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Protein quality of raw and processed sorrel (*Hibiscus sabdariffa* L.) seed in Wistar rats

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ABSTRACT: The use of non-conventional feed resources either as replacement or as a supplement in the nutrition of livestock cannot be overemphasised. The shortage of feed resources for livestock and poultry feeding diverted the majority of research in the field of animal nutrition to look into possibilities to overcome this crisis; however, a possible and perhaps the most viable proposition could be the inclusion of non-conventional feed resources in livestock rations with suitable and complete feed technology that can utilise the feed sources with maximum efficiency. Therefore, this study was carried out to estimate the protein quality of raw and processed sorrel (*Hibiscus sabdariffa* L.) seed, and how well the protein is digested, absorbed and utilised in Wistar rats, before it can be utilised and applied in feeding rabbits. Thirty 3-4-week-old weanling Wistar rats weighing 67.00 ± 0.7 g were randomly allotted into six dietary treatments of five rats each and fed the experimental diets for 28 days. The dietary layout was: T_1 = (control) casein, T_2 = raw sorrel seed, T_3 = boiled sorrel seed, T_4 = soaked sorrel seed, T_5 = fermented sorrel seed, T_6 = nitrogen-free diet. Growth performance, protein quality indices, serum biochemical indices and relative organ weights were assessed. The results showed that the average daily weight gain value (1.73 g) recorded in the casein-based diet (Control) was similar to the values recorded in boiled (1.18 g), soaked (1.34 g), and fermented sorrel seed meal (1.26 g) when compared with rats fed raw sorrel seeds (0.68 g) and that of the nitrogen-free diet groups (-1.36 g). Feed conversion ratio and protein efficiency ratio, however, showed a similar trend for both rats on the control and processed sorrel seed-based groups. There were, however, no significant differences ($p>0.05$) in the values recorded for crude protein digestibility, biological value, Net protein ratio and nitrogen retention of the casein-based diet and that of the soaked sorrel seed meal group. The total protein recorded in rats fed the casein-based diet did not differ significantly ($p>0.05$) from the rats fed the processed sorrel seed meal groups. In addition, albumin and globulin values showed a similar trend. The Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP) and Urea levels recorded in rats fed casein-based diets did not differ significantly ($p>0.05$) from the values recorded in rats fed processed sorrel seed meal. The relative organ weights of Wistar rats fed a casein-based diet, raw, processed sorrel seed and a nitrogen-free diet on kidney, spleen, liver, lungs and heart were recorded. However, there were no significant ($p>0.05$) differences in the values recorded in liver and lungs, while the values obtained for the kidney, spleen and heart differed significantly ($p<0.05$) among the treatment groups. It was concluded that rats fed soaked sorrel seed meal gave similar results to the control (casein-based diet) because it enhanced average daily weight gain, crude protein digestibility, biological value, and net protein utilisation.

Keywords: Casein, processing, protein quality, rats, sorrel seed.

INTRODUCTION

Hibiscus sabdariffa L., commonly referred to as sorrel, is primarily cultivated for its calyces; however, its leaves and seeds also serve as important culinary ingredients. The leaves and seeds are utilised in the preparation of soups and as a pot herb (Adigun, 2003). Certain varieties of sorrel are cultivated specifically for their fibre content

(Adigun, 2003). According to Duke (2000), sorrel is a valuable source of a red beverage that contains citric acid and various salts, with notable diuretic properties. Additionally, sorrel is used in the production of jams, jellies, sauces, wines, gelatin, puddings, and cakes. The seeds have also been evaluated as a potential feed ingredient for livestock and poultry (Hansawadi and Kawabata, 2000; Kwari, 2009; Igwebuike *et al*., 2010; Kwari *et al*., 2011; Duwa *et al*., 2012; Nyameh *et al*., 2012). The calyx of *Hibiscus sabdariffa* is notably rich in vitamin C, riboflavin, and several essential minerals (Babalola, 2000). Sorrel calyces are indigenous to tropical regions and are usually used to produce a local drink (Zobo) in Nigeria (Mohammed and Ismail, 2014). Zobo is known by different names in various regions: in English-speaking countries and among Arabic-speaking nations, it is referred to as karkade, while in Nigeria, it is called Isapa by the Yoruba, Zobo by the Hausa, and Aukan by the Igbo (Mohammed and Ismail, 2014).

Additionally, Mohammed and Ismail (2014) described sorrel as a shrub belonging to a large genus of low, succulent plants, or any plant with leaves containing an acidic sap that imparts a sour flavour. The acidity in sorrel is attributed to the presence of one or more organic acids, including citric, malic, and oxalic acids (Mohammed and Ismail, 2014). However, raw sorrel seed possesses a bitter taste, which is attributed to the presence of anti-nutritional factors. The unprocessed seed has also been reported to contain phenols, tannins, and phytic acid as common phytonutrients, and these have been shown to have detrimental effects on the health and performance of animals (Dairo *et al*., 2011; Keyembe, 2011). The effective utilisation of sorrel seeds by non-ruminant animals necessitates processing such as boiling, fermentation, sprouting, etc. to inactivate the anti–nutritional factors (Soetan and Oyewole, 2009). Therefore, this study was aimed at evaluating the protein quality of raw and differently processed sorrel seed in Wistar rats.

MATERIALS AND METHODS

Experimental location

This study was carried out in the Rat House, Animal Science, University of Ibadan, Ibadan, Oyo State, Nigeria. As a pre-trial or acclimatisation period, rats were housed in 32 x 14 x 11 cm, all-wire individual hutches in an open, ventilated house for seven days before the research work began. University of Ibadan Animal Care and Use Research Ethics Committee approved experimental procedures before the commencement of the study and assigned the number: UI-ACUREC /035-0423/26).

Sources and processing of sorrel seeds

The sorrel seeds were purchased from Gajigana Farm, Borno State during the harvesting period. The debris in the raw sorrel seeds was carefully removed, and the processing techniques adopted were according to (Ari *et al*., 2012). Sorrel seeds were poured into boiled water (100°C) for 30 min at the rate of 500g/L, sieved, sundried, milled and tagged as boiled sorrel seed meal (BSSM). Sorrel seeds were soaked in water for 12 hours (Duwa *et al*., 2012), sieved, sundried, milled and tagged as soaked sorrel seed meal (SSSM). Fermented sorrel seed meal (FSSM) was prepared by pouring the sorrel seed into boiled water (100°C) for 30 min, sieved and covered in an airtight bucket for three days (Kwari *et al*., 2011). All the processed sorrel samples were sundried, tagged and chemically analysed appropriately.

Animals, experimental design and diets

Thirty weanling Wistar rats (67.00 ± 0.7g), three to four weeks of age, were randomly allotted into six dietary treatments of five rats each; the experiment lasted for 28 days. The dietary layout is as follows T_1 = (control) casein, T_2 = raw sorrel seed, T_3 = boiled sorrel seed T_4 = soaked sorrel seed, T_5 = Fermented sorrel seed, T_6 = nitrogen-free diet. The experimental diet for Wistar rats is presented in Table 1. The following parameters were measured, growth performance, daily feed intake, daily weight gain, feed conversion ratio, and protein efficiency ratio), protein quality, (Biological value, crude protein digestibility, net protein ratio and nitrogen retention) serum biochemistry indices (Total protein, albumin, globulin, ALT, AST.ALP and Urea) and relative organ weights of kidney, liver, lungs, spleen and heart were measured using a senstive digital weighing balance.

Data collection

The mean weekly feed intake and the body weights were recorded throughout the experimental period. From the mean body weight and feed intake, feed conversion ratio was calculated. Protein quality was also determined by calculating Net protein ratio, crude protein digestibility, biological value and nitrogen retention. Serum biochemical indices and relative organ weights were also determined. Data collection was subjected to analysis of variance (ANOVA at $\alpha_{0.05}$ using SAS version 9.4 (2020), and where significant differences occurred, means were separated by Duncan's new multiple range test.

Statistical analysis

Data were analysed using the analysis of variance (ANOVA) procedure of SAS (2020). The differences among treatments were compared using Duncan's Multiple Range Test (Duncan, 1955) when the treatment effect was observed. Significance was taken at $p<0.05$.

Table 1. Gross composition of experimental diets for Wistar rats.

Ingredient (%)	Control	Raw SS	Boiled SS	Soaked SS	Fermented SS	Nitrogen-free diet
Corn starch	54.25	21.42	28.60	28.71	24.80	66.75
Groundnut oil	10.00	10.00	10.00	10.00	10.00	10.00
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00
Casein	12.50	0.00	0.00	0.00	0.00	0.00
Processed SSM	0.00	45.33	38.15	37.96	41.95	0.00
Non-nutritive cellulose	5.00	5.00	5.00	5.00	5.00	5.00
Glucose	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	2.00	2.00	2.00	2.00	2.00	2.00
NaCl	0.25	0.25	0.25	0.25	0.25	0.25
Premix	1.00	1.00	1.00	1.00	1.00	1.00
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calculated Nutrient						
Crude protein (%)	10.00	10.00	10.00	10.00	10.00	0.00
Energy ME (Kcal/Kg)	2150.76	3879.94	3788.28	3777.46	3822.12	2519.76

Premix: Vitamin and Trace Minerals Supplied the following additional Micro and Macro nutrient Premix: Vitamin A, 10,000,000.00 I.U, D3 2,000,000.00 I.U, E, 20,000.00 mg, Vitamin K3 mg, Vitamin B1 3,000.00 mg, B2 5,000.00 mg, Niacin 45,000.00 mg, Calcium Pantothenate 10,000.00 mg, Vitamin B6 4,000.00 mg, Vitamin B12 20.00 mg Chlorine chloride 300,000.00 mg, Folic acid I,000.00 mg, Biotin 50.00 mg, Manganese 300,000.00 mg, Iron 120,000.00 mg, Zinc 80,000.00 mg, Copper 8,500.00 mg, Iodine 1,500.00 mg, Cobalt 300,00 mg, Selenium 120.00 mg, Anti-oxidant 120,000.00 mg; SSM=Sorrel seed meal; SS=Sorrel seed.

RESULTS

Growth performance of Wistar rats fed casein-based diet, protein-free diet, raw and differently processed sorrel seed meal

Growth performance of Wistar rats fed processed sorrel seed meals is presented in Table 2. Rats fed casein-based diets had the highest average daily weight gain of 1.73 g compared to other treatment groups. However, the average daily weight gain values of 1.18, 1.34 and 1.26 g recorded in boiled, soaked, and fermented sorrel seed meal, respectively, were closer to that of the control. While the average daily weight gain recorded in raw sorrel seed was lower compared to the values recorded in processed sorrel seed, likewise in the nitrogen-free diet, the lowest value (-1.13 g) was recorded. Feed conversion ratio and protein efficiency ratio followed the same trends.

Protein quality of rats fed casein-based diet, nitrogen-free diet, raw and differently processed sorrel seed meal

The protein quality of rats fed casein-based diet, raw and differently processed sorrel seed meal is presented in Table 3. The crude protein digestibility value (97.67%) recorded in soaked sorrel seed meal did not differ significantly from the control (98.68%). However, values obtained in boiled and fermented sorrel seed meal (97.67 and 97.71%) did not differ significantly, followed by raw sorrel seed meal and the nitrogen-free diet group (97.09 and 96.97%), respectively. There were no significant differences in the biological values of the casein-based diet and that of soaked sorrel seed meal (98.68 and 98.36%), respectively, followed by fermented and boiled sorrel seed meal (98.01 and 97.43%), respectively; the least biological value of 94.21% was recorded in raw sorrel seed.

The net protein utilisation followed a similar trend to that of biological values, where there were no significant differences in the values (96.77 and 96.37%) for casein-based diet and soaked sorrel seed meal, respectively, followed by fermented and boiled sorrel seed meal values (95.17 and 94.48%), respectively, and the lowest value (92.01%) was recorded in raw sorrel seed. Nitrogen retention and protein utilisation followed a similar trend. The values (92.95 and 84.45%) recorded in casein-based diet and soaked sorrel seed meal, respectively, did not differ significantly compared to the values (79.20 and 76.80%) recorded for fermented and boiled sorrel seed meal.

Serum biochemical indices of rats fed casein-based diet, nitrogen-free diet, raw and differently processed sorrel seed meal.

The serum biochemical indices of rats fed a casein-based diet, a nitrogen-free diet, raw and differently processed sorrel seed meal are shown in Table 4. There were no significant differences in total protein values (6.08,6.17 and 6.51 g/dL) of boiled, soaked and fermented sorrel seed meal, respectively and that of the casein-based diet

Table 2. Growth performance of Wistar rats fed raw and differently processed sorrel seed meal.

Parameters	CBD	RSS	BSS	SSS	FSS	NFD	SEM	P- Value
ADFI (g)	9.57^{a}	8.89^{a}	8.81^{a}	9.58^{a}	9.81^{a}	5.51^{b}	33.99	0.000
ADWG (g)	1.73^{a}	0.68^{b}	1.18^{ab}	1.34^{ab}	1.26^{ab}	-1.13^{c}	0.19	0.000
FCR	5.33^{b}	15.57^{a}	7.09^{ab}	6.77^{ab}	8.04^{ab}	-5.93^{c}	1.51	0.000
PER	0.43^{a}	0.05^{b}	0.08^{b}	0.10^{b}	0.09^{b}	-	0.03	0.000
NPR	0.009^{a}	-0.000^{b}	-0.000^{b}	0.008^{b}	0.006^{b}	-	0.00	0.000

^{a,b,c} Means with different superscripts on the same row significantly differs (P<0.05), ADFI= average feed intake, ADWG= average daily weight gain, FCR= feed conversion ratio, PER= protein efficiency ratio, NPR= net protein ratio, CBD= casein-based diet, RSS= raw sorrel seed BSS= boiled sorrel seed, SSS= soaked sorrel seed, FSS= fermented sorrel seed, PFD= protein free diet.

Table 3. Protein quality indices of Wistar rats fed raw and processed sorrel seeds.

Parameters	CBD	RSS	BSS	SSS	FSS	SEM	P- Value
CP Dig (%)	88.68^{a}	56.75^{c}	65.40^{bc}	70.07^{b}	64.76^{bc}	3.10	0.000
BV (%)	90.93^{a}	44.71^{d}	58.43^{c}	66.96^{b}	62.40^{bc}	4.12	0.000
NPU (%)	80.64^{a}	25.70^{c}	38.32^{b}	47.02^{b}	40.49^{b}	5.07	0.000
NR (%)	80.64^{a}	25.70^{c}	38.32^{b}	47.02^{b}	40.49^{b}	5.07	0.000

^{abc} Means in the same row with different superscripts differ (p<0.05), SEM= Standard Error of Mean, CP = crude protein digestibility, BV = biological value, NPU = net protein utilisation, NR = nitrogen retention, CBD = casein-based diet, RSS = raw sorrel seed, BSS = boiled sorrel seed, SSS= soaked sorrel seed, FSS = fermented sorrel seed.

Table 4. Serum biochemical indices of Wistar rats fed raw and differently processed sorrel seed meal

Parameters	CBD	RSS	BSS	SSS	FSS	NFD	SEM
TP (g/dL)	6.64^{a}	5 .68^{b}	6.08^{a}	6.17^{a}	6.51^{a}	4.91^{b}	0.03
ALB (g/dL)	3.30^{b}	2.80^{b}	4.49^{a}	4.47^{a}	4.37^{a}	2.62^{b}	0.03
GLO (g/dL)	3.34^{a}	2.88^{ab}	1.60^{b}	1.70^{b}	2.14^{ab}	2.29^{ab}	0.04
AST (IU/L)	33.64^{ab}	24.11^{b}	38.68^{a}	26.23^{b}	31.01^{ab}	31.14^{ab}	0.28
ALT (IU/L)	26.54^{b}	32.63^{ab}	36.48^{a}	33.00^{ab}	33.46^{ab}	38.59^{a}	0.23
ALP (IU/L)	32.33^{a}	27.00^{a}	27.00^{a}	35.00^{a}	13.00^{b}	31.00^{a}	0.24
Urea (mg/dL)	15.55^{b}	17.90^{ab}	18.34^{ab}	16.06^{b}	20.00^{a}	20.05^{a}	0.09

^{a,b,c} Means with different superscripts on the same row significantly differs (p<0.05), TP = total protein, ALB = albumin, GLO = globulin, AST= aspartate amino transferase, ALT = alanine amino transferase, ALP = alkaline phosphatase, CBD = casein-based diet, RSS= raw sorrel seed, BSS= boiled sorrel seed, SSS= soaked sorrel seed, FSS = fermented sorel seed, PFD = protein free diet, SEM = standard error of mean.

(6.64 g/dL). The albumin values (4.49,4.47 and 4.37 g/dL) did not differ significantly in boiled, soaked and fermented sorrel seed meal, respectively, which were higher than the values (3.30,2.80 and 2.62 g/dL) in the casein-based diet, raw and nitrogen-free diet, respectively. The highest globulin level (3.24 g/dL) was recorded in the casein-based diet, compared to other treatment groups, followed by the values (2.88,2.29 and 2.14 g/dL) recorded in raw, nitrogen-free diet and fermented sorrel seed meal, respectively; the lowest albumin levels (1.70 and 1.60 g/dL) were recorded in soaked and boiled sorrel seed meal.

For the Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP) and Urea, they were all significantly (p<0.05) different. The highest value for Aspartate Transferase (38.68 IU/L) was recorded in boiled sorrel seed. However, values (31.14,31.01, 26.23 and 24.11 IU/L) recorded in nitrogen-free diet, fermented, soaked and raw sorrel seed meal were closer to the value (33.64 IU/L) recorded in casein-based diet. The highest values (38.59 and 36.48 IU/L) for Alanine Amino Transferase were recorded in nitrogen-free diet and boiled sorrel seed meal, while values (33.46,33.00 and 32.63 IU/L) for the fermented, soaked and raw, respectively, were closer to the value (26.54 IU/L) recorded in the casein-based diet.

There were no significant (p<0.05) differences in alkaline phosphatase values (32.33,27.00,27.00,35.00 and 31.00 IU/L) for casein-based diet, raw, boiled, soaked and nitrogen-free diet, respectively, while the lowest ALP value (13.00 IU/L) was recorded in fermented sorrel seed meal. The higher Urea values (20.86,20.05 and 20.00 IU/L) were

Table 5. Relative organ weight of Wistar rats fed raw and differently processed sorrel seed meal.

Parameters (%)	CBD	RSS	BSS	SSS	FSS	NFD	SEM
Kidney	0.77^{bc}	0.81^{b}	0.71^{bc}	0.77^{bc}	0.68^{c}	0.92^{a}	0.00
Spleen	0.21^{ab}	0.23^{a}	0.18^{bc}	0.23^{a}	0.18^{bc}	0.16^{c}	0.00
Liver	2.95	3.55	3.62	3.61	2.97	3.26	0.03
Lungs	0.97	1.03	0.99	0.96	0.98	1.13	0.01
Heart	0.48^{ab}	0.42^{ab}	0.44^{ab}	0.47^{ab}	0.40^{b}	0.56^{a}	0.00

^{a,b,c} Means with different superscripts on the same row significantly differ (p<0.05), CBD = casein-based diet, RSS = raw sorrel seed, BSS = boiled sorrel seed, SSS= soaked sorrel seed, FSS = fermented sorrel seed, NFD = nitrogen-free diet.

recorded in soaked, nitrogen-free diet and fermented sorrel seed meal, respectively, followed by casein-based diet, raw and boiled sorrel seed meal with urea values of 15.55,17.90 and 18.34 IU/L, respectively.

Relative organ weights of Wistar rats fed casein-based diet, nitrogen-free diet, raw and differently processed sorrel seed meal

The relative organ weights of Wistar rats fed a casein-based diet, nitrogen-free diet, raw and differently processed sorrel seed meal are shown in Table 5. There were no significant (p<0.05) differences in the values (2.95, 3.55,3.62, 3.61, 2.97 and 3.26%) and (0.97,1.03,0.99,0.96,0.98 and 1.13) for liver and lungs, respectively; however, kidney, spleen and heart differed significantly. A higher value (0.92%) was recorded in the nitrogen-free diet group compared to the other treatment groups' values of 0.77, 0.81, 0.71 and 0.88% for casein-based diet, raw, boiled, soaked and fermented sorrel seed meal, respectively. The spleen values of 0.21, 0.23 and 0.23% for casein-based diet, raw and soaked sorrel seed meal, respectively, were also closer; boiled sorrel seed meal, fermented sorrel seed meal and nitrogen-free diet values (0.18,0.11 and 0.16%, respectively) were closer. In the case of heart, there were no significant (p<0.05) differences in the values (0.48, 0.42, 0.44 and 0.47%) for casein-based diet, raw, boiled and soaked sorrel seed meal, respectively; however, the nitrogen-free diet value (0.56%) was the highest among the treatment groups.

DISCUSSION

Processing of sorrel seed had no influence on feed intake of Wistar rats. Based on the findings, processed sorrel seed had been shown to improve the protein quality of the sorrel seeds, which is similar to that of the control (casein-based diet). The increment in the body weights, PER, and NPR values of soaked sorrel seed meal was higher than that of raw sorrel seed meal, but closer to boiled and fermented sorrel seed meal. The results were in line with Rao (1996), who reported that food intake, gain in body weight, PER, and NPU values of rats fed with cooked Roselle (*Hibiscus sabdariffa*) seed diet were significantly higher than raw sorrel seed diets, which was in contrast with the result reported by Abu *et al.* (2020) who reported that, all the processing methods carried out on dehulled jack bean were ineffective when compared to the performance of rat fed casein based diets.

This could be due to the inhibition of anti-nutritional activities. Seeds and legumes commonly contain anti-nutritional components such as phenolics, enzyme inhibitors, lectins, hemagglutinin, alkaloids, tannins and phytic acids which may reduce their nutritional values and protein digestibility (Hernandez-Infante *et al*., 1979; Giami, 2005; Agbede and Aletor, 2005). Although anti- nutritional factors are found to largely affect the protein quality of food, many of them can be eliminated or inactivated, to a large extent, by appropriate heating and processing during food preparation (Mwasaru *et al*., 1999). Manjula *et al*. (2016) studied the feed intake and weight gain of a *Spirulina* and rice diet in Wistar rats and reported that the feed consumption rate is very low in the *Spirulina* diet but gained more weight when compared to stock and rice diets; this might be due to high biological value protein in *Spirulina*, which is in agreement with the report of Rajkumar *et al.* (2021). However, in the present study, there were no significant (p<0.05) differences in average daily feed intake of the control (casein-based diet), raw and processed sorrel seed meal, compared to the nitrogen-free diet group. This shows that processing of sorrel seed had no effect on feed intake. However, Halimatul *et al*. (2007) reported that the protein quality of raw roselle seed was similar to the boiled roselle seed at 100°C for 30minutes, which was in disagreement with the present study; the present result showed that there were significant differences in protein quality parameters.

The average daily weight gain of Wistar rats fed raw sorrel seed meal was lower than that of those fed a casein-based diet but similar to that of those fed processed sorrel seed meal. This could be due to the higher alkaloid content of raw sorrel seed, which could have interfered with nutrient absorption. A similar trend was observed for feed conversion ratio. The crude protein digestibility of Wistar rats fed processed sorrel seed meal was higher than that of rats fed raw sorrel seed. This suggests that processing of sorrel seed could have increased the surface area of the seed, exposing it to chemical and enzymatic activities in the gut, thereby aiding its digestion.

Serum biochemistry of animals was carried out to evaluate the nutritional and health status of animals. The total protein and its fractions of Wistar rats fed boiled water sorrel seed meal, room temperature-soaked sorrel seed meal, and boiled water fermented sorrel seed meal were higher than those fed raw sorrel seed meal and the nitrogen-free diet group. This suggests that the processing of the seed improved the availability of nutrients for absorption in the blood (Sahlu *et al*., 2004). This finding is in line with the observation of Ferrare *et al.* (2006) who reported a positive correlation between dietary protein and plasma protein concentration when livestock are fed a high-protein diet. The values obtained in this study were within the normal range of serum protein (5.00 -7.00 g/dL) reported by Anonymous (2012), 4.55 -6.46 g/dL reported by Udoyong *et al*. (2010) and (5.64 -7.72 g/dL) reported by Duwa *et al.* (2012) but lower than 8.32 -8.70 g/dL reported by Njidda *et al*. (2006). Higher values indicate that there is hydrolysis of enzymes of dietary proteins and explain that the blood serves as a major source of amino acids needed for the synthesis of proteins (Njidda *et al*. 2006). This observation showed that protein levels in the diets, apart from the nitrogen-free diet group, were sufficient to sustain normal blood protein levels. Blood albumin values of BWSSM, RTSSSM and BWFSSM did not differ significantly (4.49,4.47 and 4.37 g/dL) compared to the raw and nitrogen-free diet group (2.80 and 2.62 g/dL), respectively. Blood globulin, calculated by subtracting albumin from total serum protein, showed comparable protein utilisation across diets, as reflected by the feed conversion ratio. However, there were significant differences in Aspartate Amino Transferase (AST), Alanine Amino Transferase, alkaline phosphatase and urea across the treatment groups.

There were no significant ($p>0.05$) differences in relative organ weights for liver and lungs; however, kidney, spleen and heart differed ($p<0.05$). The highest kidney value recorded in raw sorrel seed meal could be as a result of presence of ANFs present in the raw sorrel seed meal, because, it has been reported by Iyakutye *et al.* (2023) that any material contains anti-nutritionals in the feed, will tend having abnormal kidney because of increased metabolic rate of the organs in an attempt to reduce these toxic elements or anti-nutritional factors to non-toxic metabolite.

Conclusion

Processing by boiling, soaking and fermentation of sorrel (*Hibiscus sabdariffa* L.) seed in this study had shown to be similar to casein-based diets compared to raw sorrel seed meal and nitrogen-free diets, respectively. However, soaked sorrel seed meal enhanced average daily weight gain, crude protein digestibility, biological value, net protein ratio, among others.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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