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Effects of enriching sorghum-wheat bun diet with snail meat powder on blood glucose and glycemic responses in healthy rats

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KEY CONTRIBUTION
The sorghum-wheat bun diet was enriched with snail meat powder as a protein source. An enriched diet significantly reduced blood glucose levels in healthy rats. Glycemic responses were improved compared to the control diet. Findings suggest the potential of snail meat as a functional food ingredient. The study demonstrates a novel approach to enhancing glycemic control through diet.

ABSTRACT
Protein diets play a critical role in regulating energy intake and metabolism, which influences the glycemic response of the body to carbohydrates by lowering insulin activity and reducing fat deposition in adipose tissue. This study aimed to determine the effects of enriching the sorghum-wheat bun diet with snail meat powder on blood glucose and glycemic responses in healthy rats. Buns were prepared by replacing portions of the composite sorghum-wheat flour with 5%, 10%, 15%, 20%, and 25% snail meat powder (SMP). Postprandial blood glucose responses were monitored by sampling blood from the tail tips of healthy male Sprague-Dawley rats at intervals of 0, 15, 30, 45, 60, 90, and 120 minutes, using a glucose testing device. The glycemic index (GI) for each diet was determined by calculating the incremental area under the blood glucose response curve over two hours. The glycemic load (GL) was then calculated by multiplying the glycemic index percentage by the total grams of carbohydrates in the diets consumed. Enriching at 25% with SMP significantly reduced the total area under the curve for blood glucose responses, lowered the dietary glycemic index from 79 to 58, and decreased the glycemic load from a high of 26 to an intermediate level of 17. Sorghum-wheat buns enriched with SMP have the potential to reduce protein deficiencies, address hidden hunger, regulate blood glucose responses, and enhance blood glucose control in healthy rats. Diets that promote stable, low blood glucose levels provide benefits in regulating metabolic responses.

Introduction

Protein-rich diets play a vital role in regulating energy intake and metabolism, modulating glycemic responses to carbohydrates by reducing insulin activity, thereby lowering fat deposition in adipose tissues (Krul, 2012). Besides, they promote satiety and improve blood glucose control by activating satiety hormones and enhancing a feeling of fullness (Leidy et al., 2015). However, in sub-Saharan Africa, the diets of resource-underprivileged households are predominantly cereals or starchy staples with minimal or no animal protein intake (Food and Agriculture/United Nations - Economic Commission for Africa/African Union Commission (FAO/ECA/AUC, 2021). Such diets often fail to meet the nutritional needs for proper growth, cognitive development, and neurological function in children, leading to Protein-Energy Malnutrition (PEM) (Srikanth et al., 2014). Epidemiological studies have linked PEM with an increased risk of pancreatic beta-cell destruction, resulting in insulin deficiency and increased blood and urine glucose levels (Reusens et al., 2011; Yi and Hui, 2015). This deficiency also predisposes to disproportionate adipose tissue growth and a heightened risk of cardiovascular diseases, such as hypertension and hypercholesterolemia, later in life (Van Abeelen et al., 2012).

In recent decades, developing countries have witnessed a shift in disease patterns, marked by a rise in non-communicable diseases (NCDs) (Mudie et al., 2019). This shift has been attributed to rapid urbanization, which has led to increased demand for convenient foods, including baked goods such as buns (Adubofuor and AmoafoMensah, 2012). Refined wheat flour is the main ingredient in baking due to its unique rheological properties that enable it to hold and retain water vapour and carbon dioxide, creating a spongy texture in baked products (Odedeji et al.,2014). However, excessive consumption of refined wheat products has been associated with reduced satiety, alterations in the gut microbiome, and elevated blood glucose and lipid levels (Fulleret al., 2016). This has led to the exploration of composite flours made from locally available drought-resistant crops, such as sorghum, to partially replace wheat in baking (Amir et al., 2015; Agengo et al., 2020a). Sorghum is an excellent source of dietary fibre (Stefoska-Needham et al., 2015) and has the potential to lower the GI of composite flour (Singh et al., 2012). However, the protein content in sorghum exhibits poor digestibility in wet cooking (Duodu et al., 2003).

Lorusso et al. (2017) proposed the enrichment as a promising technique to improve the nutritional quality of composite flour by improving its functional and biological properties. This approach supports the development of low GI diets, which help regulate appetite, increase satiety, and reduce overall energy intake, thus playing a role in weight management (Warren et al., 2003). Snails are excellent sources of animal protein (Jummai and Okoli, 2013), whose consumption has been promoted in some parts of sub-Saharan Africa to combat protein-energy malnutrition (PEM) and hidden hunger (Engmann et al., 2013). Snail meat powder averages 60 to 70 g protein/100 g on a dry matter basis (Adeyeye and Afolabi, 2004) and is a rich source of high-quality, indispensable amino acid lysine that promotes the release of growth hormones in young children (Ebenebe, 2000; Uauy et al., 2015). In addition, it provides essential vitamins and minerals, such as iron, zinc, and calcium (Engmann et al., 2013; Marcel et al., 2020). Hence, incorporating SMP into sorghum-wheat composite flour could enhance the protein quality in the diet (Agengo et al., 2020b), and improve blood glucose control (Leidy et al., 2015). Buns, with their appealing taste, affordability, diverse flavours, and longer shelf life, are ideal vehicles for delivering these nutrients to young children (Ayo and Olawale, 2003).

Several factors can influence postprandial blood glucose responses, including the rate of gastric emptying, the hydrolysis of food, and the diffusion of hydrolyzed products in the small intestine (Carreira et al., 2004). Likewise, the presence of nutrients such as proteins, lipids, and fibres in the diet has been

shown to lower the GI of foods (Murillo et al., 2022), contribute to more stable post-meal plasma glucose (Lunde et al., 2011) and promote a healthy lipoprotein profile (Mumford et al., 2011). However, the effect of consuming sorghum-wheat buns enriched with SMP on immediate (up to 2 hours) blood glucose responses is unknown. An animal assay that closely resembles the human model is most appropriate for studying the interactions between diet and blood glucose regulation, which can help in proposing dietary guidelines for managing lifestyle diseases (Bolsinger et al., 2017). No previous studies have investigated the blood glucose and glycemic responses in healthy rats fed a sorghum-wheat bun diet. Therefore, this study evaluated the effects of enriching the sorghum-wheat bun diet with snail meat powder on blood glucose and glycemic responses in healthy rats.

Materials and methods

Materials for buns

Giant African Land Snails (*Achatina fulica*) were purchased from Prime-Cuts Ltd, Nairobi-Kenya, while white non-tannin sorghum (*Sorghum bicolor*) was obtained from the University of Eldoret farm, Eldoret-Kenya. Other ingredients included all-purpose wheat flour "Exe" (Unga Millers (U) Limited, Nairobi - Kenya), pure white sugar (Mumias Sugar Company Ltd, Mumias - Kenya), salt (Kensalt Limited, Nairobi - Kenya), shortening (Blue band - Unilever Kenya Limited, Nairobi - Kenya), non - fat dry milk "Miksi" (Promasidor Kenya Ltd, Nairobi, Kenya) and yeast "Sifa Fresh - Wet Yeast" (Agro-Chemicals and Food Company LTD, Muhoroni - Kenya) that were bought from supermarkets in Eldoret, Kenya.

Processing of snail meat and sorghum grains

Snails were rinsed in distilled water and boiled for 20 minutes to aid evisceration, eliminate intestinal tract contaminants harmful to consumers, and reduce the microbial load. After cooling, the viscera were separated from the shell, and the meat was cut into small pieces, dried at 105 °C for 2 hours, and cooled at room temperature before milling to a fine powder using a kitchen blender with a grinder (Wahl ZX805, 450w 3 Pint 2 Speed Silver). The snail meat powder was then stored in an airtight plastic container at room temperature until required for use. Sorghum grains were cleaned by sorting out materials such as sand, sticks, and leaves, washed, oven-dried, milled, and properly stored in airtight containers until required for use. Moisture content in the processed sorghum flour was 9.04 g/100 g.

Formulation and preparation of buns

Buns evaluated in this study were prepared as described in Agengo et al. (2020a). Sorghum-wheat composite flour was formulated in line with an acceptable cereal blend at a ratio of 7:3 (Ayo and Nkama, 2003). Flour for buns was prepared by replacing part of the composite flour with 5, 10, 15, 20, and 25% SMP. Six types of buns were developed according to the procedures of Pyler (1988). The buns were later allowed to cool at room temperature, weighed, and packed in separate zip-lock plastic bags.

Postprandial blood glucose responses

Twenty-eight (28) healthy male Sprague-Dawley rats aged 8 to 9 weeks, initially weighing between 110 g and 127 g, were obtained from the Biological Department at the University of Eldoret, Kenya. Each rat was housed individually in a wire mesh bottom cage measuring 25 x 22 x 20 cm, equipped with a faecal collection tray underneath, and placed in a well-ventilated room. Environmental conditions were maintained at an alternating 12-hour light/dark cycle, with temperatures averaging between 22 °C and 25 °C and humidity levels between 40% and 60%. Prior to the experiments, the rats were acclimatized

for 7 days, during which the rats were fed on standardized irradiated laboratory rat pellets (Hindustan Animal Feeds, Gujarat, India). This was meant to provide each rat with a 15 g daily meal, and deionized distilled water was provided *ad libitum*. Following acclimatization, the rats were individually weighed and then divided into seven (7) groups consisting of four (4) rats each. Thereafter, the rats were fasted overnight (from 6 pm to 6 am) and tested for blood glucose at zero time before receiving a test diet using a glucose tester device (On Call, Johnson and Johnson Co., Lifescon, USA). This was followed by the determination of blood glucose after 15, 30, 45, 60, 90, and 120-minute intervals post-test diet administration. A comparative procedure was also conducted with a group of rats provided with 0.15 standard glucose (reference diet), dissolved in 100 ml of deionized distilled water. Blood samples were obtained from the rats' tail tipping, and the animals were maintained following the US National Research Council (NRC) Guide for Care and Use of Laboratory Animals (NRC, 2011).

Glycemic index and Glycemic load

The GI for each diet was determined by calculating the Incremental Area Under two hours of blood glucose response or Curve (IAUC) and compared with the IAUC for the standard glucose solution according to the method described by FAO/WHO (1997) using the following equation:

$$\{\%\} = \frac{\text{Incremental area under 2 hours blood glucose curve for test diet}}{\text{Incremental area under 2 hours blood glucose curve for reference diet}} \text{ x } 100 \quad (1)$$

The GL was calculated by multiplying the percentage GI by grams of carbohydrate in the food serving consumed. The carbohydrate content presented in Table 1 is based on the proximate composition of buns reported by Agengo et al. (2020a):

$$\text{GL} = \frac{\text{GI x Available Carbohydrate (g)}}{100 \text{ g}} \quad (2)$$

GL accounts for the carbohydrate content of a diet and how each gram affects blood glucose levels. GL is classified as low (<10), intermediate (11-19), and high (> 20) (Eleazu, 2016).

Table 1. Effect of compositing sorghum-wheat with SMP on proximate composition of buns (g/ 100 g dry matter).

	Buns					
Proximate	**0%**	**5%**	**10%**	**15%**	**20%**	**25%**
Protein	9.93	11.97	13.63	15.58	17.75	20.51
Ash	3.08	3.34	3.42	3.52	4.01	4.12
Fat	10.46	10.47	11.10	11.38	11.43	11.89
Crude fibre	3.00	2.99	2.97	2.88	2.67	2.35
Moisture	7.82	7.43	7.12	6.73	6.66	6.55
Carbohydrate	65.68	64.84	63.48	62.19	60.44	58.01

Data analysis

Each analysis was conducted on three independent samples, and the data were analysed using a one-way analysis of variance (ANOVA). A Randomized Complete Block Design (RCBD) was used to determine the variations between groups, and means were determined using the Least Significant Difference (LSD) at $p<0.05$.

Results

Postprandial blood glucose response of buns

Table 2 presents the findings on the effect of enriching sorghum-wheat buns with SMP on postprandial blood glucose responses.

Table 2. Effect of compositing sorghum-wheat buns with SMP on postprandial blood glucose response (mmol/L).

Diets	Blood Glucose Response, mmol/L (Time in minutes)						
	0	15	30	45	60	90	120
Glucose	$3.8^{f}\pm0.13$	$5.5^{e}\pm0.05$	$6.3^{c}\pm0.06$	$6.8^{a}\pm0.13$	$6.6^{b}\pm0.25$	$6.2^{d}\pm0.19$	$5.5^{e}\pm0.17$
S-WB 0% SMP	$3.6^{g}\pm0.10$	$4.7^{e}\pm0.16$	$5.2^{c}\pm0.13$	$5.5^{a}\pm0.21$	$5.3^{b}\pm0.37$	$4.8^{d}\pm0.41$	$4.4^{f}\pm0.21$
S-WB 5% SMP	$3.8^{g}\pm0.30$	$4.4^{d}\pm0.36$	$4.8^{b}\pm0.18$	$5.1^{a}\pm0.33$	$4.5^{c}\pm0.32$	$4.2^{e}\pm0.23$	$4.0^{f}\pm0.14$
S-WB 10% SMP	$3.5^{g}\pm0.22$	$4.3^{d}\pm0.21$	$4.7^{b}\pm0.43$	$4.8^{a}\pm0.18$	$4.6^{c}\pm0.29$	$4.2^{e}\pm0.15$	$3.6^{f}\pm0.14$
S-WB 15% SMP	$3.5^{g}\pm0.33$	$4.1^{d}\pm0.22$	$4.4^{b}\pm0.14$	$4.5^{a}\pm0.23$	$4.2^{c}\pm0.07$	$3.9^{e}\pm0.12$	$3.6^{f}\pm0.11$
S-WB 20% SMP	$3.4^{g}\pm0.11$	$3.7^{d}\pm0.03$	$4.2^{b}\pm0.19$	$4.3^{a}\pm0.45$	$3.9^{c}\pm0.28$	$3.6^{e}\pm0.34$	$3.5^{f}\pm0.09$
S-WB 25% SMP	$3.4^{g}\pm0.09$	$3.7^{d}\pm0.14$	$4.0^{b}\pm0.32$	$4.2^{a}\pm0.09$	$3.9^{c}\pm0.10$	$3.6^{e}\pm0.17$	$3.5^{f}\pm0.10$

Values are means ± standard deviations. Values followed by the same letter superscripts in the same row are not significantly different at (p<0.05) as assessed by the Least Significant Difference.

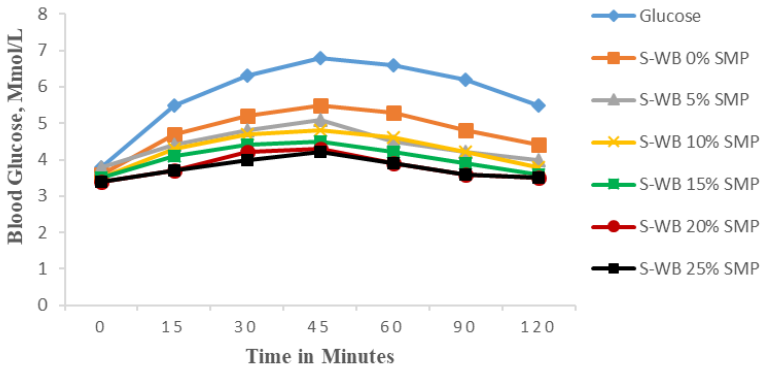

Figure 1. Postprandial blood glucose concentration 2 (two) hours after the consumption of reference and test sorghum-wheat bun meals (diets).

Glycemic index (GI) and Glycemic load (GL) of buns

Table 3 indicates the GI and GL values, along with their respective classifications, for each test diet.

Table 3. Effect of compositing sorghum-wheat buns with SMP on glycemic index and load.

Diet	Glycemic index	Classification	Glycemic load	Classification
Glucose	100	High	-	-
S-WB 0% SMP	79	High	26	High
S-WB 5% SMP	70	High	23	High
S-WB 10% SMP	65	Medium	21	High
S-WB 15% SMP	62	Medium	19	Intermediate
S-WB 20% SMP	59	Medium	18	Intermediate
S-WB 25% SMP	58	Medium	17	Intermediate

Glycemic indexes are classified as high (≥70), medium (56-69), and low (≤55) (Kouame et al., 2017); glycemic loads as high (≥20), intermediate (11-19), and low (≤10) (Eleazu, 2016).

Discussion

The plasma glucose responses in rats fed sorghum-wheat bun diet varied significantly from one another and the reference diet, with maximum peak values observed at 45 minutes (Table 2). This variation could be attributed to increased protein content (Table 1), achieved by blending sorghum-wheat buns with varying proportions of SMP. Nutrients like protein, lipid, and fibre in the diet have been shown to reduce the glycemic index (GI) of foods and stabilize post-meal blood glucose levels (Leidy et al., 2015; Lunde et al., 2011). Likewise, enriching sorghum-wheat bun diets with SMP reduced the peak blood glucose responses (Figure 1). This may be due to the optimal fat composition (10-25 g/100 g) and the recommended fibre content (2.0 g/100 g) for baked products (FAO/WHO, 1994; Olaoye and Onilude, 2008). Fats enhance satiety and regulate blood glucose responses (Jariyah et al., 2018), while fibre slows glucose absorption in the digestive tract, ultimately lowering blood glucose responses (Astawan and Widowati, 2011). Therefore, enriching sorghum-wheat bun diets with SMP could lower the risk of chronic diseases such as type II diabetes and cardiovascular diseases.

Figure 1 illustrates the peak postprandial blood glucose responses following the consumption of both reference and test sorghum-wheat bun diets. Rats fed the reference diet displayed rapid, high-onset peak plasma glucose levels, reaching a mean peak of 6.8 mmol/L within the first 45 min. Subsequently, 15 minutes later (at 60 min), it began to decline, reaching a final mean value of 5.5 mmol/L at 120 min. These results are consistent with previous studies by Thannoun and Al-Kubati (2010), Mlotha et al., (2016) and Kouame et al. (2017). The rapid increase in plasma glucose responses can be attributed to the quick absorption and utilization of pure glucose in the body (Rajamani and Raajeswari, 2016). In contrast, enriching sorghum-wheat bun diets with SMP significantly lowered their glycemic indices, resulting in a reduction of both the area under the curve and the overall blood glucose responses. Lanzerstorfer et al. (2018) found that enhancing the protein quality of white bread significantly reduced peak postprandial blood glucose responses in healthy individuals. Thus, diets that elicit rapid, high-onset peak blood glucose responses can be detrimental to individuals with elevated fasting glucose levels, such as those who are obese or diabetic (Chiavaroli et al., 2021).

Substituting 25% of the sorghum-wheat bun diet with SMP reduced the GI from 79 in the unenriched diet to 58 (Table 3). This reduction can be attributed to the increased protein content in the diet (Table 1). Thannoun and Al-Kubati (2010) previously reported a decrease in GI from 70 to 46 when white bread was enriched with lentils, chickpeas, and kidney beans. Similarly, Bonnema et al. (2016) established that consuming a high-protein breakfast lowered post-meal blood glucose responses and improved glucose regulation throughout the day. Protein-rich diets promote insulin secretion without causing a corresponding rise in glucose levels, thereby reducing the GI of the diet (Leidy et al., 2015). The GI of foods measures the blood glucose responses following the consumption of carbohydrate-rich foods (Ebere et al., 2021). Therefore, a diet high in GI is associated with an increased risk of insulin resistance, type II diabetes, and coronary heart disease (Akanbi and Ikujenlola, 2016).

Notwithstanding, low-GI diets can sometimes provoke high blood glucose responses, while high-GI foods may result in lower responses, depending on serving sizes (Ebere et al., 2021). This phenomenon is better explained by the GL of foods, which accounts for both the quality and quantity of carbohydrates in influencing blood glucose responses (Jariyah et al., 2018). Enriching a sorghum-wheat bun diet at 25% with SMP reduced its GL from a high level of 26 to an intermediate level of 17 (Table 3). High GL diets can increase the risk of diabetes by continually raising insulin demand, which can lead to β-cell exhaustion, dysfunction, and apoptosis (Kouame et al., 2017). To achieve a low GL diet, one can either opt for smaller portions of high-carbohydrate foods or choose protein-rich alternatives (Eleazu, 2016).

Hence, replacing part of the sorghum-wheat bun diet with SMP could effectively manage metabolic responses, providing an alternative to only reducing carbohydrate portions.

Conclusions

The buns evaluated in this study have the potential to combat protein deficiencies and hidden hunger. Enriching sorghum-wheat buns with SMP led to a reduction in both the GI and GL of the diets. As a result, there was a decrease in the area under the curve for blood glucose responses, which significantly improved blood glucose control in healthy rats. Diets promoting stable, low blood glucose levels are beneficial in regulating metabolic responses.

Author Contributions: A.F.B. designed, conducted the research, and wrote the paper. O.A.N. conceptualized the study, provided leadership for the research activities, and edited the manuscript. S.C.A. developed the research model, analyzed the data, and contributed to the editing of the paper. J.K.O. reviewed and edited the manuscript. All authors reviewed and approved the final version of the manuscript.

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***Conflicts of Interest*:** The authors declare no conflict of interest.

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