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Research Article

Rat bioassay for evaluation of protein nutritional quality of wheat and wheat-sorghum biscuits fortified with longhorn grasshopper (*Ruspolia differens*) powder

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Abstract: Protein-energy malnutrition (PEM) is a persistent public health challenge in most developing countries. This study investigated the protein nutritional value of wheat and wheat-sorghum biscuits fortified with longhorn grasshopper (*Ruspolia differens*) to determine their suitability as a supplementary food. Fourteen diets were fed to male weanling Sprague-Dawley rats, including 11 isonitrogenous diets with 10% protein prepared from 10 biscuit variations and skimmed milk powder as a reference, 1 protein-free diet, and 2 rehabilitation diets made with wheat and wheat-sorghum biscuits fortified with 40% *Ruspolia differens* powder (RDP). The protein efficiency ratio, food efficiency ratio, true and apparent protein digestibility, and net protein retention ratio results for the fortified biscuit diets were all negative, with the exception of wheat biscuits supplemented with RDP 40%. The isonitrogenous diets maintained the rats with no substantial weight gain or loss. Rats on rehabilitation diets gained weight rapidly, by 61% and 69% for wheat-sorghum and wheat diets, respectively. Weight increase during rehabilitation was considerably higher ($P < 0.05$) by 50% and 54.6% in wheat-sorghum and wheat diet groups compared to normal growth phases. The rehabilitated rats had a greater percentage of body weight than the experimental groups. The protein digestibility-corrected amino acid score (PDCAAS) of fortified biscuits ranged from 26% to 33% for wheat biscuits and 22% to 32% for wheat-sorghum biscuits. The digestible indispensable amino acid score (DIAAS) of the fortified biscuits varied from 47% to 60% for wheat biscuits and 44% to 60% for wheat-sorghum biscuits. As a result, the fortified biscuits fell short of the minimum requirements of 70% for PDCAAS and 75% for DIAAS in fortified protein diets. Furthermore, it appears that substituting 10% RDP to wheat-sorghum and wheat biscuits does not promote rat growth. Fortification with 40% RDP, on the other hand, dramatically improves rat's growth and rehabilitation. Because of their high protein nutritional content, the biscuits fortified with 40% RDP could be used as a supplementary to help rehabilitate malnourished children.

Keywords: biscuit; rat bioassay; wheat; sorghum; fortification; protein efficiency ratio; protein-energy malnutrition

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1 Introduction

Protein-energy malnutrition (PEM) resulting from undernutrition is a severe public health burden in many resource limited countries[1]. Undernutrition is implicated for poor health and up to 45% mortality in children under the age of 5 years[2]. It is also associated with limited cognitive development, delayed motor abilities, and lower educational attainment among young children[3]. Food-to-food fortification of commonly consumed cereals with easily available foods high in protein has been suggested as one of the sustainable strategies for addressing undernutrition in developing nations[4–5]. Sorghum, an important cereal in the diets of young children in developing countries has low protein quality[6]. However, it can be processed into different food products such as wheat-sorghum biscuits fortified with high protein foods, making it a potential food vehicle for delivery of quality proteins to alleviate PEM in young children[7–8].

In order to accomplish this, biscuits were developed from wheat and wheat-sorghum flours fortified with *Ruspolia differens* powder (RDP) at varying quantities[9]. They were considered important as supplementary foods to alleviate PEM as they showed improved protein, reactive lysine contents and increased *in vitro* protein digestibility[9]. Fortification of the biscuits with RDP improved the protein content by 20% to 118% and 15% to 116% in wheat-sorghum and wheat biscuits, respectively. The *in vitro* protein digestibility improved by 14.6% to 42.5% in wheat-sorghum biscuits and 8.6% to 65.8% in wheat biscuits while the reactive lysine content increased by 25.4% to 218.6% in wheat-sorghum and 27.3% to 291% in wheat biscuits respectively compared to the control biscuits. This improvement was attributed to the longhorn grasshopper which is an edible insect found along the Lake Victoria region of East Africa[10]. It is rich in proteins ranging between 34.2%–45.8%[11–12] with amino acid scores of between 0.8 and 2[11], which are sufficient for the daily needs of children based on the human amino acid reference standard established by Food and Agriculture Organization of the United Nations (FAO)[11]. Due to these nutritional qualities, the use of the insect protein in the formulation of high energy and protein supplementary foods to alleviate PEM in young children has been suggested[10,13–14]. For instance, researchers who developed an extruded composite flour enriched with RDP found that the quantities of protein and energy sufficiently met the needs of fast-growing infants and young children[13]. In another study, cookies fortified with blanched, boiled and toasted RDP were shown to be richer in proteins and amino acids than the unfortified cookies[15].

The protein content of a food may not effectively indicate how beneficial the protein is for growth and development, particularly in young children[16]. The use of clinical studies among humans is best but animal assays are recommended as the most suitable alternative method in determining protein quality specifically for children's food products[17]. Animal models such as rats are commonly used because they can be controlled more efficiently than humans over longer periods of time, and the results can be extrapolated to human requirements[17]. These assays have been used to assess food protein quality, depending either on the ability to promote growth in young rats (protein efficiency ratio (PER)) or nitrogen retention (net protein utilization)[18]. The computation is corrected by the ileal or fecal protein digestibility in the protein digestibility-corrected amino acid score (PDCAAS), or the ileal amino acid digestibility in the digestible indispensable amino acid score (DIAAS)[19].

Though edible insects have been shown to have high protein quality, amino acid composition, and high *in vitro* protein digestibility[20–21], there is need to assess their protein quality based on metabolic and growth indicators[22]. Such studies have been conducted by several researchers. For instance, a clinical study in which infants were fed on cereal porridge fortified with caterpillar powder reported no effect on stunting, wasting and underweight[23]. *In vivo* rat assays further demonstrate variations in insect protein digestibility depending on species, stage of development of the insect[22,24], degree and techniques of processing[25]. Reduction of nitrogen retention in rats fed on diets formulated with oven-cooked or autoclaved insects was attributed to negative modification following heat treatment[25]. Insect processing can result in Maillard glycation, thereby decreasing the protein digestibility of insect powder *in vivo*[26]. In addition, adult insects from classes such as Blattodea and Orthoptera have been reported to have low digestibility[20,27]. For example, locust which belongs to Orthoptera enhanced nitrogen excretion[27] and was resistant to gut pepsin digestion[28] when fed to rats. This is attributed to high chitin content in the locust, which rats are unable to digest. Consequently, determining the *in vivo* protein digestibility of edible insect protein would show differences in the protein quality of fortified products. Despite their high protein content, it was considered necessary to use a rat bioassay to evaluate the efficacy of the formulated RDP-fortified wheat-sorghum and wheat biscuits as protein supplements to alleviate PEM. Therefore, the objective of this study was to determine the protein nutritional quality and the effect on growth of wheat and wheat-sorghum biscuits fortified with RDP compared to unfortified biscuits using a rat bioassay.

2 Materials and methods

2.1 Formulation and preparation of biscuit samples

The concept behind developing the biscuit for school-aged children was drawn from a previous study[8] intending to fulfill approximately half of the protein needs for school going children aged 3 to 10 years. According to the acceptable macronutrient distribution range (AMDR) recommended for this age group to prevent PEM, the protein and energy requirement ranges from 10 to 30 g of protein per day[29]. Hence, the study aimed at providing a minimum of almost half that amount, that equates to 13 g of protein per day, with each biscuit containing 6.5 g of protein. Based on the results of proximate analysis, preliminary computations were made to establish formulations of both the RDP and the cereal flours that would provide 6.5 g of protein in each biscuit.

Ten types of biscuits were used in this study. These included 5 wheat and 5 wheat-sorghum formulations fortified with RDP at 0%, 5%, 15%, 25%, and 40%. The basic ingredients for the 100% wheat and wheat-sorghum (50:50, *m*/*m*) were 100 g of flour, 25 g of sugar, 30 g of sunflower oil, and 6 g of vanilla essence. Water was dependent on the treatment and ranged from 17.8% (40% RPD + 60% wheat biscuits) to 27.1% (40% RPD + 60% wheat-sorghum biscuits) of total weight of ingredients. The biscuits were stored in airtight plastic containers in the freezer at −18 °C until required. The method of preparing and the nutritional composition of the biscuits has been described elsewhere[9].

2.2 Diet formulation

The diets utilized in the present study were developed using the Association of Official Analytical Chemists (AOAC) International method 960.48[30] with modifications. The final diets formulated had 10% crude protein by dry weight since the biscuit with the lowest protein content (wheat-sorghum biscuit with 0% RDP) had 11.60 g/100 g, which was more than 10%. The biscuits used in the diets were milled at medium speed for 3 min in a high-powered mill (Kenwood Chef KMC 200; Kenwood Co., Ltd., Havant, UK). The percentages of ingredients in the diets were determined from the proximate compositions of the biscuits and skimmed milk powder (Miksi, Promasidor (Kenya) Ltd., Nairobi, Kenya) (Table 1).

This study involved the preparation of 14 different diets. Eleven diets were isonitrogenous, including 10% protein. The first 10 were developed using wheat and wheat-sorghum biscuits supplemented with 0%, 5%, 15%, 25%, and 40% RDP. The 11th was a reference diet made from skimmed milk powder, as described by Chapman et al.[31]. In order to achieve a 100% diet composition, biscuits and skimmed milk powder were used instead of the basal diet's 1:1 corn starch-sucrose mixture (Table 2). The 12th, baseline (protein-free) diet, designed to assess the rats' endogenous nitrogen excretion, was made by replacing the test food with a corn starch-sucrose mixture. The 13th and 14th diets were prepared for rehabilitation with the goal of providing 20% protein for catch-up growth using wheat and wheat-sorghum biscuit diets fortified with 40% RDP (the highest RDP level), in accordance with formulations for fortified complementary foods blends for young children[32]. All of the diets were designed to supply sufficient nourishment through the incorporation of 1% vitamins, 1% cellulose, 5% minerals, and 9% fat content adjusted using corn oil. To formulate each diet, all of the dry ingredients were mixed together for exactly 10 min in a Kenwood food mixer (Kenwood Chef KMC200; Kenwood Co., Ltd., Havant, UK) set to moderate speed to ensure even distribution. The oil was then added and mixed for another 10 min. Each diet was kept in a separate zip-lock polyethylene bag in the refrigerator at 4 °C until used. Before feeding, 15 g of dry feed from each diet was mixed with 5 g of distilled water to moisten the feed and make it less difficult for the rats to consume.

Table 1 Proximate composition of wheat and wheat-sorghum biscuits fortified with *Ruspolia differens* powder (RDP) and skimmed milk powder (g/100 g).

Group	Moisture	Protein	Fat	Ash	Crude fiber	Carbohydrate
Wheat-RDP biscuits						
0% RDP	6.41 ± 0.41^{d}	12.97 ± 0.19^{i}	19.30 ± 0.90^{h}	0.76 ± 0.12^{g}	0.84 ± 0.00^{i}	59.72 ± 0.78^{b}
5% RDP	4.31 ± 0.12^{ef}	14.90 ± 0.38^{g}	20.97 ± 0.57^{g}	0.81 ± 0.11^{g}	1.38 ± 0.11^{h}	57.64 ± 0.92^{c}
15% RDP	4.08 ± 0.00^{fg}	18.27 ± 0.32^{e}	23.87 ± 0.48^{d}	0.96 ± 0.11^{fg}	2.58 ± 0.12^{e}	50.25 ± 0.29^{e}
25% RDP	3.73 ± 0.00^{gh}	25.13 ± 0.21^{c}	24.66 ± 0.21^{b}	1.16 ± 0.19^{ef}	3.51 ± 0.28^{d}	41.80 ± 0.52^{g}
40% RDP	3.23 ± 0.12^{i}	28.01 ± 0.23^{b}	26.38 ± 0.31^{a}	1.28 ± 0.23^{de}	5.65 ± 0.21^{b}	35.47 ± 0.69^{i}
Wheat-sorghum-RDP biscuits						
0% RDP	4.56 ± 0.00^{e}	11.60 ± 0.49^{j}	23.27 ± 1.41^{e}	1.06 ± 0.12^{ef}	1.64 ± 0.11^{g}	57.87 ± 1.31^{c}
5% RDP	4.48 ± 0.00^{e}	13.91 ± 0.31^{h}	23.27 ± 0.11^{e}	1.15 ± 0.11^{eg}	2.21 ± 0.00^{f}	54.97 ± 0.32^{d}
15% RDP	3.84 ± 0.00^{gh}	17.44 ± 0.52^{f}	23.87 ± 0.48^{d}	1.23 ± 0.11^{e}	3.51 ± 0.00^{d}	50.41 ± 0.48^{e}
25% RDP	3.54 ± 0.00^{hi}	20.33 ± 0.69^{d}	23.93 ± 0.10^{cd}	1.46 ± 0.10^{cd}	4.30 ± 0.12^{c}	46.44 ± 0.78^{f}
40% RDP	3.17 ± 0.12^{i}	25.29 ± 0.71^{c}	24.47 ± 0.11^{bc}	1.77 ± 0.21^{b}	5.60 ± 0.01^{b}	39.70 ± 0.82^{h}
Skimmed milk powder	3.00	20.00	30.00	5.50	0.00	41.50

Note: The results were expressed as mean ± standard deviation; values with the different superscript letter in the same column indicated significantly differences at $P < 0.05$ as assessed by Fisher's least significant difference.

Table 2 Formulation of the experimental diets (g/kg dry basis).

Ingredients	Wheat-RDP biscuits					Wheat-sorghum-RDP biscuits					Reference diet	Basal diet	Rehab diet-WH	Rehab diet-WS
	0%	5%	15%	25%	40%	0%	5%	15%	25%	40%				
100% WH + 0% RDP	616.80		0	0	0	0	0	0	0	0	0	0	0	0
95% WH + 5% RDP	0	536.90	0	0	0	0	0	0	0	0	0	0	0	0
85% WH + 15% RDP	0	0	437.88	0	0	0	0	0	0	0	0	0	0	0
75% WH + 25% RDP	0	0	0	318.34	0	0	0	0	0	0	0	0	0	0
60% WH + 40% RDP	0	0	0	0	285.62	0	0	0	0	0	0	0	714.04	0
100% WS + 0% RPD	0	0	0	0	0	689.66	0	0	0	0	0	0	0	0
95% WS + 5% RDP	0	0	0	0	0	0	575.14	0	0	0	0	0	0	0
85% WS + 15% RDP	0	0	0	0	0	0	0	458.72	0	0	0	0	0	0
75% WS + 25% RDP	0	0	0	0	0	0	0	0	393.52	0	0	0	0	0
60%WS + 40% RDP	0	0	0	0	0	0	0	0	0	316.32	0	0	0	790.82
Skimmed milk powder	0	0	0	0	0	0	0	0	0	0	400	0	0	0
Corn oil	90	90	90	90	90	90	90	90	90	90	90	90	90	90
Mineral mix	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Vitamin mix	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Cellulose	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Sucrose	111.60	151.55	201.06	260.83	277.19	75.17	132.43	190.64	223.24	261.84	220	420	62.98	24.59
Corn flour	111.60	151.55	201.06	260.83	277.19	75.17	132.43	190.64	223.24	261.84	220	420	62.98	24.59
Total	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000

Note: Vitamin and mineral mix (Chick Start, Vetcare Africa, Nairobi, Kenya); cellulose (wheat bran, locally milled), sucrose (Nzoia Sugar Company Ltd., Bungoma, Kenya); corn flour (Clovers, Tri Clover Industries Ltd., Nairobi, Kenya); corn oil (Elianto, BIDCO Africa Ltd., Thika, Kenya); Rehab diet-WH, rehabilitation diet made from 40% RDP wheat biscuit; Rehab diet-WS, rehabilitation diet made from 40% RDP wheat-sorghum biscuit; RDP, *Ruspolia differens* powder.

2.3 Animals and housing

Fifty-two weanling male Sprague-Dawley rats aged 3 to 5 weeks were obtained from the University of Nairobi's Zoology Department in Kenya. The rats had an initial weight ranging from 35 to 94 g. They were confined in individual cages with wire mesh bottomed screens to separate the faecal matter. An alternating 12-hour light/dark cycle with a mean temperature of 22 to 25 °C and relative humidity of 40% to 60% was maintained. The animals were maintained according to the National Research Council's guide for the care and use of laboratory animals[33].

The rats were acclimatized for 3 days from their arrival on October 15 to October 17, 2021. During this time, the rats were fed standardized laboratory irradiated rat pellets (Hindustan Animal Feeds, Gujarat, India) in a 1:1 ratio to the designed diets. Each rat was given 15 g meal per day. On the final day of acclimatization (18 October, 2021), the rats began to feed only on the experimental diets. After acclimatization on day 4, the rats were randomly divided into 13 groups of 4 rats, with the condition that the average weight of rats in any one group did not exceed 5 g of the average weight of rats in another group.

The growth study lasted 28 days, from day 4 (18 October to 14 November, 2021). Before the experiment began, the rats' weights were taken on an electronic balance (Gebr. Bosch PE 625, Germany) and repeated on alternate days throughout the study. The first 8 groups were provided meals containing wheat and wheat-sorghum fortified with RDP at doses of 5%, 15%, 25%, and 40%. The 9th and 10th groups were fed unfortified wheat and wheat-sorghum diets containing 0% RDP. The 11th group received skimmed milk powder (a reference diet). The 12th and 13th groups were fed 2 diets. First, they were both provided a protein-free diet for 11 days. On the 12th day of the growth study, the protein-free diet was discontinued, and rehabilitation diets began. This is because the rats had lost 20% of their initial weight. The 12th group was fed wheat (40% RDP), whereas the 13th group was fed wheat-sorghum (40% RDP). Throughout the study, the animals had *ad libitum* access to experimental diets and clean water. Daily records of each rat's food consumption were maintained. Each rat was fed 15 g of food per day. Protein quality indices such as PER, feed efficiency ratio (FER), and net protein retention ratio (NPRR) were calculated using FAO/World Health Organization (WHO)[34] formulae.

The protein digestibility study ran for 5 days, from October 18 to October 22, 2021. Each rat's feces were collected daily in polyethylene bags and stored at −18 °C until needed. NPRR, FER, food intake, protein intake, and body weight gain or loss were determined based on each rat's daily food consumption and weight gain/loss data.

2.4 Chemical analysis of faecal matter

Each rat's faeces were collected and dried overnight at 100 °C in an air circulation oven before being weighed and pulverized with a laboratory mortar and pestle. Faeces from 4 rats fed the same diet were mixed. Nitrogen in the feces was measured using the micro Kjeldahl method[35]. Endogenous nitrogen losses were determined from the faecal matter of rats fed a protein-free diet. The test diet's faecal nitrogen and nitrogen intake were used to determine apparent protein digestibility (APD), faecal protein, and true protein digestibility (TPD).

2.5 Computations

The following protein quality indices were calculated from the data collected[36] according to Eqs. (1)–(5):

$$\mathrm{PER}\,(\%) = \frac{\text{Weight gain (g)}}{\text{Protein consumed (g)}} \times 100 \tag{1}$$

$$\mathrm{NPRR} = \frac{\text{Weight gain (g)} + \text{Weight loss in protein free diet (g)}}{\text{Protein consumed (g)}} \tag{2}$$

$$\mathrm{APD}\,(\%) = \frac{I - F}{I} \times 100 \tag{3}$$

$$\mathrm{TPD}\,(\%) = \frac{I - (F - F_0)}{I} \times 100 \tag{4}$$

$$\text{Faecal protein}\,(\%) = \frac{F - F_0}{I} \times 100 \tag{5}$$

Where I represented nitrogen intake of the test diet (g); F represented faecal nitrogen loss on the test diet (g); F_0 represented faecal nitrogen loss on a protein-free diet (g).

2.6 PDCAAS determination

The PDCAAS is the standard method for assessing protein quality in food using human amino acid requirements[36]. In the present study, amino acid composition data for the respective biscuit samples and their real digestibility values were utilized to calculate the PDCAAS using the Eqs. (6)–(7)[36–37]. Amino acid scores for 9 essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) were calculated using a human pattern for amino acid requirements in children aged 3 to 10.

$$\text{Amino acid score} = \frac{\text{Amino acid in 1 g test protein (mg)}}{\text{Amino acid in requirement pattern (children 3−10 years old) (mg)}} \tag{6}$$

$$\mathrm{PDCASS} = \mathrm{TPD} \times \text{Lysine score or limiting amino acid score} \tag{7}$$

2.7 DIAAS determination

DIAAS measures the available protein quality for regulatory reasons and is best computed using ileal digestibility data[37]. Nonetheless, faecal crude protein digestibility can be employed when other options are unavailable. In this study, faecal nitrogen was utilized to calculate DIAAS as follows:

$$\mathrm{DIAAS} = \frac{\text{Weight of the same dietary indispensable amino acid in 1 g of the dietary protein (mg)}}{\text{Weight of the same dietary indispensable amino acid in 1 g of the reference protein (mg)}} \tag{8}$$

2.8 Statistical analysis

Mean ± standard deviation was used to present the results. All data were subjected to one-way analysis of variance (ANOVA) and means separated using Fisher's least significant difference. $P < 0.05$ was considered significant. Statistical analysis were performed using Minitab Release 18 Software (Minitab Inc., Pennsylvania, USA).

3 Results and discussion

3.1 Growth and rehabilitation studies

3.1.1 Protein nutritional quality

The PER, FER and NPRR values for the fortified wheat and wheat-sorghum biscuit diets were negative except the wheat biscuit diet fortified with RDP at 40% and the wheat-sorghum biscuit diets fortified with RDP at 5% and 15% (Table 3). PER reflects protein digestibility and amino acid bioavailability of a test protein. The negative PER values for the fortified diets is attributed to the fact that sulfur containing amino acids were limited in them that support body tissue building and growth in rats and presence of chitin. Similarly, negative PER values have been found in rats fed on a grasshopper diet[22]. The negative NPRR values as the levels of RDP in the diets increased may be attributed to low digestibility, and loss of biological activity of amino acids a result of processing. Because rats are unable to digest chitin from insect powder, the amount of chitin in the diets may have had a negative effect on digestibility[27].

This therefore implies for the diets that resulted in the negative values, more nitrogen was lost from the bodies of the rats compared to what was retained. Researchers have demonstrated that the nitrogen retention of diets formulated with oven-cooked or autoclaved insects reduced in rats because the amino acid profile was negatively modified by heat treatment[25]. These factors may also explain the significant weight loss in the rats. Similarly, a high protein diet formulated from grasshoppers (*Sphenarium purpurascens*) did not cause an increase in body size, abdominal circumference, or weight gain when fed to rats[38]. Another possible explanation for the lack of increase in weight as the levels of RDP increased in the diets is that there was an increase in the amount of fecal lipids (results not shown). This increase could have led to a low energy balance in the body of the rats as lipids are calorie dense. The increase in the fecal lipid loss can be attributed to increased

Table 3 Growth indices of body weight, protein efficiency ratio (PER), food efficiency ratio (FER), and net protein retention ratio (NPRR) of rats fed on diets based on 10 variations of biscuits, a reference diet, and a basal diet in the first 10 days of the rat bioassay study.

Diet formulation	PER (%)	FER (%)	NPPR	Body weight gain (g)
Wheat biscuits				
0% RDP	−0.28 ± 0.00^{e}	−0.03 ± 0.00^{bcd}	−0.69 ± 0.00^{c}	−2.43 ± 0.00^{bcd}
5% RDP	−0.57 ± 0.00^{g}	−0.06 ± 0.00^{def}	−1.09 ± 0.10^{e}	−3.90 ± 0.00^{d}
15% RDP	−0.41 ± 0.00^{f}	−0.04 ± 0.00^{cde}	−0.94 ± 0.00^{d}	−2.82 ± 0.00^{cd}
25% RDP	−0.14 ± 0.01^{d}	−0.01 ± 0.00^{bc}	−0.58 ± 0.00^{bc}	−1.12 ± 0.00^{bc}
40% RDP	0.68 ± 0.12^{a}	0.07 ± 0.00^{ef}	0.05 ± 0.00^{a}	3.93 ± 0.00^{a}
Wheat-sorghum biscuits				
0% RDP	−0.98 ± 0.00^{j}	−0.09 ± 0.00^{g}	−1.35 ± 0.12^{f}	−9.48 ± 0.00^{f}
5% RDP	0.87 ± 0.13^{i}	−0.08 ± 0.00^{fg}	−1.36 ± 0.11^{f}	−6.40 ± 0.00^{e}
15% RDP	0.07 ± 0.00^{d}	−0.01 ± 0.00^{b}	−0.59 ± 0.10^{bc}	−0.47 ± 0.00^{b}
25% RDP	−0.08 ± 0.00^{d}	−0.00 ± 0.00^{bc}	−0.50 ± 0.11^{b}	−0.77 ± 0.00^{c}
40% RDP	−0.66 ± 0.12^{h}	−0.06 ± 0.13^{ef}	−1.26 ± 0.20^{f}	−3.97 ± 0.00^{d}
Reference diet	0.44 ± 0.00^{f}	0.04 ± 0.01^{a}	0.02 ± 0.00^{a}	3.85 ± 0.00^{a}
Basal diet		−0.07 ± 0.00^{eg}		−7.11 ± 3.70^{e}

Note: The results were expressed as mean ± standard deviation (n = 4); values with the different letter superscripts in the same column indicated significantly differences at P < 0.05 as assessed by least significant difference. RDP, *Ruspolia differens* powder.

amount of crude fiber from the grasshopper's exoskeleton in the diets as fortification levels increased (Table 1). Studies have shown that feeding rats chitin derived from the exoskeleton of edible insects leads to a reduction in intestinal lipid absorption. Also, feeding Zucker rats with chitin from *Tenebrio molitor* exerted antilipidemic effects on the rats[39].

3.1.2 Rehabilitation studies

Figure 1 shows the effect of the diets on growth of rats. The isonitrogenous diets with 10% protein, both fortified and unfortified, did not have an effect on rat growth and acted as a maintenance diet, as the rats did not gain or lose weight. This kind of response has been observed in diets that do not have adequate protein. Previous studies have established that sorghum only diets fed to rats caused weight loss, and they attributed it to poor quality of protein in the diets that did not support any growth[40–41]. For the fortified diets, this finding suggested that for RDP diets to have a significant effect on growth, RDP levels ought to be higher than the 10% used in this study. Furthermore, the deficiency of sulfur containing amino acids may have limited the growth of the rats as explained earlier.

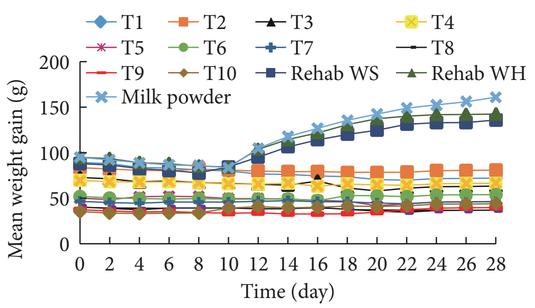

Figure 1 Mean weight increases in rats on unfortified and fortified wheat and wheat-sorghum biscuit diets for 28 days. T1, 100% wheat-sorghum; T2, 100% wheat; T3, 5% RDP wheat-sorghum; T4, 5% RDP wheat; T5, 15% RDP wheat-sorghum; T6, 15% RDP wheat; T7, 25% RDP wheat-sorghum; T8, 25% RDP wheat; T9, 40% RDP wheat-sorghum; T10, 40% RDP wheat; Rehab WS, rehabilitation diet made from 40% RDP wheat-sorghum; Rehab WH, rehabilitation diet made from 40% RDP wheat biscuit; RDP, *Ruspolia differens* powder.

The rats on protein-free diets lost weight by 10.8% up to the 10th day. When fed the rehabilitation diets they rapidly gained weight from a mean group weight of 84.3 to 135.9 g for wheat-sorghum and 84.2 to 142.4 g for wheat diets, respectively. The rate of weight gain during rehabilitation was significantly higher (P < 0.05) by 50% and 54.6% in the groups fed on wheat-sorghum and wheat diets respectively compared to the unfortified cereal diets. At the end of the study, the rats on the rehabilitation diet had higher weight than the other experimental groups. This is attributed to the high protein content (20%) of the diets which provided enough proteins for catch-up growth. The rats fed on the skimmed milk powder showed the highest growth compared to the rehabilitation diets formulated from the wheat and wheat-sorghum biscuit diets fortified with 40% RDP. Comparably, diets supplemented with cricket and termite powders caused rapid growth in malnourished rats[42], suggesting that edible insects may provide an alternative nutrient-dense protein source for combating the effects of early-life malnutrition in humans.

The growth pattern seen among rats fed on the rehabilitation diets has been observed in malnourished children. In a similar manner, rapid weight gain and increase in other growth parameters when undernourished children are rehabilitated with high energy, high protein supplementary diets has been reported[43–44]. In this study, the rates of catch-up growth were 2 to 3 times the average daily rate of growth. Accelerating catch-up growth is advantageous because it reduces the length of hospitalization or community-based nutrition rehabilitation centers therefore reducing the cost of treatment[45].

The wheat based diets caused higher growth than the wheat-sorghum based diets. Studies have shown that wheat protein is more digestible compared to sorghum proteins. Wheat has been found to have a higher digestibility (81%) compared to wheat (46%)[46]. The high digestibility of wheat is attributed to low crude fiber and absence of tannins[47]. On the other hand, researchers have demonstrated that the poor digestibility of sorghum protein is caused by the dense internal grain structure, presence of polyphenols and phytic acid, formation of disulfide and non-disulfide crosslinks, protein hydrophobicity and changes in secondary structure induced during wet cooking[48–49]. Such protein crosslinks may reduce the digestibility and biological value of food proteins, as a protein with high digestibility has a higher nutritional value than one with low digestibility because it provides more amino acids for absorption during proteolysis[50], explaining the high growth rate in the wheat based diets than the wheat-sorghum based diets.

3.2 *In vivo* protein digestibility

The food intake of the rats significantly reduced with increase in the level of fortification (Table 4). The wheat and wheat-sorghum biscuit diets had reductions of 18.3% to 37.3% and 11.7% to 37.4% respectively compared to the unfortified biscuit diets. Additionally, the food intake was lowest (27.85 and 27.88 g) when both wheat and wheat-sorghum were replaced with 40% RDP. The reduced food intake as the levels of RDP increased in the diets may be attributed to the presence of compounds in the insect powder, which negatively affect palatability. Similarly, diets supplemented with termite, grasshopper and moth caterpillar powders had reduced intake compared to cricket supplemented diets attributed to differences in palatability of the insect diets[22]. It is possible that in the wild, many insect species sequester compounds from their food plants, which cause them to be unpalatable[51]. The low food intake might also be explained by deficiency or imbalance of amino acids in the protein source[52]. For instance, in this study, the diets were deficient in sulfur amino acids, methionine + cysteine (Table 5), therefore did not meet the high nutritional requirements of the rats. However, the amino acid quantities in the diets adequately met the requirements for young children (Table 5).

The presence of chitin may also have led to reduction in food intake in the rats by suppressing appetite[53], increasing intestinal food volume as it is indigestible and probably inducing satiety[54]. Food consumption in rats could be modulated by the indigestible food volume reaching the small intestine[55]. A previous study reported reduced food intake in Wistar rats fed on diets rich in insoluble fiber[56]. Among human subjects, pancakes fortified with 30% *Alphitobius diaperinus* and addition of 20% and 30% *Acheta domesticus* powders were highly satiating and caused reduction in the amount of food intake[57].

Fortification with RDP increased lysine content (Table 5) by 2.9% to 32.8% and 6.7% to 47.9% in the wheat and wheat-sorghum diets respectively compared to the 100% cereal diets, which in the presence of sugar and high baking temperature may increase the quantities of Maillard reaction products (MRPs). Studies have shown that feeding rats with diets rich in MRPs causes a reduction in the amount of food intake. Researchers have demonstrated that consumption of diets containing bread crust fractions and infant formula supplemented with MRPs decreased food intake in rats[58–59].

The highest food intake was by rats fed with wheat and wheat-sorghum diets with 0% RDP, which were the same as the skimmed milk powder and basal diets (Table 4). Low protein diets may increase total food intake in order to satisfy protein requirements[60]. Similarly, feeding rats a low protein diet increased the daily food intake, consequently increasing the protein supply[61].

The fecal weight of rats fed on 100% wheat-sorghum biscuit diet was 297% and 170% higher than that for the skimmed milk powder and 100% wheat biscuits (Table 4). This may be explained by the formation of enzyme-resistant starch and available kafirins during thermal processing of sorghum making the diets less digestible[62–63]. Higher faecal bulk of 52% to 62% for rats fed a diet with 100% sorghum compared to casein and sorghum-soy diet fed rats, respectively has also been reported[40]. Similarly, faecal weight of rats fed an unfortified wheat-sorghum bun diet was 59% higher than in rats fed a reference diet have been reported[41]. In the fortified wheat biscuit diets, fortification at 40% RDP led to the least faecal matter, while fortification at 25% produced the highest fecal matter. This trend was similar in the wheat-sorghum biscuit diets. This may be explained by the fact that the diets with the 40% RDP diet contained higher protein content than 25% RDP diets, and therefore were more digestible.

Table 4 shows that increase of RDP in both wheat and wheat-sorghum biscuit diets reduce protein intake by 18.2% to 37.2% and 11.7% to 37.4% respectively compared to the unfortified diets. The protein faecal output also increase by 100% to 245.6% and 39.7% to 167.9% in wheat and wheat-sorghum biscuits respectively as fortification levels increased compared to the 100%

Table 4 Food intake, fecal weight, protein intake, protein fecal output, and protein retention of rats fed diets based on 10 variations of biscuits, a reference diet, and a basal diet.

Diet formulation	Food intake (g)	Fecal weight (g)	Protein intake (g)	Protein fecal output (g)	Protein retention (g)
Wheat biscuits					
0% RDP	44.42 ± 2.13[a]	1.15 ± 0.57[bcdef]	4.44 ± 0.22[a]	0.68 ± 0.10[f]	3.76 ± 0.11[a]
5% RDP	34.58 ± 1.80[bc]	1.25 ± 0.18[bcdef]	3.46 ± 0.23[bc]	1.65 ± 0.10[c]	1.81 ± 0.32[cd]
15% RDP	32.27 ± 7.42[bc]	0.95 ± 0.51[def]	3.23 ± 0.68[cd]	1.36 ± 0.00[d]	1.86 ± 0.67[bcd]
25% RDP	36.28 ± 1.30[abc]	1.53 ± 0.60[bcd]	3.63 ± 0.13[bc]	2.35 ± 0.00[a]	1.28 ± 0.11[de]
40% RDP	27.85 ± 5.10[c]	0.88 ± 0.21[ef]	2.79 ± 0.47[d]	2.04 ± 0.00[b]	0.74 ± 0.50[e]
Wheat-sorghum biscuits					
0% RDP	44.55 ± 1.17[a]	3.10 ± 0.60[a]	4.46 ± 0.19[a]	0.78 ± 0.20[f]	3.67 ± 0.37[a]
5% RDP	35.77 ± 3.61[bc]	1.44 ± 0.76[bcde]	3.58 ± 0.42[bc]	1.75 ± 0.09[c]	1.89 ± 0.42[cd]
15% RDP	33.30 ± 6.00[bc]	1.58 ± 0.39[bc]	3.33 ± 0.61[cd]	1.09 ± 0.00[e]	2.23 ± 0.61[bc]
25% RDP	39.35 ± 5.42[ab]	1.70 ± 0.11[b]	3.94 ± 0.52[ab]	1.47 ± 0.00[d]	2.46 ± 0.53[b]
40% RDP	27.88 ± 1.58[c]	1.10 ± 0.10[cdef]	2.79 ± 0.20[d]	2.09 ± 0.00[b]	0.69 ± 0.22[e]
Reference diet	44.25 ± 6.71[a]	0.78 ± 0.11[f]	4.43 ± 0.68[a]	0.44 ± 0.00[g]	3.99 ± 0.68[a]
Basal diet	43.97 ± 9.42[a]	0.95 ± 0.19[ef]			

Note: The results were expressed as mean ± standard deviation ($n = 4$); values with the different letter superscripts in the same column indicated significantly differences at $P < 0.05$ as assessed by least significant difference. RDP, *Ruspolia differens* powder.

Table 5 Amino acid score for control and test diets (mg/g protein) with FAO requirement pattern for children aged 3–10 years and NRC amino acid reference pattern for rats.

Amino acid	Skimmed milk powder	0WH	5WH	15WH	25WH	40WH	0WS	5WS	15WS	25WS	40WS	FAO (2013)[1]	NRC (2011)[2]
Histidine	27.13	22.29	24.76	26.54	29.32	33.59	23.56	25.21	27.67	30.61	35.78	16	19
Isoleucine	60.51	37.87	39.56	41.36	43.21	46.55	35.78	38.65	42.58	46.88	50.69	30	41
Leucine	97.95	115.32	117.56	120.11	122.67	127.33	110.34	115.21	118.45	121.45	126.11	61	71
Lysine	79.31	25.94	26.71	27.89	31.21	34.45	23.32	24.89	27.23	31.43	34.48	48	61
Met + Cys	34.32	33.78	34.67	36.12	37.33	39.43	32.76	34.87	37.62	38.21	39.9	23	65
Phe + Tyr	69.58	71.48	71.99	73.67	75	76.87	71.32	73.44	74.88	76.98	80.32	41	68
Threonine	45.13	37.67	38.43	39.81	42.51	43.33	36.98	37.67	39.21	42.88	46.32	25	41
Tryptophan	14.10	14.01	15.01	16.23	18.43	19.22	14.32	15.88	17.89	22.98	25.21	6.6	13
Valine	66.92	51.46	52.41	55.94	56.77	60.23	49.87	51.36	56.82	61.67	63.85	40	49
Protein (%)	36.16	12.97	14.9	18.27	25.13	28.01	11.6	13.91	17.44	20.33	25.29		
Total	494.95	409.82	421.1	437.67	456.45	481	398.25	417.18	442.35	473.09	502.66		
TPD (%)	96.63	51.17	46.97	50.93	33.11	46.41	47.14	43.35	54.98	42.47	44.42		
Limiting amino acid (3–10 years)	None	Lysine	Lysine	Lysine	Lysine	Lysine	Lysine	Lysine	Lysine	Lysine	Lysine		
Lysine score (3–10 years)	1.65	0.54	0.56	0.58	0.65	0.72	0.49	0.52	0.57	0.65	0.72		
PDCAAS (3–10 years)	1.00	0.28	0.26	0.30	0.22	0.33	0.23	0.22	0.31	0.28	0.32		
Limiting amino acid (rat)	Met + Cys	Met + Cys	Met + Cys	Met + Cys	Met + Cys	Met + Cys	Met + Cys	Met + Cys	Met + Cys	Met + Cys	Met + Cys		
Lysine score (rat)	0.53	0.52	0.53	0.56	0.57	0.61	0.50	0.54	0.58	0.59	0.61		
PDCAAS (rat)	1.00	0.28	0.26	0.30	0.22	0.33	0.23	0.22	0.31	0.28	0.32		

Note: [1] Amino acid reference pattern for children aged 3–10 years were obtained from FAO (2013); [2] Amino acid reference pattern for rats were obtained from National Research Council (NRC) amino acid reference pattern for rats (NRC, 2011); indispensable amino acid scores for the skimmed milk powder were obtained from USDA (2018); Met + Cys, methionine + cysteine (sulfur amino acid); Phe + Tyr, phenylalanine + tyrosine (aromatic amino acid); PDCAAS, protein digestibility corrected amino acid score; TPD, true protein digestibility. Acronyms for wheat-RDP biscuits: 0WH, 100% wheat, 5WH, 5% RDP wheat, 15WH, 15% RDP wheat, 25WH, 25% RDP wheat diet, 40WH, 40% RDP wheat diet. Acronyms for wheat-sorghum biscuits: 0WS, 100% wheat-sorghum diet, 5WS, 5% RDP wheat-sorghum diet, 15WS, 15% RDP wheat-sorghum diet, 25WS, 25% RDP wheat-sorghum diet, 40WS, 40% RDP wheat-sorghum diet.

cereal biscuit diets. As a result, protein retention decreased by 50.5% to 80.3% in wheat and 32.9% to 81.2% in wheat-sorghum biscuit diets as RDP levels increased. Protein intake from unfortified diets was similar to intake from the reference diet. This may possibly be explained by the low food intake as the fortification levels increased. Also, the amount of chitin in the diets may have had an influence on digestibility of the diets since rats are unable to digest chitin from insect powder[27]. This implies that the high levels of indigestible proteins in the fecal matter indicate that higher amounts of amino acids are bound to chitin and therefore were not digested[64]. Also, migratory locust protein has been found to be resistant to gut pepsin but digestible by intestinal trypsin and chymotrypsin[28]. Because of this, the protein retention decreased significantly as the fortification levels with RDP increased. The reduction in the protein retention may have been possibly caused by imbalance in the sulfur containing amino acids as explained earlier. For instance, low nitrogen retention in rats fed on *Tenebrio molitor* and *Acheta domesticus* powders supplemented diets compared with control casein diet has been reported[25].

The APD of the fortified biscuit diets reduced with increase in RDP levels (Table 6). Compared to the unfortified diets, there was reduction in APD by 13% to 29% and 9% to 29% in wheat and wheat-sorghum biscuit diets, respectively. The APD of the control biscuit diets was similar to the reference diet. The TPD of the fortified biscuits diet reduced with increase in RDP levels. There was reduction in TPD by 45% to 64% and 41% to 54% in wheat biscuit and wheat-sorghum biscuit diets respectively when compared to the reference diets. TPD indicates how well a protein is digested which is a measure of the bioavailability of amino acids present in a protein[52]. The lower TPD of the insects compared to skimmed milk powder may be a result of the presence of chitin, and high temperature processing and formation of MRP.

Table 6 Indices of apparent protein digestibility (APD) and true protein digestibility (TPD) of diets based on 10 variations of biscuits, and a reference diet.

Diet formulation	APD (%)	TPD (%)
Wheat biscuits		
0% RDP	92.32 ± 0.89[a]	51.17 ± 3.71[bc]
5% RDP	76.05 ± 0.71[d]	46.97 ± 3.70[cd]
15% RDP	80.21 ± 0.30[d]	50.93 ± 0.52[bc]
25% RDP	71.51 ± 0.61[e]	33.11 ± 0.41[f]
40% RDP	65.93 ± 3.87[f]	46.41 ± 3.86[de]
Wheat-sorghum biscuits		
0% RDP	92.08 ± 1.72[a]	47.14 ± 3.51[cd]
5% RDP	76.21 ± 2.86[d]	43.35 ± 1.87[de]
15% RDP	84.09 ± 2.01[b]	54.98 ± 5.28[b]
25% RDP	83.06 ± 2.39[bc]	42.47 ± 3.49[e]
40% RDP	65.34 ± 5.42[f]	44.42 ± 4.87[de]
Reference diet	92.64 ± 0.00[a]	93.04 ± 0.88[a]

Note: The results were expressed as mean ± standard deviation (n = 4); values with the different letter superscripts in the same column indicated significantly differences at $P < 0.05$ as assessed by least significant difference. RDP, *Ruspolia differens* powder.

3.3 Protein evaluation from PDCAAS

The effect of compositing wheat and wheat-sorghum with RDP on essential amino acid composition of the experimental biscuits has been published elsewhere[9]. Table 5 shows the quantities of the indispensable amino acids in the skimmed milk powder and experimental food products relative to the FAO reference patterns for 3 to 10 and 1 to 2 years old children[37] and the National Research Council (NRC) reference pattern for growing rats[65]. The PDCAAS reflects the estimated ability for the food product to meet the protein needs of an individual[40]. The 3 to 10 years old amino acid scoring pattern is recommended by WHO/FAO/UNU Expert Consultation[36] for evaluating protein quality for school children and adolescents. The casein diet had an amino acid pattern that is considered adequate for both preschool and school children. Fortification with RDP increased the PDCAAS of the fortified compared to the unfortified biscuits. For instance, there was an 18% increase in the PDCASS of both wheat and wheat-sorghum diets. The PDCAAS of the diets ranged between 0.26 and 0.33 for wheat diets, and 0.22 and 0.32 for wheat-sorghum diets. In these diets, the most limiting indispensable amino acid was lysine. However, it is important to note that none of the biscuits met the minimum score of 0.7 of skimmed milk powder recommended by FAO/WHO Codex Alimentarius Committee[4]. The control biscuits were deficient in lysine, which is required for growth and development of young children. A PDCAAS of 0.46 has been reported in rats fed with grasshopper powder[22]. According to the NRC[65], young rats require more lysine, sulfur-containing amino acids, and all other essential amino acids than children aged 3 to 10 (Table 5). The skimmed milk powder was insufficient in sulfur-containing amino acids, as evidenced by a low PDCAAS based on the most limiting amino acids. Rats require higher levels of these amino acids to maintain hair development[65]. Because of their high requirements, sulphur amino acids (methionine + cysteine) were the first limiting amino acids for rat growth in all of the experimental diets studied. Previous studies have demonstrated that *Ruspolia differens* is deficient of these amino acids[11,66]. Edible insects such as mealworms, house cricket, Mormon cricket and Eastern tent caterpillar are deficient in sulfur containing amino acids[25,67]. The results in this study imply that since the amino acid requirements of the rat are much higher than those of children, growth patterns observed in this study would probably be higher in preschool and school children if they consumed the fortified wheat-sorghum and wheat biscuits. A protein source that supports modest growth in rats would promote optimal growth in children[68].

3.4 Protein evaluation from DIAAS

The DIAAS were calculated using the lysine score from the reference and test diet, as it is the first limiting amino acid for children aged 6 months to 3 years[37]. Table 7 demonstrates that the DIAAS for fortified wheat biscuits varied from 46% in unfortified biscuits to 60% in 40% RDP fortified biscuits. In wheat-sorghum biscuits, unfortified biscuits had a DIAAS of 41%, whereas 40% RDP fortified biscuits had a value of 60%. These diets, however, did not achieve the minimum DIAAS of 75% proposed for dietary protein quality evaluation in human nutrition. This result is consistent with the previous research that reported DIAAS of 44% to 69% when sorghum buns were fortified with snail meat powder[41]. This suggests that the lysine content of the biscuits should be enhanced to meet the indispensable amino acid requirements of children aged 6 months to 3 years old by fortifying with RDP in amounts above 40%. Furthermore, the amino acid score could be enhanced by extracting chitin from the insect powder or utilizing a protein isolate from it.

Table 7 Protein intake of the control and test diets (mg/g protein) with the amino acid requirement based on pattern for children 6 months to 3 years.

Amino acid	Milk powder	0WH	5WH	15WH	25WH	40WH	0WS	5WS	15WS	25WS	40WS
Histidine	1.36	1.11	1.24	1.33	1.47	1.68	1.18	1.26	1.38	1.53	1.79
Isoleucine	1.89	1.18	1.24	1.29	1.35	1.45	1.12	1.21	1.33	1.47	1.58
Leucine	1.48	1.75	1.78	1.82	1.86	1.93	1.67	1.75	1.79	1.84	1.91
Lysine	1.39	0.46	0.47	0.49	0.55	0.60	0.41	0.44	0.48	0.55	0.60
Met + Cys	1.27	1.25	1.28	1.34	1.38	1.46	1.21	1.29	1.39	1.42	1.48
Phe + Tyr	1.34	1.37	1.38	1.42	1.44	1.48	1.37	1.41	1.44	1.48	1.54
Threonine	1.46	1.22	1.24	1.28	1.37	1.40	1.19	1.22	1.26	1.38	1.49
Tryptophan	1.66	1.65	1.77	1.91	2.17	2.26	1.68	1.87	2.10	2.70	2.97
Valine	1.56	1.20	1.22	1.30	1.32	1.40	1.16	1.19	1.32	1.43	1.48
IAA ref ratio	1.39	0.46	0.47	0.49	0.55	0.60	0.41	0.44	0.48	0.55	0.60
DIAAS (%)	127 (SAA)	46 (Lys)	47 (Lys)	49 (Lys)	55 (Lys)	60 (Lys)	41 (Lys)	44 (Lys)	48 (Lys)	55 (Lys)	60 (Lys)

Note: The amino acid requirements are based on pattern for children 6 months to 3 years according to FAO[17]. DIAAS were determined based on the least value of 'digestible indispensable amino acid (IAA) reference ratio' expressed as percentage for each reference pattern. Met + Cys, methionine + cysteine (sulfur amino acids); Phe + Tyr, phenylalanine + tyrosine (aromatic amino acids). Acronyms for wheat-RDP biscuits: 0WH, 100% wheat; 5WH, 5% RDP wheat; 15WH, 15% RDP wheat; 25WH, 25% RDP wheat diet; 40WH, 40% RDP wheat diet. Acronyms for wheat-sorghum biscuits: 0WS, 100% wheat-sorghum diet; 5WS, 5% RDP wheat-sorghum diet; 15WS, 15% RDP wheat-sorghum diet; 25WS, 25% RDP wheat-sorghum diet; 40WS, 40% RDP wheat-sorghum diet. RDP, *Ruspolia differens* powder.

4 Conclusion

Fortification of wheat and wheat-sorghum biscuits with 40% RDP promotes rat growth. At 10% protein content, the fortified diets functioned as maintenance diets and did not promote growth. Supplementation with RDP lowers NPR, PER, and TPD in wheat and wheat-sorghum biscuits while enhancing PDCAAS and DIAAS. The diets failed to meet the recommended minimum PDCAAS of 70% and DIAAS of 75% for assessing protein quality in diets. However, wheat and wheat-sorghum biscuits supplemented with 40% RDP have the greatest potential to facilitate rehabilitation in malnourished children due to their high protein content.

Ethical approval

The rats' maintenance was conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals[33]. At the end of the study, the rats were euthanized using ether according to the American Veterinary Medical (AVMA) guidelines on euthanization of laboratory of rodents[69] and disposed by burying. A research license was granted by the National Commission for Science, Technology and Innovation (NACOSTI) (License Number: NACOSTI/P/22/16130).

Availability of data and material

The datasets analysed for this study are available from the corresponding author on reasonable request.

Declaration of competing interest

The authors declare that they have no known competing interests. The study sponsors were not involved in any part of the work.

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References

[1] F. T. Manjong, V. S. Verla, T. O. Egbe, et al., Risk factors of under nutrition among indigenous children under five years in developing countries: a scoping review, J. Public Health Epidemiol. 12 (2020) 340–348. https://doi.org/10.5897/JPHE2020.1279.

[2] UNICEF/WHO, World Bank Group, UNICEF-WHO-The World Bank. Joint child malnutrition estimates-levels and trends-2021 edition, United Nation Children's Fund, Washington, DC, 2021. https://data.unicef.org/resources/jme-report-2021/.

[3] O. R. Katoch, Determinants of malnutrition among children: a systematic review, Nutrition 96 (2022) 111565. https://doi.org/10.1016/j.nut.2021.111565.

[4] FAO/WHO, Codex Alimentarius: foods for special dietary uses (including foods for infants and children). Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, 1994. Available from: https://www.fao.org/3/ca2329en/ca2329en.pdf.

[5] S. N. Serrano-Sandoval, D. Guardado-Felix, J. A. Gutiérrez-Uribe, Legumes in human health and nutrition, in: C. Rauh (Ed.), Reference module in food science, Elsevier, 2022, pp. 430–437. https://doi.org/10.1016/B978-0-12-821848-8.00061-5.

[6] J. R. N. Taylor, K. G. Duodu, Sorghum and millets: grain-quality characteristics and management of quality requirements, in: C. Wrigley, I. Batey, D. Miskelly (Eds.), Cereal grains, Elsevier, 2017, pp. 317–351. https://doi.org/10.1016/B978-0-08-100719-8.00013-9.

[7] M. Goubgou, L. T. Songré-Ouattara, F. Bationo, et al., Biscuits: a systematic review and meta-analysis of improving the nutritional quality and health benefits, Food Prod. Process. Nutr. 3 (2021) 26. https://doi.org/10.1186/s43014-021-00071-z.

[8] C. A. Serrem, H. L. de Kock, J. R. N. Taylor, Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour, Int. J. Food Sci. Technol. 46 (2011) 74–83. https://doi.org/10.1111/j.1365-2621.2010.02451.x.

[9] A. K. Ronoh, C. A. Serrem, S. B. Tumwebaze, et al., Effect of fortifying sorghum and wheat with longhorn grasshopper (*Ruspolia differens*) powder on nutritional composition and consumer acceptability of biscuits, Food Sci. Nutr. 12 (2024) 3492–3507. https://doi.org/10.1002/fsn3.4018.

[10] M. W. Mmari, J. N. Kinyuru, H. S. Laswai, et al., Traditions, beliefs and indigenous technologies in connection with the edible longhorn grasshopper *Ruspolia differens* (Serville 1838) in Tanzania, J. Ethnobiol. Ethnomed. 13 (2017) 60. https://doi.org/10.1186/s13002-017-0191-6.

[11] G. Ssepuuya, R. Smets, D. Nakimbugwe, et al., Nutrient composition of the long-horned grasshopper *Ruspolia differens* Serville: effect of swarming season and sourcing geographical area, Food Chem. 301 (2019) 125305. https://doi.org/10.1016/j.foodchem.2019.125305.

[12] N. Siulapwa, A. Mwambungu, E. Lungu, et al., Nutritional value of four common edible insects in Zambia, Int. J. Sci. Res. 3 (2014) 876–884.

[13] E. P. Shabo, E. Owaga, J. Kinyuru, Physico-chemical characterization, acceptability and shelf stability of extruded composite flour enriched with long-horned grasshopper (*Ruspolia differens*), J. Agric. Sci. Technol. 21 (2022) 4–32. https://doi.org/10.4314/jagst.v21i2.2.

[14] C. Chuwa, T. Ngendello, P. Saidia, et al., Edible grasshoppers (*Ruspolia differens*) as alternative source of protein from insects to combat malnutrition, Afr. J. Food Agric. Nutr. Dev. 23 (2023) 23576–23589. https://doi.org/10.18697/ajfand.121.23680.

[15] B. O. Ochieng, J. O. Anyango, F. M. Khamis, et al., Nutritional characteristics, microbial loads and consumer acceptability of cookies enriched with insect (*Ruspolia differens*) meal, LWT-Food Sci. Technol. 184 (2023) 115012. https://doi.org/10.1016/j.lwt.2023.115012.

[16] N. Shaheen, S. Islam, S. Munmun, et al., Amino acid profiles and digestible indispensable amino acid scores of proteins from the prioritized key foods in Bangladesh, Food Chem. 213 (2016) 83–89. https://doi.org/10.1016/j.foodchem.2016.06.057.

[17] FAO, Dietary protein quality evaluation in human nutrition: report of an FAO Expert Consultation, Food and Agriculture Organization of the United Nations, Rome, Italy, 2011. Available from: https://www.fao.org/3/i3124e/i3124e.pdf.

[18] Z. A. Bhutta, K. Sadiq, Protein digestion and bioavailability, in: B. Caballero (Ed.), Encyclopaedia of human nutrition, 3rd ed, Academic Press, 2013, pp. 116–122. https://doi.org/10.1016/B978-0-12-375083-9.00240-3.

[19] D. Tomé, Protein quality and sources, in: B. Caballero (Ed.), Reference module in food science, Elsevier, 2021, pp. 559-567. https://doi.org/10.1016/B978-0-12-821848-8.00028-7.

[20] M. Rodríguez-Rodríguez, F. G. Barroso, D. Fabrikov, et al., *In vitro* crude protein digestibility of insects: a review, Insects 13 (2022) 682. https://doi.org/10.3390/insects13080682.

[21] P. Nolan, A. E. Mahmoud, R. R. Kavle, et al., Edible insects: protein composition, digestibility, and biofunctionalities, in: Z. F. Bhat, J. D. Morton, A. E.-D. A. Bekhit, et al. (Eds.), Processing technologies and food protein digestion, Academic Press, 2023, pp. 429–494. https://doi.org/10.1016/B978-0-323-95052-7.00020-0.

[22] F. I. Oibiokpa, H. O. Akanya, A. A. Jigam, et al., Protein quality of four indigenous edible insect species in Nigeria, Food Sci. Hum. Wellness 7 (2018) 175–183. https://doi.org/10.1016/j.fshw.2018.05.003.

[23] M. Bauserman, A. Lokangaka, J. Gado, et al., A cluster-randomized trial determining the efficacy of caterpillar cereal as a locally available and sustainable complementary food to prevent

stunting and anaemia, Public Health Nutr. 18 (2015) 1785–1792. https://doi.org/10.1017/S1368980014003334.
[24] C. Dexter, G. T. Matanhire, T. Jombo, et al., Protein quality of commonly consumed edible insects in Zimbabwe, Afr. J. Food Agric. Nutr. Dev. 19(3) (2019) 14674–14689. https://doi.org/10.18697/ajfand.86.17645.
[25] C. Poelaert, F. Francis, T. Alabi, et al., Protein value of two insects, subjected to various heat treatments, using growing rats and the protein digestibility-corrected amino acid score, J. Insects Food Feed 4 (2018) 77–87. https://doi.org/10.3920/JIFF2017.0003.
[26] Y. Singh, M. Cullere, A. Kovitvadhi, et al., Effect of different killing methods on physicochemical traits, nutritional characteristics, *in vitro* human digestibility and oxidative stability during storage of the house cricket (*Acheta domesticus* L.), Innov. Food Sci. Emerg. Technol. 65 (2020) 102444. https://doi.org/10.1016/j.ifset.2020.102444.
[27] M. Ochiai, M. Inada, Powdered edible locust enhances nitrogen excretion and presents a low-energy value in growing rats, J. Asia-Pac. Entomol. 23 (2020) 1138–1143. https://doi.org/10.1016/j.aspen.2020.08.019.
[28] M. Ochiai, K. Tezuka, H. Yoshida, et al., Edible insect *Locusta migratoria* shows intestinal protein digestibility and improves plasma and hepatic lipid metabolism in male rats, Food Chem. 396 (2022) 133701. https://doi.org/10.1016/j.foodchem.2022.133701.
[29] Institute of Medicine, Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids (macronutrients), The National Academies Press, Washington, DC, 2005.
[30] AOAC, Official methods of analysis. Method 960-48, 17th ed, The Association, Washington, DC, 2000.
[31] D. G. Chapman, R. Castillo, J. A. Campbell, Evaluation of protein in foods: I. a method for the determination of protein efficiency ratios, Can. J. Biochem. Physiol. 37 (1959) 679–686. https://doi.org/10.1139/o59-074.
[32] K. G. Dewey, Formulations for fortified complementary foods and supplements: review of successful products for improving the nutritional status of infants and young children, Food Nutr. Bull. 30 (2009) S239–S255. https://doi.org/10.1177/15648265090302S209.
[33] National Research Council of the National Academies, Guide for the care and use of laboratory animals, 8th ed, The National Academies Press, Washington, DC, 2011. https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf.
[34] FAO/WHO, Protein quality evaluation: report of the joint FAO/WHO expert consultation, FAO, Rome, Italy, 1991. http://www.fao.org/docrep/013/t0501e/t0501e00.pdf.
[35] L. W. George, Official methods of analysis of AOAC INTERNATIONAL, 22nd ed, New York, AOAC Publications, 2023. https://doi.org/10.1093/9780197610145.001.0001.
[36] WHO/FAO/UNU Expert Consultation, Protein and amino acids requirements in human nutrition: a report of a joint FAO/WHO/UNU expert consoultation, WHO Technical Report Series Report No. 935, WHO Media Centre, Geneva, Switzerland, 2007.
[37] FAO, Dietary protein quality evaluation in human nutrition: report of an FAO expert consultation, Food and Agriculture Organization of the United Nations, Rome, 2013. Available from: https://www.fao.org/documents/card/en/c/ab5c9fca-dd15-58e0-93a8-d71e028c8282/.
[38] A. M. M. Sanchez, A. M. C. Nuñez, L. T. Meza-Cureño, et al., Biochemical and morphometric parameters support nutritional benefits in laboratory rats fed with an orthopteran-based (*Sphenarium purpurascens*) Diet, Food Nutr. J. 7 (2022) 1–14. https://doi.org/10.29011/2575-7091.100236.
[39] D. K. Gessner, A. Schwarz, S. Meyer, et al., Insect meal as alternative protein source exerts pronounced lipid-lowering effects in hyperlipidemic obese Zucker rats, J. Nutr. 149 (2019) 566–577. https://doi.org/10.1093/jn/nxy256.
[40] C. A. Serrem, H. L. de Kock, A. Oelofse, et al., Rat bioassay of the protein nutritional quality of soy-fortified sorghum biscuits for supplementary feeding of school-age children, J. Sci. Food Agr. 91 (2011) 1814–1821. https://doi.org/10.1002/jsfa.4389.
[41] F. B. Agengo, A. N. Onyango, C. A. Serrem, et al., Efficacy of compositing with snail meat powder on protein nutritional quality of sorghum-wheat buns using a rat bioassay, J. Sci. Food Agr. 100 (2020) 2963–2970. https://doi.org/10.1002/jsfa.10324.
[42] I. Agbemafle, N. Hanson, A. E. Bries, et al., Alternative protein and iron sources from edible insects but not *Solanum torvum* improved body composition and iron status in Malnourished rats, Nutrients 11 (2019) E2481. https://doi.org/10.3390/nu11102481.
[43] F. Azimi, A. Esmaillzadeh, E. Alipoor, et al., Effect of a newly developed ready-to-use supplementary food on growth indicators in children with mild to moderate malnutrition, Public Health 185 (2020) 290–297. https://doi.org/10.1016/j.puhe.2020.06.025.
[44] W. M. López-Alonso, J. Gallegos-Martínez, J. Reyes-Hernández, Impact of a nutritional intervention based on amaranth flour consumption to recovery undernourished children, Curr. Res. Nutr. Food Sci. J. 9 (2021) 222–232. https://doi.org/10.12944/CRNFSJ.9.1.22.
[45] T. C. E. Mosha, M. R. Bennink, P. K. W. Ng, Nutritional quality of drum-processed and extruded composite supplementary foods, J. Food Sci. 70 (2005) C138–C144. https://doi.org/10.1111/j.1365-2621.2005.tb07074.x.
[46] J. D. Axtell, A. W. Kirleis, M. M. Hassen, et al., Digestibility of sorghum proteins, Proc. Natl. Acad. Sci. 78 (1981) 1333–1335. https://doi.org/10.1073/pnas.78.3.1333.
[47] B. O. Eggum, I. Kreft, B. Javornik, Chemical composition and protein quality of buckwheat (*Fagopyrum esculentum* Moench), Plant Foods Hum. Nutr. 30 (1980) 175–179. https://doi.org/10.1007/BF01094020.
[48] K. G. Duodu, A. Nunes, I. Delgadillo, et al., Effect of grain structure and cooking on sorghum and maize *in vitro* protein digestibility, J. Cereal Sci. 35 (2002) 161–174. https://doi.org/10.1006/jcrs.2001.0411.
[49] K. G. Duodu, J. R. N. Taylor, P. S. Belton, et al., Factors affecting sorghum protein digestibility, J. Cereal Sci. 38 (2003) 117–131. https://doi.org/10.1016/S0733-5210(03)00016-X.
[50] J. R. N. Taylor, H. L. de Kock, E. Makule, et al., Opportunities and challenges for wholegrain staple foods in sub-Saharan Africa, J. Cereal Sci. 104 (2022) 103438. https://doi.org/10.1016/j.jcs.2022.103438.
[51] J. P. Williams, J. R. Williams, A. Kirabo, et al. , Nutrient content and health benefits of insects, in: A. T. Dossey, J. A. Morales-Ramos, M. Guadalupe Rojas (Eds.), Insects as sustainable food ingredients, Elsevier, 2016, pp. 61–84. https://doi.org/10.1016/B978-0-12-802856-8.00003-X.
[52] K. E. Ekpo, Effect of processing on the protein quality of four popular insects consumed in Southern Nigeria, Arch. Appl. Sci. Res. 3 (2011) 307–326.
[53] R. A. Samra, G. H. Anderson, Insoluble cereal fiber reduces appetite and short-term food intake and glycemic response to food consumed 75 min later by healthy men, Am. J. Clin. Nutr. 86 (2007) 972–979. https://doi.org/10.1093/ajcn/86.4.972.
[54] S. Fuller, E. Beck, H. Salman, et al., New horizons for the study of dietary fiber and health: a review, Plant Foods Hum. Nutr. Dordr. Neth. 71 (2016) 1–12. https://doi.org/10.1007/s11130-016-0529-6.
[55] H. S. Koopmans, An integrated organismic response to lower gut stimulation, Scand. J. Gastroenterol. Suppl. 82 (1983) 143–153.
[56] L. M. Zem, C. V. Helm, G. S. Henriques, et al., Pereskia aculeata: biological analysis on wistar rats, Food Sci. Technol. 37 (2017) 42–47. https://doi.org/10.1590/1678-457x.29816.
[57] M. Skotnicka, A. Mazurek, K. Karwowska, et al., Satiety of edible insect-based food products as a component of body weight control, Nutrients 14 (2022) 2147. https://doi.org/10.3390/nu14102147.

[58] C. Delgado-Andrade, J. A. Rufián-Henares, F. J. Morales, Lysine availability is diminished in commercial fibre-enriched breakfast cereals, Food Chem. 100 (2007) 725–731. https://doi.org/10.1016/j.foodchem.2005.10.031.

[59] B. Sarriá, R. López-Fandiño, M. P. Vaquero, Does processing of a powder or in-bottle-sterilized liquid infant formula affect calcium bioavailability?, Nutrition 17 (2001) 326–331. https://doi.org/10.1016/s0899-9007(00)00585-2.

[60] B. D. White, F. Du, D. A. Higginbotham, Low dietary protein is associated with an increase in food intake and a decrease in the *in vitro* release of radiolabeled glutamate and GABA from the lateral hypothalamus, Nutr. Neurosci. 6 (2003) 361–367. https://doi.org/10.1080/1028415031000164036[5].

[61] S. Aparecida de França, M. P. dos Santos, M. A. R. Garófalo, et al., Low protein diet changes the energetic balance and sympathetic activity in brown adipose tissue of growing rats, Nutrition 25 (2009) 1186–1192. https://doi.org/10.1016/j.nut.2009.03.011.

[62] J. Taylor, G. Duodu, Resistant-type starch in sorghum foods-factors involved and health implications, Starch-Starke 75(9/10) (2022). https://doi.org/10.1002/star.202100296.

[63] T. H. Vu, S. Bean, C. F. Hsieh, et al., Changes in protein and starch digestibility in sorghum flour during heat-moisture treatments, J. Sci. Food Agr. 97 (2017) 4770–4779. https://doi.org/10.1002/jsfa.8346.

[64] M. D. Finke, D. Oonincx, Insects as food for insectivores, in: J. A. Morales-Ramos, M. Guadalupe Rojas, D. I. Shapiro-Ilan (Eds), Mass production of beneficial organisms, 2nd ed, Academic Press, 2014, pp. 583–616. https://doi.org/10.1016/B978-0-12-391453-8.00017-0.

[65] National Research Council, Nutrient requirements of laboratory animals, National Academy of Sciences Press, Washington, DC, 2011.

[66] F. T. Fombong, M. Van Der Borght, J. Vanden broeck, influence of freeze-drying and oven-drying post blanching on the nutrient composition of the edible insect *Ruspolia differens*, Insects 8 (2017) 102. https://doi.org/10.3390/insects8030102.

[67] M. D. Finke, G. R. DeFoliart, N. J. Benevenga, Use of a four-parameter logistic model to evaluate the quality of the protein from three insect species when fed to rats, J. Nutr. 119 (1989) 864–871. https://doi.org/10.1093/jn/119.6.864.

[68] T. C. Mosha, M. R. Bennink, Protein quality of drum-processed cereal-bean-sardine composite supplementary foods for preschool-age children, J. Sci. Food Agr. 84 (2004) 1111–1118. https://doi.org/10.1002/jsfa.1756.

[69] AVMA, AVMA Guidelines on euthanasia of animals: 2020 edition, American Veterinary Medical Association, Schaumburg, Illinois, 2020. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf.