

Running head: soy fortified sorghum biscuits for supplementary feeding

ORIGINAL ARTICLE

Rat bioassay of the protein nutritional quality of soy fortified sorghum biscuits for supplementary feeding of school-age children

Charlotte A. Serrem^{1,2}, Henriëtte L. de Kock¹, André Oelofse,³ John R. N. Taylor¹

¹Department of Food Science, University of Pretoria

South Africa

²Moi University, Kenya

³Centre for Nutrition, University of Pretoria

South Africa

Correspondence: Prof. J R N Taylor

Department of Food Science, University of Pretoria, Private Bag X20, Hatfield 0028,

South Africa

Tel +27 12 4204296, Fax +27 12 4202839

e-mail: john.taylor@up.ac.za

ABSTRACT

BACKGROUND: Protein-Energy Malnutrition (PEM) remains a major deficiency disease among children in developing countries. The protein nutritional quality of soy fortified sorghum biscuits was evaluated with respect to their potential as a protein-rich supplementary food. Three isonitrogenous diets based on: 50:50 ratio decorticated sorghum: defatted soy flour biscuits, 100% sorghum biscuits, casein, and a protein-free diet were fed to male Sprague Dowley weanling rats.

RESULTS: Protein Efficiency Ratio (PER) for the sorghum-soy biscuit diet was equivalent to the reference casein diet, and zero for the 100% sorghum diet. Faecal bulk for 100% sorghum diet was 1.5 times higher than sorghum-soy and casein diets. True protein digestibility of the three diets was high, 88 to 95%, agreeing with previous rat studies with sorghum. Biological Value and Net Protein Utilization of the sorghum-soy biscuit diet were similar to the casein diet, but lower than the 100% sorghum biscuit diet

CONCLUSION: Notwithstanding limitations of rat bioassay for assessing sorghum food protein quality, the high PER of defatted soy flour fortified sorghum biscuits (sorghum: soy 50:50 ratio) indicates they have considerable potential as a supplementary food to young children to alleviate PEM.

Key words: Protein Efficiency Ratio; Protein-Energy Malnutrition; rat bioassay; school-age children; sorghum; supplementary feeding.

INTRODUCTION

Protein-Energy Malnutrition (PEM) resulting from under-nutrition continues to be a major deficiency disease among children in Africa and other developing countries.^{1,2} The Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) epidemic has

aggravated the situation in sub-Saharan Africa, which accounts for 91% of new infections among children worldwide and has an estimated 14.1 million AIDS orphans,³ by increasing the number of children vulnerable to PEM. Sorghum, a widely cultivated dietary staple in the semi-arid and arid zones of Africa,⁴ could be used in protein-rich supplementary foods for children, such as legume fortified sorghum biscuits, to help alleviate PEM.

To help achieve this, we formulated biscuits made from sorghum flour fortified with defatted soy flour (DSF) at varying levels.⁵ Substituting sorghum with 50% DSF dramatically improved the protein quality of biscuits. For example, compared to 100% sorghum biscuits the protein content and in vitro protein digestibility of the 50:50 sorghum: DSF biscuits were 168%, and 170% higher. Lysine and reactive lysine contents increased by 221% and 257% per 100 g protein compared to the 100% sorghum biscuit. However, the most sensitive assessment of protein quality is achieved by clinical studies or animal assays that measure growth or metabolic indicators.⁶ Animal models are most commonly used because animals can be controlled more effectively than humans over long periods.⁷

Studies on sorghum protein quality using in vivo assays in both humans and animals have shown that the overall effect of cooking sorghum is reduction in digestibility and enlargement of faecal volume. For example, cooked whole grain sorghum fed to pre-school children in Peru did not support growth, had apparent digestibility of 46% and gave stool weight three times higher than the control casein diet.⁸ A reduction in True Digestibility (TD) and increase in Biological Value (BV) of products made from cooked, compared to uncooked sorghum foods was demonstrated using rat study.⁹ However, high apparent protein digestibilities of 81% and 74% were achieved when sorghum grain was decorticated and extruded¹⁰ and fermented,¹¹ respectively.

A problem with rat bioassay of sorghum foods is that it has been found that the young rat is more efficient in digesting sorghum protein than children. For example, with the same samples of sorghum, protein digestibility was 80% by rat bioassay¹², and as stated above, only 46% in a study with young children.⁸ However, the value of rat bioassay for assessing the protein quality of legume fortified foods is demonstrated by the fact this assay showed that heat treatment inactivates antinutrients in soy beans. They were found to have a TD of 51-60% when raw, and 78.3% after cooking¹³, indicating that rat bioassay does show differences in protein digestibility.

Notwithstanding possible drawbacks, the use of a rat model was deemed desirable in order to evaluate the efficacy of the formulated soy fortified sorghum biscuits as protein supplements to alleviate PEM. Therefore, the objective of this study was to determine the protein nutritional quality of soy fortified sorghum biscuits compared to unfortified sorghum biscuits using a rat bioassay.

MATERIALS AND METHODS

Formulation and preparation of biscuits

Two types of sorghum biscuits were used in this study, one made of 100% sorghum and the other with 50:50 sorghum: DSF. The soy fortified sorghum biscuit was formulated to provide 7 g protein in one biscuit of 28 g. Two such biscuits could provide 50% of the required dietary intake of protein for 3 to 10 year olds. The biscuit basic formulation comprised 225 g sorghum flour (decorticated red, non-tannin sorghum (Nola Mabela, Tiger Brands, Potchefstroom, South Africa), 56 g sugar, baking powder, 66 g sunflower oil, 80 ml water and 13.5 g vanilla essence.⁵ In the soy fortified biscuits, DSF was substituted for sorghum flour at a 50:50 ratio and water was increased 120 ml to produce a workable dough. Sorghum

flour was milled to a particle size of not more than 500 µm using a laboratory hammer mill. Biscuits were prepared as described.⁵ After preparation, biscuits were stored at 10°C until required.

Diet preparation

The biscuits were pulverized using a Waring Commercial® laboratory blender (New Hartford, CT), set at medium speed for 2 minutes. Three isonitrogenous diets were prepared from the two types of sorghum biscuits and from Animal Nutrition Research Council Reference Casein (ANRC) (calcium caseinate, Fonterra, New Zealand), to provide 8% crude protein (N x 6.25) in the final diet, dry weight basis. The percentages of ingredients in the diet formulation were calculated based on the composition of the biscuit samples (Table 1). The casein diet was the reference. The biscuits and casein were incorporated into the basal diet (Table 2) at the expense of corn starch-sucrose mixture (1:1 ratio). The diets also contained 1% vitamin and 5% mineral fortification mixes, both from ADVIT Animal Nutrition (Kempton Park, South Africa). A basal (protein-free) diet was prepared in which the test food was replaced by the corn starch-sucrose mixture in order to estimate the endogenous nitrogen excretion by the rats. The oil content in all four diets was adjusted to 9% using sunflower oil. The formulations of the diets are shown in Table 2.

Animals and housing

Twenty four weanling male Sprague Dowley rats (South African Vaccine Producers, Johannesburg, South Africa), four to five weeks old, weighing 80 to 120 g were used in this study. The study was conducted at the Biomedical Research Centre, Onderstepoort, University of Pretoria, according to AOAC¹⁴ method 960-48, with modifications. Approval by the University of Pretoria Faculty of Natural and Agricultural Sciences Ethics Committee was granted for this study. Maintenance of animals was conducted in accordance with the US

National Research Council Guide for the Care and Use of Laboratory Animals.¹⁵ The animals were housed individually in stainless steel metabolic cages (Tecniplast®, Tecniplast Group, Varese, Italy) equipped to separate urine and faeces. Temperature was maintained at 21 to 24°C with alternate periods of light and dark of 12 hours and relative humidity between 40 and 70%.

Growth study

The animals were fed on standardized laboratory irradiated rat pellets (EPOL, Pretoria, South Africa) for an initial acclimatization period of 7 days during which rats gained a mean weight of 40 g across groups, showing that they were in positive nitrogen balance. The animals were then distributed randomly into 4 groups of 6 rats each, with the mean weight of each groups not differing by more than 5 g. One group of rats was fed the protein-free diet, another, the casein, and the third and fourth groups the 100% sorghum biscuit and 50:50 sorghum: DFS biscuit diets, respectively. Food and sterilized water were provided ad libitum for the entire study period. Records of food intake for each rat were maintained daily. Rats were weighed on alternate days. The growth study lasted 28 days. The Net Protein Ratio (NPR) study lasted days 1 to 10 of the growth study, Protein Efficiency Ratio (PER), days 8 to 13 and the protein digestibility study, days 5 to 9. The rats fed the protein-free diet were euthanized on day 10, after the NPR and protein digestibility studies, and the remaining three groups on day 28 after the growth study. Euthanasia was performed by placing the rats in an inhalation chamber containing a high dose of anaesthetic (isoflurane). NPR and PER were calculated.¹⁶ PER and NPR were computed for 5 days (days 8 to 13 of the study) because the Sprague Dowley rats on the casein diet unexpectedly lost weight after the 13th day, possibly due to nutrient deficiencies and imbalances. In rat bioassay, it has been found that the effect of imbalances can be considerable in diets that contain sub-optimal concentrations of protein, as with the

8% protein in these diets, and the immediate response is decreased food intake¹⁷. The problem may also have been exacerbated by the fact that due to the rats having to be rehabilitated, the measurement of PER only commenced when they were 63 days old. Bender *et al.*¹⁸ showed that TD of cooked haricot beans with 5, 10 and 20% protein was 80, 74 and 67% , respectively in 23 day old rats, but reduced to 63, 55 and 51% , respectively in 63 day old rats.”

Protein digestibility study

The protein digestibility study lasted 5 days, days 5 to 9 of the growth study. During the study, the food consumed was calculated from the food supplied minus the weight of uneaten food daily. Faeces for each rat were collected into polyethylene coated paper bags daily, as was urine which was collected into plastic containers containing 1 ml 0.5 M sulphuric acid. Faeces and urine were frozen at -20° C until required.

Protein quality indices

The protein quality indices calculated from the data collected included PER, NPR, Net Protein Utilization (NPU), TD, BV and apparent protein digestibility, as described by WHO⁷ and FAO/WHO.¹⁶

Chemical analyses

The total faeces from each rat were dried overnight at 100° C in an air circulation oven, weighed, pulverized using a laboratory blender and faeces from six rats fed the same diet were pooled. The urine samples from each rat were pooled and freeze dried. Nitrogen in the faeces and urine was determined by Dumas combustion method 46-30¹⁹ and protein content (N x 6.25) calculated. The moisture content of the food and faeces was determined by a one stage air oven procedure, method 44-15A.¹⁹ Faecal and urine nitrogen of rats fed the protein-

free diet was used to calculate endogenous nitrogen loss. Six individual values per diet for TD, BV and NPU were computed from nitrogen intake, faecal nitrogen, urinary nitrogen and endogenous faecal and urinary nitrogen.¹⁶ Apparent digestibility, BV and NPU were also computed excluding the endogenous nitrogen.

Protein Digestibility Corrected Amino Acid Score (PDCAAS) determination

The PDCAAS is the official method for predicting protein quality for food based on human amino acid requirements.⁶ The parameters considered are essential amino acid profile of the test protein, its digestibility and ability to supply the amino acid in sufficient quantity.⁷ Indispensable amino acid profiles for the biscuits were determined using the Pico-Tag method²⁰ by acid hydrolysis, separation and quantification by reverse phase HPLC. True digestibility values were used to compute the PDCAAS using the following equation⁷:

$$\text{PDCAAS} = \text{True Faecal N Digestibility} \times \text{Lysine score (or the amino acid with the lowest ratio)}$$

Amino acid scores for indispensable amino acids were computed using a reference pattern for humans to establish suitability of the diet for pre-school and school age children.⁷

Statistical analysis

Chemical analysis of each sample was repeated three times. Results are presented as mean values and standard deviations. Data was subjected to one-way analysis of variance (ANOVA) using Statistica Software version 8.0 (Statsoft, Tulsa, OK). Means were separated using Fisher's Least Significant Difference test (LSD).

RESULTS AND DISCUSSION

Growth study

After acclimatization for 7 days when the three groups of animals were fed the experimental diets of casein, 100% sorghum, and 50:50 sorghum-soy containing only 8% protein and one group the protein-free diet for 6 days, they all lost weight. The 8% level of protein for the experimental diet was chosen because the 100% sorghum biscuit protein content was lower than 10% therefore to prepare isonitrogenous diets that were comparable, 8% protein was used. The weight loss may be explained by the lower protein content of 8% of the experimental diet to which rats had to adjust, compared to the 18% protein content of the laboratory diet. The laboratory diet was reintroduced for 7 days to rehabilitate them, and the rats gained a mean weight of 41 g, ranging from 37 g to 45 g between groups.. Consequently, when the 28 day growth study was started, the animals on the casein diet had approximately 15 g higher mean weight than the two biscuit diet groups because they lost less weight than the other three groups. When the 7 day rehabilitation diet was introduced, all the rats gained weight but the casein diet fed rats were already heavier than the other three groups. The four groups of animals all lost weight again. On day 10 the protein-free diet fed rats were euthanized. On day 8 the groups fed the casein, 100% sorghum biscuit and 50:50 sorghum: DFS biscuit diets started gaining weight. However, on day 14 the casein diet fed rats started losing weight again and this trend continued until the last day of the study, so that after 20 days the group had lost 21 g in weight (Table 3). This weight loss may have been caused by deficiency of a nutrient with these larger rats or an imbalance of amino acids or toxic proportions of a specific nutrient as stated earlier,. However, the 50:50 sorghum: DFS diet fed rats steadily gained weight until day 28 of the growth study, with a group weight gain of 14 g. Consequently, PER and NPR were calculated using weight gain data from the 5 day period (days 8 to 13 of the growth study) when the casein diet fed rats gained weight, against days 1 to 5 of the study for the protein-free group for NPR.

The food intake of the protein-free diet group was only half of the other three diets and there was large weight loss of 19 g (Table 3). The low food intake was probably related to the absence of protein in the basal diet. Low protein diets result in reduced food intake, and protein deficiency in weanling rats, which causes reduced growth, muscular wasting, emaciation and death if sufficiently severe.²¹ In contrast, the rats fed the 50:50 sorghum: DFS biscuit diet gained weight, approx. 6 g, the same amount as the rats fed the reference casein diet over the 5 day study period. However, the rats fed the 100% sorghum biscuit diet did not gain weight over the 5 days. Thus, during the 5 day period used to compute PER and NPR, the rats fed the casein diet had a mean daily weight gain of 1.25 g, 50:50 sorghum: DFS 1.17 g, 100% sorghum 0.06 g and protein-free -3.80 g.

Table 3 shows that the actual PER of 50:50 sorghum: DFS biscuits, approx. 1.5, was the same as the PER of the reference casein. In contrast, the 100% sorghum biscuits had a PER of essentially zero. The Table further shows that during the 20 day period from days 8 to 28 of the growth study, the 50:50 sorghum: DFS biscuit diet fed rats gained 10% weight, as compared to the 100% sorghum biscuit diet fed rats that did not gain weight.

It is probable that the growth of the 50:50 sorghum DFS biscuit diet fed rats was due to much higher lysine content contributed by the soya. It has been shown that increasing the lysine content of a wheat gluten diet resulted in 56% higher growth rate with rats compared to pure gluten diet.²² Similarly, PERs for maize-soybean-cowpea-peanut composite weaning foods of 84-96% compared to casein²³, and up to 80% for bean-rice diets compared to 67% for a rice only diet²⁴ have been reported. Also, rats fed a diet of sorghum flour substituted with 46% bambara groundnut with 19% protein had the same growth rate as with a casein diet.²⁵ Also

as found here, other studies have shown that cereal only diets do not support growth. Mosha and Bennink²⁶ showed that a maize meal only diet fed to rats caused weight loss. In another study, children fed whole grain sorghum gruel showed a dramatic slowing of weight gain or weight loss of approximately 5.9 g/kg body weight per day.⁸ This was ascribed to inadequate quality and quantity of dietary protein.

Table 3 also shows that the 50:50 sorghum: DFS diet had the highest NPR. However, the NPRs of all three diets were quite similar, as a result of the large weight loss in the protein-free group. In contrast to PER, NPR takes into account the weight loss of the rats on the protein-free diet¹⁶. The NPR of the 50:50 sorghum: DFS diet was slightly higher than that of the casein diet, probably because of the slightly, but not statistically significantly, lower protein/food intake of the former group.

Digestibility study

The faecal nitrogen digestibility method used in this study is an internationally approved method.¹⁴ It has been used by several researchers working on food products fortified with legumes^{23,24,25,26} to establish differences in protein quality by rat bioassay. Some researchers have further used the protein free diet as described¹⁴ to determine endogenous nitrogen excretion^{24,25}, as was done in this study, and have obtained results. The faecal bulk for rats fed the 100% sorghum biscuit diet was 50% and 62% higher than for the casein 50:50 sorghum: DFS biscuit diets, respectively (Table 4). This can be attributed to the formation of enzyme-resistant starch and unavailable kafirin protein during thermal processing of sorghum, resulting in starch being recovered in the faeces of rats fed a sorghum diet²⁷. Similarly, children fed a sorghum diet were found to have stool volumes 2.5 times higher than the control casein fed group,⁸ and in another study stool weights for 10 to 15 year old boys doubled when sorghum replaced rice in their diets.²⁸

Faecal nitrogen, expressed as a percentage of nitrogen intake, for rats fed the 100% sorghum biscuit diet was some 57% and 29% higher than the casein and 50:50 sorghum: DFS biscuit diets, respectively (Table 4). The higher faecal nitrogen content may be accounted for by the unavailable sorghum endosperm proteins, the kafirins,²⁷ as discussed. Reduced digestibility of cooked sorghum proteins is attributed to disulphide mediated polymerization, making them less susceptible to attack by proteolytic enzymes.²⁹ In general, most faecal nitrogen is from undigested food, and the rest is from cells shed from intestinal mucosa and residues of digestive juices.³⁰ The faecal nitrogen loss of rats fed the 50:50 sorghum: DFS biscuit diet was also higher than the loss from rats fed the reference casein diet. This may be due to the unavailable sorghum endosperm protein because of the 50% sorghum in the biscuit. The soy in the diet may also have contributed to nitrogen excretion. Fairweather-Tait *et al.*³¹ comparing faecal nitrogen excretion from rats fed a casein control diet and a bean diet, attributed the higher nitrogen excretion of the bean diet to increased microbial activity in the intestines. In a similar study, the increase was ascribed to enhancement of mucosal cell turnover through consumption of a bean diet.¹⁸

Table 5 shows that apparent protein digestibility and TD of the casein, 100% sorghum biscuit and 50:50 sorghum: DFS biscuit diets were all high (82% and above) with only slight differences between diets. The slightly higher protein digestibility (4%) for the 50:50 sorghum: DFS biscuit diet compared to the 100% sorghum biscuit diet may be due to replacement of the less digestible kafirins with the more digestible soy proteins the globulins. As mentioned, sorghum substituted with bambara groundnut had higher apparent protein digestibility.²⁵

These results show that the rat is very efficient in its digestion of sorghum proteins, and is likely to have overestimated the true protein digestibility of 100% sorghum containing biscuits. In vitro protein digestibility showed that sorghum and sorghum-soy biscuits had digestibility of 30% and 81%, respectively.⁵ The pepsin digestion method used in the in vitro study agrees with the digestion values found with children for sorghum³². Axtell *et al.*¹² similarly reported high protein digestibility (81%) of whole grain sorghum gruel and concluded that the rat was more efficient than children in digesting sorghum protein. High TD of 87.5% was also reported for *ugali*, a stiff porridge, made from a low polyphenol (non-tannin) sorghum variety Dabar in a rat feeding trial.⁹ The high protein digestibility of sorghum found in this present study and other rat feeding studies contrast sharply with the finding of low sorghum protein digestibility using human subjects. Protein digestibility of 46% and nitrogen retention of 14% was reported for children fed whole grain sorghum gruels made from high lysine and normal cultivars.⁸ It was concluded that sorghum is a poor source of dietary protein for infants and children. Similarly, other workers have found apparent protein digestibilities of only 69% and 66% for sorghum roti and cooked decorticated sorghum diets, respectively consumed by pre-school children,³⁰ and 54% for a high sorghum diet consumed by teenage boys.²⁸

Table 5 also shows that the 100% sorghum biscuit diet had a higher BV than the casein and 50:50 sorghum: DFS biscuit diets, 16% and 31% higher, respectively. The 100% sorghum biscuit diet also had a 26% higher NPU than the 50:50 sorghum: DFS biscuit diet. The high BV and NPU for the 100% sorghum biscuit diet may be because microbial fermentation of uncooked starch in the sorghum biscuits may have changed the routes of nitrogen excretion from urine to the faeces.²⁷ Sorghum starch granules are encapsulated by hydrophobic cross-linked kafirins,³³ which do not absorb adequate water for expansion and probably remained

uncooked in the biscuits. In vivo, the resistant starch-kafirin complex is believed to pass through the small intestines undigested but is exposed to microbial degradation in the hind gut where resistant starch is used as substrate for energy and undigested kafirin as nitrogen source.²⁷ This effect was not as pronounced in the soy fortified biscuit diet (Table 5), presumably because the level of sorghum was only 50% that in the 100% sorghum diet. Similarly, it has been found that addition of raw potato starch to diets fed to rats increased BV and reduced digestibility.³⁴ It was suggested that less nitrogen was secreted in the urine and more in faeces due to fermentation of raw starch in the lower intestine and microflora obtaining nitrogen from urea diffusing from blood to the caecum and colon.

Protein Digestibility Corrected Amino Acid Score

Table 6 shows the quantities of the indispensable amino acids in the casein and experimental food products relative to reference patterns for 3 to 10 and 1 to 2 year old children⁷, PDCAAS indicates the effectiveness of a food product to meet an individual's protein needs. The 3 to 10 year old amino acid scoring pattern is recommended for judging protein quality for school children and adolescents.⁷

The casein diet had an amino acid pattern that is regarded adequate for both pre-school and school children. The 100% sorghum biscuit diet was highly deficient in the indispensable amino acid lysine with respect to the requirements of pre-school and school age children. As a consequence of this, the PDCAAS of the 100% sorghum biscuit diet were very much lower than the recommended minimum for school and pre-school children, respectively.^{7,35} Complementing sorghum with DSF increased the total amount of indispensable amino acids in biscuits including lysine, the first limiting amino acid.⁵ At the 50% DSF replacement level, the PDCAAS for sorghum biscuits improved more than 3-fold. In fact, the PDCAAS for the

50:50 sorghum: DFS biscuit diet was 0.87, substantially higher than the minimum score of 0.70 of casein as recommended by Codex Alimentarius Commission.³⁵

Researchers have conducted studies on other types of supplementary foods and used the PDCAAS index to provide information about the complementation potential of processed plant food protein sources. For instance, the PDCAAS of a rice-bean infant weaning food was found to be increased by 34% compared to beans alone.³⁶ Acceptable PDCAAS values ranging between 0.72 and 0.82 were reported for extruded cowpea-soy-maize foods for 2 to 5 year olds.²³ Composites of rice, maize, bean and sardine meals for pre-school children had values ranging from 0.77 to 0.90.²⁶

Conclusions

Although the rat is not an ideal model for the determination of the protein quality of sorghum food, it is clear that 50:50 sorghum: DFS biscuit has a protein nutritional quality that is similar to animal protein as measured by the parameter of PER. Hence, such soy fortified sorghum biscuits have the great potential for use as a protein supplementary food to alleviate PEM in young children.

Acknowledgements

Dr. Tamsyn Pulker and Ms Ilse Jansen Van Rensburg of Biomedical research Centre, Onderstepoort and Dr Janet Taylor of the Department of Food Science, University of Pretoria are gratefully acknowledged for their assistance in this study.

Charlotte Serrem acknowledges VLIR-OUS and Moi University for a scholarship.

REFERENCES

- 1 Walker AF, The contribution of weaning foods to Protein-Energy Malnutrition. *Nutr Res Rev* **3**:25-47 (1990).
- 2 Muller O, and Krawinkel M, Malnutrition and health in developing countries. *Can Med Assoc J* **173**:279-286 (2005).
- 3 Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO). Aids epidemic update. Accessed March 2010 at <http://www.unaids.org> (2009).
- 4 International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Crops: Sorghum. Accessed November 2009@ <http://www.icrisat.org/sorghum/sorghum.htm> (2009).
- 5 Serrem CA, De Kock HL, Oelofse A and Taylor JRN, Nutritional Quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. *Int J. Food Sci. Technol.* **46**:74-83 (2011)
- 6 Boutrif E, Recent developments in protein quality. *Food Nutr Agric* **2**:3 (1991).
- 7 World Health Organization (WHO), Protein and amino acid requirements in human nutrition: Report of a joint WHO/FAO/UNU Expert Consultation (2002). WHO Technical report series No.935. World Health Organization, Geneva, (2007).
- 8 MacLean WC, De Romana GL, Placko RP and Graham GG, Protein quality and digestibility of sorghum in preschool children: Balance studies and plasma amino acids. *J Nutr* **111**:1928-1936 (1981).
- 9 Eggum BO Monowar L, Bach Knudsen KE, Munk L, and Axtell J, Nutritional quality of sorghum and sorghum foods from Sudan. *J Cereal Sci* **1**:127-137 (1983).

- 10 MacLean WC, De Romana GL, Placko and Graham GG, The effect of decortication and extrusion on the digestibility of sorghum by preschool children. *J Nutr* **113**:2171-2177 (1983).
- 11 Graham GG, MacLean WC, Morales E, Hamaker BR, Kirleis AW, Mertz ET, and Axtell JD, Digestibility and utilization of protein and energy from nasha, a traditional fermented Sudanese weaning food. *J Nutr* **116**:978-984 (1986).
- 12 Axtell JD, Kirleis AW, Hassen MM, Mason ND, Mertz ET, and Munk L, Digestibility of sorghum proteins. *Proc Natl Acad Sci USA* **78**:1333-1335 (1981).
- 13 Vasconcelos IM, Maia AA B, Siebra EA, Oliveira JTA, Carvalho AFFU, Melo, VMM, Carlini CR, and Castelar LI, Nutritional study of two Brazilian soybean (*Glycine max*) cultivars differing in contents of antinutritional and toxic proteins. *J Nutr Biochem* **12**:55-62 (2001).
- 14 Association of Official Analytical Chemists International (AOAC), Method 960-48. Official Methods of Analysis of the Association of Analytical Chemists International, 17th edn. AOAC International: Gaithersburg, ML (2003)
- 15 National Research Council (NRC), Guide for the Care and Use of Laboratory Animals. National Academy of Sciences, Washington, (1993).
- 16 FAO/WHO, Protein quality evaluation. The report of the joint FAO/WHO Expert Consultation. FAO. Rome (1991).
- 17 Harper AE. Effects of disproportionate amounts of amino acids. Pg 138-166 in: Improvement of Protein Nutriture. A. E. Harper and D. M Hegsted, eds. National Academy of Sciences: Washington DC. (1974).
- 18 Bender AE, Mohammadiha H, and Almas K., Digestibility of legumes and available lysine content. *Plant Food Hum Nutr* **29**:219 (1979).

- 19 AACC International, Approved Methods of the American Association of Cereal Chemists, 10th edn. Methods 46-30, 44-15. The Association: St. Paul, MN (2000)..
- 20 Bidlingmeyer BA, Cohen SA and Tarvin TL, Rapid analysis of amino acids using pre-column derivatization. J. Chromatogr **336**:93-104 (1984).
- 21 National Research Council, Nutrient requirements of laboratory animals. National Academy of Sciences Press: Washington. Pp 11-58 (1995).
- 22 Ashley VM and Anderson GH, 1975. Food intake regulation in the weanling rat: Effects of the most limiting essential amino acids of gluten, casein, and zein on the self-selection of protein and energy. J Nutr **105**:1405-1411(1975)
- 23 Mensa-Wilmot Y, Phillips RD, and Hargrove JL, Protein Quality Evaluation of cow-pea based extrusion cooked cereal/legume weaning mixtures. Nutr Res **21**:849-857 (2001).
- 24 Joseph E and Swanson BG, Growth and nitrogen retention of rats fed bean (*Phaseolus vulgaris*) and bean and rice diets. Food Res Int **26**:261-269 (1993).
- 25 Nnam NM, Comparison of the protein nutritional value of food blends based on sorghum, bambara groundnut and sweet potatoes. Int. J Food Sci Nutr **52**:25-29 (2001).
- 26 Mosha TCE, and Bennink MR, Protein quality of drum-processed cereal-bean-sardine composite supplementary foods for pre-school age children. J Sci Food Agric **84**:1111-1118 (2004).
- 27 Bach Knudsen KE, Kirleis AW, Eggum BO and Munck L, Carbohydrate composition and nutritional quality for rats of sorghum tô prepared from decorticated white and whole grain red flour. J Nutr **118**:588-597 (1988).
- 28 Kurien PP, Narayanao M, Swaminathan M, and Subramanyan V, The metabolism of nitrogen, calcium and phosphorus in undernourished children; the

- effect of partial or complete replacement of rice in poor vegetarian diets by kaffir corn (*Sorghum vulgare*). Br J Nutr **14**: 339-345 (1960).
- 29 Duodu KG, Taylor, JRN, Belton PS, and Hamaker BR, Mini review: Factors affecting sorghum protein digestibility. J. Cereal Sci. **38**:17-131 (2003).
- 30 Kavithaparna S, Geervani P, and Sumathi S, Digestibility and retention of proteins of sorghum products by preschool children and adults. Nutr Rep Int **38**:1231-1238 (1988).
- 31 Fairweather-Tait SJ, Gee JM and Johnson IT, The influence of cooked kidney beans (*Phaseolus vulgaris*) on intestinal cell turnover and faecal nitrogen excretion in the rat. Br J Nutr **49**:303-312 (1983).
- 32 Mertz ET, Hassen MM, Cairns-Whittern C, Kirleis AW, Tu L, and Axtell JD. Pepsin digestibility of proteins in sorghum and other major cereals. Proc. Nat. Acad. Sci. USA **81**:1-2 (1984).
- 33 Ezeogu LI, Duodu KG, Emmambux MN and Taylor JRN, Influence of cooking conditions on the protein matrix of sorghum and maize endosperm flours. Cereal Chem **85**: 397-402 (2008).
- 34 Beames RM, and Eggum BO, The effect of type and level of protein, fibre and starch on nitrogen excretion patterns in rats. Br J Nutr **46**:301-313 (1981).
- 35 FAO/WHO, Codex Alimentarius: Foods for special dietary uses (including foods for infants and children). Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Vol 4, 2nd edn, FAO, Rome (1994).
- 36 Kannan S, Nielsen SS, and Mason AC, Protein Digestibility-Corrected Amino Acid Scores of beans and bean-rice infant weaning food products. J. Agric Food Chem **49**:5070-5074 (2001).

37 Rutherford SM and Moughan PJ, The digestible amino acid composition of several milk proteins: Application of a new bioassay. *J Dairy Sci* **81**:909-917 (1998).

Table 1 Proximate composition (g kg⁻¹) of the biscuit samples (100% sorghum biscuits, 50:50 sorghum:defatted soy flour biscuits) and reference protein (casein)

	Protein (N x 6.25)	Fat	Moisture	Carbohydrates by difference	Ash	Crude fibre
Casein	901.0	10.0	41.0	1.0	47.0	0.0
100% sorghum biscuits	92.3	210.0	3.25	635.0	13.9	16.1
50:50 sorghum: defatted soy flour biscuits	247.1	196.8	3.82	456.0	27.8	36.6

Table 2: Formulations of the four experimental diets (g kg⁻¹ dry basis)

Ingredients	Diets			
	Basal (protein-free)	Casein (reference)	100% sorghum	50:50 Sorghum: soy
Casein ¹	0	90.0	0	0
100% sorghum biscuits	0	0	850.0	0
50:50 sorghum: defatted soy flour biscuits	0	0	0	332.0
Sunflower oil ²	90.0	90.0	90.0	90.0
Mineral mixture ³	50.0	50.0	50.0	50.0
Vitamin mixture ⁴	10.0	10.0	10.0	10.0
Cellulose powder ⁵	10.0	10.0	0	10.0
Corn starch ⁶	420.0	375.0	0	254.0
Sucrose	420.0	375.0	0	254.0
Total	1000	1000	1000	1000

¹Casein – New Zealand Milk Products (Clover Fonterra, Johannesburg, South Africa); ²Sunflower oil “Sunfoil” (Willowton Oil, Pietermaritzburg, South Africa); ³Mineral mixture and ⁴Vitamin mixture composition according to AOAC method 960.48 (ADVIT Animal Nutrition S. A. (Pty) Ltd, BASF, Kempton Park, South Africa); ⁵Cellulose powder (W& R, Balston Ltd. England); ⁶Corn starch (Tongaat Hulett, Johannesburg, South Africa).

Table 3 Growth of rats, Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) for consumption of 100% sorghum and 50:50 sorghum: soy biscuit diets.

Indices	Diet groups			
	Basal (protein-free)	Casein (reference)	100% Sorghum	50: 50 sorghum: soy
Initial weight (g)	142.00 ^a ± 8.60	164.25 ^b ± 13.65	145.00 ^a ±17.58	143.83 ^a ±10.00
Weight after 5 day experimental period (g)	123.00 ^a ± 7.91	170.50 ^c ±15.06	145.33 ^b ±18.20	149.67 ^b ±9.20
Weight gain after 5 day experimental period (g)	-19.00 ^a ± 2.00	6.25 ^c ± 5.51	0.33 ^b ±1.50	5.83 ^c ±3.63
Weight after 20 days (g)	112.50 ^a ±7.53**	143.25 ^b ±12.09	143.50 ^b ±16.4	157.83 ^c ±11.27
Weight gain after 20 days (g)	-29.5±2.60**	-21.00 ^a ± 11.21	-1.50 ^b ±3.21	14.00 ^c ±3.32
Food intake 5 day experimental period (g)	24.57 ^a ± 3.50	54.55 ^b ±8.35	49.54 ^b ± 1.88	48.53 ^b ± 5.14
Protein intake (g)	nd	4.36 ^a ±0.67	3.96 ^a ±0.15	3.88 ^a ±0.41
PER	nd	1.43	0.08	1.50
Relative PER (%)	nd	100*	5.17	99.79
NPR	nd	5.79	4.42	6.40

Values are means±standard deviations for 6 rats. Values in a row followed by different letter superscripts are significantly different at P≤0.05 as assessed by Fisher's least significant difference test. NPR values all negative so considered zero. nd= not determined. *= values of 2.5 and 100% are assumed values for casein. **=weight and weight loss for protein-free diet fed rats for 9 days.

Table 4 Effect of consumption by rats of 100% sorghum and 50:50 sorghum: soy biscuit diets on food and protein intake, protein output and retention

Indices	Diet groups			
	Basal (protein free)	Casein (reference)	100% sorghum	50:50 sorghum: soy
Food intake (g)	24.57 ^a ± 3.50	54.55 ^b ±8.35	49.54 ^b ± 1.88	48.53 ^b ± 5.14
Protein intake (g)	nd	4.36 ^a ±0.67	3.96 ^a ±0.15	3.88 ^a ±0.41
Faecal excretion (g)	1.62 ^a ±0.26	3.14 ^b ±0.37	4.70 ^c ±0.57	2.89 ^b ±0.32
Faecal nitrogen (N x 6.25) output (g)	0.23 ^a ± 0.04	0.46 ^b ± 0.05	0.72 ^d ± 0.09	0.56 ^c ± 0.06
Faecal nitrogen (%)	nd	5.25 ^a ±1.19	12.25 ^c ±1.87	8.64 ^b ±2.03
Urine excretion (g)	15.25 ^a ±8.05	20.00 ^{ab} ±2.90	16.00 ^{ab} ±4.08	22.60 ^b ±2.97
Urine nitrogen (N x 6.25) output (g)	0.72 ^a ±0.42	1.21 ^b ±0.17	0.68 ^a ±0.71	1.29 ^b ±0.17
Urine nitrogen (%)	nd	11.58 ^b ±5.04	-1.11 ^a ±4.2	14.92 ^b ±4.81
Protein (N) Balance (g)	nd	3.65 ^a ±0.74	3.52 ^a ±0.24	2.98 ^a ±0.52

Values are means±standard deviations based on data for 6 rats for 5 days (day 4 to 8). Values in a row followed by different letter superscripts are significantly different at $P \leq 0.05$ as assessed by Fisher's least significant difference test. nd= not determined. Food and protein intake, and faecal excretion and faecal nitrogen output (dry basis). Urine excretion (as is).

Table 5 Indices of protein quality for 100% sorghum and 50:50 sorghum: soy biscuits as measured by rat bioassay

Indices	Diet groups		
	Casein (reference)	100% Sorghum	50:50 sorghum: soy
Apparent protein digestibility (%)	89.4 ^c ±1.6	81.9 ^a ±1.8	85.4 ^b ±2.5
True protein digestibility (TD) (%)	94.8 ^c ±1.2	87.8 ^a ±1.9	91.4 ^b ± 2.0
Apparent protein Biological Value (BV) (%)	68.2 ^a ±8.0	79.1 ^b ±5.5	60.4 ^a ±8.0
True protein BV (%)	89.5 ^a ±5.2	103.3 ^b ±4.8	85.6 ^a ±5.2
Apparent Net Protein Utilization (NPU) (%)	61.0 ^{ab} ±7.1	64.9 ^b ±5.4	51.7 ^a ±8.0
True NPU (%)	84.8 ^{ab} ± 4.9	90.6 ^b ± 5.5	78.3 ^a ± 5.8

Values are means±standard deviations for 6 rats fed for 5 days (days 4 to 8). Values in a row followed by different letter superscripts are significantly different at $P \leq 0.05$ as assessed by Fisher's least significant difference test.

Table 6: Comparison of amino acid composition (mg/g protein) of diet protein sources with WHO⁷ requirement pattern for pre-school and school children

Amino acid	Diet protein sources			WHO ^b	WHO ^c
	Casein ^a	100% sorghum	50:50 sorghum: soy		
Lysine	73	14	45	48	52
Leucine	92	127	83	61	63
Phenylalanine + tyrosine	102	84	83	41	46
Valine	64	47	48	40	42
Tryptophan	16	12	40	6.6	7.4
Methionine + Cysteine	33	28	58	24	26
Threonine	39	21	28	25	27
Histidine	33	19	24	16	18
Isoleucine	50	38	45	31	31
Total	502	380	415	293	312
True protein digestibility (%)	94.75	87.75	91.36		
Lysine score (3-10 yrs)	1.52	0.29	0.95		
PDCAAS ^d (3-10 yrs)	1.0	0.26	0.87		
Limiting amino acid (3-10 yrs)	None	Lysine	Lysine		
Lysine score (1-2 yrs)	1.40	0.26	0.87		
PDCAAS (1-2 yrs)	1.0	0.24	0.80		
Limiting amino acid (1-2 yrs)	None	Lysine	Lysine		

^aCasein amino acid profile as determined for calcium caseinate of New Zealand Milk Board.³⁷

^bAmino acid reference pattern for children aged 3 to 10 years.⁷

^cAmino acid reference pattern for children aged 1 to 2 years.⁷

^d.Protein digestibility-corrected amino acid score (PDCAAS) based on lysine score.