabdariffa* Seed (*Hibiscus sabdariffa* L.) (Karkadeh) on Carcass Characteristics of Sudan Desert Sheep. *Asian Journal of Animal Sciences*, **6**: 240-248.
21. I.A.R. (2021). Institute for Agricultural Research. Meteorological Data Information of Samaru and its Environs. Zaria: Ahmadu Bello University Zaria.
22. AOAC. (2005). Official methods of Analysis, 15th ed. Association of Official Analytical Chemistry, Washington, D.C.
23. VAN SOEST, P.J. & WINE, R.H. (1967). Use of Detergents in the Analysis of Fibrous Feed. IV. Determination of Plant Cell-Wall Constituents. *Journal of the Association of Official Analytical Chemists*, **50**: 50-55.
24. YOKO, U., KEIKO, H. KAZUO, S. & ICHIRO, K. (2000). Spectrometric Determination of Saponin in Yucca Extract Used as Food Additive. *Journal of AOAC International*, **83**(6): 1451-4. DOI:10.1093/jaoac/83.6.1451.
25. PAUZENGA, U. (1985). Feeding parent stock. *Zootecnica International*, 22-24.
26. SAS (2002). Statistical Analysis Institution Users Guide Version 9, edition SAS Institute Inc. Calorina, USA.
27. ARI, M.M., ALU, E.S., LONGTEP, B.D., ALUM, I., USMAN, A.H., KWASSAU, M.S. & ONU, J.P. (2015). Nutrient composition and phtyochemical screening of crushed, toasted and fermented roselle (*Hibiscus sabdariffa*) seeds. *Annual Research and Review in Biology*. **7**(1): 39-44.
28. ABU EL GASIM, MOHAMMED, A.Y., MOHAMMED, A. & ASMA, A.A. (2008). Effect of soaking, sprouting and cooking on chemical composition, bioavailability of minerals and in vitro protein digestibility of roselle (*Hibiscus sabdariffa* L.) seed. *Pakistan Journal of Nutrition,* **7**(1): 50-56.
29. RESEARCH ANIMAL RESOURCE (2009). Reference values for laboratory animals: Normal haematological values. RAR Websites, Research Animal Resources (RAR),

University of Minnesota. Available at: http://www.ahc.umn.edu/rar/refvalues.html.

30. MAHADEVAN, N., SHIVALI & KAMBOJ, P. (2009). *Hibiscus sabdariffa* Linn. An overview. *Natural Product Radiance*, **8**(1): 77–83.
31. OLUSOLA, A.O. (2011). Evaluation of the antioxidant effects of *Hibiscus Sabdariffa* Calyx Extracts on 2, 4-Dinitrophenylhydrazine-Induced Oxidative Damage in Rabbits. *Webmedcentral Biochemistry*, **2**(10), WMC002283.
32. GARBA, Y. & ABUBAKAR, A.S. (2012). Haematological response and blood chemistry of yankasa rams fed graded levels of *Tamarindus indica* (Tamarind) leaves. *Nigerian Journal of Basic and Applied Science*, **20**(1): 44-48.
33. AL-HADITH, H.A. & BADAWI, N.M. (2015). Determination of serum proteins and glucose concentrations in clinically normal and anemic Awassi sheep. *World's Veterinary Journal*. **5**(1): 01-06.
34. DUNCAN, D.B. (1955). Multiple Range and Multiple F-Test. Biometrics, 11, 1-5.
35. ITODO, J.I., REKWOT, P.I., ALUWONG, T.T., RWUAAN, J.S., ABAH, K.O., BABASHANI, M., UBAH, S.A., BUGAU, J.S., ATE, U.I. & KUJE, A.A. (2020). Effect of feeding graded levels of whole cottonseed on haemato-biochemical parameters of red Sokoto bucks. *Sokoto Journal of Veterinary Sciences*, **18**(2): 83-90.
36. DEEBA, F., QURESHI, A.S., ALI, L., KHAN, A.N. & USMAN, M. (2023). Cottonseed cake effect on Hematology, Biochemical and Histological Changes in Liver and Testes in Rabbits. *Concept of Dairy & Veterinary Sciences*, **5**(3): 2023.
37. CÂMARA, A.C., DO VALE, A.M., MATTOSO, C.R., MELO, M.M., & SOTO-BLANCO, B. (2016). Effects of gossypol from cottonseed cake on the blood profile in sheep. *Trop Animal Health Production*, **48**(5): 1037-42. doi: 10.1007/s11250-016-1039-0.