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Nutrient utilisation and blood chemistry of Red Sokoto bucks fed on diets with different inclusion levels of raw and soaked roselle (*Hibiscus sabdariffa* L.) seeds

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Abstract

This study evaluated nutrient utilisation and blood chemistry of Red Sokoto bucks fed a 10 and 20 % inclusion level of raw, water- and lime-soaked *Hibiscus sabdariffa* L. seeds in rice bran based diets. 21 Red Sokoto bucks aged 8–10 months and weighing 9–13 kg were randomly allotted into six treatments with three bucks each, while a seventh dietary treatment with zero inclusion of seeds served as control in a 2×3 factorial arrangement using a complete randomised design. The results indicated that increase in dietary inclusion levels of soaked *H. sabdariffa* seeds increased ($P < 0.05$) the nutrient utilisation of bucks as compared to the control, while a decrease was observed with increasing dietary inclusion levels of raw seeds. Dietary inclusion of both raw and water-soaked *H. sabdariffa* seeds increased ($P < 0.05$) the packed cell volume. Soaking also influenced the white blood cell value which increased with increasing inclusion levels of *H. sabdariffa* seeds. However, values of haemoglobin and red blood cells were only affected by 20 % inclusion of raw and water-soaked *H. sabdariffa* seeds ($P < 0.05$) compared to control. Inclusion of *H. sabdariffa* seeds furthermore reduced serum protein, albumin, globulin, glucose and urea levels compared to control. It is therefore concluded that *H. sabdariffa* seeds support haematopoiesis in Red Sokoto bucks. While both inclusion levels of water-soaked and 10 % raw *H. sabdariffa* seeds improved nutrient utilisation compared to control and 20 % inclusion of raw seeds, the 20 % inclusion of water-soaked *H. sabdariffa* seeds recorded the best nitrogen utilisation efficiency.

Keywords: *Hibiscus sabdariffa* seeds, soaking, Red Sokoto bucks, nutrient utilisation, blood chemistry, goats

1 Introduction

Nigeria hosts an estimated 19.5 million cattle, 41.3 million sheep and 72.5 million goats (National Agricultural Sample Survey, 2011). From this estimate, goats represent about 54.4 % of total ruminant livestock. The indigenous goat breeds in order of importance are Red Sokoto (50 %), West African Dwarf (45 %) and Sahel (5 %) (Ajala *et al.*, 2008). Goats contribute about 24 % of meat supply in Nigeria (Oni, 2002).

Goats, like other herbivores in the tropics and sub-tropics, experience marked seasonal fluctuations in feed supply which results in a seasonal pattern of wet season live weight gains and dry season live weight losses until animals reach marketable weight (Poppi & McLennan, 1995). This is due to the scarcity of good quality feed during the dry season. Feed intake is one of the important factors that influence animals' lifetime productivity, health and carcass characteristics (Bawa *et al.*, 2003). The increased demand and high cost of conventional animal feed ingredients like soybean or ground nut cake makes it necessary to search for alternative indigenous feed resources which are readily available and cheaper than the conventional feed ingredients (Sodeinde

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et al., 2007). The search for alternative feed resources has over the past decades rekindled research interest in the use of tropical browses, herbs and medicinal plants as nutrient sources for ruminants (Okoli *et al.*, 2002).

H. sabdariffa L. or roselle is one of these alternative feed resources that is cultivated on a wide range of tropical soil sand performs satisfactorily well on relatively infertile soils (Adanlawo & Ajibade, 2006). It is popularly called "Yakuwa" in Hausa and belongs to the family of Malvaceae; it is a popular vegetable in Indonesia, India, West Africa and many other tropical countries (Tindall, 1986; Babatunde, 2003). The vegetable is widely grown in the North-Eastern and middle belt regions of Nigeria (Akanya *et al.*, 1997).

H. sabdariffa is cultivated for its pleasant red colour calyx, used for making a local drink (*sobo*) and wine (Al-Wandawi *et al.*, 1984). Although abundant seeds are produced, they are highly underutilised. The seeds have been reported to have a high content of oil and protein. Al-Wandawi *et al.* (1984) reported that seeds contain 25.20 % crude protein (CP), while Abdu *et al.* (2008) reported a value of 23.46 % CP. However, the utilisation of *H. sabdariffa* seeds as an alternative feed source for ruminant livestock may be limited due to the presence of some anti-nutritional factors such as tannins, phytic acid, and trypsin inhibitor (Abu-Tarboush & Basher, 1996; Abdu *et al.*, 2008), as well as gossypol (Abu-Tarboush & Basher, 1996). Nutrient utilisation by ruminants is limited if the anti-nutrient concentration in the feed offered is above a certain threshold, despite the detoxifying activities of rumen microbes on many anti-nutrients (Kama, 2005; Isah *et al.*, 2011). Hence, there is a need for feed processing in order to improve the quality and utilisation of such plant materials by ruminants. Different processing methods have been carried out on roselle seeds with quite a number of successes recorded (Abdu *et al.*, 2008; Hambidge *et al.*, 2005; Ibrahim & Yashim, 2014) while the economic efficiency of feeding water-soaked *H. sabdariffa* seeds to Red Sokoto bucks has been reported (Ibrahim *et al.*, 2016).

According to Afolabi *et al.* (2011), changes in haematological parameters are often used to determine the physiological status of the body and to determine stresses due to environmental, nutritional and/or pathological factors. Haematological components are valuable in monitoring feed toxicity especially with feed constituents that affect blood formation (Oyawoye & Ogunkunle, 2004). It was reported that roselle calyces have a haematopoietic effect in farm animals (Mahadevan *et al.*, 2009; Olusola, 2011).

The objective of this study was to evaluate the growth performance and selected blood parameters of Red Sokoto bucks fed different inclusion levels of raw and soaked *H. sabdariffa* seeds in rice bran based diets, so as to establish baseline information on the nutrient utilisation of roselle seeds in bucks.

2 Materials and methods

2.1 Study site

The study was conducted at the Small Ruminant Unit of the Department of Animal Science, Teaching and Research Farm, Ahmadu Bello University, Zaria. Zaria is located in the northern Guinea Savanna Zone of Nigeria, on latitude 11°14′44″ N and longitude 7°38′65″ E, at an altitude of 610 m a.s.l. The climate is relatively dry with annual rainfall of 700–1400 mm occurring between the months of April and September (Ovimaps, 2014).

2.2 Sourcing and processing of Hibiscus sabdariffa seeds

The seeds of the red variety of *H. sabdariffa* were purchased on a public market in Yobe state during the harvest period (October / November); they were cleaned to remove impurities before processing. A first batch of 20 kg of *H. sabdariffa* seeds was soaked in 50-litre plastic containers containing a 6 % lime solution (0.06 g CaO per g of water) for 24 h. The seeds were removed, washed and sundried for 72 h. A second batch of 20 kg of *H. sabdariffa* seeds were completely submerged and soaked in 60 litres clean water and stored in a 100-litres plastic container. The water was drained every 24 h and again 60 litres of clean water were added. On the fourth day the seeds were removed, washed and sundried for 72 h. The dried seeds of both water and lime treatments were stored in airtight polythene containers until required for diet formulation.

A third batch of 20 kg cleaned but untreated *H. sabdariffa* seeds was also stored in an airtight polythene container for diet formulation.

2.3 Experimental setup

The raw and treated *H. sabdariffa* seeds were each included at a level of 10 and 20 % into a diet consisting of cotton seed cake, maize bran, rice bran, bone meal and common salt to obtain iso-nitrogenous and iso-caloric diets (Table 1). The diets were fed as a total mixed ration for a period of 8 weeks for the haematological study and an additional 7 days for the digestibility study, in a 2×3 factorial arrangement using a complete randomised design with three different diet types (i.e. raw, water- and lime-soaked roselle) and two inclusion levels (10 and 20 %).

The experimental animals were purchased from a public market in Anchau, Kaduna State. Twenty one growing Red

Sokoto bucks aged 8–10 months and weighing 9–13 kg were randomly allotted to 7 groups with 3 animals per group. The animals were treated against endo- and ecto-parasites using Albendazole® and acaricides respectively, according to the manufacturers' recommendation.

The animals were housed in individual metabolic crates for total faecal and urine collection. Prior to the experiment they were allowed 14 days to adjust to the metabolic crates and to the experimental diets before the commencement of the digestibility trial. The experimental diets were offered at 3 % of their live weight in a single meal at 8:00 a.m. daily. During the 7-day digestibility study, the daily faecal output was quantified (amount of fresh matter); 10 g of the homogenized fresh faeces were sub-sampled daily and oven-dried at 60 °C for 48 h for dry matter determination. After that, the daily samples of each animal were pooled for laboratory analysis. The daily urine output was collected into a plastic container placed under the metabolic crates and containing 100 ml of 0.1 NH_2SO_4 to prevent nitrogen loss by volatilisation. The collected urine was strained through a layer of glass wool to remove detached hair fragments and/or other solid contaminants. A 10 % aliquot of the total daily urine output of each animal was stored in a refrigerator at 4 °C for nitrogen determination (Osuji *et al.*, 1993). All the aliquots per animal were pooled after the experimental period for nitrogen assay.

2.4 Sample analysis

The proximate analysis of samples of the experimental diets, faeces, raw, water-soaked and lime-soaked *H. sabdariffa* seeds and nitrogen determination in urine was conducted according to standard methods (AOAC, 2005). The residual dry matter of the samples was determined by oven-drying at 60 °C for 48 h. Nitrogen was determined by the micro Kjeldahl method with Tecator Product apparatus (KjeltecTM2100), while crude protein was calculated by multiplying $N \times 6.25$. The Soxhlet extraction procedure was used for determination of crude fat (ether extract) using electromantle ME. The ash was measured by combustion of the dried material in a muffle furnace at 600 °C for 8 h. Crude fibre, sequential neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using Tecator Line (FT 122 FibertecTM) according to the method described by Van Soest & Wine (1967). The concentration of phytic acid was determined according to Wheeler & Ferrel (1971). A standard curve of ferric nitrate was plotted. Phytate phosphorus was calculated from the standard curve assuming a 4 : 6 Fe to P molar ratio. The concentration of total tannins present in raw and soaked roselle seeds was determined colorimetrically as described in AOAC (2005), whereby tannic acid was used as a reference standard. The total oxalates concentration was determined by calcium oxalate precipitation (titrimetric method) of Oke (1966); the method involved titration of acidic aqueous extracts of the sample with a standard solution of potassium permanganate. The analysis of minerals (calcium, phosphorus, potassium, iron and magnesium) was carried out using atomic absorption spectrophotometry in wet feed samples digested by concentrated nitric and pelrchloric acids, using PG instrument AA500 model. The content of metabolisable energy (ME) in each diet was determined using the equation of Pauzenga (1985).

$$\text{ME (Kcal / kg DM)} = 37 \times \%\text{CP} + 81.8 \times \%\text{EE} + 35 \times \%\text{NFE}$$

The digestibility coefficients (d) of nutrients were calculated as follows:

$$d = \frac{\text{Nutrient intake (g/d)} - \text{Faecal nutrient excretion (g/d)}}{\text{Nutrient intake (g/d)}}$$

The animals' nitrogen balance was calculated as follows:

$$\text{N-balance (g/d)} = \text{N-intake (g/d)} - \text{Faecal N-excretion (g/d)} - \text{Urine N-excretion (g/d)}$$

2.5 Haematological analysis

Blood samples of 20 ml volume were taken from the jugular vein of each animal using a hypodermic needle after eight weeks since the start of the feeding trial (and before the commencement of the digestibility trial) into two types of test tubes; 10 ml into sterile plain test tubes for serum preparation and the remnant 10 ml into Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulant bottles containing 0.5 ml EDTA for determination of haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs), white blood cells (WBCs), lymphocytes and neutrophils according to standard methods (Coles, 1986). The blood in EDTA anticoagulant bottles was immediately inserted in ice containers. RBCs count was done in a haemocytometer chamber with Natt and Herdrics diluents to obtain a 1 : 200 blood dilution. The number of leucocytes was estimated as total $\text{WBC} \times 200$. PCV was determined as micro haematocrit with 75×16 mm capillary tubes filled with blood and centrifuged at 3000 rpm for 5 minutes. The differential count of leucocytes was obtained from blood stained with Wrights dye and the neutrophil and lymphocyte cells were counted with a laboratory counter while the Hb concentration was also calculated. The serum biochemical parameters determined were blood urea nitrogen, glucose, total serum protein, globulin, albumin, creatinine and cholesterol. The blood in the plain test tubes was immediately centrifuged for serum preparation so as to ensure optimum glucose de-

Table 1: *Ingredients and calculated nutritional values of the experimental diets.*

Ingredients (% of DM)	*Control*	*Raw*		*WSS*		*LSS*	
		10 %	20 %	10 %	20 %	10 %	20 %
H. sabdariffa seeds	0	10.0	20.0	10.0	20.0	10.0	20.0
Cotton seed cake	39.0	29.0	19.0	24.0	9.0	25.0	13.0
Maize bran	19.5	19.5	19.5	24.5	29.5	23.5	25.5
Rice bran	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Bone meal	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Common salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100	100
Calculated composition (% of DM)							
Crude protein	14.10	14.10	14.10	14.10	14.10	14.04	14.26
Crude fibre	21.35	23.10	24.85	23.15	24.95	23.14	24.91
ME (MJ / kg DM)	8.21	8.09	7.98	8.13	8.05	8.12	8.01
Cost (Naira / kg DM)	34.95	33.90	32.90	32.80	30.70	34.20	34.10

WSS: Water-soaked seeds, LSS: Lime-soaked seeds; DM: Dry matter.

termination. Blood glucose was measured by the glucose oxidase method. It involved the production of a coloured compound from the activities of glucose oxidase enzyme on Beta-D-glucose and the colorimetric measurement of the coloured compound (Miller, 1959). Total serum protein and serum globulin were determined using the burette method as described by Doumas (1975); urea nitrogen was analysed by the di-methyl monoxide method as described by Varley & Bell (1980). Creatinine was determined by the Jaffe reaction method (Jaffe, 1886). Albumin was measured using dye–binding technique with bromocresol green as described by Doumas & Biggs (1972), while serum cholesterol was assessed according to the reaction described by Bernhard (1918).

2.6 Statistical analysis

All data collected during the experiment were subjected to statistical analysis using the general linear models (GLM) procedure of SAS version 9.13 (SAS, 2002) according to a completely randomized model in 2×3 factorial arrangement as:

$$Y_{ijk} = \mu + P_i + G_j + (PG)_{ij} + e_{ijk}$$

where Y_{ijk} = dependent variable, μ = over all mean, P_i = effect of ith seed processing method, G_j = effect of jth inclusion level, $(PG)_{ij}$ = interaction effects of ith processing method and jth inclusion level, and e_{ijk} = random error. Significance was declared at $P < 0.05$. Significantly different means were compared using Duncan multiple range test (Duncan, 1955). As normal distribution of the individual variables was not tested, the results of statistical analysis are only indicative.

3 Results

3.1 Chemical composition of experimental diets

Table 2 illustrates the proximate components of raw and soaked *H. sabdariffa* seeds. The crude protein concentrations of water-soaked and lime-soaked seeds as well as their NFE and crude ash concentrations were numerically higher than of the raw seeds, while a decrease was observed in the concentrations of crude fibre, fibre fractions and the ether extract of water-soaked and lime-soaked seeds.

The concentrations of anti-nutrients and minerals in raw and soaked *H. sabdariffa* seeds is shown in Table 3. Soaking

Table 2: *Chemical composition of raw and soaked H. sabdariffa seeds.*

Proximate constituents (% of DM)	*Raw*	*WSS*	*LSS*
Dry matter[†]	94.67	94.09	92.90
Crude protein	25.18	27.88	30.98
Crude fibre	27.26	26.22	23.97
Ether extract	15.18	9.22	8.97
Ash	9.18	12.00	10.66
NFE	21.40	29.12	23.42
ADF	35.98	21.32	20.33
NDF	62.89	48.81	45.40
Hemicellulose	11.59	9.01	8.41
Processing cost (Naira / kg)	0.00	7.00	15.00

[†] in % of air dry matter, WSS = Water-soaked seeds, LSS = Lime-soaked seeds; DM = Dry matter; NFE = Nitrogen free extract, ADF = Acid detergent fibre, NDF = Neutral detergent fibre.

Table 3: *Concentrations of anti-nutrients and minerals in raw and soaked H. sabdariffa seeds.*

Component (% of DM)	*Raw*	*WSS*	*LSS*
Phytate	0.17	0.15	0.16
Total tannins	2.40	1.17	1.58
Oxalate	1.46	0.87	1.04
Calcium	1.10	1.73	2.57
Phosphorus	4.30	5.56	7.71
Magnesium	0.56	0.41	0.44
Iron	0.36	1.80	2.16
Potassium	11.98	8.30	3.83

WSS = Water-soaked seeds, LSS = Lime-soaked seeds.

reduced the concentration of anti-nutrients, magnesium and potassium but increased the calcium, phosphorus and iron concentrations of *H. sabdariffa* seeds.

Table 4 presents the chemical composition of the experimental diets. For all dietary treatments, the analysed crude protein concentration of the diets was about 2 % lower than the calculated concentrations, but the analyses verified the iso-nitrogenous and iso-energetic nature of the formulated diets.

3.2 Nutrient digestibility and nitrogen balance

Table 5 shows the interaction between processing methods and inclusion levels of *H. sabdariffa* seeds in rice bran based diets on nutrient digestibility in Red Sokoto bucks. The diet containing 10 % of lime-soaked *H. sabdariffa* seeds had a significantly higher ($P < 0.05$) dry matter digestibility than all other processing methods and inclusion levels. The crude protein digestibility for the 20 % dietary inclusion levels of water-soaked (82.4 %) and 10 % inclusion level of lime-soaked (79.9 %) *H. sabdariffa* seeds were not significantly different, but were significantly higher ($P < 0.05$) than in all other treatments. The crude fibre digestibility at 20 % inclusion of lime-soaked seeds was significantly higher ($P < 0.05$) than in all other treatments. The digestibility of the ether extract was similar ($P > 0.05$) for the 10 % inclusions level of water-soaked and lime-soaked seeds but significantly higher ($P < 0.05$) than of all other treatments and the control, while the digestibility of soluble carbohydrates (NFE-fraction) was higher ($P < 0.05$) for the 20 % inclusion of water-soaked seeds. The ADF, NDF and hemicelluloses digestibility for both dietary inclusion levels of water-soaked and lime-soaked seeds was higher ($P < 0.05$) compared to the control and the diets containing raw seeds.

Table 6 summarizes the interaction between processing method and inclusion level of raw, water-soaked and lime-soaked *H. sabdariffa* seeds on the nitrogen balance of Red Sokoto bucks. For the 10 % inclusion level of raw seeds, higher ($P < 0.05$) values for nitrogen intake, urinary nitrogen excretion, total nitrogen excretion and absorbed nitrogen were found. Across all treatments and the control, the highest ($P < 0.05$) values for retained nitrogen, both absolute and in percent of ingested nitrogen, was determined at 20 % inclusion of water-soaked seeds.

3.3 Haematology and serum chemistry

Table 7 shows the impact of processing method and inclusion level of *H. sabdariffa* seeds on the haematology and serum chemistry of Red Sokoto bucks. The value for PCV increased with increasing inclusion of the raw and water-soaked seeds ($P < 0.05$), while there was only a numerical increase with increasing inclusion level of lime-soaked seeds. The WBC values also increased ($P < 0.05$) with increasing inclusion level of both raw and soaked seeds, whereby the highest value was determined at 20 % inclusion of raw seeds ($P < 0.05$). Total serum protein ranged from 4.8 to 7.6 g / 100 ml, with lowest values ($P < 0.05$) obtained for the 20 % inclusion level of raw seeds. The concentration of albumin, blood glucose and blood urea of the control was higher ($P < 0.05$) than of all inclusion levels of raw and soaked seeds, whereas the globulin value was similar to the values measured in bucks receiving water-soaked seeds. At 20 % inclusion of raw seeds, the cholesterol was lower ($P < 0.05$) than with a 20 % inclusion of water-soaked seeds. Creatinine ranged from 47.0 to 82.7 mmol / l and increased ($P < 0.05$) with increasing inclusion of both water-soaked and lime-soaked seeds but not with raw seeds ($P > 0.05$). Blood urea was unaffected by soaking method and inclusion level.

4 Discussion

4.1 Nutrient digestibility

The inclusion of water-soaked and lime-soaked *H. sabdariffa* seeds effectively increased nutrient digestibility compared to the inclusion of raw *H. sabdariffa* seeds and the control diet only. However, while the 10 % inclusion of raw *H. sabdariffa* seeds competed favourably with the control diets, the nutrient digestibility, but not the dry matter digestibility declined at a 20 % inclusion. Increasing dietary inclusion level of water-soak *H. sabdariffa* seeds had no effects on dry matter digestibility, while a reducing effect was recorded for lime-soaked *H. sabdariffa* seeds. The crude protein digestibility was positively influenced by 10 and 20 % inclusion of water-soaked *H. sabdariffa* seeds but negatively affected by the inclusion of raw and lime-soaked *H. sabdariffa* seeds. This may be attributed to an improved

Table 4: *Chemical composition of the experimental diets.*

Constituent (% of DM)	*Control*	*Raw*		*WSS*		*LSS*	
		10 %	20 %	10 %	20 %	10 %	20 %
Dry matter	89.60	90.30	89.93	89.53	90.23	90.20	89.80
Crude protein	12.60	12.38	12.50	12.88	12.31	12.98	12.64
Crude fibre	22.70	23.80	22.90	24.80	20.90	18.70	25.50
Ash	8.90	8.70	9.00	7.90	8.00	9.80	7.80
Ether extract	4.00	4.20	4.40	4.10	4.20	4.40	4.60
NFE	51.90	50.80	51.20	50.40	54.60	54.20	49.40
ADF	27.40	29.50	27.80	28.00	29.60	28.80	30.00
NDF	46.60	48.20	49.00	44.80	50.00	49.30	49.00
Hemicellulose	10.60	11.10	13.10	12.80	11.90	10.00	10.20
ME (MJ / kg DM)	10.90	10.80	10.90	10.80	11.30	11.40	10.80

WSS: Water-soaked seeds, LSS: Lime-soaked seeds; NFE: Nitrogen free extract, ADF: Acid detergent fibre, NDF: Neutral detergent fibre.

Table 5: *Effects of soaking method and inclusion level of H. sabdariffa seeds on nutrient digestibility in Red Sokoto bucks.*

Digestibility (%)	*Control*	*Raw*		*WSS*		*LSS*		*SEM*
		10 %	20 %	10 %	20 %	10 %	20 %	
Dry matter	58.12^{c}	59.4^{c}	58.2^{c}	68.2^{b}	70.0^{b}	74.4^{a}	68.6^{b}	1.38
Crude protein	70.8^{c}	71.7^{bc}	65.8^{d}	75.7^{b}	82.4^{a}	79.9^{a}	74.2^{b}	1.33
Crude fibre	54.1^{e}	57.9^{d}	56.3^{de}	68.1^{b}	64.4^{c}	66.0^{bc}	72.3^{a}	1.48
Ether extract	41.6^{e}	44.8^{e}	48.9^{d}	59.9^{a}	54.6^{c}	58.6^{ab}	55.4^{bc}	1.75
NFE	69.3^{d}	73.5^{bc}	62.1^{e}	75.8^{ab}	77.3^{a}	74.1^{bc}	72.5^{c}	1.18
ADF	64.4^{c}	60.7^{d}	52.2^{e}	69.0^{ab}	70.0^{a}	66.6^{bc}	68.0^{ab}	1.50
NDF	59.4^{d}	63.7^{cd}	61.0^{d}	67.2^{ab}	68.2^{a}	65.3^{ab}	64.8^{bc}	1.49
Hemicellulose	65.2^{b}	59.7^{c}	54.0^{d}	71.1^{a}	72.2^{a}	63.9^{b}	69.6^{a}	1.45

$^{a-e}$ Means with different superscript within rows are significantly different ($P < 0.05$), SEM: Standard Error of Mean, WSS: Water-soaked seeds, LSS: Lime-soaked seeds; NFE: Nitrogen free extract, ADF: Acid detergent fibre, NDF: Neutral detergent fibre.

Table 6: *Effects of soaking method and inclusion level of H. sabdariffa seeds on the nitrogen balance of Red Sokoto bucks.*

Parameter (g / day)	*Control*	*Raw*		*WSS*		*LSS*		*SEM*
		10 %	20 %	10 %	20 %	10 %	20 %	
Nitrogen intake	31.9^{f}	40.9^{a}	19.0^{g}	38.7^{b}	37.3^{d}	34.8^{e}	38.4^{c}	0.12
Urinary nitrogen	1.9^{c}	5.6^{a}	3.5^{b}	1.3^{e}	1.3^{e}	1.1^{f}	1.5^{d}	0.04
Faecal nitrogen	9.5^{a}	5.4^{c}	4.2^{e}	7.0^{b}	4.7^{d}	7.0^{b}	9.5^{a}	0.07
Total nitrogen excretion	11.4^{b}	11.5^{a}	7.6^{f}	8.3^{d}	6.0^{g}	8.1^{e}	11.0^{c}	0.07
Nitrogen absorbed	22.4^{f}	35.5^{a}	14.8^{g}	31.6^{c}	32.6^{b}	27.8^{e}	28.9^{d}	0.13
Nitrogen retained	20.5^{f}	29.9^{c}	11.4^{g}	30.3^{b}	31.3^{a}	26.7^{e}	27.3^{d}	0.13
Nitrogen retained (% of N-intake)	64.9^{d}	73.2^{c}	59.9^{e}	78.4^{b}	83.9^{a}	76.6^{b}	70.1^{c}	1.68

$^{a-g}$ Means with different superscript within rows are significantly different ($P < 0.05$), SEM: Standard Error of Mean, WSS: Water-soaked seeds, LSS: Lime-soaked seeds.

Table 7: *Effects of soaking method and inclusion level of raw and soaked H. sabdariffa seeds in rice bran based diets on haematology and serum chemistry of Red Sokoto bucks.*

Parameter	*Control*	*Raw*		*WSS*		*LSS*		*SEM*
		10 %	20 %	10 %	20 %	10 %	20 %	
Packed cell volume (%)	32.0^{c}	35.3^{b}	39.3^{a}	36.0^{b}	40.3^{a}	33.7^{bc}	35.7^{b}	1.40
Haemoglobin (g / 100 ml)	10.6^{bc}	11.8^{b}	13.7^{a}	13.1^{ab}	13.9^{a}	11.0^{bc}	10.0^{c}	0.76
Red blood cells (g / 100 ml)	5.3^{b}	6.0^{ab}	7.0^{a}	7.8^{a}	7.9^{a}	5.8^{b}	5.6^{b}	0.50
White blood cells ($\times 10^3$ / mm^3)	5.7^{d}	10.1^{b}	12.0^{a}	7.0^{c}	9.3^{b}	7.4^{c}	8.7^{b}	0.62
Neutrophils (%)	17.7^{c}	23.7^{a}	24.3^{a}	15.3^{d}	21.7^{b}	13.7^{d}	17.7^{c}	0.86
Lymphocytes (%)	78.0^{a}	53.0^{d}	68.7^{c}	71.7^{b}	71.0^{bc}	78.0^{a}	66.0^{c}	1.42
Total protein (g / 100 ml)	7.4^{a}	5.7^{c}	4.8^{d}	6.2^{bc}	7.6^{a}	6.6^{b}	6.6^{b}	0.38
Albumin (% of TP)	48.3^{a}	41.0^{b}	42.0^{b}	35.7^{d}	38.0^{c}	41.3^{b}	38.3^{c}	1.09
Globulin (% of TP)	39.0^{a}	35.2^{b}	36.0^{b}	38.0^{ab}	38.9^{a}	35.9^{b}	36.3^{b}	1.07
Glucose (mmol / l)	30.3^{a}	27.0^{b}	25.0^{bc}	23.3^{cd}	23.7^{c}	21.3^{d}	24.0^{c}	1.05
Cholesterol (mmol / l)	4.8^{ab}	4.6^{ab}	4.0^{b}	4.6^{ab}	4.9^{a}	4.5^{ab}	4.5^{ab}	0.42
Creatinine (mmol / l)	56.3^{c}	47.0^{d}	49.0^{d}	57.3^{c}	62.7^{b}	54.7^{c}	72.7^{a}	1.45
Blood urea (mmol / l)	4.5^{a}	3.5^{b}	2.7^{b}	2.9^{b}	2.9^{b}	3.2^{b}	3.2^{b}	0.39

$^{a-e}$ Means with different superscript within rows are significantly different ($P < 0.05$), SEM: Standard Error of Mean, WSS: Water-soaked seeds, LSS: Lime-soaked seeds.

diet quality – in physical forms and chemical terms due to reduced antinutriens – with the water-soaked *H. sabdariffa* seeds. Also, the economic efficiency of feeding water-soaked *H. sabdariffa* seeds has been reported (Ibrahim *et al.*, 2016). No effect of inclusion level and seed treatment was observed with regard to the digestibility of soluble carbohydrates (NFE fraction), whereas inclusion of raw seeds had a reducing effect. While there was negative effect associated with the digestibility coefficient of ADF and hemicelluloses with increasing inclusion of raw *H. sabdariffa* seeds, no effect was noted for both inclusion levels of the water-soaked seeds and an almost similar trend was observed for lime-soaked seeds. Elaigwu (2008) reported a declined trend of the digestibility of dry matter, crude protein and crude fibre in weaner rabbits with increasing dietary inclusion of raw *H. sabdariffa* seeds from 10 to 30 % as a replacement for groundnut cake.

4.2 Nitrogen balance

The 20 % inclusion level of raw *H. sabdariffa* seeds had a significantly ($P < 0.05$) negative effect on the animals' nitrogen balance, whereas the 10 % inclusion of raw seeds improved nitrogen utilisation as compared to the control. Both inclusion levels of lime-soaked seeds improved nitrogen utilisation. However, nitrogen retained as a percentage of intake, which is a major indicator for protein nutrition (Owen & Zinn, 1988) tended to be lower with 20 % inclusion level of raw *H. sabdariffa* seeds. The authors reported that high protein retention in animals is related to high biological value of the protein (good amino acids profile with less antinutrients), readily digestible and absorbable. Both dietary inclusion levels of water-soaked *H. sabdariffa* seeds reduced nitrogen intake, faecal nitrogen and total nitrogen output but favoured the absorption and retention of nitrogen. Especially the 20 % inclusion of water-soaked seeds yielded the highest nitrogen utilisation efficiency. These results illustrate that both inclusion levels of the two soaking methods as well as the 10 % inclusion of raw *H. sabdariffa* seeds improved nitrogen utilisation when compared to the control diet. This may be due to complementary/synergistic effects of the combination of the two protein sources: *H. sabdariffa* seeds and cotton seed cake, which resulted in better digestibility (Gibson *et al.*, 1998; Dewey, 2003). The poor nitrogen utilisation at 20 % inclusion of raw seeds might be attributed to low nitrogen intake and low digestibility of crude protein of the raw seeds. Also, Yankugh *et al.* (1986) observed a decrease in nitrogen intake and nitrogen retention in pigs when fed high levels of brewer's dried grain, a feed with a high tannin concentration, as a replacement for maize.

4.3 Haematology and serum chemistry

The dietary inclusion of both the raw and soaked *H. sabdariffa* seeds increased pack cell volume, haemoglobin, red blood cells, white blood cells and neutrophils both significantly and numerically, except for haemoglobin and red blood cells, which were unaffected by lime-soaked seed inclusions. However, these parameters are all within the range reported by Research Animal Resources (2009). The increase in PCV with dietary inclusion of *H. sabdariffa* seeds

may be due to the increase in the number of red blood cells, which was similar to the values reported by Isaac *et al.* (2013). These observations suggested that a chemical component in raw and water-soaked *H. sabdariffa* seeds might support haemopoiesis, since the RBC value depends on those of haemoglobin and PCV. The findings are also in agreement with the work of Mahadevan *et al.* (2009) who discovered the presence of iron minerals and vitamins in roselle calyxes, and the study of Olusola (2011) who reported an antioxidative potency of roselle calyxes in rabbits, resulting in a gradual increase in PCV, Hb and RBC as the dietary roselle concentration increased. The increase in PCV with increasing dietary inclusion of treated *H. sabdariffa* seeds will probably improve nutrient absorption and transportation and thus result in an increased primary and secondary polycythemia as earlier reported by Isaac *et al.* (2013). The higher values of white blood cells and neutrophils for the 20 % inclusion of raw *H. sabdariffa* seeds as compared to the other treatments, suggest the presence of antinutritional factors to which the animals reacted by increasing phagocytising blood cells and antibodies (Soetan *et al.*, 2013). Dietary nutrients are an important factor affecting haematological parameters in farm animals (Yeong, 1999; Iheukwumere & Herbert, 2002).The increase in blood creatinine levels with inclusion of treated *H. sabdariffa* seeds suggests an increase in the animals' muscular activities which may be due to increase breakdown of creatine in muscle or decrease excretion in the urine (Barcelos *et al.*, 2016).

5 Conclusions

(1) Water-soaked and lime-soaked *H. sabdariffa* seeds can replace cotton seed cake in rice offal based diets up to 20 % for better nutrient utilisation while the raw seeds can be included up to 10 % in such a diet.

(2) The 20 % inclusion of water-soaked *H. sabdariffa* seeds as a replacement for cotton seed cake recorded the best nitrogen utilisation efficiency.

(3) Inclusion levels of *H. sabdariffa* seeds in rice offal based diets as a replacement for cotton seed cakes was noted to support haematopoiesis in Red Sokoto bucks while reduction in serum protein, albumin, globulin, glucose and urea levels was observed.

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