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Finger Millet and Defatted Sesame Seeds Flours as Complementary Foods: Nutritional Evaluation and Protein Quality

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The role of germination and fermentation on nutritional protein quality indices of finger millet and defatted sesame seeds complementary foods was studied comprising of non-germinated non-fermented finger millet and defatted sesame seeds (NGNFFS), non-germinated fermented finger millet and defatted sesame seeds (NGFFS), germinated non-fermented finger millet and defatted sesame seeds (GNFFS) and germinated fermented finger millet and defatted sesame seeds (GFFS).
All amino acids composition increased with germination and fermentation except for lysine and tryptophan which were limiting essential amino acids. Notable amino acid increments were

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Methionine 0.88- 3.13, arginine 6.15- 6.66 and leucine 9.67- 10.66, isoleucine, histidine, threonine, phenylalanine and valine increased from 2.96- 4.05 respectively. The protein efficiency ratio (PER) for NGNFFS was higher (1.17) than the control (0.52), net protein retention (NPR) for NGNFFS was more (0.65) than control (0.23). Their relative values R-PER and R-PER were within a similar range as control. The mean weekly weight changes of animals varied from 56.4- 85.2g (NGNFFS), 56.4- 86.7g (GNFFS), 57.0- 84.1g (NGFFS), 56.2- 80.4 (GFFS), 56.0- 79.0g (nutrend) and 57.0- 40.4g (basal diet) respectively.

Keywords: Complementary foods; fermentation; germination; nutrients.

1. INTRODUCTION

Infancy and early childhood require adequate nutrition which is fundamental to growth and development. This is a critical stage in the lives of children because it is when behavioural development, health and optimal growth take place. Complementary foods are foods other than breast milk or infant formula (liquids, semisolids, and solids) introduced to an infant to provide nutrients [1]. These foods are formulated from a combination of food materials comprising of cereals, legumes, tubers, roots and seeds. Irrespective of their sources, complementary foods are expected to be high in energy density, rich in protein quality and possess low levels of anti-nutritional components [2]. Animal proteins such as meat and milk are important for complementary feeding. However, these are expensive and unaffordable for most of the population in Nigeria.

Protein-energy malnutrition (PEM) is a major concern in Nigeria especially among children and expectant mothers in rural communities and internally displaced persons (IDP) camps. Milk, meat and fish are good sources of protein but are quite expensive and out of reach of vulnerable groups in Nigeria. Cereals like sorghum and maize constitute the major source of complementary foods in developing countries like Nigeria [3]. These products may be inadequate in protein, essential amino acids and mineral quality and quantity, thus the need for complementary foods of which are available and affordable [3].

Finger millet contains a substantial amount of essential amino acids, which include arginine, leucine, threonine and valine [4]. This makes finger millet highly important and a good source of complementary and functional foods.

Sesame is grown mainly for its seeds, which contain approximately 50% oil and 25% protein [5]. It is an important oil seed that is incorporated in several products due to its fiber and protein quality [6].

Germination and fermentation enhance the quality of complementary food products. Hence, proper processing and judicious blending of locally available foods could result in improved intake of nutrients [7]. Therefore, the aim of the study is to ascertain the quality of protein, essential amino acids and minerals of finger millet and defatted sesame seeds.

2. MATERIALS AND METHODS

2.1 Sources of Raw Materials and Preliminary Handling

Essentially 5kg of red finger millet (*Eleusine coracana*) and white sesame seeds (*Sesamum indicum*) were bought from Wadata market, Makurdi (yes but at the point I can't change this). The raw materials were cleaned and sorted to remove defective and unwholesome particles. The basal diet ingredients were bought from a local shop in Makurdi. Rice husk was obtained from Mikap Nig. Ltd. Gboko road, Makurdi (even though Mikap is not a standard research institute, it is a certified and standard organization in Nigeria). A 25kg acclimatization starter feed (chicken feed, Crown Flour Mill Ltd) was purchased from Wurukum market, Makurdi. Thirty Wister albino rats (male) were bought from the Department of Animal Science, Benue State University Teaching Hospital, Makurdi, Benue State, Nigeria.

2.2 Production of Flour Samples

2.2.1 Preparation of germinated and non-germinated finger millet flour

The process for the production of finger millet flours is shown in Fig. 1 as described by Ariahu et al., 1999(b). Finger millet grains were washed and steeped in water at room temperature using the ratio of 1:3 (w/v grain: water) in a bucket. The steep water was replaced every 4 hours for a period of 12 hours and then drained. Wet grains

were spread out to germinate while water was continuously sprinkled on them. The non-germinated and germinated grains were removed at 0 and 72 hours and dried. The dried seeds were rubbed between the palms to separate testa and rootlets from cotyledons and then the seeds winnowed. The resulting dried seeds were milled using a blender and sieved with recycling of the seeds to improve output. The non-germinated finger millet (NGF) and germinated finger millet (GF) flours were sealed and stored properly for analysis.

2.2.2 Preparation of fermented finger millet flour

The fermented finger millet was obtained by natural lactic acid fermentation [8] as also shown in Fig 1. 100g each of the germinated (GF) and non-germinated (NGF) finger millet was mixed with distilled water to form slurry and then allowed to ferment naturally for 24 hours. At the end of the period, 50% of the fermented mixture was used as a starter culture for a new fermentation cycle. pH and titratable acidity (an index of lactic acid bacteria activity) were monitored during this period. The process was allowed to continue to enable a stable and constant pH. The resultant concentrates were dried in an oven and milled in a blender to obtain germinated and fermented finger millet (GFF) and non-germinated fermented finger millet (NGFF).

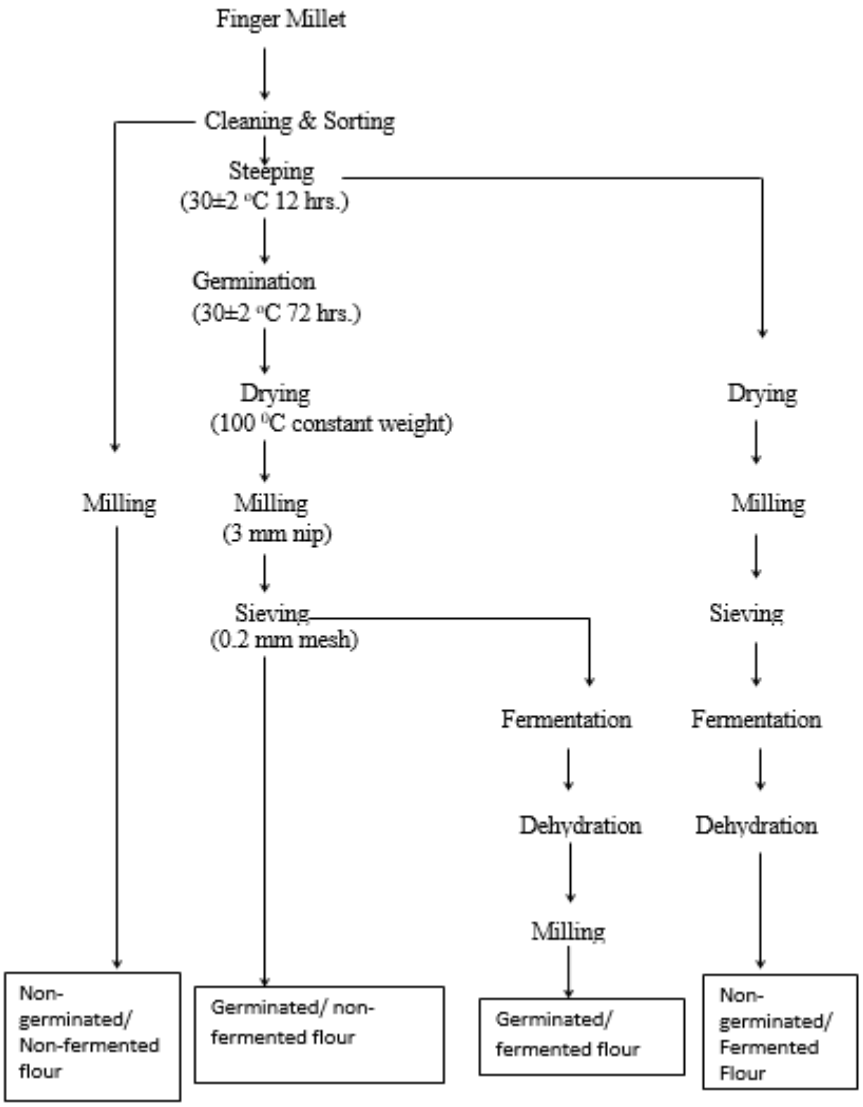

Fig. 1. Flow chart for the preparation of germinated and fermented finger millet flour

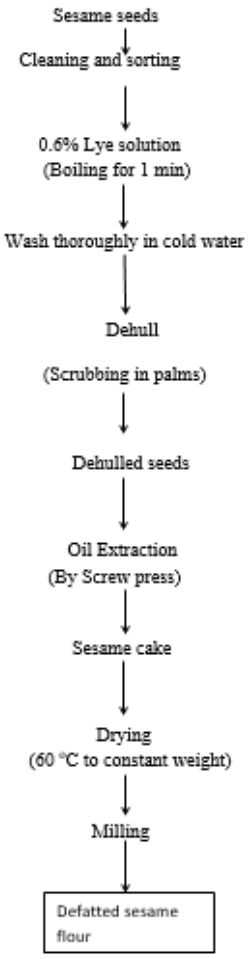

Fig. 2. Flowchart for the production of defatted sesame
Source: Gernah et al. [3]

2.2.3 Preparation of defatted sesame flour

The process for sesame seeds flour formulation by Ravindra, [4] is shown in Fig. 2. The seeds were sorted and cleaned to remove debris and stones. The seeds were soaked in warm water to soften the seed coat and dehulled by rubbing between palms followed by washing. The resultant seeds were dried, milled and defatted by a screw press method as described by Muthamilarasan,[6]. Defatted flour was analyzed for fat until a fat content of 15% in the cake. The cake was dried in an oven and milled.

2.3 Food Products Development

The effect of germination and fermentation on four different food formulations comprising non-germinated finger millet + defatted sesame (NGNFFS), germinated finger millet+ defatted sesame (GNFFS), non-germinated fermented finger millet + defatted sesame (NGFFS) and germinated fermented finger millet + defatted sesame (GFFS) were made by incorporating finger millet flours with defatted sesame flour to obtain 16g protein and 9g fat/100g as recommended by the Protein Advisory Group (PAG). Material balancing was used to obtain the amount of the four blends of finger millet/sesame seeds flour from their respective proximate compositions [9].

2.4 Feeding Tests

Animal feeding experiment was performed using Wister male rats with a modification of the method described by Pellet et al., [10]. A complete randomized design (CRD) was used, and 30 Wister rats were weighed into six wooden cages with five animals per cage at the beginning

of each feeding trial. The cages were placed on a platform for the collection of faeces. The animals were acclimatized by feeding poultry starter feed for one week. After acclimatization, test diets were formulated, and control was mixed with a basal diet to obtain a protein level (9g) while the basal diet was fed directly. The rats were fed the diets and water *ad libitum* for 28 days. The average food intake of each group was determined daily while average weight gain was determined at 2 days interval. The amount of Protein consumed was calculated by material balance from food intake. Faeces collected from day 10-28 were dried, weighed and milled into fine powder for analysis. Protein efficiency ratio (PER) and net protein ratio were estimated from average feed intake and average weight gain at 14 and 28 days of feeding.

2.5 Evaluation of Protein Quality Indices

2.5.1 Amino acids determinations

Amino acid composition of products was evaluated using an amino acid analyzer based on the high-performance liquid chromatography technique described by Rhon et al.,[11]. Samples of hydrolysate were prepared as described by Pellet and Young [10]. A mixture of standard amino acids was carried through the hydrolysis and incubation process.

HPLC Analysis: An aliquot of 150ul of each hydrolysate was injected into the amino acid analyzer (Skykam S7130) using a cation separation glass column at 130 ^{o}C.

The reaction mixture was detected at 570nm and 440nm on a dual-channel photometer. The amino acid composition was calculated from areas of standards obtained from the integrator and expressed as a percentage of total protein. The amino acid scores were obtained relative to FAO/WHO reference patterns.

2.5.2 In-vivo protein evaluation

Protein quality indices were determined using standard methods. From the values of Mean Daily Feed Intake (MFDI) and Mean Daily Weight Gain (MDWG) obtained, Protein Efficiency Ratio (PER), and net Protein Retention (NPR) were estimated by the method of Jena *et al*., 2020 as follows:

$$NPR = \frac{Wt-Wb}{Wp} \quad (8)$$

$$PER = \frac{Wt}{Wp} \quad (9)$$

$W_{t\,=}$ average weight gain of test animals on a given diet, W_b = average weight loss of animals on basal diet, W_p= protein consumed by test animals on the given diet

The relative PER (R-PER) and relative and relative NPR (R-NPR) were obtained by relating the PER and NPR values respectively to those of Animal Nutrition Council (ANCR) casein which are 2.5 for PER [2] and 4.02 for NPR [2] as follows

$$R-PER = \frac{PER\ of\ diet}{PER\ of\ ANCR-CASEIN} \quad (10)$$

$$R-NPR = \frac{NPR\ of\ diet}{NPR\ of\ ANCR-CASEIN} \quad (11)$$

2.5.3 Feeding experiments

The four test diets were formulated using material balancing [9] by blending 43.75 g of the basal (nitrogen-free) diet with 56.25g of NGNFFS, GNFFS, NGFFS and GFFS, respectively, to give 9 g protein/100g of each test diet. The basal diet consisted of corn starch: 80 g/100 g, corn oil: 10 g/100 g, common table salt: 4 g/100 g, Sugar: 1 g/100 g, vitamin: 1 g/100 g and non-nutritive fiber (rice husk): 4 g/100 g.

3. RESULTS AND DISCUSSION

3.1 Protein Quality Indices

3.1.1 Essential amino acid profile of complementary foods

Essential amino acids composition is shown in Table 1 and the FAO/WHO reference pattern was used as a means of comparing the result. The result of the essential amino acids composition of the formulated products was within the FAO/WHO/UNU standards. It was observed that leucine had the highest (10.66) mean value compared to other amino acids. GNFFS and NGNFFS had the highest amounts of arginine, isoleucine, lysine, histidine, threonine, phenylalanine and valine having values ranging from 2.96- 4.05, while tryptophan and methionine values ranged from 0.88-3.13. The total amino acids (TAA) of food samples were above average recommended values. This indicates that the complementary foods are nutritionally adequate to meet the essential amino acid demand of children. In comparison, the total amino acid profiles of GNFFS and GFFS

in the current study were higher compared to melon (53.4 g/100 g) and pumpkin (38.3 g/100 g), respectively as reported by Davidson et al., [12-14] The values for amino acids in the formulated diets were superior to those of popcorn-based diets [15,16]. Cereals are deficient in lysine and tryptophan (Mensah et al.,2023) but in addition to legumes, which are rich in tryptophan and lysine but deficient in sulphur-containing amino acids, a desirable pattern of essential amino acids comparable to or higher than the reference protein is obtained [17-19]. The use of finger millet and sesame-based foods are therefore suggested as alternative protein and energy sources for infant and adult food products (Mensah et al., 2023).

3.1.2 *In-vivo* protein quality indices

A comparison of the NPR and PER mean values of the formulated food with the corresponding ANRC (casein) resulted in the R-NPR and R-PER data which ranged from 0.21-0.95 and 0.85-0.94 as well as 0.06– 0.13 and 0.33–0.475, as shown in Table 2 respectively. The PER values of GFFS and GNFFS were high (2.36 & 2.25) compared to the control diet, which had the lowest value (2.12). The PER relates weight gain in the test animals and the corresponding protein intake while NPR indicates the relationship between weight changes in the animals fed the test diets relative to those fed the basal diet [20,21].

As shown in Figs. 1 and 2, there was a steady increase in the feed intake by the test animals with the control diet being consumed higher (108.16g) than the basal diet (43g-39g) notably with a decrease in consumption to the other test diets. The high daily feed intake by animals fed with a control diet compared with the experimental food samples might be due to the fortification of the formula with quality protein like casein or other quality protein food materials. The GNFFS recorded a consumption rate of 56.37g – 85.70g. This was the most consumed diet among the test diets, which could be due to the formation of better flavours and taste by germination [22,23].

Table 1. Essential amino acid profile of finger millet and defatted sesame based complementary food

Nutrients	NGNFFS	Products GNFFS	NGFFS	GFFS	*RDA
Lysine	2.96	3.16	2.88	3.25	5.8
Histidine	3.21	3.76	3.06	3.18	1.9
Arginine	6.66	7.32	6.15	6.82	2.0
Threonine	3.55	3.66	3.45	3.33	3.4
Valine	3.75	3.81	3.56	3.48	3.5
Methionine	1.96	2.44	2.37	3.13	2.2
Isoleucine	4.16	4.05	4.28	4.18	2.8
Leucine	10.66	9.78	9.92	9.67	6.6
Tryptophan	0.88	0.92	1.13	0.98	1.1
Phenylalanine	3.69	4.11	4.28	4.34	2.8

Results are means ± standard deviation of triplicate determinations. Key: NGNFFS= non-germinated non-fermented finger millet and defatted sesame flour, GFFS= germinated fermented finger millet and defatted sesame flour, NGFFS= non-germinated fermented finger millet and defatted sesame flour, GNFFS= germinated non-fermented finger millet and defatted sesame flour

Table 2. *In-vivo* protein quality of test diets

Parameter	NGNFFS	Products NGFFS	GNFFS	GFFS	NUTREND	LSD
NPR	3.45^{c}±0.01	3.46^{c} ±0.07	3.51^{d} ±0.02	3.81^{b} ±0.02	3.23^{a}±0.02	0.046
PER	2.17^{c} ±0.07	2.21^{c} ±0.01	2.25^{c} ±0.07	2.36^{b} ±0.07	2.12^{a}±0.10	0.124
R-NPR	0.85^{b} ±0.02	0.86^{b} ±0.02	0.21^{b}±0.07	0.95^{a} ±0.07	0.80^{a}±0.01	0.453
R-PER	0.87^{c} ±0.00	0.88^{c} ±0.07	0.90^{c} ±0.07	0.94^{b}±0.07	0.85^{a}±0.01	0.023

Results are means of ± standard deviation of duplicate expressed on dry weight. Values along each row with superscripts are not significantly ($p<0.05$) different.

Key: GNFFS= germinated non-fermented finger millet and defatted sesame flour, GFFS= germinated fermented finger millet, NGFFS= non-germinated fermented finger millet and defatted sesame flour

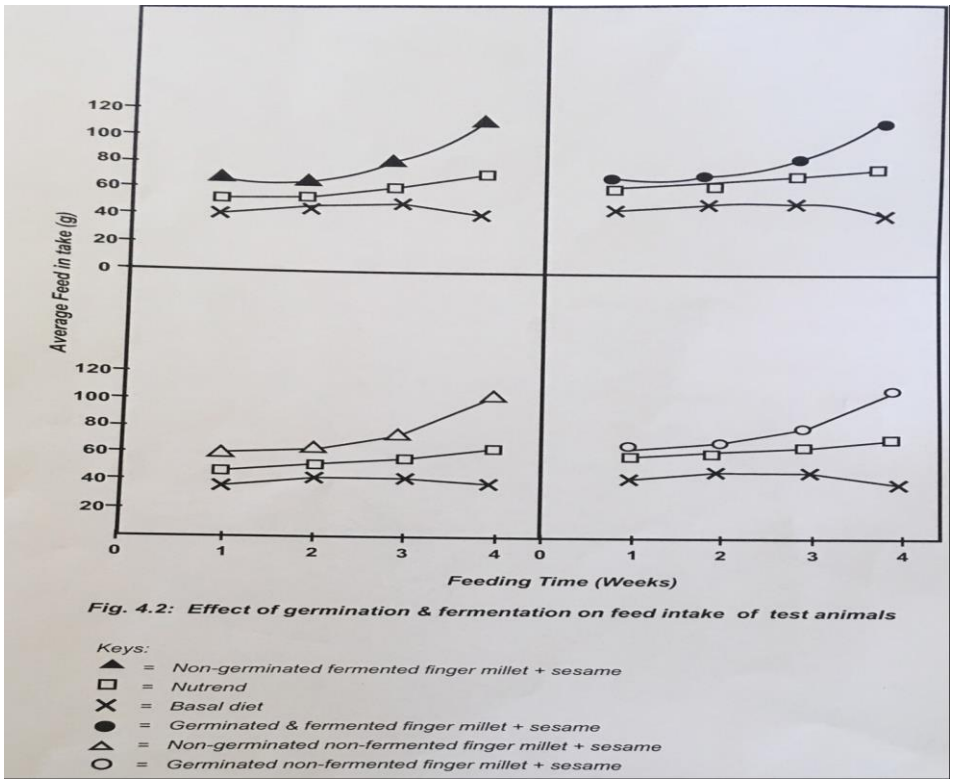

Fig. 3. Effect of germination & fermentation on feed intake of test animals

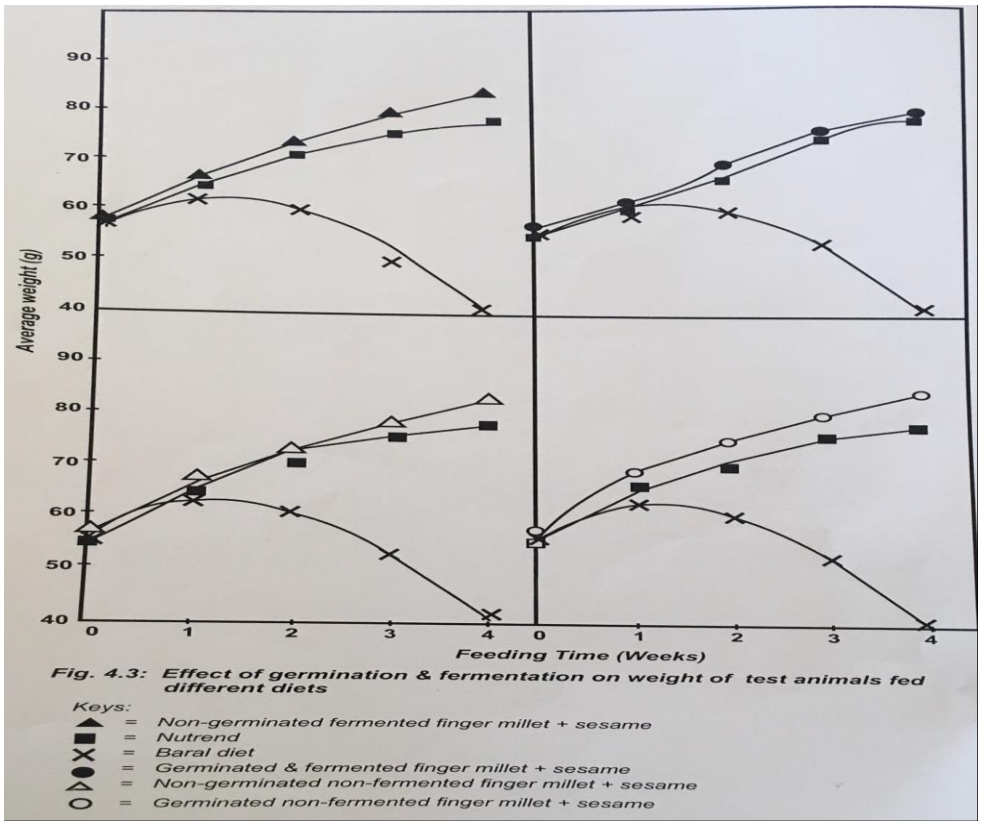

Fig. 4. Effect of germination & fermentation on weight of test animals fed different diets

4. CONCLUSION

Germination and fermentation of finger millet improved the essential amino acids composition, protein efficiency ratio, and the overall nutritional content of the products. The essential amino acid of the test diets was above the recommended values of FAO/WHO [24]. There was an increase in the weight of the test animals; this may be due to the bioavailability of nutrients made accessible by germination and fermentation.

There was better nutrient quality demonstrated by growth performance of test animals as well as reduction in bulk. This is ideal for children as they require little food with high protein, less bulk and high energy because of the capacity of their stomach.

Results obtained from this study are recommended as baseline data for standardization and regulatory policies of finger millet and defatted sesame seeds based complementary foods.

AVAILABILITY OF DATA AND MATERIAL

The data supporting the findings of this study are available from the corresponding author, upon reasonable request.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) herby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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