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Protein Quality Evaluation of Raw and Processed Seeds of *Cadaba farinosa* Forssk and Growth Performance of Albino Rats Fed with the Products

I. M. Inuwa[1*], M. K. Atiku[2] and J. A. Mashi[2]

[1]Department of Food Technology, Kaduna Polytechnic, P.M.B. 2021, Kaduna, Nigeria.
[2]Department of Biochemistry, Bayero University, P.M.B. 3011, Kano, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author IMI designed the study and carried out the laboratory analyses. Author MKA supervised the entire work and drafted the manuscript. Author JAM contributed in literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Cadaba farinosa Forssk is a wild shrub whose seeds are eaten as a famine food in North Eastern Nigeria when there is poor harvest of conventional crops. This research was carried out in order to evaluate the protein quality of the seeds and to carry out the growth performance of experimental animals fed on seeds processed by different methods. The processed methods include cooking, cooking with the addition of potash, fermentation and sprouting. PTH-Amino acid analyzer was used to determine the amino acid profile and milk reference protein was used for scoring the essential amino acids. The products were fed to weaning albino rats for 28 days growth performance studies. The determination of nitrogen content of the feed, faeces, urine and the carcass of the animals were carried out by Kjeldahl method. Protein quality evaluation of the processed products was carried out by calculating the Biological Value (BV), Protein Efficiency Ratio (PER), Net Protein Utilization (NPU) and The Protein Digestibility-Corrected Amino Acids Score (PDCAAS). Results of Amino acid analysis showed that the sample cooked with addition of potash contain higher levels of amino acids. Glutamic acid was found to the most abundant amino

**Corresponding author: Email: iminuwapkm@gmail.com;*

acid with a value of 11.96 g/ 100 g and 13.96 g/ 100 g in the sprouted and raw seeds respectively. Chemical score of amino acids revealed higher score for protein of seeds cooked with addition of potash except tryptophan which was 219.29% for seeds cooked without potash. The highest PDCAAS (49.84%) was found in the diet cooked with potash, however, it showed lower biological value (95%) and protein efficiency ratio (0.32). The group fed with sprouted diet showed significant increase (*P=.05*) in body weight (52.13%). Though cooking with addition of potash showed higher distribution of amino acids, but sprouting and fermentation were more promising in terms of promoting the growth of the experimental animals.

Keywords: Cadaba farinosa; processed seeds; protein quality; growth performance.

1. INTRODUCTION

Since the year 2009, North Eastern Nigeria has faced insecurity due to the conflict with boko haram insurgent group, which has caused deepening humanitarian crisis and devastated civilian population. About 7.7 million people need assistance and 1.6 million are internally displaced. Children under-five in Nigeria experience high malnutrition rates: 43.6% are stunted, 10.8% are wasted [1]. Current level of food insecurity in north eastern Nigeria, Somalia, South Sudan and Yemen reflect continued underinvestment in agriculture and livelihoods within the wider humanitarian and development fields. Conflict and drought are forcing people to abandon their homes and lands. As agricultural seasons are repeatedly missed and livelihoods abandoned, the humanitarian caseloads and number of people on the brink of famine rises [2]. In Borno, Yobe and Adamawa states of North Eastern Nigeria, over 5 million people are facing hunger and 450,000 children are severely malnourished. Food production is grossly hindered due to persistent insurgency and climatic changes [3]. The most recent analysis from famine early warning system network indicate that, across the part of the Chad basin encompassing North East Nigeria, acute food insecurity is at either a crisis or worse, in emergency stage [4]. Many women and children are forced to rely on some wild species for food [3]. FAO conference on forests for food security highlighted the importance of wild foods for the food and nutrition security of millions of people [5]. It is clear that wild food plants and animals continue to form a significant proportion of the global food basket, and while a variety of social and ecological drivers are acting to reduce wild food use, their importance may be set to grow as pressures on agricultural productivity increases [6].

Cadaba farinosa Forssk is called 'Bagayi' or 'Anza' in Hausa speaking area of Northern Nigeria [7]. It is a wild shrub belonging to the family *Capparaceae*. Food uses of the plant include cooking the young leaves and adding it to couscous. They can also be used; in both fresh and dried form to spice and flavor foods. The pounded leaves and twigs are prepared with cereals and made into a cake or pudding called "farsa" or "balambo" by Kanuri people of North Eastern Nigeria. The macerated flowers are used as a sweetener [8]. Also, in Borno and Yobe states, the seeds of the plant are cooked and eaten like beans or ground to flour for meal preparation and eaten with a soup [9]. Protein-energy malnutrition is still a major public health issue in developing countries. It is associated with as much as 50-60% of under-five mortality in poor countries and a myriad of morbidities [10]. Since protein serve important and essential functions in the body and these proteins can be obtained solely from dietary intake, it is very obvious that the quality and quantity of protein and peoples' daily diet, knowledge of best food sources and metabolism of proteins in the body should be matters of uttermost importance to health care professionals [11]. Protein quality evaluation aims to determine the capacity of food protein sources and diet to meet the protein and essential amino-nitrogen requirements, i.e. to satisfy the metabolic needs for amino acids and nitrogen [12]. The protein content of raw seeds of *C. farinosa* was reported as 26.72% [9]. Therefore, the objective of this research is to evaluate the quality of protein in the seeds in terms of levels of essential amino acids present and supporting the growth of experimental animals, and also to determine the effects of different methods of processing on the quality of the nutrient present in the seeds.

2. MATERIALS AND METHODS

2.1 Sample Collection

Ripe fruits of *Cadaba farinosa* Forssk were collected from a bush in Fune Local Government

Area of Yobe state, North Eastern Nigeria. The plant was authenticated in the Herbarium of Department of Botany, Ahmadu Bello University, Zaria, Nigeria (Voucher Number: ABU 06921). The fruits were air-dried under ambient temperature, seed coats were removed manually and then air-dried further.

2.2 Experimental Animals

Thirty five weaning albino rats who were between 27 and 30 days old, with an average weight of 39.97±3.75g consisting of males and females were obtained from an animal breeder in the Department of Pharmacology, Ahmadu Bello University, Zaria.

2.3 Processing of *C. farinosa* Seeds

The seeds of *C. farinosa* were processed according to the method described by Atiku and Inuwa [9]:

2.3.1 Cooking

Exactly 3 kg of the seeds were submerged in a tap water and placed over a kerosene stove for 2 hrs. They were then collected from the pot with a scoop, air-dried for 4 days and milled in a hammer mill to the average particle size of 280µm particle size.

2.3.2 Cooking with addition of potash

Exactly 3 kg of the seeds were submerged in a tap water and boiled over a kerosene stove for 1 hrs, 40 min with addition of 10 g of potash. They were air-dried and milled in a hammer mill to the average of 280µm particle size.

2.3.3 Fermentation

After cooking 3 kg of the seeds for 2 hrs over a kerosene stove, it was transferred into a low density plastic container lined with a banana leaves that was imbued with natural starter. The leaves were also used to cover the cooked seeds. Fermentation took place for 2 days at ambient temperature of 30-35^{o}C. They were then air-dried, milled in a hammer mill to the average of 270µm particle size.

2.3.4 Sprouting

After steeping in low density plastic container for 2 days, the seeds were then spread over a tray and covered with a cheese clothes for 5 days. The seeds germinated to the average of 5mm sprout length. They were then dried and milled in a hammer mill to the average of 270µm particle size.

2.4 Analyses

2.4.1 Determination of amino acids profile

Amino acids profile was determined according to the method of Stage 5-proposal [13] using model 120A PTH-amino acid high performance liquid chromatography (HPLC) analyzer. The process involves drying the sample, defatting, acid hydrolysis, evaporation of the hydrolysate and loading the sample into the analyzer.

2.4.1.1 Drying

The sample was dried to a constant weight at 100^{0}C in a vacuum oven.

2.4.1.2 Defatting

Exactly 5g of the oven-dried sample was defatted by Soxhlet extraction method. Mixture of chloroform and methanol (1:1) was used as the solvent for the extraction.

2.4.1.3 Hydrolysis

Exactly 0.346g of the defatted sample was transferred into a glass ampoule. Five milliliter of 6N HCl containing 0.5% phenol, and 1ml of thioglycolic acid reducing agent were added to prevent oxidation of tryptophan. Oxygen was expelled from the ampoule by passing nitrogen into the ampoule to prevent oxidation of methionine and cystine. The ampoule was sealed and placed in an oven at 105^{0}C for 24 hours. It was then cooled and filtered to remove humins. The sample was then evaporated in a rotary evaporator under high vacuum for 15 minutes to remove residual acids.

2.4.1.4 Sample loading

Exactly 60µL of the evaporated hydrolysate was measured in a micropipette and injected into 2.11D x 220mm catridge analyzer that was designed to separate and analyze free acidic, neutral and basic amino acids in the hydrolysate. The column solvents consists of 5% aqueous tetrahydrofuran and acetonitrile, and two sodium acetate buffer of pH 3.8 and pH 4.6. These solvents and buffer solutions were used to form

the mobile phase required for the gradient elution of PTH-amino acids from the column. The PTH-amino acids eluted from the column were monitored at 254nm. Norleucine internal standard was analyzed as a control which provides the calibration for the amino acids.

2.4.2 Chemical score for essential amino acids

Milk protein for amino acids reference pattern [14] was used to calculate the amino acids scores according to the method of Ihekoronye and Ngoddy [15] as follows:

$$\text{Amino acid score} = \frac{mg\ of\ amino\ acid\ in\ 1g\ of\ test\ protein}{mg\ of\ amino\ acid\ in\ 1g\ reference\ protein} \times 100$$

2.4.3 Growth performance of experimental animals

The growth performance of weanling albino rats was carried out according to the method described by Alagbaoso *et al.* [16]. Thirty five weanling rats who were between 27 and 30 days old, with an average weight of 39.97±3.75g consisting of males and females were randomly divided into seven groups with five animals per group. The groups were fed with the raw, steeped, cooked, cooked with addition of potash, fermented or sprouted seeds of *C. farinosa* after mixing the flours with cold water to form paste, while the last group was fed with cassava flour which was used as the protein-free diet for the study. The animals were provided with constant supply of water where they eat and drink *ad libitum*.

Serving the animals with the feed was carried out in such a way that the feed was weighed in the morning before serving them, and the remnants was weighed the following morning in order to determine the quantity of feed consumed each day. Each animal in a group was weighed every week before serving them with the feed in order to determine the weekly change in their body weight.

2.4.3.1 Total feed intake

The total feed intake was calculated by taking the total of feed consumed for 28 days.

2.4.3.2 Body weight gain

The percent body weight gain was calculated as follows:

$$\%\ BWG = \frac{Final\ weight - initial\ weight}{Final\ weight} \times 100$$

2.4.3.3 Feed efficiency ratio

The feed efficiency ratio was calculated by dividing the percent body weight gain by feed intake.

$$\text{FER} = \frac{\%\ BWG}{FI}$$

2.4.4 Protein quality evaluation

Each group of the experimental animals were transferred into metabolic cage after 28 days of growth performance. Their faeces dropped into a container, and the urine drains through a separate tube into a different plastic bottle for 7 days. The animals were then sacrificed in order to obtain their carcass. The percent nitrogen content of the carcass, faeces and the urine of animals fed with the test diets and those fed with the protein-free diet were determined by Kjeldahl method [17].

The calculations for the biological value (BV), protein efficiency ratio (PER|) and the net protein utilization (NPU) was carried out according to the method of Pellet and Young [18], while calculation for protein digestibility-corrected amino acid score (PDCAAS) was carried out according to the method of Gilani and Sepehr [19] as follows:

2.4.4.1 Biological value (BV)

$$\%BV = \frac{Nitrogen\ retained}{Nitrogen\ absorbed} \times 100 = \left(\frac{In - (Fn - Fe) - (Un - Ue)}{In - (Fn - Fe)}\right) \times 100$$

Where: In = N ingested (Food intake N)
Fn = Faecal N of animals on test diet
Fe = Faecal N of animals on protein-free diet
Un = Urinary N of animals on test diet
Ue = Urinary N of animals on protein-free diet

2.4.4.2 Protein efficiency ratio (PER)

$$\text{PER} = \frac{Body\ weight\ gain}{Protein\ consumed}$$

Where: Protein consumed =

$$\frac{Total\ feed\ intake \times \%\ Protein}{100}$$

2.4.4.3 Net protein utilization (NPU)

$$\%\text{NPU} = \frac{Nitrogen\ retained}{Nitrogen\ intake} \times 100 = \frac{B-Bk}{I} \times 100$$

Where: B = Carcass N of animals on test diet for 7 days
Bk = Carcass N of animals on protein-free diet for 7 days
= N intake of animals on test diet for 7 days.

2.4.4.4 Protein digestibility-corrected amino acid score

$$\%\text{PDCAAS} = \frac{mg\ of\ limiting\ amino\ acid\ in\ 1g\ of\ test\ food}{mg\ of\ the\ same\ amino\ acid\ in\ 1g\ of\ reference\ protein} \times Protein\ Digestibility \times 100$$

$$\text{Protein Digestibility} = \frac{PI-(Fp-MFp)}{PI}$$

Where: PI = Protein intake for 7 days
Fp = Faecal N of animals on test diet for 7 days
MFp = Faecal N of animals on protein-free diet for 7 days.

3. RESULTS

3.1 Amino Acids Profile of Raw and Processed Seeds of *C. farinosa* Forssk.

The result showed that there was general decrease (*P=.05*) in amino acids content following different methods of processing (Table 1). Glutamic acid is the most abundant amino acids in the samples. Seeds cooked with addition of potash have relatively higher level of amino acids than the remaining samples, except tryptophan which is higher in the cooked seeds (3.07g/100g protein).

3.2 Chemical Scores of Amino Acids

The chemical scores of the essential amino acids are presented in Table 2. The amino acids scores decrease (*P=.05*) following different methods of processing. Amino acids scores of the sample cooked with addition of potash were significantly higher (*P=.05*) than the remaining samples.

3.3 Growth Performance of Albino Rats and Protein Quality Evaluation

The growth performance of weaning albino rats and protein quality evaluation of processed seeds of *C. farinosa* are presented in Table 3, while Fig. 1. showed the growth pattern of experimental animals fed with processed products. There was significant increase in (*P=.05*) weight gain in animals on the sprouted diet within 28 days (52.13%), while the slowest body weight gain was in animals on the diet cooked with addition of potash. Proteins of the sample cooked with addition of potash have lower biological value, protein efficiency ratio and net protein utilization. The protein digestibility-corrected amino acid score of the sample cooked with addition of potash was significantly higher (*P=.05*) than the remaining samples.

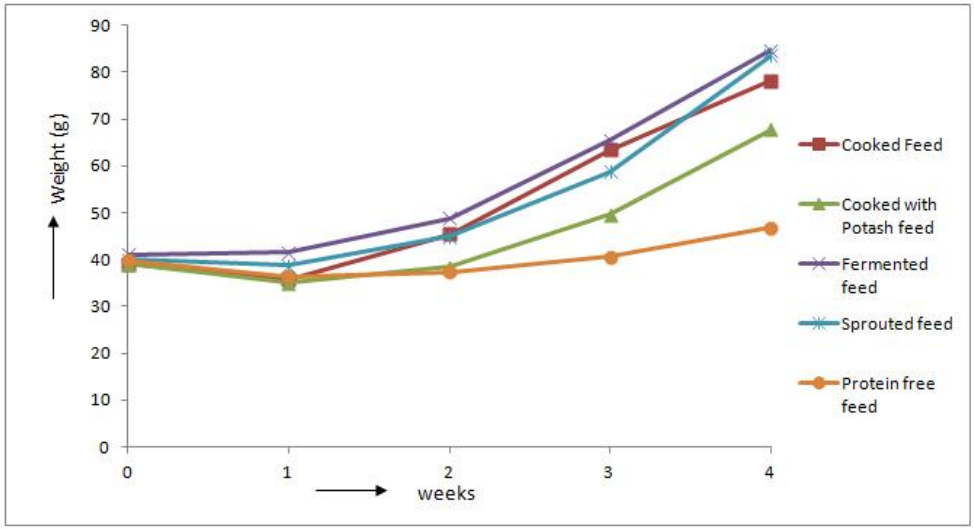

Fig. 1. Growth pattern of experimental animals fed with tests and protein-free diet

Table 1. Amino acids profile (g/100g protein) of raw and processed seeds of *C. farinosa* Forssk

Amino acids concentration	Raw	Steeped	Cooked	Cooked with potash	Fermented	Sprouted
Leucine	8.14	7.79	4.20	7.30	5.90	5.49
Lysine	4.64	4.51	3.02	4.35	4.03	3.98
Isoleucine	3.21	3.14	2.03	3.08	3.01	2.95
Phenylalanine	4.17	3.99	3.02	3.73	3.55	3.19
Tryptophan	0.95	0.92	3.07	0.89	0.81	0.68
Valine	3.60	3.45	2.89	3.33	3.16	2.98
Methionine	1.55	1.50	1.15	1.44	1.28	1.28
Proline	3.25	3.05	2.23	2.84	2.64	2.44
Arginine	5.59	5.33	4.13	4.99	4.82	4.56
Tyrosine	2.58	2.58	2.06	2.41	2.41	2.24
Histidine	2.24	2.20	1.88	2.11	1.98	1.92
Cystine	1.03	0.97	0.85	0.91	0.91	0.91
Alanine	3.41	3.41	3.00	3.19	3.11	3.26
Glutamic acid	13.63	13.33	10.52	13.17	12.26	11.96
Glycine	4.09	3.99	3.21	3.92	3.75	3.52
Threonine	3.28	3.19	2.50	3.05	2.89	2.89
Serine	3.59	3.46	2.84	3.19	3.08	3.00
Aspartic acid	9.49	9.30	7.81	8.81	8.81	8.31

Table 2. Chemical scores of essential amino acids of raw and processed seeds of *C. farinosa* Forssk

Amino acids concentration	Raw (%)	Steeped (%)	Cooked (%)	Cooked with potash (%)	Fermented (%)	Sprouted (%)
Isoleucine	68.30	66.80	43.19	65.53	64.04	62.77
Leucine	85.68	82.00	44.21	76.84	62.11	57.79
Lysine	59.49	57.82	38.72	55.77	51.67	51.03
Methionine + cystine	78.18	74.45	60.61	71.21	66.36	66.36
Phenylalanine + tyrosine	66.18	64.41	49.80	60.20	58.43	53.24
Threonine	74.55	72.50	56.82	69.32	65.68	65.69
Tryptophan	67.86	65.71	219.29	63.57	57.86	48.57
Valine	56.25	53.91	45.16	52.03	49.38	46.56

**Scoring was based on the FAO/WHO (1973) reference pattern for essential amino acids for milk protein.*

Table 3. Protein quality of processed seeds of *C. farinosa* forssk and growth performance of albino rats fed with the products

Growth/Protein quality Parameters	Tests groups					
	Raw	Steeped	Cooked	Cooked with potash	Fermented	Sprouted
EQW						
FI(g)	32	59	699	901	709	702
BWG (%)	-	-	50.13	42.48	51.65	52.13
FER	-	-	0.07	0.05	0.07	0.07
BV(%)	-	-	98.0	95.0	98.0	98.0
PER	-	-	0.45	0.32	0.45	0.41
NPU(%)	-	-	32.0	28.0	56.0	38.0
PDCAAS(%)	-	-	37.37	49.84	47.32	46.41

FI= feed intake; BWG= body weight gain; FER= feed efficiency ratio; BV= biological value; PER= protein efficiency ratio; NPU= net protein utilization; PDCAAS= protein digestibility- corrected amino acid score.

4. DISCUSSION

The results revealed that seeds cooked with potash showed higher distribution of amino acids which might be due to its effect on the plant matrix, hence improving the abundance of the amino acids. Glutamic acid was the most abundant amino acid in both raw and processed seeds of *C. farinosa*. It was reported that glutamic acid was the most abundant amino acid in the white melon flour with values ranging from 12.82g/100g protein to 15.64g/100g protein [20]. Leucine content of the raw seeds of *C. farinosa* (8.14g/100g protein) was similar to 8.1g/100g protein isolate of cowpea [21]. The tryptophan level of the products cooked without potash (3.07g/100g protein) was higher than 0.95g/100g protein of the raw seeds. This may be due to hydrolysis of the cell walls during cooking and other methods of processing which might have destructive effect on the amino acid. Lysine was reduced by 25.2% and histidine by 36.7% in oze seed flour after 60 minutes boiling [22]. Also, analysis of raw and processed seeds of *C. farinosa* showed that the seeds contain appreciable levels of sulphur-containing amino acids. The raw seed contain 1.55g/100g protein and 1.03g/100g protein of methionine and cystine respectively [23]. Overall, there were decreases in the amino acids level after different methods of processing of seeds of *C. farinosa*. However, there were relatively higher distributions of essential amino acids when compared to much other plant protein [24]. Hence, the products would be of high nutritional quality among many plant foods.

Protein quality of a food can be evaluated by comparing its amino acid content with that of a reference protein [25]. The chemical scores of essential amino acids of raw and processed *C. farinosa* seeds ranged from 38.72% for lysine in cooked seeds to 219.29% for tryptophan in the same sample based on the milk amino acid reference pattern [14]. Score for tryptophan of cooked sample was much higher than 144.44 of oyster mushroom [24]. Lysine is the limiting amino acids in the raw and processed seeds of *C. farinosa*. Lysine was also the limiting amino acid in cereal grains [15,26]. This value is lower than what was obtained in cooked seeds of *C. farinosa*. Addition of potash during cooking increased the amino acid scores of all the essential amino acids except tryptophan. However, it was reported that the chemical score for phenylalanine alone was 51.0% of white melon flour [20] which is higher than what was obtained in processed seeds of *C. farinosa*. Yet, *C. farinosa* can also provides appreciable amount of essential amino acids in the diet like most other protein-rich plant foods.

The total feed intake by the groups after 28 days were 32g, 59g, 699g, 901g, 709g and 702g of raw, steeped, cooked, cooked with addition of potash, fermented and sprouted seeds respectively. The animals on raw seeds died after 3 days, while those on the steeped seeds died after 5 days. This may be due high concentration of toxicants such as cyanide [9]. Group on the diet cooked with addition of potash consumed higher quantity of the feed, yet they showed slower body weight gain (42.48%) within the period of study. This finding is in agreement with the report that chronic intake of 'kanwa' has adverse effect on the growth rate and blood parameters [27]. The fastest body weight gain (52.1%) was recorded in the group fed with sprouted seeds. However, this was slower than the body weight gain of 32.18g to 80.76g of albino rats fed with 50% noodles and 50% rat pellets for 28 days while the total feed intake was 257.76g [28].

The biological value of proteins of processed seeds of *C. farinosa* showed that the seeds cooked with addition of potash was 95% which was lower than 98% in the remaining samples. These results were higher than 61.50% of cooked *Mucuna pruviens seeds* [29]. Also, the net protein utilization (NPU) of cooked seeds of *Mucuna pruviens* was 40.20% [29]. This value is lower than the value for the fermented seeds of *C. farinosa* (56%), however, it is higher than the results obtained from the remaining samples. The protein efficiency ratio (PER) of processed seeds of *C. farinosa* were 0.45, 0.32, 0.45 and 0.41 for cooked, cooked with potash, fermented and sprouted seeds respectively. These values were less than 1.45, 3.30, 3.15 and 2.94 for groundnut-ogi, crayfish-groundnut-ogi, crayfish-ogi and commercial nutrend respectively [29,30]. Hence, quality of proteins of processed seeds of *C. farinosa* is lower than those of groundnut-ogi, crayfish and the commercial nutrent.

The results of Protein Digestibility-Corrected Amino Acid Score (PDCAAS) obtained from the processed seeds of *C. farinosa* served to the experimental animals revealed 37.37%, 49.84%, 47.32% and 46.31% for the cooked, cooked with addition of potash, fermented and sprouted seeds respectively. Hence, cooking with addition of potash showed higher PDCAAS value than the

remaining samples as a result of higher amino acid profile. However, the values for fermented and sprouted samples were higher than 45.72% of raw black beans [31]. It was also reported the PDCAAS of 0.500 and 0.534 (i.e. 50.0% and 53.4%) of split green peas and black beans respectively [32]. This implies that fermented and sprouted products are good for feeding children because the promotes faster growth.

5. CONCLUSION

Cooking with addition of potash revealed higher distribution of amino acids profile in the sample. However, addition of potash during cooking caused lowering of tryptophan than the seeds cooked without potash. The biological value, protein efficiency ratio and net protein utilization of samples cooked with potash were lower than the results obtained from the remaining samples. Experimental animals consumed larger quantity of the seeds cooked with potash, but they showed slower body weight gain. Sprouting and fermentation were more promising in terms of supporting growth of the experimental animals, hence they can be adopted for processing the seeds especially when meant for consumption by the children.

ETHICAL APPROVAL

All authors hereby declare that the principle of laboratory animals care (NHI publication number 829 revised 1985) were followed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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