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Soy flour snack bars lower glycaemic response in type 2 diabetes mellitus subjects: A randomised cross-over design

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ABSTRACT

Introduction: Low glycaemic snacks may help to improve blood glucose control. However, data on the effect of soybean snack bars on postprandial glucose levels of the diabetic population is scarce. Therefore, the current study aimed to examine the effect of consuming soy flour snack bars on glycaemic response (GR) over a 180-minute period in individuals with diabetes by estimating postprandial glucose levels variation and total area under the curve (AUC). **Methods:** Nine subjects (age: 54.6±4.0 years; BMI: 25.0±2.5 kg/m^2) with type 2 diabetes mellitus (T2DM) diagnoses without complication enrolled in this randomised, open-label, cross-over trial. On three separate sessions, they consumed glucose standard solution, soy flour snack bar (SF), and wheat flour snack bar (WF) containing 25 g of available carbohydrate, respectively. Finger prick capillary method was executed to measure blood glucose levels at 30, 60, 90, 120, 150, 180 minutes after test product ingestion. **Results:** Overall, significantly lower postprandial glucose levels were observed at 30, 60, 90, and 120 minutes (122.3±17.6, 136.3±24.9, 125.7±25.3, and 107.2±24.1 mg/dL; p<0.001) in those who consumed SF snack bars than WF snack bars (147.9±41.3, 168.0±43.6, 152.6±30.0, and 140.6±33.4 mg/dL). The AUC level after the ingestion of SF snack bar was 2044.8±503.1 mg.min/dL, >20% lower compared to ingestion of WF snack bar (4735.0±666.8 mg.min/dL), p<0.001. These glycaemic control benefits can be explained due to the high fibre and protein content linked to the physicochemical properties of SF. **Conclusion:** With high nutritional properties, SF snack bar has a low GR and might help control blood glucose in T2DM subjects.

Keywords: glycaemic response, hyperglycaemia, snack, soy food, T2DM diet

INTRODUCTION

Diabetes is a prevalent public health disease. Nearly 90% of diabetic cases are type 2 diabetes mellitus (T2DM) (Goyal & Jialal, 2020). According to the latest data, the prevalence of diabetes was 10.9% or equivalent to more than 10 million Indonesian people in 2018 (MOH Indonesia, 2018). It is estimated that this number will sharply increase to 16.6 million in 2045, among whom 7.9 million are undiagnosed (IDF, 2019). The development of T2DM is caused by the reduction of insulin release and glucose

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utilisation, as well as elevation of glucose synthesis, which lead to significant changes in postprandial blood glucose on a frequent basis (Mouri & Badireddy, 2020). Moreover, the HbA1c level in T2DM patients is higher than normal, and it is positively correlated with body mass index (BMI) (Babikr *et al.*, 2016). A previous study found that T2DM subjects with an excess weight status have a higher risk of poor blood glucose control (Bae *et al.,* 2016). However, a study by Wang *et al.* (2017) showed that blood glucose levels at 30 and 120 minutes (min) postpandrial were higher in T2DM patients who were underweight or normal weight (BMI <24 kg/m^2) than those who were overweight or obese (BMI ≥24 kg/m^2).

In individuals with diabetes, hyperglycaemia is related to poor glucoregulation (glycaemic control) and the development of serious complications, including diabetic retinopathy (eye), nephropathy (kidney), neuropathy (nerves), and cardiomyopathy (heart) (Mouri & Badireddy, 2020). The current review recommends new efforts to control hyperglycaemia, i.e. by selecting foods with low glycaemic index (GI) and glycaemic load (GL) (Yari *et al.,* 2020). Evidence widely documents that consuming low GI foods is capable of attenuating 24-hour blood glucose profile and decrease postprandial glucose level (Kaur *et al.,* 2016). Moreover, the type and amount of carbohydrates consumed and the physical form of food are the other known factors associated with postprandial blood glucose fluctuation level (Franz *et al.,* 2002). Thus, glycaemic control through diet modification is a well-known strategy for managing postprandial blood glucose, while limiting the risk of hyperglycaemia or elevated blood glucose greater than normal level [>140 mg/dL (7.8 mmol/L)] (Soelistijo, Lindarto & Decroli, 2019). In addition, regular practice of an eating plan such as having small and frequent portions, and accounting for snack time between meals, would also improve blood sugar control in subjects living with T2DM (Gray & Threlkeld, 2019).

Soy foods have long been a staple of the Asian cuisine, and their popularity is now spreading worldwide and reflecting today's global health, nutrition and lifestyle trends (Dukariya *et al.,* 2020). A wide variety of soy foods are available, such as traditional soy foods (soy milk, tofu, *tempeh*, natto) and other foods that use soy flour as a functional ingredient (bakery, pasta, snack bar) (Jideani, 2011). For the past 25 years, nutritional and health benefits of soy foods have been extensively explored, especially for their role in managing postprandial blood glucose in diabetic cases (Dukariya *et al.,* 2020; Lecerf *et al.,* 2020). *Tempeh*, one of the most widely consumed soy-based foods in Indonesia, has been found to be negatively correlated with insulin resistance (Febrianti *et al.*, 2019).

In the last few years, investigation on alternatives from wheat flour to soy flour has been conducted (Mohammed, 2019). The findings showed that soy flour has better nutritional properties than wheat flour, indicated by high levels of fibre, protein, healthy fat, isoflavones, as well as low carbohydrate and moisture content, which may make soy flour more beneficial to control blood glucose level than wheat flour. For instance, the latest study investigating the consumption of soy food products in the form of snack bars has been scientifically proven effective on controlling postprandial glucose levels, where significantly lower blood glucose levels and reduction in glycaemic response (GR) were observed (Nurdin *et al.,* 2020; Urita *et al.*, 2012). Also, studies by Nurdin *et al.* (2020) and Urita *et al.* (2012) observed that the consumption of wheat flour snack

bar was linked to significantly higher postprandial glycaemia than soy flour snack bar consumption.

Consequently, this study used soy flour snack bar as an alternative food form to investigate the effect of soy flour on postprandial glucose level. Moreover, to date, snack bars made from soy flour have not been previously explored in Indonesia. Following the previous study finding, which has been conducted among healthy subjects, we found that soy flour (SF) snack bar consumption was more effective to control blood glucose than wheat flour snack bar (WF); and SF snack bar can be recommended as a potential snack alternative for healthy subjects with blood glucose concerns (Nurdin *et al.,* 2020). Therefore, the present study is a follow-up study aiming to examine the effect of consuming SF snack bar on postprandial glucose levels in subjects with T2DM, in which variations in postprandial glucose levels were monitored at several time points over a 180-minute period. Also, different BMIs may give glycaemic variability; thus, as a secondary analysis, this study also compared GR by estimating the total area under the curve (AUC) of blood glucose according to BMI. It was hypothesised that consumption of SF snack bar would attenuate postprandial glucose level by having lower blood glucose level and low GR due to its low GI value, high fibre and protein content. Moreover, an increase in BMI would increase the risk of having poor GR, indicating impaired postprandial glucose levels.

MATERIALS AND METHODS

Study design and population

A cross-over randomised controlled trial with a seven-day wash-out period on the glycaemic control benefit of soy flour snack bar was executed in October 2019 to January 2020 at IPB University, Indonesia. A non-blinded (open-label) design was carried out to obtain additional scientific evidence about the GR of soy flour-based snack bar in people with T2DM. An available soy flour-based snack bar in the Indonesian market (SOYJOY®) was used as test food. The study procedure had three phases.

Phase 1 (Selection and enrolment phase)

Phase 1 was conducted one week prior to the first meal glucose tolerance test. In this selection and enrolment phase, subjects were enrolled through the Indonesian Diabetes Association (PERSADIA) chapter Bogor, IPB Bogor Clinic, and social media announcement. The inclusion criteria were 1) men or women aged 40 – 60 years, previously diagnosed with T2DM without complication, 2) blood glucose was controlled by one or two types of antidiabetic drugs (metformin and/or insulin secretagogue), 3) Haemoglobin A1c (HbA1c) level ≤8%, 4) no bowel issues (diarrhoea or soy allergy/intolerance), 5) no alcohol and smoking, and 6) able and comply with the study. A total of 96 subjects were enrolled and further screened by healthcare practitioners for health, including physical examinations; laboratory tests [HbA1c, aspartate aminotransaminase (AST), and alanine aminotransaminase (ALT)]; health interviews and family medical history. Most of the screened subjects were excluded for not meeting the inclusion criteria, had insulin therapy, had food allergy/intolerance history, phobia towards needles, or were not willing to be pricked on the fingers. Among those enrolled, only nine subjects passed the screening. Each subject gave his/her written consent for the study during the selection visit. Approval of the study protocol was obtained from the Human Research Ethics Committee of IPB University No. 142/IT3.KEPMSM-

IPB/SK/2019. Figure 1 explains the CONSORT study diagram.

Phase 2 (Preparation)
The preparation phase was the process of allocating the subjects into their respected groups for intervention purposes. This phase included two types of preparation, i.e. room and subject. A room with ambient temperature (maximum 20°C) was prepared for the process of blood sample withdrawal. A 10-hour overnight fast and no vigorous exercise were prerequisites for the subjects. The investigator instructed the subjects to start fasting at 8 p.m. the previous night until morning. In the fasting state, only plain water was permitted. Fasting blood glucose was taken between 8 to 10 a.m.

Phase 3 (Blood glucose measurements)
Subjects were allocated in an open-label trial to consume the test product, i.e. glucose standard solution (glucose anhydrous, D-glucose MERCK®, SG) (reference food), soy flour-based snack bar (SOYJOY®, strawberry flavour, SF), and wheat flour-based snack bar (WF) (test food). A healthcare practitioner was in charge of taking the subjects' blood samples. Before subjects consumed the test product, fasting blood glucose was withdrawn at 0 minute time point (baseline). After the baseline blood was drawn, subjects ingested one of the test foods, i.e. SF, WF or SG reference food, with portions equivalent to 25 g of available carbohdyrate. The subjects were required to consume the reference food within 10 minutes, and for test food between 10 – 15 minutes. Following the study procedure, the test products were provided within a seven-day wash-out period. On three sessions, subjects consumed a glucose standard solution (25.0 g) on session 1, and either soy flour (SF, ±47.0 g) or wheat flour snack bar (WF, ±37.5 g) on sessions 2 and 3. Within a 180-minute observation time after ingestion of test product, as much as 2 μL of blood sample was withdrawn at 30, 60, 90, 120, 150, and 180 minutes via finger prick capillary method using *Accu-chek Active*® glucometer (Roche, Germany).

Outcome parameters
Baseline characteristics measurements
A standardised questionnaire was used to interview the subjects' personal and family health history. A physical examination, including body height and weight, was also conducted. Stadiometer and digital weighing scale (Omron BF508) were used to measure body height and weight, respectively. Both assessments were completed twice, and the mean value was used in the analysis. Laboratory tests using blood samples were also taken to measure HbA1c concentration and liver function tests (AST and ALT). Analysis of blood samples was conducted at an accredited clinical laboratory - Prodia Laboratory, Bogor.

Glycaemic response (GR)
This study used finger prick capillary blood samples by *Accu-chek Active*® to assess blood glucose concentration. The GR curve was plotted using a line graph with time interval (minutes) on the x-axis and blood glucose concentration (mg/dL) on the y-axis. It was illustrated using glucose AUC, which was calculated using the trapezoidal method with fasting blood glucose serving as the baseline for calculation.

Test food product
The test food product was a commercial snack bar (SOYJOY®, strawberry flavour, SF) made by PT. Amerta Indah Otsuka, Indonesia. SF is registered in the Indonesia National Agency for Drug and Food Control (NADFC) under BPOM RI

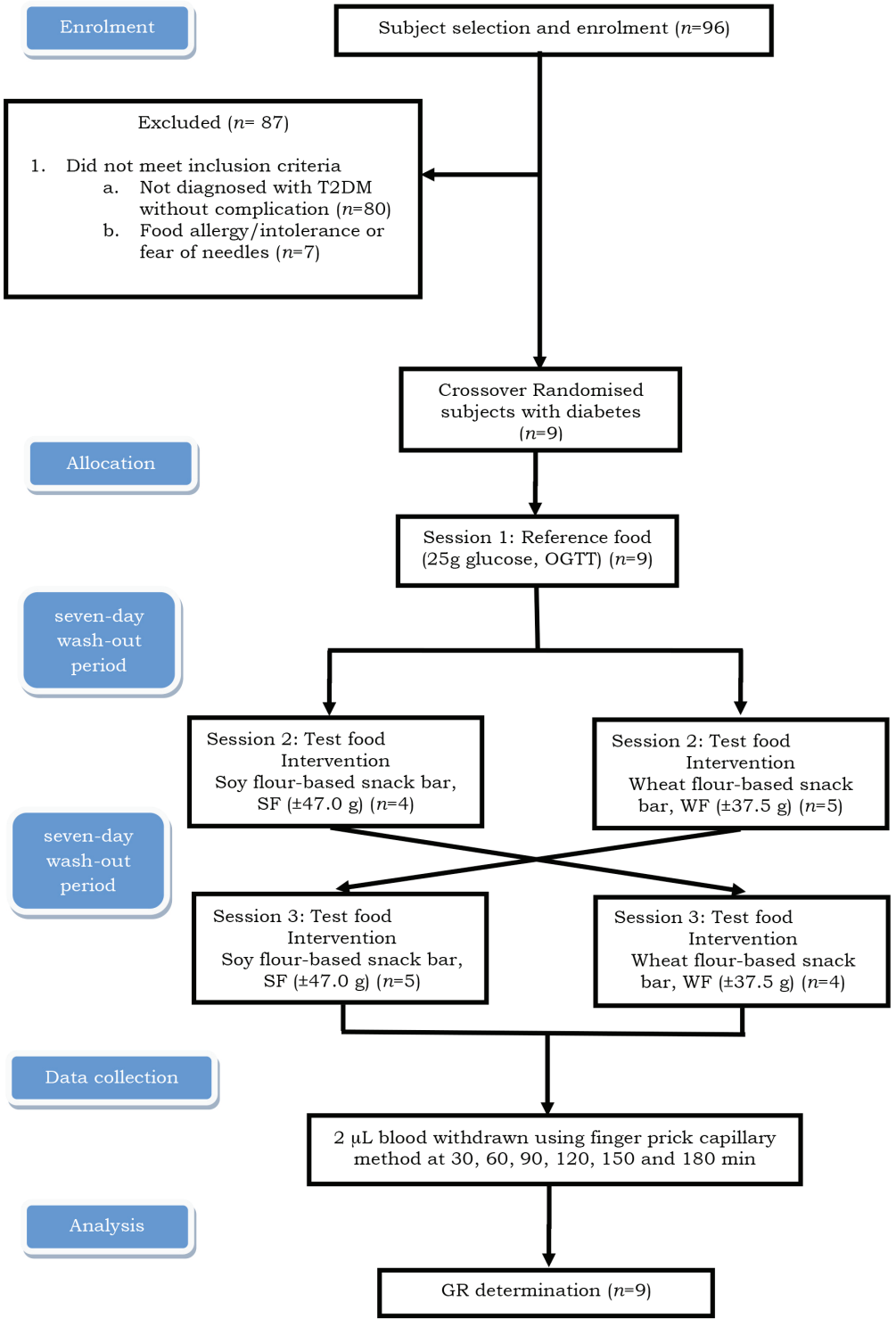

Figure 1. CONSORT flowchart of randomised controlled trial with a crossover design. Oral Glucose Tolerance Test (OGTT); GR (Glycaemic Response)

MD 636013005275 and is certified halal under LPPOM MUI No.00100086950118. In contrast, the comparison product, an unregistered and handmade snack bar was developed using similar formula and materials with SF, apart from soy flour that was changed into wheat flour (WF). The chosen justification of WF snack bar as one of the test products is described elsewhere (Nurdin *et al.,* 2020). Nutrient composition analysis of the test products was done in an accredited laboratory (PT. Saraswanti Indo Genetech, Indonesia). Table 1 summarises the nutrient composition of the test foods per 100 g.

Statistical analysis

IBM SPSS version 20.0 was used to analyse the data. The Shapiro-Wilk test was executed to see the normality in data distribution. It was used because the study sample size was less than 50. Two-way repeated measures analysis of variance (ANOVA) was mainly used to analyse the significant differences between postprandial glucose level at each time (0-180 minutes) and GR estimated by blood glucose AUC using the trapezoidal method. Bonferroni test was used to account for various comparisons to assess the significant magnitude difference of blood glucose peak. Significant difference was described as *p*-value less than 0.05. The sample size was set at 9, at a level of $p<0.05$, based on study references by Kim *et al.* (2020) and Urita *et al.* (2012).

RESULTS

Table 2 describes the general characteristics of the study population. In total, the present study recruited nine subjects with diabetes aged 54.6±4.0 years on average. The mean height and

Table 1. Nutritional composition of test food per 100 g

	Soy flour-based snack bar (SF)[†]	*Wheat flour-based snack bar (WF)*[‡]
Product name	SOYJOY	-
Form	Snack bar	Snack bar
Composition		
Serving size 100 g containing		
Energy, kcal	433	414
Protein, %	12.1	4.8
Total fat, %	15.2	11.9
Carbohydrate, %	61.9	71.9
Sugar, %	36.2	36.4
Fibre, %	8.9	5.1
Sodium, mg	98.9	119.1
Potassium, mg	408.1	75.5
Isoflavone, mg	48.9	N/A
Soy flour		
Moisture content, %	6	N/A

[†]Ingredients: soy flour (29%), pineapple and strawberry (14%), butter, egg, sugar, soluble food fibre, skimmed milk, salt, and synthetic flavour

[‡]WF was developed using similar formula and materials with SF, apart from soy flour that was changed into wheat flour.

Snack bar serving size (30g)

Soy flour (29%): 29%*30=8.7 g per serving size

body weight were 154.9±7.3 cm and 60.1±7.6 kg, respectively. Accordingly, mean BMI was 25.0±2.5 kg/m^2, with four subjects having normal BMI (18.5 – 22.9 kg/m^2) and five subjects having BMI over 23.0 kg/m^2, thus categorised as overweight.

The subjects had HbA1c <8% (7.1±0.9%, average), AST 10-35 U/L (21.3±5.1 U/L, average), and ALT 10-40 U/L (19.6±9.3%, average). Antidiabetic drugs consumed by subjects were metformin (50.0%), glimepiride (25.0%), Gliclazide (8.3%), Linagliptin (8.3%), and Pioglitazone (8.3%). There were 55.6% of subjects who consumed a single antidiabetic drug and 44.4% who consumed two types of antidiabetic drugs. There were no significant differences between groups on health parameters (HbA1c, AST, ALT).

Postprandial blood glucose over 180 minutes was influenced by time (p<0.001) and test products (p=0.025), but no significant effect was found for BMI (p=0.070). Figure 2 shows the variations in postprandial blood glucose after the ingestion of test products. Following the ingestion of SF snack bar, WF snack bar, and glucose standard test products, blood glucose levels peaked simultaneously at 60 minutes, with SF

Table 2. General characteristics of the study population (n= 9), mean±*SD*

Characteristics	*Overall (n=9)*	*Body mass index (kg/m^2)*	
		Normal weight (BMI 18.5 – 22.9) (n=4)	*Excess weight (BMI ≥23.0) (n=5)*
Age, year	54.6±4.0	54.8±3.7	54.4±4.7
Height, cm	154.9±7.3	155.5±10.7	154.4±4.6
Weight, kg	60.1±7.6	55.1±7.6	64.0±5.3
Body mass index, kg/m^2	25.0±2.5	22.7±0.2	26.9±1.6
Sex, *n* (%)			
Men	1 (11.1)	1 (25.0)	-
Women	8 (88.9)	3 (75.0)	5 (100.0)
Visceral fat, %	7.4±2.9	7.3±1.5	7.6±3.9
Body fat, %	31.9±5.3	28.9±6.7	34.3±2.3
Laboratory test			
HbA1c level, %	7.1±0.9	7.3±0.8	7.1±1.1
AST level, U/L	21.3±5.1	19.0±1.2	23.2±6.4
ALT level, U/L	19.6±9.3	15.8±2.3	22.6±11.9
Diabetes prevalence, *n* (%)			
45-54 years	4 (44.4)	1 (25.0)	3 (60.0)
55-64 years	5 (55.6)	3 (75.0)	2 (40.0)
Antidiabetic drugs intake, tablet *n* (%)			
Metformin	6 (50.0)	3 (60.0)	3 (42.9)
Glimepiride	3 (25.0)	1 (20.0)	2 (28.5)
Gliclazide	1 (8.3)	0 (0.0)	1 (14.3)
Linagliptin	1 (8.3)	1 (20.0)	0 (0.0)
Piolitazone	1 (8.3)	0 (0.0)	1 (14.3)
Controlled antidiabetic drugs status, *n* (%)			
Single type	5 (55.6)	2 (50.0)	3 (60.0)
Combination type (2 drugs type)	4 (44.4)	2 (50.0)	2 (40.0)

snack bar having a considerably lower peak (140.0±21.9 mg/dL, p<0.001) than WF snack bar (171.4±38.9 mg/dL) and glucose standard (217.8±33.7 mg/dL). Moreover, significantly lower blood glucose levels were also found for SF snack bar at 30, 60, 90, and 120 minutes (p<0.001) (Figure 2A). A consistent decrease in blood glucose levels was noticed in both groups after a 60-minute observation period, with a steady decrease in the average blood glucose level in those who consumed SF snack bar. According to secondary analysis by BMI, similar trends were observed, where SF snack bar had lower

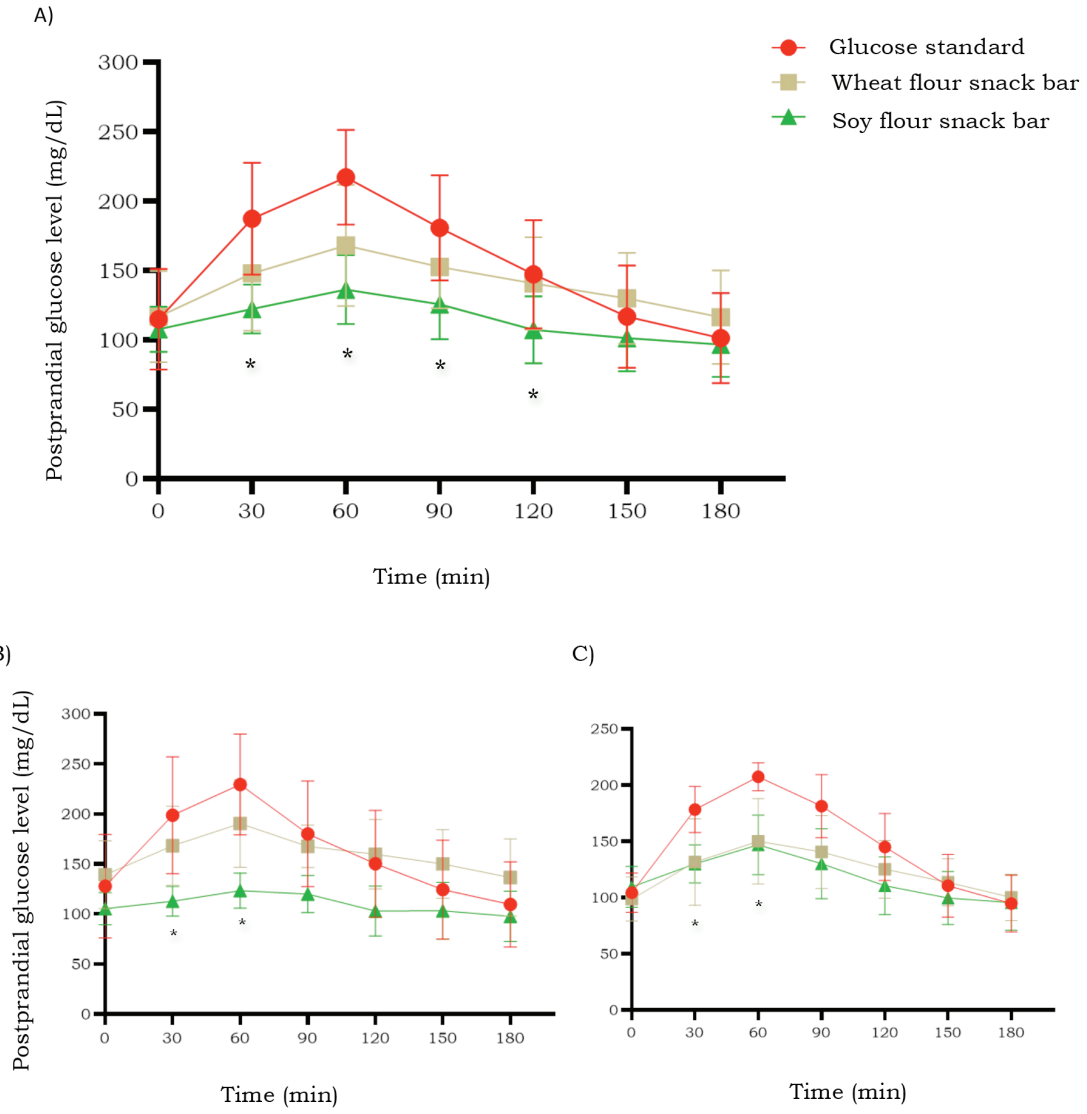

Figure 2. Variations in the postprandial glucose level after ingestion of test products at several time points over 180 minutes period [A (overall), B (BMI 18.5 – 22.9 kg/m^2), C (BMI ≥23.0 kg/m^2)]. SF snack bar induced significantly lower blood glucose at 30, 60, 90 and 120 min (p<0.05) than WF snack bar or glucose standard
*denotes statistical significance (p<0.05).

blood glucose levels than WF over a 180-minute observation period (Figures 2B and 2C). Lower blood glucose levels were found for SF snack bar at 30 and 60 minutes in subjects with normal BMI (18.5 – 22.9 kg/m^2) compared with WF snack bar (Figure 2B). Also, there were no differences in postprandial blood glucose variations between those who consumed SF and WF in overweight subjects with diabetes (BMI ≥23.0 kg/m^2), but there was a tendency for lower blood glucose levels to occur in SF snack bar than WF snack bar (Figure 2C).

Figure 3 describes the three-hour blood glucose AUC. After ingestion of SF snack bar, the AUC was significantly lower (2044.8±503.1 mg.min/dL, p<0.001) than WF snack bar (4735.0±666.8 mg.min/dL) and glucose standard (8392.3±654.2 mg.min/dL), which indicated a considerable >20% reduction in GR after ingestion of SF snack bar. When compared between BMI group, SF snack bar had lower blood glucose level AUC for both normal BMI (2132.0±975.1 mg.min/dL, p=0.038) and overweight BMI (1975.0±1957.0 mg.min/dL, p=0.362), respectively, than WF snack bar (5398.8±1051.1 mg.min/dL and 4204.0±2526.2 mg.min/dL).

DISCUSSION

The present study found significantly lower blood glucose level and low GR with a maximum peak of 140.0±21.9 mg/dL and >20% lower AUC after the ingestion of SF snack bar in T2DM subjects. This glycaemic control benefit is most probably related to soy flour's nutritional properties, i.e. low in carbohydrate and high in fibre, protein and isoflavones content (Mohammed, 2019), which distinguishes it from wheat flour. Although the mechanism of action is unknown, it is believed that the glycaemic control benefit may be linked to dietary fibre (DF), protein (DP) and isoflavones' physicochemical properties (Rivero-Pino, Espejo-Carpio & Guadix, 2020; Kurylowicz, 2020; Goff *et al.*, 2018).

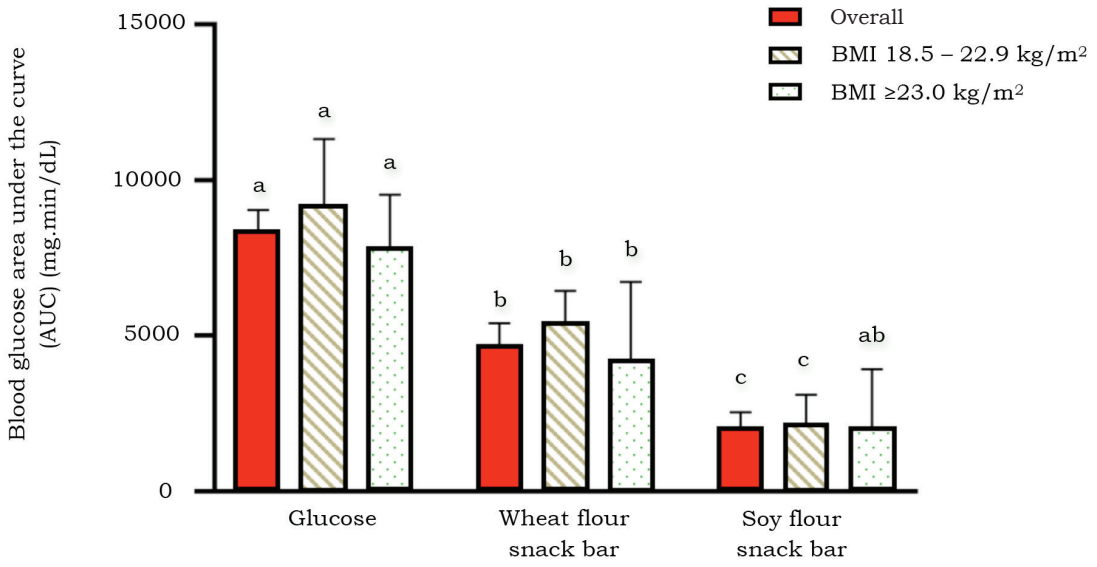

Figure 3. Blood glucose area under the curve (AUC). SF snack bar has lower blood glucose AUC level (p<0.05) than WF snack bar or glucose standard.
[abc] Different letters within the same bar column indicates difference between test product

In individuals with diabetes, DF intake can help improve glycaemic control in several mechanisms of action, i.e. by delaying gastric emptying, modifying the secretion of digestion- and fermentation-related hormones, inhibiting the activity of amylase and delayed starch hydrolysis, altering amylolysis progress, and improving the development of an absorptive barrier layer through interactions with the mucosa (Goff *et al.,* 2018). According to evidence-based data, to some extent, all of these mechanisms contribute to enhanced gut viscosity and as a consequence, slower glucose molecules absorption and greater insulin function. The mechanism of action caused by DP intake is associated with the elevation of gut endocrine cell hormone level, incretins (e.g. glucagon-like peptide-1, GLP-1) (Rivero-Pino *et al.*, 2020). Ultimately, GLP-1 stimulates insulin release whilst inhibiting glucagon release (incretin effect). By these mechanisms, blood glucose level is effectively controlled.

Furthermore, the latest review by Kurylowicz (2020) explained the action mechanism behind isoflavones' role in controlling blood glucose. The findings described that isoflavones are known to have anti-diabetic properties, indicated by their role in β-cell destruction prevention and insulin release stimulation, while contributing to glucose homeostasis by decreasing glucagon release and liver's lipolysis and inflammation. These mechanisms lead to enhanced insulin sensitivity, better glucose absorption in the muscles, and improved adipose tissue metabolism and secretory activity. The review also mentioned that isoflavones enhance hypothalamic appetite regulation, augment incretin effect, positively alter the gut microbiota composition, and affect glucose reabsorption. The reported findings, however, were based on distinct experimental models and circumstances, and the isoflavones concentrations administered in preclinical investigations were significantly greater than those found naturally in living organisms. Therefore, these mechanisms of action support our hypothesis that SF snack bar consumption per serving size (100g) containing fibre (8.9%), protein (12.1%) and isoflavones content (48.9 mg) would not cause any significant blood spike (hyperglycaemia) in individuals with T2DM. The magnitude of a 180-minute blood glucose profile attenuation in the present study, however, is small compared to a previous study on soy flour snack bar that reported a maximum peak of 136.9 mg/dL (7.6 mmol/L) and nearly 50% lower blood glucose AUC after the ingestion of SF snack bar in individuals with T2DM (Urita *et al.*, 2012).

To date, evidence have shown that the nutritional properties of soybean are not the only factor affecting postprandial blood glucose fluctuation level (Franz *et al.,* 2002). Findings have highlighted that the type and amount of carbohydrates consumed and the physical form of food are deemed equally relevant when evaluating its influence on postprandial blood glucose fluctuation level. The first evidence agreed by medical practitioners concluded that "soybean in some way causes a reduction in the percentage and total quantity of sugar passed in diabetic subjects on the usual dietary restrictions" (Holt, Muntyan & Likver, 1996). This finding might be due to soybean's low carbohydrate content, which differentiates it from other legumes (except peanuts) that contain mostly carbohydrates. Recently, an updated recommendation about the type and amount of carbohydrates consumed for T2DM management is explained by Yari *et al.* (2020). They suggested that particularly for diabetic subjects without complication, the strategic objective of nutrition treatment is to improve glycaemic control by selecting

foods with low GI/GL value. Looking at its characteristics (slow digestion), low GI/GL foods contribute to a slower and smaller rise in postprandial glucose level (Vlachos *et al.,* 2020).

Furthermore, several prior studies have investigated various forms of soy snack alternatives for people with diabetes, such as soy cookies and biscuits (Maya, Sulaeman & Sinaga, 2020; Kim *et al.,* 2020). The present study is the first to prove the glycaemic control benefit of consuming soy flour-based snack bars in T2DM subjects in Indonesia. Also, this study is the continuation of a larger study on the effect of SF snack bar consumption on postprandial blood glucose of healthy subjects (Nurdin *et al.,* 2020). The findings recommend soy flour snack bar as a low GI/GL source food that is potentially favourable for controlling blood glucose level and can be an alternative snack for healthy people with blood glucose concerns. Moreover, studies by Kim *et al.* (2020) and Yan *et al.* (2017) reported lower glycaemic impact after consuming snack bars in healthy older adults. The results showed comparable efficacy with our findings due to its high fibre or protein content. In the current study which involved individuals with diabetes, the glycaemic control benefit of consuming SF snack bar was shown to be better than WF snack bar, with GR fluctuation in the range of 100-140 mg/dL, which was considered to be well-controlled for individuals with diabetes 1-2 hours after the start of a snack (180 mg/dl).

Views vary on the link between weight status and glycaemic control on individuals with T2DM, but there is evidence supporting their association (Bae *et al.,* 2016). The finding concluded that T2DM individuals with excess body weight (overweight, obese class I, II, or III) have higher risk of poor glycaemic control and are linked to substantially increased likelihood of having high HbA1c levels. Hypothetically, poor glycaemic control can increase the probability of impaired postprandial glucose levels, which in turn covers the food effect under investigation. This hypothesis is in accordance with the current study finding, where abnormal postprandial glucose levels were observed in those who consumed SF snack bar and were overweight. The fact is that when an individual predisposed to diabetes has excess weight, the cells in the body become less responsive to insulin (insulin resistance) due to an increased amount of non-esterified fatty acids, glycerol, hormones, cytokines, proinflammatory substances, and other substances that lead to the impairment of β-islet cells of the pancreas, causing poor glycaemic control (Al-Goblan, Al-Alfi & Khan, 2014).

There are unique characteristics and drawbacks that must be acknowledged within this study. Firstly, equal amounts of available carbohydrate in snack bars as an alternative food form showed that glycaemic control of postprandial glucose levels is achievable by substituting wheat flour with soy flour snack bar. This study has confirmed the short-term effect of SF snack bar on GR of individuals with T2DM. However, future research should focus on evaluating the effect of long-term consumption of SF snack bar on glycaemic control in individuals with T2DM using HbA1c test as an outcome under investigation. Secondly, despite the statistical significance that those who consumed SF snack bar had lower postprandial glucose levels and low GR with blood glucose peak, the limited magnitude of the blood glucose AUC attenuation (>20%) may be attributable to the small sample size (n= 9). In future research, a larger sample size needs to be considered in order to obtain better precision and confidence in the results for clinical reasons. Thirdly, taking into account prior published study

on healthy (Nurdin *et al.,* 2020) and diabetic subjects (Urita *et al.*, 2012), the current study confirmed the feasibility of utilising SF snack bar to help blood glucose management in both healthy and diabetic subjects. Nonetheless, the findings of this study also strengthened the available evidence that weight status, particularly overweight, is associated with distinct metabolic responses. Future studies could investigate the effect of SF snack bar on overweight subjects with additional subject characteristics, such as age, race and co-morbidities for glycaemic control (Bae *et al.,* 2016).

CONCLUSION

To conclude, the current study found that consumption of snack bar made from soy flour contributed to lowering postprandial glucose levels over a 180-minute observation period, with a statistically significant attenuation of >20% lower GR (AUCs) and blood glucose peak than wheat flour snack bar. Overall, this study confirmed our hypothesis that consuming soy flour snack bar maintained postprandial glucose levels in normal range due to its nutritional properties (high fibre, protein, isoflavones and low carbohydrate). This finding might help control the blood glucose of T2DM subjects through diet modification by including SF snack bar as part of a regular eating plan practice (2-3 snack bars in a day as a snack alternative) in a well-balanced nutritious diet.

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Authors' contributions

NMN, principal investigator, conceptualised and designed the study, assisted in drafting of the manuscript, reviewed the manuscript; HFN, co-principal investigator, contributed in study design, advised on data analysis and interpretation, and reviewed the manuscript; KRE, prepared the draft of the manuscript, led the data collection in IPB University, data analysis and interpretation, reviewed the manuscript; MYK, reviewed and finalised the manuscript.

Conflict of interest

NMN, HFN and KRE declare no conflict of interest. MYK is the scientific supervisor at PT. Amerta Indah Otsuka. This study was funded by PT. Amerta Indah Otsuka. All authors disclose that the sponsor company had no influence in the execution of the study, including no input into the study design, data collection, analyses, or interpretation of the data, in the writing of the manuscript, and in the decision to publish the results.

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Determinants of diet quality among mothers of young children in an urban slum area in Jakarta: Mother's age, vegetables availability, and eating out frequency

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ABSTRACT

Introduction: Poor diet quality is a major issue, and health concerns may be related to diet. Mothers with young children usually have their meals at home; thus, home food environment plays a role in determining dietary behaviours. This study examined the association between health concerns and diet quality among mothers; additionally, the effect of home food environment on this relationship was assessed. **Methods:** This cross-sectional study comprising 229 mothers (aged 19–49 years) with young children was conducted in an urban slum area in North Jakarta. Data were collected via interviews using a structured questionnaire. The General Health Interest Scale and Consumer Behaviour Questionnaire were used to assess health concern and home food environment, respectively. The 24-hour dietary recall method was used to calculate the Diet Quality Index-International (DQI-I) score. Spearman's correlation, multiple linear regression, and path analysis were used to analyse the data. **Results:** Majority of the mothers had poor diet quality (mean DQI-I score, 41.44/100). No significant correlation between health concern and diet quality was observed. After adjusting for age, the relationship between health concern and diet quality was not mediated by vegetables availability or eating out frequency (indirect effect=0.012; p=0.096). Multiple linear regression analysis revealed age as a significant predictor of diet quality (B=0.196; p=0.024). **Conclusion:** Diet quality among mothers of young children differed with age and was related to both health concerns and home food environment. Thus, the development of strategies to promote healthy eating based on different age groups is warranted.

Keywords: diet quality, healthy diet, home environment, mothers

INTRODUCTION

Presently, Indonesia is facing the triple burden of malnutrition, namely stunting, micronutrient deficiencies such as anaemia in women of reproductive age (WRA), and overweight. These nutrition-related problems may be related to diet. Suboptimal dietary habits comprising high consumption of sugar-sweetened beverages (SSB) and processed meats, as well as inadequate consumption of healthy foods such as fruits and vegetables, whole grains and legumes have been observed worldwide (GBD 2017

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Diet Collaborators, 2019). Unfortunately, the understanding regarding nutrition is widely associated with quantity, rather than quality. In a recent study, the diet quality of Indonesian adult women from two different ethnic groups (Sundanese and Minang) was reported to be poor (Stefani *et al.*, 2018).

Diet quality refers to the intake of foods by an individual, and it is based on several indicators such as diversity and adequacy, which can be used to assess compliance with existing dietary recommendations (Alkerwi, 2014). Poor diet quality has been associated with a higher risk of obesity and non-communicable diseases (NCDs), particularly in women (Sundararajan *et al.*, 2014). Thus, a healthy, high quality diet that leads to optimal health and nutritional status should be considered as an important step for WRA who are going to be mothers in the future. Maternal diet is known to be a predictor of a child's diet. A recent study showed a positive association between mother's and child's intake of foods from the same food groups such as fruits, vegetables, saturated fats, and added sugars (Kueppers *et al.*, 2018). Thus, it was highlighted that promoting a mother's diet might prove beneficial in improving the diet of a child.

Health concern is one of several factors used to determine diet quality and refers to an individual's perception about health-related issues, including nutrition. A study in Luxemburg reported a direct association between nutritional awareness and diet quality among adults (Alkerwi *et al.*, 2015). Additionally, a study among Taiwanese college students showed that the relationship between health concern and healthy eating attitudes was partially mediated by food choice motives (Sun, 2008). Presently, the food environment is considered a crucial factor that can influence diet. It consists of several aspects (physical, political, economic, and socio-cultural) that exist at both the macro-level (community) and micro-level (household) (Rosenkranz, 2008). In developed countries, modifications of the home food environment have been proposed as a strategy for obesity prevention and management, especially in children (Robson *et al.*, 2019). Food availability at home was reported as a mediator between the children's diet and maternal nutrition knowledge (Campbell *et al.*, 2013), as well as maternal healthy eating schema, i.e. how mothers define themselves in terms of healthy eating (Kueppers *et al.*, 2018). However, this relationship is not well understood among adults, particularly among mothers in developing countries and specifically among those living in urban slum areas. In 2018, 30.6% of the Indonesian urban population were reported to be living in slums (World Bank, n.d.). In Jakarta, 445 out of 520 screened hamlets were classified as slum areas (BPS Provinsi DKI Jakarta, 2017). These findings indicated that there is a large proportion of low socioeconomic communities living in the urban areas of Indonesia. Urban slum areas in Indonesia, particularly Jakarta, are known to have abundant food stores that could promote unhealthy eating (Sufyan *et al.*, 2019).

To the best of our knowledge, the degree of health concern or health consciousness among mothers of young children, particularly in urban slum settings, is rarely discussed. Understanding the level of importance given to health and nutrition among mothers would aid in delivering the appropriate level of education to improve their dietary behaviours. Thus, this study aimed to examine the association between health concern and diet quality among mothers of young children, and to determine the effect of home food environment on this relationship.

MATERIALS AND METHODS

Study design, sampling procedures and sample size estimation

This observational cross-sectional study was conducted in Indonesia from November 2019 to March 2020. The Jakarta province was chosen as a representative of urban areas in Indonesia because of high prevalence of overweight and obesity, as well as a large proportion of slum areas. The sub-district of North Jakarta and the urban village of Pejagalan were randomly selected for this study. An obesity assessment conducted in North Jakarta revealed that 44.9% of women aged 15 years and older were obese. Moreover, North Jakarta had the least number of food vendors who supply food in compliance with the health requirements set by the primary healthcare centre (Provincial Health Office of DKI Jakarta, 2018). Pejagalan, an urban village located in North Jakarta, is densely populated and characterised as a slum area because of its low socioeconomic situation and unfavourable housing and environment. The subjects enrolled in this study comprised of mothers with children under five years of age and pre-school children. The list of subjects was obtained from randomly selected community health posts (*Posyandu*), and consecutive sampling was applied. Mothers aged 19–49 years who were apparently healthy, fluent in Bahasa Indonesia, maintained the role of primary food provider at home, and agreed to participate by signing the informed consent were included in the study. Pregnant and lactating mothers, and those who were on a specialised or restrictive diet were excluded.

The estimated sample size was determined using a correlation formula with an *r* coefficient of 0.3, based on previous studies regarding health concern, home food environment, and dietary behaviour, along with a confidence level of 95% and a power of 80%. A design effect of two was applied to increase the variation because of the non-probability sampling technique used. A 20% increase in the sample size was applied in consideration of non-response rate, resulting in a total of 204 subjects. This number was considered to be sufficient for path analysis, which requires a minimum of 100 subjects.

Ethical approval for this study was obtained from the Ethical Committee of Faculty of Medicine, Universitas Indonesia (No. ND-6/UN2.F1/ETIK/PPM.00.02/2020 dated 6 January 2020). Written consent to participate in the study was obtained from the mothers.

Research tools and data collection

The subjects were visited for a face-to-face interview using a structured questionnaire consisting of four sections. Before data collection, all instruments were pre-tested among 30 individuals with characteristics that were similar to those of the subjects. Subsequently, necessary corrections and modifications were made, particularly in the third and fourth sections of the questionnaire. The first section pertained to the sociodemographic and individual characteristics of the subjects, which included the following: age; level of education (elementary school, junior high school, senior high school or university); employment status ('unemployed' including housewives, 'partially employed' defined as working half-day and working at home [e.g. owning a small store at home], 'full-time employed' defined as working full-day or at least for eight hours a day); average monthly household income; and self-reported medical history.

The second section comprised of dietary assessment to measure diet quality, which was calculated using the Diet Quality Index-International

(DQI-I). DQI-I is a global tool that is used internationally to monitor diet that relates to nutrition transition issues (Kim *et al.*, 2003). The element of adequacy in the DQI-I was modified according to the Indonesian dietary guidelines in this study. Dietary assessment was conducted via a 24-hour dietary recall over two non-consecutive days. The interviewer was trained to conduct the procedure of a 24-hour dietary recall using multiple-pass method as follows: (i) information about the overall foods consumed by the subjects were collected; (ii) details about the ingredients and cooking methods used were noted, and an estimation of the amount of foods consumed was made; (iii) and finally, the data collected were reviewed from the beginning. The interviewer used a food photo book published by the Indonesian Ministry of Health to assist subjects in estimating the amount of foods consumed. Dietary data were analysed using NutriSurvey for Windows, Version 2007, with the Indonesian Food Composition Table published by the Indonesian Ministry of Health, Singapore Energy and Nutrition Composition of Food, and USDA Food Data Central used for nutrients that were unavailable in the Indonesian food database. Underreporting and over-reporting of energy intake was considered using a cut-off of 500–3500 kcal per day. Hence, subjects with an energy intake of <500 or >3500 kcal were excluded from further analysis in the DQI-I. Likewise, those who were not able to complete the second day of their 24-hour dietary recall were excluded from the analysis. The average result from 24-hour dietary recall was used to compute the scores for DQI-I, which ranged from 0 to 100; a higher score indicated better diet quality.

The third section was used to assess the home food environment. The questionnaire used a modified version of the National Health and Nutrition Examination Survey Consumer Behaviour Questionnaire (NHANES CBQ) (CDC, 2010), which consisted of questions pertaining to the availability of the five food groups (vegetables, fruits, salty snacks, low-fat milk, and soft drinks), the characteristics of family meals (cooking frequency, eating out frequency, having take-out food frequency, and eating together frequency), and food expenditure (groceries, eating out, and take-out food). Food availability, cooking frequency, and the frequency of eating together were expressed by the number of days in the past week. For food availability, low-fat milk from the original questionnaire was substituted with sweet snacks because of the low consumption of dairy products among the Indonesian population. Soft drinks referred to any SSB, including homemade drinks with added sugar or manufactured products. The frequency of eating out and ordering a take-out was expressed as the number of days over the past month. The original response for the frequency of eating out, having a take-out, and cooking at home comprised a five-level response ranging from 'never' to 'always'. We modified this response using the number of days to reduce bias among subjects who provided neutral answers (such as 'rarely' or 'sometimes'). Food expenditure was defined as the money spent on food over the past month and was expressed in Indonesian Rupiah (IDR). In this study, food expenditure referred to the expenditure per capita, considering variations in the sizes of households.

The last section was used to assess health concern utilising the General Health Interest Scale (GHIS) (Roininen, Lähteenmäki & Tuorila, 1999). The original questionnaire consisted of eight statements related to dietary behaviours, with responses ranging from 0 to 6 on a 7-point Likert scale, wherein the subjects

had to declare whether they 'strongly disagreed' or 'strongly agreed' with each statement. However, in the present study, the procedure was modified and interviews were conducted, wherein the interviewer read the statements and asked the subjects to rate each of them. The original GHIS questionnaire was translated from English to Bahasa Indonesia, followed by a preliminary interview comprising of mothers with similar characteristics; a Cronbach's alpha value of 0.71 was obtained. The GHIS score ranged from 0 to 48. Higher score indicated greater health concern perceived by the subjects. The scores were categorised further according to the 33rd and 66th percentiles to determine low, moderate, and high groups.

Statistical analysis

Data analysis was conducted using SPSS Version 20. For numerical data, a normality test was conducted to determine the data distribution using the indicators of Kolmogorov–Smirnov: p>0.05, coefficient of variance of <20% or skewness and kurtosis z-values of ±1.96. Normally distributed data were presented as mean±standard deviation (*SD*), whereas the asymmetrically distributed data were presented as median and interquartile range. Multiple linear regression was conducted to determine the predictors of diet quality. The level of significance was set at <0.05. Mediation analysis was conducted with path analysis using SPSS AMOS Version 23 by calculating indirect effect (IE), which was calculated by multiplying the regression standardised coefficients of the independent and mediating variables. Because of data normality issues, bootstrapping was applied and set at 5,000. IE estimates of 95% CI not containing zero were interpreted as statistically significant and indicated as potential mediation.

RESULTS

Sociodemographic characteristics, health concern and the home food environment

In total, 254 subjects completed all the sections of the interview. After calculating for underreporting of energy intake and excluding subjects who were unable to complete the second day of their 24-hour dietary recall, 229 subjects were retained for diet quality analysis. Table 1 presents the characteristics of the subjects. Mean age of the mothers was 32.6 years. Majority of the subjects were married (98.3%), held a high school degree (34.9%), and were unemployed (68.6%). The median monthly household income was IDR 3 million (approximately USD 214, as of February 2021), which was lower than the minimum wage in the Jakarta province, as of 2020. According to self-reported medical history, most subjects claimed to have never been diagnosed with obesity, diabetes, hypertension and other NCDs. However, family medical history indicated that hypertension was the most common disease.

The median total GHIS score among the subjects was 32. Furthermore, 27.5%, 37.1%, and 35.4% of the subjects were presented with low, moderate, and high health concerns, respectively. More than half of the subjects reported that vegetables (67.2%), sweet snacks (59.4%), and SSB (86.5%) were always available at home. Majority of the subjects reported never eating out (62%); having a take-out at home was more common (up to 70.3% of subjects had a take-out at least once a week). The subjects usually cooked meals at home, and eating meals together with family members at home was common during dinner time almost every day. The median total food expenditure during the past month for groceries, eating

Table 1. General characteristics, health concern, and home food environment of mothers of young children (*n*=229)

Characteristics	*Median (Q1–Q3)*	*n*	*%*
Sociodemographic characteristics			
Age (years)	32.6 ± 6.1[†]		
Education level			
Less than elementary school		12	5.2
Elementary school graduate		64	27.9
Junior high school graduate		66	28.8
Senior high school graduate		80	34.9
Diploma/bachelor graduate		7	3.1
Household income (IDR)[‡]	3,000 (2,300–4,275)		
Employment status			
Unemployed		157	68.6
Partially employed		46	20.1
Fully employed		26	11.4
Marital status			
Single or previously married		4	1.7
Married		225	98.3
Individual medical history			
Overweight/obesity		44	19.2
Diabetes mellitus		2	0.9
Hypertension		22	9.6
High level of cholesterol		11	4.8
Other NCDs (gastritis, gout, etc.)		17	7.4
Family medical history			
Overweight/obesity		17	7.4
Diabetes mellitus		54	19.7
Hypertension		93	40.6
High level of cholesterol		30	13.1
Heart disease		26	11.4
Stroke		23	10.0
Other NCDs (gastritis, gout, etc.)		22	9.6
Health concern			
GHIS score	32 (27–37)		
Low health concern		63	27.5
Moderate health concern		85	37.1
High health concern		81	35.4
Home food environment			
Food availability (days)[§]			
Vegetables	7 (4.5–7)		
Fruits	3 (1–5)		
Salty snack	4 (0–7)		
Sweet snack	7 (2–7)		
SSB	7 (7–7)		
Family meal characteristics			
Eating out frequency[¶]	0 (0–3)		
Having take-out food frequency[¶]	8 (3–20.5)		
Cooking frequency[§]	6 (2–7)		
Eating dinner together frequency[§]	7 (3–7)		
Food expenditure per capita (IDR)[††]			
Eating out	0 (0–30.8)		
Take-out	41.7 (12.3–150)		
Groceries	225 (120–375)		

GHIS: General Health Interest Scale; IDR: Indonesian rupiah; NCDs: Non-communicable diseases; SSB: Sugar-sweetened beverages

[†]Mean (±*SD*)

[‡]Income expressed in '000 (1 USD = Rp14,067.29 – as of 19 February 2021)

[§]Number of days in the past week

[¶]Number of days in the past month

[††]Amount of money spent for food in the past month expressed in '000 (1 USD = Rp14,067.29 – as of 19 February 2021)

Table 2. Diet quality of the mothers of young children (*n*=229)

DQI-I component score	*Score range (points)*	*Median (Q1–Q3)*
Total score	0–100	41.4±7.4†
Variety	0–20	12.6±3.5†
Overall food group variety	0–15	9.6±2.7†
Within-group variety for protein source	0–5	3 (1–5)
Adequacy	0–40	16 (13–19)
Vegetable group	0–5	1 (1–1)
Fruit group	0–5	0 (0–1)
Grain group	0–5	3.3±1.2†
Fibre	0–5	1 (1–1)
Protein	0–5	5 (5–5)
Iron	0–5	1 (1–3)
Calcium	0–5	1 (1–3)
Vitamin C	0–5	1 (1–3)
Moderation	0–30	11.7±4.3†
Total fat	0–6	0 (0–3)
Saturated fat	0–6	0 (0–0)
Cholesterol	0–6	6 (3–6)
Sodium	0–6	6 (3–6)
Empty-calorie food	0–6	0 (0–3)
Overall balance	0–10	0 (0–0)
Macronutrient ratio (CHO: Protein: Fat)	0–6	0 (0–0)
Fatty acid ratio (PUFA: MUFA: SFA)	0–4	0 (0–0)

CHO: Carbohydrates; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; SFA: Saturated fatty acids
†Mean±*SD*

out, and having a take-out was IDR 1.5 million (approximately USD 107). The median food expenditure per capita was IDR 41,670 (approximately USD 3) for take-out food and IDR 225,000 (approximately USD 16) for groceries.

Diet quality of the subjects

The mean DQI-I score was 41.4 out of 100 (Table 2), which highlighted that the diet quality of the subjects was poor. Majority of the subjects had low-quality diets, with a total DQI-I score of less than 60 (98.7%). Among the four elements of DQI-I, adequacy and moderation had relatively lower scores than variety, and the score for overall balance was 0. These findings indicated that poor diet among the subjects was attributed to the low consumption of fruits and vegetables, inadequate intake of fibre, iron, calcium and vitamin C, and a lack of macronutrient balance. Moreover, the intake of fats exceeded the recommended amount. Spearman's correlation analysis revealed no significant relationship between health concern and diet quality (r=0.092; p=0.166). Likewise, the correlations between health concern and other DQI-I elements were not statistically significant.

Predictors of diet quality

Table 3 shows the results of the multivariate analysis conducted using multiple linear regression with the enter method. The variables included in the analysis were age, education level, household income, employment

Table 3. Multiple linear regression analysis for diet quality predictors among mothers of young children (*n*=229)

Variable	*B*	*SE*	*p-value*
Age (years)	0.196	0.087	0.024*
Education level†	–0.608	0.537	0.768
Average monthly household income (IDR)	<0.001	<0.001	0.522
Employment status‡	–0.608	0.823	0.461
Health concern	0.053	0.067	0.431
Food availability (days) §			
Vegetables	0.521	0.261	0.047*
Fruits	0.370	0.224	0.101
Salty snack	0.152	0.175	0.385
Sweet snack	–0.211	0.174	0.226
Sugar-sweetened beverages	0.169	0.271	0.534
Family meals characteristics			
Eating out frequency (days)¶	0.250	0.107	0.021*
Having take-out food frequency (days)¶	–0.033	0.072	0.649
Cooking at home frequency (days)§	0.023	0.243	0.924
Food expenditure per capita (IDR) ††			
Food groceries	<0.001	0.003	0.901
Eating out	–0.018	0.009	0.037*
Having take-out food	0.007	0.007	0.298

Statistical analysis using multiple linear regression with enter method
†Education level (1=bachelor/diploma graduate, 2=senior high school graduate, 3=junior high school graduate, 4=elementary school graduate, 5=less than elementary school)
‡Employment status (1=fully employed, 2=partially employed, 3=unemployed)
§Number of days in the past week
¶Number of days in the past one month
††Amount of money spent for food in the past month
*p<0.05

status, health concern, food availability (vegetables, fruits, salty snacks, sweet snacks, and SSB), family meal characteristics (frequency of eating out, having take-out food, and cooking at home), and food expenditure per capita (groceries, eating out, and take-out food). Our model implied that older age, frequent availability of vegetables at home, and frequently eating out were associated with a higher DQI-I score. In contrast, an increase in the eating out expenditure was associated with a lower DQI-I score.

Mediating factors between health concern and diet quality

The variables included for path analysis were the significant predictors according to the results from the multiple linear regression analysis. Because of a strong correlation between the variables, the eating out expenditure was omitted from path analysis, and health concern was included as the main independent variable. Before adjusting for age, several direct effects were observed between health concern with the availability of vegetables (β= 0.115; p=0.034)

and frequency of eating out (β=–0.152; p=0.027), but not with the quality of the diet (β=0.101; p=0.179). A significant IE between health concern and diet quality through the availability of vegetables was observed (IE=0.021; p=0.019). However, after adjusting for age, several associations lost their significance, indicating that age might have a stronger effect on these variables. Only the availability of vegetables had a significant direct effect on diet quality (β=0.167; p=0.002). However, its IE on diet quality lost its significance (IE= 0.012; p=0.096; Table 4). Thus, the relationship between health concern and diet quality among mothers of young children in the urban slum areas of the present study was not mediated by the availability of vegetables. Nonetheless, this model satisfied the assumptions of the goodness of fit tests, such as the Chi-square, Goodness of Fit Index (GFI), Comparative Fit Index (CFI), and Normed Fit Index (NFI).

DISCUSSION

In the present study, less than 3% of the subjects consumed a good diet (DQI-I total score of >60). This finding was consistent with those of previous studies not only among Indonesian women (Stefani *et al.*, 2018), but also in the African–American (Hartman *et al.*, 2015) and Malaysian indigenous population (Chong, Appannah & Sulaiman, 2019), which underlines the fact that poor diet quality among WRA

Table 4. Path analysis estimates after adjusting for age

Variable	*Direct effect*			*Indirect effect*		
	Coeff.	*SE*	*p-value*	*Coeff.*	*95%CI (lower–upper)*	*p-value*
Age → Health concern	0.243	0.083	0.001**			
Age → Diet quality	0.191	0.081	0.008**			
Age → Vegetable availability	0.153	0.023	0.020*			
Age → Eating out frequency	–0.140	0.079	0.049*			
Health concern → Diet quality	0.061	0.062	0.434			
Health concern → Vegetable availability	0.078	0.018	0.163			
Health concern → Eating out frequency	–0.118	0.061	0.096			
Vegetable availability → Diet quality	0.167	0.231	0.002**			
Eating out frequency → Diet quality	0.060	0.067	0.273			
Health concern → Vegetable availability → Diet quality				0.012	–0.002–0.043	0.096
Health concern → Eating out → Diet quality				–0.007	–0.035–0.003	0.171

Chi-square (df. 1=3.841) = 3.154, p=0.076
RMSEA (root mean square error of approximation) = 0.097>0.08, GFI = 0.995>0.9, CFI = 0.952>0.9, NFI = 0.942>0.9
*p<0.05, with 95% bootstrapped bias-corrected CI does not contain 0

requires immediate attention. Among those with a better quality of diet in the present study, the consumption of foods with protein was adequate. The DQI-I score on protein adequacy was relatively high, indicating that at least 10% of energy intake was obtained from protein sources. Affordable protein-rich foods included eggs, fish, and soybean products (e.g. tofu and *tempeh*). Additionally, tofu and *tempeh* are easily available as fried snacks or side dishes that are usually sold in small food stores in the neighbourhood. Similar to a qualitative study in East Jakarta, the consumption of '*kerongkongan*' (chicken neck and bones with less meat) was common among the subjects in the present study (Sufyan *et al.*, 2019), which were used as another affordable alternative to animal proteins. Nonetheless, despite the high score for protein, intakes of key nutrients, such as iron and calcium, did not meet the necessary recommendations.

Our findings also suggested that adequate consumption of fruits contributed to better quality diet. Fruits, such as bananas, *dukuh* and rambutan, were commonly consumed. The consumption of fruits in the form of *rujak* (eaten along with palm sugar) contributed to a higher score for the adequacy of fruits intake. Vegetable soup was one of the most common dishes cooked at home. Conveniently packaged vegetables used for certain dishes were easily available at affordable prices in traditional markets around the neighbourhood. However, the quantity of the vegetables was little and probably inadequate to satisfy the recommended intake for adults, particularly in households with more than two members. A previous Indonesian study highlighted that the majority of the Indonesian population did consume vegetables; however, the amount of vegetables consumed in grams per day was relatively low regardless of age group, sex, and socioeconomic status. Thus, over 90% of the population failed to meet the Indonesian dietary guidelines (Hermina & Prihatini, 2016).

Furthermore, limiting the consumption of SSB and other empty-calorie foods plays an essential role. The score for empty-calorie foods was low in the present study; at least 3% of the energy intake among the subjects was obtained from nutrient-poor foods, which was mostly SSB, such as homemade tea with sugar. This result was similar to that reported in a previous study comprising urban adults in Indonesia (Khusun, Wiradnyani & Siagian, 2017). Additionally, the diet was typically similar among the subjects in the present study, hence all resulted in poor quality. Regardless of income, education level, and employment status, no significant difference in diet quality was observed.

The findings of the present study indicated that an increase in health concern was associated with better diet quality. Previous studies among female adolescents (Jezewska-Zychowicz *et al.*, 2017), college students (Botchway, Wiafe-Akenteng & Atefoe, 2015), and adults in Luxemburg (Alkerwi *et al.*, 2015) presented with similar results. However, the insignificant direct effect of health concern on diet quality was not aligned with a previous study, which found a direct association between nutritional awareness and diet quality among adults aged 18–69 years old (Alkerwi *et al.*, 2015). This might be attributed to the different instrument used in their study. Alkerwi *et al.* (2015) referred to nutritional awareness as 'the importance of eating balanced meals' and used a single statement to classify the responses of subjects into three levels: 'high importance', 'moderate importance', and 'little importance'. Alternatively, in the present study, multiple statements from the GHIS were

used, comprising of eating behaviours, food choices, and balanced diet.

The home food environment has been explored as a mediator of food intake and diet, particularly among children. According to Campbell *et al.* (2013), home food availability was a significant mediator between the mother's level of knowledge about nutrition and the children's food intake. Mothers with children were reported to generally eat at home (Raskind *et al.*, 2017); hence, we assumed that home food availability might influence the mother's diet. In the present study, our findings suggested that the availability of fruits and vegetables at home could potentially act as a mediator between health concern and diet quality. It is assumed that adults have more access to foods; thus, they are not dependent on food availability and eating-related activities at home, despite having an increased health interest. As mothers of young children, the motivation to ensure good nutrition for the sake of their children plays an important role in shaping the home food environment. Moreover, when mothers recognise their unhealthy diet, they purchase healthier foods to improve their eating behaviours (Raskind *et al.*, 2017). This practice determines the provision of FV at home as a parental modelling effort to promote healthy eating among their children (Dave *et al.*, 2010). FV intake has been included in the promotion of nutrition in terms of a balanced diet in Indonesia. Nonetheless, as the primary food provider, mothers would always consider the preferences of their children or husband (Raskind *et al.*, 2017; Sufyan *et al.*, 2019), which might explain the wide availability of SSB and sweet snacks at home, regardless of the level of health concern.

Healthy eating is often perceived as costly in low-income communities. This is likely due to financial instability, which enhances the perception of unhealthy diets being more affordable (Munt, Partridge & Allman-Farinelli, 2017). Although family meal characteristics did not appear to be significant mediators, a significant correlation between health concern and cooking frequency was observed in the present study. Greater health concern was associated with more frequent cooking and fewer takeaways. Furthermore, more frequent cooking was correlated with better diet quality, as supported in a previous study (Farmer *et al.*, 2019). However, the prominent practice of purchasing take-outs could be due to economic reasons. Usually, mothers cook rice at home and purchase ready-to-eat side dishes because they are affordable and could be consumed by other family members. Eating out was not common in the present study, except among full-time working mothers. It is important to note that the results of the multivariate analysis in the present study must be cautiously interpreted because it implies that eating out could contribute to better diet quality. In urban slum areas, people usually eat at local food stalls or from street food vendors, which offer cooked meals, including vegetarian and protein-rich dishes. Therefore, improving the knowledge about healthy eating might prove useful and encourage consumers to choose their food wisely.

Age was found to be a significant predictor of diet quality in the present study. A previous study in Japan found that women aged ≥40 years presented with better diet quality (Kurotani, Ishikawa-Takata & Takimoto, 2020). In the present study, subjects under the age of 30 years were more likely to have lower DQI-I and GHIS scores. They ate out more often and cooked less frequently. Age is believed to be related to the familiarity with certain food items. As suggested in a study on Mediterranean diet, ageing was associated with increased familiarity with vegetables, fruits, fish, legumes

and other typical Mediterranean foods; those belonging to the younger age group were more familiar with sweets, soft drinks, and red meat (Predieri *et al.*, 2020). The findings of the present study suggested a significant direct effect of age on health concern. As the risk of NCDs increase along with age, older individuals are encouraged to change their diet to lead a healthier life (Delaney & McCarthy, 2011). Unfortunately, the instrument used in this study to assess health concern was unable to measure the degree of importance of health in relation to the risk of developing NCDs.

This study had some limitations. Firstly, actual calculations based on the individual's nutritional status were not used for underreporting of energy intake. Several attempts to calculate the underreporting of energy intake using established body weight and height according to the Indonesian dietary recommendations were made; however, the results excluded more than half of the subjects. Thus, a 500–3500 kcal cut-off was used in this study instead. Secondly, we did not explore and record the kind of foods typically purchased by the subjects who frequently ate out or ordered a take-out. Given that eating out frequently was a predictor of better diet quality, this finding should be cautiously interpreted.

The findings of this study suggested that it is important to improve the understanding of health, nutrition and healthy eating behaviours among mothers to develop a greater interest in health and to create a healthier food environment at home. Furthermore, age differences among mothers should be taken into account when exploring diets and food-related behaviours, particularly during the development of interventions and education materials. The frequency of cooking, eating out, and purchasing takeaways appeared to vary with age; hence, qualitative methods that include the barriers and enabling factors related to food preparation practices should be applied to explore the understanding and importance of health and diet among younger and older mothers in future.

CONCLUSION

Diet quality among mothers of young children is a major issue. Interventions to improve diet quality should focus more on increasing the compliance towards consumption of vegetables and fruits. Furthermore, strategies and interventions to improve diet should be tailored according to age groups because of differences in factors that contribute to their diet and food choices.

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Authors' contributions

FSB, conceptualised and designed the study, conducted data collection and analysis, prepared the draft of the manuscript; DNC and LAAW, advised on the study conceptualisation, data collection procedure, analysis and interpretation, and reviewed the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest regarding the research and the publication of this article.

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Milk supplementation increases mid-upper arm circumference and haemoglobin level among pregnant women in Kupang, Indonesia: Evidence from a regression discontinuity design

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ABSTRACT

Introduction: The high prevalence of chronic energy deficiency (CED) and anaemia among pregnant women in Indonesia is worrying. Nusa Tenggara Timur (NTT) is one of the provinces in Indonesia with the highest prevalence of CED. This study aimed to determine the effect of fortified milk supplementation on changes in mid-upper arm circumference (MUAC) and haemoglobin level among pregnant women. **Methods:** This quasi-experimental study was conducted in three locations of Community Health Centers in NTT from May to August 2019. Purposive sampling was used to recruit 69 pregnant women who were divided into two groups based on haemoglobin levels; the intervention group consisted of 31 pregnant women with haemoglobin levels below 11 g/dL, and control group consisted of 38 pregnant women with haemoglobin levels above 11 g/dL. Intervention group was provided with fortified milk supplementation, while control group received education about prevention of CED and anaemia. Data were analysed using regression discontinuity design with haemoglobin of 11 g/dL as cut-off. **Results:** Using regression discontinuity method, we were able to determine the effect of milk supplementation based on haemoglobin levels and confirm the result that milk supplementation significantly increased MUAC by 4.69 cm. Despite no discontinuity found, a positive increase of 0.98 g/dL in haemoglobin level was important to note. **Conclusion:** Milk supplementation of 300 kcal/day for three months significantly increased MUAC and to some extent, increased haemoglobin level. Thus, it should be considered when planning nutrition programmes to improve the nutritional status of pregnant women.

Keywords: haemoglobin level, milk supplementation, MUAC, pregnant women, regression discontinuity design

INTRODUCTION

Chronic energy deficiency (CED) and anaemia are considered as serious public health concerns, especially among pregnant women, due to their effects on pregnancy and pregnancy outcomes. CED and anaemia in pregnant women have been found to negatively affect pregnancy outcomes, such as imposing higher risks of intrauterine growth retardation (IUGR), low birth weight, and short birth length, which can further cause undernutrition throughout childhood, as well as reduced mental

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and motor skills development (Black *et al.*, 2008; Ahmed, Hossain & Sanin, 2012).

Globally, maternal undernutrition remains common in lower- and middle-income countries (LMIC), including South East Asia (Black *et al.*, 2008). Pregnant women are known to be susceptible to iron deficiency and global data have shown minor changes in the prevalence of anaemia in women of childbearing age from 31.6% to 32.8% in 2000–2016 (Global Nutrition Report, 2018). Anaemia in pregnant women increases the risks of perinatal and neonatal mortality, as well as low birth weight (Rahman *et al.*, 2016).

The high prevalence of CED and anaemia among pregnant women in Indonesia is a worrying phenomenon. The prevalence of CED in pregnant women has decreased from 24.2% in 2013 to 17.3% in 2018, whereas in the same period, the prevalence of anaemia in pregnant women has dramatically increased from 37.1% to 48.9% (Ministry of Health of the Republic of Indonesia, 2018), possibly due to increasing prevalences of both chronic and acute deficiencies of macro- and micronutrients, as well as the high prevalence of hookworm infection. In the long term, high prevalence of low birth weight may increase the risks of children developing chronic diseases throughout adulthood and later life, such as coronary heart diseases, hypertension, type 2 diabetes, and metabolic syndrome (Marciniak *et al.,* 2017).

Data on consumption patterns of pregnant women in Indonesia have shown that there are large number of pregnant women who do not meet the Indonesia Recommended Dietary Allowance (RDA) throughout pregnancy. Approximately 70-80% of pregnant women in both village and city do not meet their energy and protein requirements (Ministry of Health of the Republic of Indonesia, 2014). This situation is exacerbated by the number of women of childbearing age who enter pregnancy with poor nutritional status. Based on the Basic Health Research data (2018), the prevalence of CED in pregnant and non-pregnant women in Indonesia is quite high at 17.3% and 14.5%, respectively (Ministry of Health of the Republic of Indonesia, 2018). If this situation is not resolved immediately, Indonesia will experience a crisis of human resources in terms of nutrition and intellectual capacities. One way to overcome CED and anaemia in pregnant women of Indonesia is to provide food supplementation containing energy and iron for this vulnerable population.

This study was conducted among pregnant women in Nusa Tenggara Timur (NTT). CED and anaemia are highly prevalent among pregnant women in NTT, which has the highest prevalence of CED in women of childbearing age in Indonesia - 36.8% in pregnant women and 32.5% in non-pregnant women (Ministry of Health of the Republic of Indonesia, 2018). The study aimed to determine the effect of fortified milk powder supplementation on changes in anaemia and CED rates in pregnant women. Supplementation of milk for pregnant women was chosen because fortified milk powder is rich in energy and iron (both are associated with anaemia), with practical preparation and manufacture, is easily consumed by pregnant women, and can be obtained at a relatively affordable price.

MATERIALS AND METHODS

The quasi-experimental study was conducted in Kupang Regency, NTT Province in the eastern part of Indonesia, with study sites in three selected Community Health Centers (Puskesmas), namely Naibonat Health Center-East Kupang District, Oesao Health Center-East Kupang District, and Tarus-Kecamatan Kupang Tengah District Health Center. The selected Health Centers were within the Sehati Midwives Group (our partner in this

study) working area and selection was advised and consulted by NTT Province Health Office. Purposive sampling was used in this study where all eligible subjects in the three health centres were included as samples. The inclusion criteria of the study were pregnant women that frequently went to these selected community health centres, did not have physical and mental disorders, did not have any specific diet such as vegan diet, and was self-reported as not having lactose intolerance or milk allergy.

The number of pregnant women recruited at the start of the study was 108 pregnant women, with 54 pregnant women in each group. Both groups comprised women with haemoglobin levels below 11 g/dL, which is based on the World Health Organization (2011) reference cut-off for anaemia among pregnant women. During the study period, the number of pregnant women was reduced to 31 in the intervention group and 38 in the control group. The drop-outs in the intervention group was caused by two people having miscarriages, two people moved houses, two gave births, two people went to another place when data were collected, and 15 pregnant women who had haemoglobin levels >11g/dL but received milk supplementation due to field situation. In the control group, the drop-outs were due to six miscarriages, one who moved to a new house, two returned home, six went to another place during data collection, and one gave birth. Per protocol analysis was conducted on a total of 69 pregnant women who completed the study.

The outcomes were post-intervention haemoglobin level and mid-upper arm circumference (MUAC) calculated as g/dL and cm, respectively. Calculated using the RD package for STATA, the power of this study was 75%, slightly lower than the expected power of 80%, due to drop-out of respondents that were mainly related to moving to other places as reasons for not continuing their participation.

In the intervention group, supplement was given to pregnant women in the form of two cups of milk (one cup contained one sachet of 35 g powdered milk diluted in 180 ml lukewarm water) containing 17 mg elemental iron per day and total energy of 300 kcal/day. In addition, the protein content of a sachet of powdered milk was 6 g, fat 3.5 g, carbohydrate 22 g, and both iron and calcium 25% of the Indonesia RDA. Milk supplementation was provided to pregnant women in person from house-to-house. Pregnant women drank the milk every morning and evening under the supervision of the cadre. In the control group, milk supplementation was not provided, but participants received education about CED and anaemia, preventive measures, and efforts to overcome them. The education was provided at the beginning of the study for both intervention and control groups. The two groups were followed for a three-month period (June–September 2019). Measurements of anthropometry (body weight, MUAC) and haemoglobin level were conducted at four time points: T0 - Baseline in June 2019, T1 - July 2019, T2 - August 2019, and T3 - Post-intervention in September 2019. Measurements were conducted by the local Puskesmas' midwives. When a mother gave birth, the weight and length of the infant were measured and recorded by the midwives.

The instruments used in this study included a pre- and post-test knowledge questionnaire for pregnant women, an intervention monitoring form, a weight measurement tool (digital scale with 0.1 kg accuracy), a MUAC flexible measuring tape, blood collection equipment for haemoglobin examination using quick-check tool, interview guide for 24-hour dietary recall, as well as measuring devices for infants' weight and length (length board). Training was provided by the research team to the data collectors, namely 21 cadres under the

care of a local partner "Sehati" midwives group. The responsibilities of cadres involved distributing and providing milk supplementation to pregnant women twice a day (morning and evening), every day for 12 weeks (three months, from June to August 2019), ensuring that milk supplementation was consumed by pregnant women, recording the monitoring form for milk drinking (throughout the intervention), and providing a monitoring form at the end of the intervention to the Puskesmas' midwives. To assess nutrient intakes, 24-hour food recalls were carried out at baseline and post-intervention by two research assistants who were D3 graduates from the State Health Polytechnic of the Ministry of Health, Kupang. Measurements of haemoglobin level, body weight, and MUAC were conducted by midwives who had been trained to use the Quick-check Haemoglobin Testing System (ACON), Camry brand step scale, and MUAC tape. Pre- and post-test trainings were provided to trainees to ensure the competency of enumerators. Prior to data collection, all prospective respondents were given information about the research mechanism and informed consent was obtained through verbal and written forms. All information collected about the respondents during the study were stored anonymously in a database protected by password.

Regression discontinuity (RD) design was used in the analysis with basic components: a score to define a cut-off point as threshold, an intervention, and outcomes. This design's main advantage is its simplicity in estimating causal effects, assuming that the threshold value distribution of intervention cannot be manipulated by subjects and covariates are evenly distributed both above and below the threshold through continuity assumption (Bor *et al.*, 2014). This design then allowed us to see the valid causal effect of milk supplementation on outcomes by giving milk supplementation to only anaemic pregnant women, while non-anaemic women received no intervention as they were part of the control group.

In a situation where intervention affects the outcomes, the RD design allows us to isolate the effect of milk supplementation on haemoglobin level and MUAC due to the use of sharp design in which our haemoglobin scores on baseline were continuously distributed and discontinuity around the threshold of 11 g/dL was found, had only one cut-off, and the distribution of intervention and control groups were clear in the threshold (Cattaneo, Idrobo & Titiunik, 2019). We used first degree local polynomial approach with CCT mean squared error minimising bandwidth choice, triangular kernel, and data were statistically analysed using STATA 14.1 (StataCorp, 2015) with rdrobust and rdmulti package along the STATA module to confirm the procedures were correct (Cattaneo *et al.*, 2019).

This study obtained ethical approval by the Institute of Research and Community Service at Atmajaya Catholic University (Letter of Approval No.0617/III/LPPM-PM.10.05/05/2019 dated 24 May 2019). Written informed consent was obtained from all subjects.

RESULTS

At baseline, the mean age of mothers in the intervention group was younger (27.3 years old) compared to the control group (30.2 years old). However, the difference was not significant and both intervention and control groups were within the same age group (according to WHO) and as a result, the age difference did not affect study findings. Table 1 shows the proportion of mother's age between intervention and control groups. Social and economic characteristics of subjects in both groups could be assumed to be relatively similar since they came from the same catchment area of health centres. This similarity was also

reflected as no significant difference was found on knowledge and attitude scores at pre-test and post-test (Table 2). The mean maternal haemoglobin level in the intervention group was 10.0±1.7 g/dl and the control group was 12.2±1.1 g/dl. Mean weight and MUAC of pregnant women in the intervention group were 48.5±9.5 kg and 23.3±3.0 cm, respectively, while in the control group, they were 54.6±7.8 kg and 26.4±2.2 cm, respectively. Weight gain in the two groups were not statistically significant (Table 2), so we decided to use MUAC as an indicator of CED since it was not as easy to change as weight. Consistent to the intention of using RD design, the control group had better values of haemoglobin, MUAC, and weight than the intervention group.

The results of nutrient consumption analysis (Table 3) showed that there were significant differences (p<0.05) in nutrient consumption between baseline

Table 1. Distribution of respondents' characteristics by treatment group

Variable	*Group*				*p-value*
	Intervention		*Control*		
	n	%	*n*	%	
Age					
<35 years	27	87.1	29	76.3	0.36
>35 years	4	12.9	9	23.7	
Infant birth weight					
<2500 gram	1	3.2	2	5.3	0.17
>2500 gram	30	96.8	36	94.7	
Infant birth length					
<46 cm	1	3.2	0	0.0	0.45
>46 cm	30	96.8	38	100.0	

Table 2. Baseline and post-intervention measurements by treatment group

Variable	*Group*				*p-value*
	Intervention		*Control*		
	n	*mean*	*n*	*mean*	
Mother's weight (kg)					
Baseline	31	50.1	38	55.2	0.03
1st measurement	31	52.7	38	57.7	0.03
2nd measurement	31	55.3	38	60.7	0.02
Post-intervention	31	55.9	38	61.9	0.01
Δ Mother's weight (kg)					
Baseline-1st measurement	31	2.5	38	2.5	0.92
1st-2nd measurement	31	2.6	38	3.0	0.47
2nd measurement- post-intervention	31	0.6	38	1.2	0.39
Knowledge (score)					
Pretest	31	13.5	38	12.9	0.56
Posttest	31	15.5	38	14.9	0.59
Attitude (score)					
Pretest	31	75.6	38	74.4	0.56
Posttest	31	76.7	38	76.4	0.87

and post-intervention in the intervention group, with an average energy difference of 306 kcal, fat 20.9 g, iron 4.1 mg, and calcium 426.4 mg; whereas in the control group, significant differences ($p<0.05$) between the values of nutrient consumption at the beginning and end of the study period were seen in energy and fat, with an average difference of 636 kcal and 24.3 g, respectively.

Figure 1a exhibits that the RD effect in haemoglobin level of the intervention group increased by 0.98 g/dL with milk supplementation. This RD effect represented an increase of 9.5% relative to the control group, but no significant discontinuity effect was found in the haemoglobin level outcome (p=0.15).

Discontinuity was found in MUAC ($p<0.05$) where RD affected the intervention group by 4.7 cm higher than the control group. The effect represented 16.4% relative to the control group above the threshold and this effect was statistically significant (p=0.035) (Figure 1b). Table 4 summarises the discontinuity of the outcomes with haemoglobin level of 11 g/dL as cut-off.

DISCUSSION

The consumption data apparently exhibited that the intervention group experienced a significant increase in the consumption of energy, carbohydrate, protein, fat, iron, and calcium; whereas in the control group, the increase was only in energy and fat consumption. This showed that giving milk to pregnant women can significantly increase the consumption of several nutrients. However, this study showed that in general, the daily nutrients consumption of pregnant mothers was very low, especially micronutrients. Based on the Indonesian RDA, the consumption of iron and calcium in pregnant mothers in the intervention group only met 42.6% and 57.4% of the RDA; whereas in the control group, it was even lower at 30.4% and 29.9% of the RDA,

Table 3. Analysis of respondents' daily nutrient consumption by treatment group

Nutrients	*Type of groups*							
	Intervention (n=31)				*Control (n=38)*			
	Baseline	*Post-intervention*	*Δ Mean*	*% RDA*	*Baseline*	*Post-intervention*	*Δ Mean*	*% RDA*
Energy (kcal)	1282±530	1588±525	306*	62.0	1404±602	2040±1763	636*	80.0
Carbohydrate (g)	192.8±83.9	210.7±79.9	17.9	52.7	226.7±99.9	224.5±101.4	2.3	56.1
Protein (g)	46.3±21.8	57.2±19.7	10.9	81.7	55.5±34.3	66.5±48.2	11.0	95.0
Fat (g)	37.1±25.1	57.9±25.8	20.9*	85.7	42.1±26.8	66.3±58.6	24.3*	98.2
Iron (mg)	6.5±3.8	10.6±3.7	4.1*	39.3	8.5±8.7	8.3±5.3	0.2	30.7
Calcium (mg)	276.3±197.5	702.7±371.3	426.4*	58.6	352.3±231.2	374.4±326.3	22.1	31.2

*$p<0.05$, paired t-test

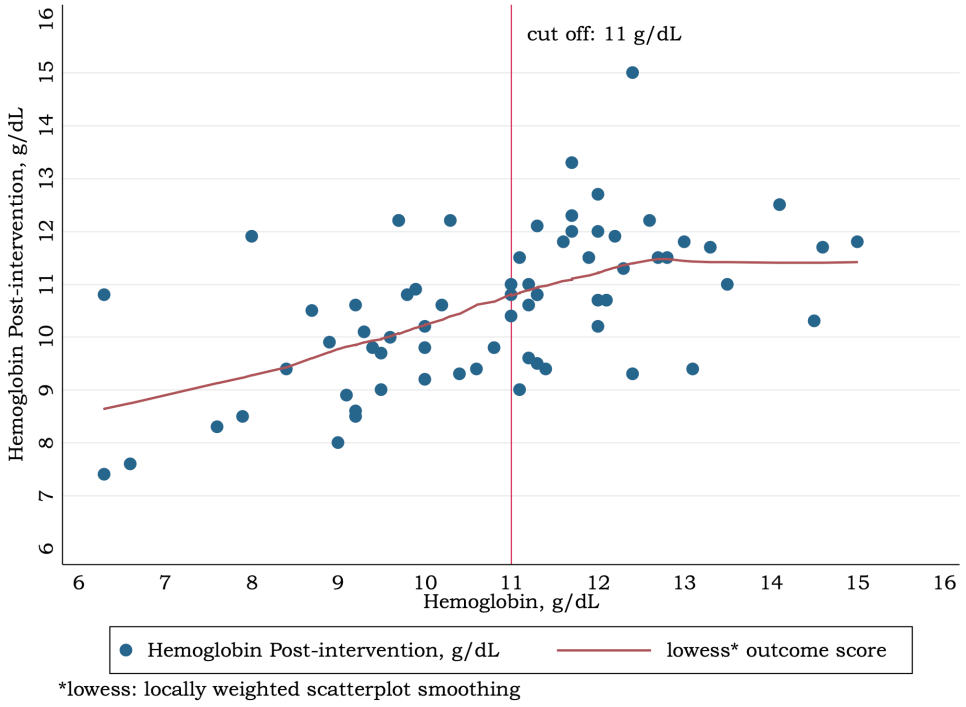

Figure 1a. Regression discontinuity analysis on post-intervention haemoglobin level (g/dL)

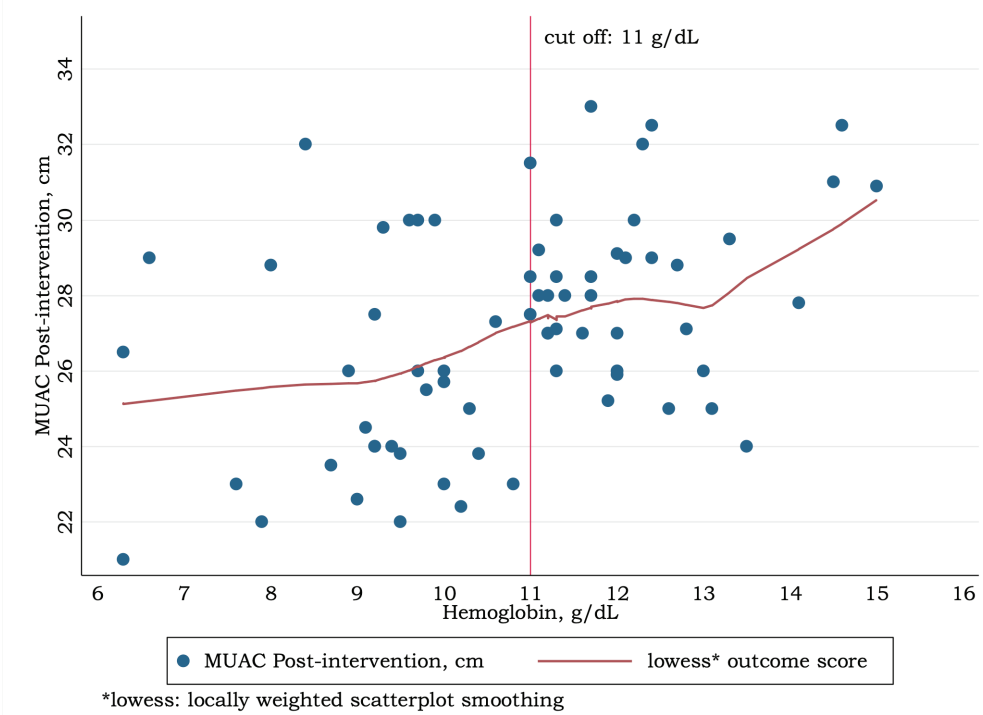

Figure 1b. Regression discontinuity analysis on post-intervention MUAC (cm)

Table 4. Effect size on outcomes based on discontinuity regression

Outcome	*Effect size (95% CI)*	*Relative percentage (%)*	*p-value*
Haemoglobin (g/dL)	0.98 (-0.35 to 2.32)	9.5	0.15
Mid-upper arm circumference (cm)	4.69 (0.33 to 9.06)	16.4	0.04

respectively. All women in this study were distributed with iron-folate tablets from the government, but the recall conducted in this study did not reveal iron supplementation reported by subjects. However, based on information from midwives, no compliance problems have been reported regarding iron-folate consumption.

The low consumption of iron in pregnant women is concerning. During pregnancy, iron is required for both mother and foetus. The urgency of increasing iron status in mothers is to maintain blood volume expansion during pregnancy. Failure to expand maternal plasma volume can result in unwanted pregnancy outcomes, such as premature birth, pre-eclampsia, and foetal growth restriction (Vricella, 2017). The lack of expansion in blood volume during pregnancy can also result in a decrease in cardiac output, which is associated with a decrease in uterine blood flow that can ultimately lead to growth retardation in the foetus (Soma-Pillay *et al.*, 2016). According to Mecacci *et al.* (2015), nutritional supplementation, such as iron in milk, for pregnant women is needed in sufficient quantities for foetal development and prevention of the effects of anaemia. In addition, iron fortified foods, including milk, are considered as a promising approach to prevent iron deficiency anaemia in pregnancy in developing countries because they are relatively accessible and cost-effective (Osungbade & Oladunjoye, 2012).

In this study, milk supplementation could increase haemoglobin level, although not significant and the increase was relatively low. The modest effect of milk supplementation on haemoglobin levels in this study could be explained in a couple of ways. Firstly, maternal anaemia in pregnancy is related to many factors including infections, inappropriate health seeking behaviour, and poverty (Stoltzfus, 2011). In this context, as pointed out by Steketee (2003) considering the tropical climate, poor sanitation and hygiene, it is very possible that hookworm infestation was prevalent among pregnant women in the study area. In 2014, it was reported that in the East Nusa Tenggara rural areas, the prevalence of hookworm infection reached 53.5% (Sungkar *et al.*, 2015). Blood loss caused by hookworm infestation can range from 0.05 mL/day to 0.25 mL/day, depending on the type of nematodes (Steketee, 2003). Secondly, the dietary pattern outside supplementation was based mostly on plant origin foods and less animal source foods. Thus, the low dosage of fortified iron provided by milk supplement (i.e. 17 mg, only 25% of RDA) might have been insufficient to give a significant effect since the iron provided by daily consumption was low, especially among the intervention group. In addition, women who entered pregnancy with undernourished condition is unlikely to improve their nutritional status due to additional demand of the foetus (Ahmed *et al.*, 2012). Thus, food supplementation for malnourished pregnant women can have a significant role in the body's physiological and metabolic requirements, both for themselves and their foetus. Although during pregnancy the body can work in such a way as to compensate for the state of deficiency or excess of certain nutrients, a pregnant woman will not be able to provide essential nutrients for her foetus if she herself is deficient (Koenig, 2017). This

can be a targeted focus for intervention in populations of pregnant women vulnerable to nutrient deficiencies.

Recently, Lipoeto, Masrul & Nindrea (2020) found in their study that after controlling for age, dietary pattern, parity, education level, iron supplementation, health knowledge, prenatal care, health status, and comorbidity, the dominant significant factor for anaemia was chronic energy deficiency. Furthermore, Lipoeto *et al.* (2020) stressed that a reduction in chronic energy deficiency may also reduce anaemia. This might explain why haemoglobin level increase was modest, but MUAC increase was significant since the correction on energy deficiency came first, followed by the increase in haemoglobin level. If a longer duration of intervention was applied, it is not impossible that we can see a higher increase in haemoglobin level.

This study found a significant increase in MUAC in both groups. This is expected and in accordance with the physiological changes in pregnancy, namely the increase in maternal weight due to the development of maternal and foetal body tissues. Regression discontinuity analysis showed that there was a significant effect of milk supplementation on MUAC with relative percentage of 16.4% and an effect size of 4.69 cm higher in intervention group compared to controls. This is in line with Papathakis, Singh & Manary (2016)'s findings that food supplementation can increase MUAC and gestational weight, which can have a positive effect on foetal growth and development. Likewise, according to Heppe *et al.* (2011), milk consumption during pregnancy is associated with an increase in foetal weight and birth weight. A longitudinal study in India showed that milk consumption in the second trimester of pregnancy was positively associated with an increase in placental weight (Rao *et al.*, 2001). There is a tendency that the best effect on foetal growth occurs when milk supplementation is provided in the last trimester. According to Melnik *et al.* (2015), milk is the strongest predictor of increased maternal weight gain during the last trimester. Increased weight in placenta because of milk consumption not only increases nutrient transfer to the foetus, but can also increase maternal blood sugar levels, which will increase foetal growth and birth weight (Melnik *et al.* 2015). Maternal diet for stimulation of smooth transfer of nutrients and uteroplacental blood supply is therefore important to be considered to support foetal growth (Burton & Jauniaux, 2018).

In this study, milk supplementation in the amount of 300 kcal/day for three months had a positive effect on pregnant women with anaemia in terms of MUAC and haemoglobin levels. The energy content of the provided supplement was relatively modest and on the lower side compared to other studies which usually ranged around 300-1000 kcal/day (Liberato, Singh & Mulholland, 2013). Moreover, Ahmed *et al.* (2012) suggested an energy content of 700 kcal/day for supplementing undernourished pregnant women. However, despite the minimal energy content, this study found that milk supplement had a significant effect on MUAC. This might be related to the fact that undernourished women will benefit more from the supplementation compared to normal women (Jackson & Robinson, 2001). Another important note is the contribution of 16% protein towards total energy of the milk supplement. Protein-energy balance in food supplement to treat CED is an important consideration for the supplement to be effective and it is supposed to be under 25% (Jackson & Robinson, 2001) or more recently, under 20%, as excessive energy contribution from protein may do more harm than good to the foetus's growth (Liberato *et al.*, 2013).

Interventions to support foetal growth are crucial for vulnerable groups with CED to avoid the potential long term impacts of prenatal growth

optimisation as a risk factor for foetal programming of metabolic syndrome such as diabetes, hypertension, and heart diseases (Marciniak *et al.*, 2017). Therefore, milk supplementation for population of pregnant women that are prone to energy and iron deficiency is considered suitable for population with high prevalence of CED.

Huynh *et al.* (2018) showed that efforts to improve the nutritional status of mothers through milk supplementation had a positive effect on the nutritional status of pregnant women preparing for the requirement of fat storage for lactation. A study by Zhang *et al.* (2018) among 228 pregnant women in Vietnam showed that there was a sustained effect of maternal milk supplementation which not only contributed to foetal growth and development, but also better exclusive breastfeeding. Two studies of milk supplementation intervention for lactating mothers for three months in Beji and Cipayung Subdistricts in Depok and East Jakarta, Indonesia showed consistently high success rates of exclusive breastfeeding for six months (more than 80%) (Fikawati, Syafiq & Mardatillah, 2017; Fikawati *et al.*, 2019). Furthermore, to support exclusive breastfeeding for six months, as well as the optimal growth and development of children, continuous milk supplementation from pregnancy to lactation is to be provided.

The limitation of this study was related to the lack of maternal height measurement and no pre-pregnancy body mass index values could be obtained as indicators of maternal nutritional status. The limited time of the study also caused the study to lack monitoring of accurate pregnancy outcome measurements, such as birth weight and infant's length. We also noted that despite similar levels of compliance among mothers regarding iron and folate supplementation in both groups, this should be included in the questionnaire and be reported in future. Another limitation was that the sample size could be increased to obtain higher power although the power of this study was considered as sufficient. The study could not reach mothers who did not come to the measurement site because of incomplete addresses. Thus, it is possible that the actual situation is even worse.

CONCLUSION

Using RD method, we were able to determine the effect of milk supplementation based on haemoglobin level and confirm the result that milk supplementation was able to significantly increase MUAC by 4.69 cm, and despite no discontinuity found, a positive increase of 0.98 g/dL in haemoglobin level was important to note. Provision of supplementation targeted to pregnant women is recommended to ensure adequate intakes of both macro- and micronutrients which could not be provided by daily food intakes.

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Authors' contributions

AS, conceptualised and designed the study, prepared the analysis and draft of the manuscript, and reviewed the manuscript; SF, advised on data analysis and interpretation, and reviewed the manuscript; NPM, data analysis and interpretation, assisted in drafting of the manuscript, and translated the manuscript; M, conducted the field study and led the data collection in Kupang District.

Conflict of interest

None.

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Knowledge, attitude and practices (KAP) towards anaemia among female university students in Malaysia: A cross-sectional survey

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ABSTRACT

Introduction: Anaemia is a common health problem in Malaysia, most common being iron deficiency anaemia (IDA). This study aimed to assess knowledge, attitude, practices (KAP), and health-seeking behaviour (HSB) towards anaemia among undergraduate female students. **Methods:** A cross-sectional study was conducted among 180 female university students from a medical university in Malaysia. Data were collected using an online questionnaire comprising KAP and HSB. SPSS version 25.0 was used for statistical analysis. **Results:** Most participants (60%) had an appropriate level of knowledge about anaemia. A positive attitude was shown with adequate awareness of anaemia. A total of 55.5% of participants reported having good health practices. However, 52.9% of the participants consumed less iron-rich foods and 81.7% reported skipping meals. The overall observed health-seeking behaviour was good. A statistically significant relationship was found between knowledge with attitude (p=0.003) and practice (p=0.005). This study observed that the study population had poor nutritional status, long menstrual duration, and vegetarianism. Moreover, infrequent consumption of vitamin C-rich fruits and low iron-folate supplementation were also observed. **Conclusion:** A good level of knowledge on IDA among students was noted. However, most of the students showed a lack of positive attitude and good practices towards preventing anaemia. University students are prone to IDA due to a lack of KAP, which can significantly affect health-seeking behaviour. This issue should not be neglected; therefore, implementing intervention programmes to educate students on the preventive measures against the risk factors of IDA is recommended.

Keywords: anaemia, health-seeking behaviour, knowledge attitude practice

INTRODUCTION

In Malaysia, anaemia has a prevalence of 21.3% and represents a health concern among all high-risk groups, including children, pregnant women, and women of reproductive age (De Benoist *et al.*, 2008; IPH, 2019). Anaemia is defined as insufficient circulating red blood cells (RBCs) essential for oxygen transmission (WHO, 2001). It is characterised by a haemoglobin (Hb) cut-off value of <12.0 g/dL for women and <13.0 g/dL for men

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(WHO, 2001). Iron deficiency anaemia (IDA) is the most common and takes up about 50% of anaemia cases (De Benoist *et al.*, 2008). Usually, IDA occurs due to low intake of iron-rich foods, periodic blood loss or poor iron absorption. IDA initially presents with non-specific signs and symptoms such as pale appearance and fatigue. However, it can cause impaired cognitive development in children and an increased risk of miscarriage in pregnant women.

According to the National Health and Morbidity Survey (NHMS) 2019, one in five Malaysians have anaemia, with the most affected being reproductive women aged 15–49 years old (IPH, 2019). Young women are considered vulnerable to IDA because of iron loss during menstruation and failure to achieve the recommended daily iron intake (IPH, 2014). IDA is a common problem in Asia; however, studies are mostly done on pregnant women and children. There is limited data available for female university students (Alzaheb & Al-Amer, 2017). Female students enrolled in higher education could be more susceptible to IDA because they prioritise academic activities than consuming adequate diets. Insufficient knowledge, attitude, and practices (KAP) on IDA prevention can be classified as risk factors because they invigorate poor dietary habits and negligence in prevention activities. Poor dietary habits, such as skipping meals and eating fast food, are considered a public health concern among university students, making them vulnerable to nutritional deficiencies (Ganasegeran *et al.*, 2012). For this purpose, health-seeking behaviour (HSB) should be emphasised as it plays a role in the early detection of anaemia. Unfortunately, many people are less likely to seek medical care as they are unaware or do not take their symptoms seriously.

Based on the National Plan of Action for Nutrition Malaysia III (NPANM III), the global nutrition target is to reduce 50% of anaemia prevalence in reproductive women by 2025 (NCCFN, 2016). Many KAP studies on anaemia from Malaysia have mainly focused on pregnant women and adolescents, when this issue is equally applied to female undergraduate students (Azma, Ainoon & Azlin, 2012). Therefore, it is crucial to collect information to assist the design of nutrition interventions and create awareness of the significance of KAP and HSB in preventing IDA among female university students. The current study aimed to determine the KAP and health-seeking behaviour towards anaemia among undergraduate female university students.

MATERIALS AND METHODS

The study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki as revised in 2013. Detailed informed consent was obtained for the inclusion of subjects in this study. This study was reviewed and approved by the International Medical University (IMU) Joint-Committee on Research and Ethics (IMUJC) # 4.2/JCM-191/2019. All information collected were strictly confidential, and anonymity was ensured.

Sample size calculation

A cross-sectional online study was conducted from 8th July to 10th August 2020 among 180 female undergraduate students aged 19–26 years old studying in various schools in IMU, Kuala Lumpur. The sample size calculated based on G*Power analysis was 180 for 7.3% (Kharel *et al.*, 2017) prevalence of anaemia at 99% confidence level. The inclusion criteria were female undergraduate students studying at IMU. Those who did not fulfil the criteria were excluded from this study.

Data collection

The online questionnaire was adapted from two validated questionnaires (Jalambo *et al.*, 2017; Shahzad *et al.*, 2017). It was developed using Google Forms and distributed through online platforms such as WhatsApp and Outlook. The questionnaire consisted of five sections: sociodemographic information, knowledge, attitude, practice, and HSB towards anaemia.

Study variables, such as age, ethnicity, height, weight, knowledge, attitude, practice, and HSB, were recorded from the data. In addition, the study participants' undergraduate programme, semester, average duration of menstrual cycle, and vegetarianism status were also obtained.

The KAP questionnaire consisted of 36 questions and was divided into four sections, namely knowledge (18 questions), attitude (8 questions), practice (6 questions), and HSB (4 questions). The knowledge section corresponded to the level of awareness the participants had on the dangers associated with IDA. The attitude section provided information on the participants' perspective and feelings towards IDA. The practice part aimed to assess the participants' dietary habits. Lastly, HSB questions were included to ascertain its correlation with KAP and demonstrate proactiveness for treatment.

Data analysis

Data analysis was performed using SPSS version 25.0. All quantitative variables were examined for normality by the Shapiro-Wilk test before analysis, whereas continuous variables were presented as mean and standard deviation. One-way ANOVA was used to determine statistically significant differences between mean and median proportions of subjects with good and poor levels of KAP. Pearson correlation coefficient was used to evaluate the relationship between each study variable. A p-value of <0.05 was considered statistically significant.

RESULTS

Demographic information

In this study, the participants' mean age was 21.6 years old and mean BMI was 20.7 kg/m^2 (Table 1). Based on the results, 23.3% (46) students were underweight and 7.8% (14) were overweight. Most participants consisted of Chinese students (81.1%), followed by Indians (7.2%), Malays (5.6%) and other ethnicities (6.1%). Regarding periodic menstruation, 19.4% (35) students had an average duration of 2–4 days menstruation, 74.4% (134) had 5–7 days, while 6.1% (11) had >7 days. Lastly, 98.9% (178) students were non-vegetarian. The descriptive statistics and characteristics of the study population are illustrated in Table 1.

Table 1. Descriptive statistics and characteristics of the study sample

Characteristics	*mean±SD*	*n*	*%*
Age group (years)	21.6±1.3		
19–20		40	22.3
21–22		89	49.4
23–24		47	26.1
25–26		4	2.3
BMI (kg/m^2)	20.7±3.3		
<18.5		46	25.6
18.5–24.9		116	64.4
25.0–29.9		14	7.8
>30.0		4	2.2
Ethnicity			
Chinese		146	81.1
Malay		10	5.6
Indian		13	7.2
Others		11	6.1
Average duration of menstruation			
2-4 days			19.4
5-7 days			74.4
>7 days			6.1
Vegetarian			
Yes			2.2
No			97.8

Knowledge

Knowledge was assessed by grading each response based on the number of correct answers and the subjects' degree of awareness. Each correct answer on understanding corresponded to one point. The participants were required to answer an additional question to verify their knowledge if they answered "Yes" to the first question. A score of >24 points (>70%) and <14 points out of a total of 34 points were considered as good and poor knowledge, respectively (9).

The mean score obtained was 60.3%, indicating a good level of knowledge on IDA among students. All respondents

Table 2. Characteristics of answers to knowledge questions

Characteristics	*n*	*%*
K.1 Do you know what is anaemia?		
Yes	180	100.0
No	0	0.0
K.2 Definition of anaemia?		
Lack of red blood cells	172	95.6
Hb levels <12g/dL	2	1.1
Related to blood	1	0.6
No answer	5	2.8
K.3 Most common type of anaemia?		
Iron deficiency anaemia	177	98.3
Vitamin deficiency anaemia	2	1.1
Aplastic anaemia	1	0.6
K.4 Harmful effects of anaemia?		
Yes	149	82.8
No	31	17.2
K.5 Most affected group in Malaysia by anaemia?		
Non-pregnant women of reproductive age	69	38.3
Pregnant women	98	54.4
Children	13	7.2
K.6 Causes of anaemia?		
Yes	162	90.0
No	18	10.0
K.7 Symptoms of anaemia?		
Yes	167	92.8
No	13	7.2
K.8 Consequences of anaemia for infants and young children?		
Yes	132	73.3
No	48	26.7
K.9 Consequences of anaemia for pregnant women?		
Yes	144	80.0
No	36	20.0
K.10 Prevention of anaemia?		
Yes	139	77.2
No	41	22.8
K.11 Identification of iron-rich foods?		
Yes	123	68.3
No	57	31.7

claimed to know about anaemia, where 96.7% defined the term correctly. The majority (98.3%) of students recognised IDA as the most prevalent type of anaemia. A high percentage of students were accurate on the function of Hb (96.1%) and aware of the harmful effects (82.8%), causes (90.0%) and symptoms (92.8%) of anaemia. Despite that, only 60%–80% of the students could correctly identify all the consequences of anaemia.

Moreover, only 38.3% answered correctly about the most affected group by anaemia: non-pregnant women. Approximately half of the students (55.6%) could recognise a person with anaemia. Most students also understood the consequences of anaemia on children (73.3%) and pregnant women (80.0%). A total of 139 respondents (77.2%) knew how to prevent anaemia, but only 68.3% could identify the effects of different foods on iron absorption. The characteristics of answers to knowledge questions are shown in Table 2.

Attitude

About 32.8% of students were unsure if they were diagnosed with anaemia (Table 3). A positive attitude was detected when 34.4% and 62.2% considered anaemia a serious or moderate health problem. Although the benefits of iron-rich foods were well known by 90.0% of respondents, 11.1% found difficulty and 20.0% were not confident in preparing these meals. Findings also showed that 72.8% were willing to take iron supplements if diagnosed with anaemia. Lastly, most respondents (87.8%) deemed nutrition education programmes beneficial for preventing IDA (Table 3).

Table 3. Characteristics of answers to attitude questions

Characteristics	*n*	*%*
A.1 How likely do you think you are to be anaemic?		
Very likely	33	18.3
Unsure	59	32.8
Not likely	88	48.9
A.2 How serious do you think anaemia is as a public health problem?		
Serious	62	34.4
Moderate	112	62.2
Not serious	6	3.3
A.3 How beneficial do you think it is to prepare meals with iron-rich foods?		
Beneficial	162	90.0
Not sure	17	9.4
Not beneficial	1	0.6
A.4 How difficult is it for you to prepare meals with iron-rich foods?		
Easy	32	17.8
Capable	128	71.1
Difficult	20	11.1
A.5 How confident do you feel in preparing meals with iron-rich foods?		
Very confident	20	11.1
Capable	124	68.9
Not confident	36	20.0
A.6 How serious do you think anaemia is as a public health problem?		
Yes	131	72.8
Maybe	37	20.6
No	12	6.7

Table 4. Characteristics of answers to practice and health-seeking behaviour (HSB) questions

Characteristics	*n*	*%*
P.1 Do you consume dietary haem iron (present in animal meats)?		
Yes	165	91.7
No	15	8.3

P.2 How frequently do you consume the following foods?	*Never*		*Not often*		*Monthly*		*Weekly*		*Daily*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Animal meats	2	1.1	17	9.4	0	0.0	28	15.6	133	73.9
Fish	2	1.1	27	15.0	12	6.7	103	57.2	36	20.0
Vitamin C rich fruits	25	13.9	68	37.8	7	3.9	80	44.4	68	37.8
Green leafy vegetables	2	1.1	8	4.4	4	2.2	23	12.8	143	79.4
Legumes	6	3.3	69	38.3	17	9.4	61	33.9	27	15.0
Coffee/Tea	9	5.0	63	35.0	14	7.8	44	24.4	50	27.8
Milk	8	4.4	54	30.0	14	7.8	57	31.7	47	26.1
Junk food	3	1.7	81	45.0	24	13.3	59	32.8	13	7.2

P.3 How much of the following foods do you consume per day?*	*Never or <1 serving/day*		*1-2 servings/day*		*>3 servings/day*	
	n	*%*	*n*	*%*	*n*	*%*
Animal meats	27	15.0	147	81.1	7	3.9
Fish	86	47.8	93	51.7	1	0.6
Vitamin C rich fruits	67	37.2	98	54.4	15	8.3
Green leafy vegetables	23	12.8	128	71.1	29	16.1
Legumes	128	71.1	50	27.8	2	1.1

P.4 How much of the following beverages do you consume per day?	*Never or <1 cup/day*		*1-2 cups/day*		*4-6 cups/day*	
	n	*%*	*n*	*%*	*n*	*%*
Coffee/Tea	111	61.7	65	36.1	4	2.2
Milk	107	59.4	72	40.0	1	0.6

Characteristics	*n*	*%*					
P.5 When do you usually consume the following foods?			*Before meal*		*During meal*		*After meal*	
			n	*%*	*n*	*%*	*n*	*%*
Vitamin C rich fruits			46	25.6	10	5.6	124	68.9
Coffee/Tea			65	36.1	51	28.3	64	35.6
Milk			76	68.9	46	25.6	58	32.2
P.6 Do you tend to skip meals?								
Often	39	21.7						
Seldom	108	60.0						
Never	33	18.3						
HSB.1 Would you seek medical attention if you suspect that you have anaemia?								
Yes	162	90						
No	18	10						
HSB. 2 Have you checked your haemoglobin levels in the past 1 year?								
Yes	80	44.4						
No	100	55.6						
HSB. 3 Have you taken iron folic acid (IFA) tablets in the past 1 year?								
Yes	25	13.9						
No	155	86.1						
HSB. 4 Have you taken deworming tablets in the past 6 months?								
Yes	3	1.7						
No	177	98.3						

Practice

Out of 180 students, 91.7% included dietary haem iron in their meals, as shown in Table 4. Daily consumption of animal meats (73.9%) and green leafy vegetables (79.4%) were the highest. Most respondents consumed vitamin C-rich fruits weekly (44.4%); however, only 5.6% ate them during meals. Furthermore, only 15.0% of the students included legumes in their daily meals. Regarding beverages, most students did not drink coffee, tea (35%) or milk (30%) often. If so, approximately half of the population never consumed or consumed <1 cup of coffee, tea (61.7%) or milk (59.4%) per day. These beverages were also commonly consumed before meals, where coffee or tea was consumed by 36.1% and milk was consumed by 68.9%. The practice of skipping meals was detected in 87.1% of subjects.

Health-seeking behaviour (HSB)

HSB was assessed by evaluating the participants' proactiveness for regular health inspection and performing activities to prevent the development of IDA. Out of a total of four activities, students who had done ≥2 activities were categorised as high HSB, <2 activities as low HSB (Asfaw, Ayanto & Aweke, 2018). Overall, the participants displayed high HSB. About half (52.8%) of the students performed at least one activity, 43.3% students did two activities, and only 4.4% did none. As observed in this study, 90.0% of students would agree to seek medical attention if they suspect anaemia. However, only 44.4% checked their Hb levels, 14.0% had taken iron-folic acid (IFA) tablets in the past year, and 2.0% of students had taken deworming tablets in the past six months. The characteristics of answers to HSB questions are shown in Table 4.

Association between KAP and HSB

Findings showed that there was statistically significant relationship between knowledge with attitude (p=0.003) and practice (p=0.005), but not with HSB (Table 5). A higher level of knowledge strongly corresponded to a better attitude in the study population, but weakly corresponded to practices towards preventing anaemia. In contrast, the variables attitude, practice, and HSB were found to have no significant associations with each other.

DISCUSSION

Anaemia is prevalent among women of reproductive age, where young female

Table 5. Associations between KAP and HSB

		Knowledge	*Attitude*	*Practice*	*HSB*
Knowledge	Pearson Correlation Sig. (2-tailed)	1	0.218 0.003*	-0.209 0.005*	0.054 0.468
Attitude	Pearson Correlation Sig. (2-tailed)		1	-0.067 0.369	0.096 0.201
Practice	Pearson Correlation Sig. (2-tailed)			1	0.052 0.485
HSB	Pearson Correlation Sig. (2-tailed)				1

*Significantly correlated at p<0.05 (2-tailed); HSB: Health-seeking behaviour

students are prone to develop IDA due to hectic schedules and academic stress, resulting in negligence on a healthy lifestyle and poor dietary habits (Ganasegeran *et al.*, 2012). IDA generally causes fatigue and weakness due to less oxygen being supplied to the body, leading to impaired work productivity and poor academic performance (Soleimani & Abbaszadeh, 2011). The prevention of IDA includes lifestyle modifications, especially consumption of iron-rich foods (WHO, 2016). Thus, improving KAP towards anaemia among female university students is necessary to garner awareness on the prevention of IDA and the adoption of HSB.

To determine knowledge level, a score of >70% and <40% were considered as good or poor knowledge, respectively. The respondents scored an average of 60.3% and elicited an appropriate awareness level on IDA. This score was slightly higher than another study conducted among students from a home economics college who scored 54% (Shahzad *et al.*, 2017). Most students correctly answered the general characteristics of anaemia, such as its symptoms, causes, and harmful effects. In our study, 54.4% perceived pregnant women as individuals most affected by IDA. Pregnant women have a higher incidence rate, but anaemia remains most prevalent among non-pregnant women in Malaysia (De Benoist *et al.*, 2008). Prevention of IDA should start early to improve pregnant women's overall health status, thus progressively facilitating the reduction of maternal and perinatal mortality. Only seven students (3.88%) answered all questions correctly about the effect of each food on iron absorption. This showed that the study sample lacked nutrition knowledge, which was strongly reflected on their practices.

The respondents' overall attitude was positive; however, 32.8% were unsure if they had anaemia, which was lower than observed in other studies (Singh, Rajoura & Honnakamble, 2019). Although the knowledge level and attitude observed were acceptable, the results obtained were unsatisfactory since this population who studied in a medical university was expected to better understand health issues and had convenient access to health facilities for medical diagnosis compared to the public.

The negative attitude was related to their ability and confidence in the preparation of meals high in iron. The present study recommends dietary counselling on food preparation to stimulate behavioural change and enhance dietary diversity and quality among the students (Lopes *et al.*, 2018). This is evident in a study where nutrition education in secondary schools increased Hb levels after KAP improvement (Yusoff, Daud & Ahmad, 2012). According to a study, iron supplements helped alleviate the undesirable effects of anaemia encountered by students (Soleimani & Abbaszadeh, 2011). Also, concentration and school performance among adolescent girls in Ghana were remarkably increased after iron and folic acid supplementation (IFAS) programme (Deshpande, Basil & Basil, 2009).

Numerous studies have reported that students' poor eating behaviour is strongly associated with stress and low self-esteem (Deshpande *et al.*, 2009). Nonetheless, the subjects demonstrated good food selection practices as they had the highest daily consumption of ideal portion-sized animal meats (73.9%) and green leafy vegetables (79.4%). Low daily consumption of vitamin C-rich fruits (37.8%) was observed in our population, which predisposes them to IDA due to decreased non-haem iron absorption secondary to low vitamin C intake.

However, more than half of the participants (52.9%) consumed less iron-rich foods. A total of 81.7% reported

skipping meals, and 63.9% drank tea or coffee during or after meals. Taking tea or coffee during or after meal decreases iron absorption and can lead to IDA. Tea consumption of >4 cups per day was related to a higher IDA prevalence, mainly due to the presence of iron absorption inhibitors (polyphenols and calcium) (Sung *et al.*, 2018). Moreover, 32.8% of participants consumed junk food weekly. These findings agree with Kannan & Ivan (2017); 25% of their study subjects regularly skipped meals, and 33% frequently consumed fried foods.

Based on the Malaysian Adult Nutrition Survey (MANS) 2014 (IPH, 2014), iron intake was the most inadequate in women where only 52% of the recommended nutrient intake was achieved, which was consistent with the previous study conducted by Loh & Khor (2010). The tendency to skip meals was found in 60% of respondents. Skipping meals and having an imbalanced diet is common among college students, as observed in Bangladesh, and this may contribute to IDA (Shill *et al.*, 2014). Many studies revealed a higher prevalence of anaemia among those skipping meals (Shill *et al.*, 2014).

Low nutritional status is a significant risk factor for anaemia, especially in underweight and overweight populations (Shill, Karmakar & Kibria, 2014). The Ministry of Health Malaysia (MOH) reported that women >19 years have an average iron loss of 1.90 mg per day during menstruation, which is double the amount of basal daily iron loss, thus increasing the risk of IDA in this age group (NCCFN, 2017). In this study, 74.4% of the students had an average menstruation duration of between five to seven days, and 6.1% of the students had an average duration of more than seven days.

Findings revealed that 52.8% of the participants had high HSB towards anaemia. Also, the participants performed at least one or two of the preventive activities mentioned in Table 5, indicating high HSB among the female students.

In the present study, the observed score for seeking medical attention when encountering health issues was higher (90.0%) than a finding in the literature, which was 77.2% (Hadaye, Dass & Lavangare, 2018). The present study revealed 55.6% of participants did not check their Hb levels in the past year. Students generally rather stay undiagnosed or practise self-medication, consistent with another reported study (El Kahi *et al.*, 2012).

IDA may result from a lack of iron folate supplementation and deworming tablets. In our study, 86.1% and 98.3% of participants did not take these tablets, putting them at higher IDA risk. A reduction in anaemia prevalence was shown in a study conducted among the Indian population after supplementation with IFA alone or with vitamin C and deworming tablets (Joseph & Ramesh, 2013).

Good knowledge and change in attitude can help achieve the necessary shift in behaviour towards healthy foods to prevent IDA (Shahzad *et al.*, 2017). The present study showed a statistically significant relationship with a positive linear correlation (p=0.003) between knowledge and attitude. Thus, a higher level of knowledge corresponds to a better attitude and vice versa. This finding was consistent with a study in Putrajaya, which observed that a high level of knowledge reflected a positive attitude towards anaemia (Adznam, Sedek & Kasim, 2018). In comparison, a weak negative correlation existed between knowledge and practice (p=0.005). This result contrasted with findings by Imunticha, Achen & Quadras (2015), who found a weak but positive correlation between knowledge and self-

reported practices on IDA prevention.

Based on the discussion above, it is imperative to strengthen students' dietary knowledge and reinforce good food-based prevention practices against IDA. The synergistic effect of improved knowledge and attitude are more likely to prompt practices against IDA and develop HSB. A recent study by Singh *et al.* (2019) noticed a statistically significant change in KAP among Delhi adolescent girls before and after providing health education (Singh, Honnakamble & Rajoura, 2019). Researchers suggest that achieving the desired change of behaviour towards nutrition and health depends on gaining sufficient knowledge, changing attitude, and developing acceptable practices related to health and nutrition (Shariff *et al.*, 2008). Therefore, nutrition interventions should be reinforced, especially since 35.1% of Malaysian adults have low health literacy on managing medical issues and disease prevention (IPH, 2019). Besides that, government and educational institutions are suggested to organise programmes to help provide information on health and nutrition for students (WHO, 2001). Such programmes are expected to assist IDA prevention through counselling on the importance of consuming iron-rich foods and taking necessary tests and supplements to strengthen positive HSB towards anaemia (WHO, 2001). Through improved KAP and HSB, there will be lower incidences of morbidity and mortality caused by IDA (Inche, Sutan & Shamsuddin, 2014). Hence, the risk of IDA in these female students should not be neglected, and more research should be conducted to raise awareness that will promote changes in KAP and HSB to combat IDA.

Some limitations were identified in this study. While it was not among the primary purposes of this study, a valuable measure of Hb level would be of great interest in determining the effects of KAP on the prevalence of anaemia among the study sample. Furthermore, a more homogenous and larger sample size may have been able to reveal clearer and more immediate differences between individuals with good and poor KAP.

CONCLUSION

The female undergraduate students demonstrated an appropriate level of knowledge; however, the majority showed a lack of positive attitude and good practice towards preventing anaemia. In addition, skipping meals was observed in most students. A statistically significant correlation was only found between knowledge with attitude and practice. The overall HSB among the students was high. This study highlighted that female university students are prone to IDA due to a lack of KAP and thus recommends intervention programmes through educational institutions to improve KAP, encourage HSB, and take preventive measures against the risk factors of IDA in this population.

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Authors' contributions

HC, led the data collection, did the analysis and prepared the draft of the manuscript; CJL, led the data collection and reviewed the manuscript; NRY, led the data collection and reviewed the manuscript; PDK, reviewed the manuscript; MS, designed the study and reviewed the manuscript; SA, corresponding investigator, conceptualised and supervised the study, and reviewed the manuscript.

Conflict of interest

The authors have no conflict of interest associated with the materials presented in this paper.

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Formulation of local food multimix sprinkle to enhance nutritional adequacy of preschool children in southernmost provinces of Thailand

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ABSTRACT

Introduction: This study aimed to formulate an effective Food Multimix-Sprinkle (FMM-S) by using locally available materials. **Methods:** Fish (Threadfin bream), cow liver, and orange fleshed sweet potato (OSP) were cooked and dried by using applicable household (HH) method and drum drying (DD). Then, the dried materials were milled and sieved through a 20 mesh screen. Fish, cow liver, and OSP sprinkles were mixed in various proportions to meet nutrient level targets [at least 30% of Thai Recommended Dietary Allowance (RDA) for 1-3 years old children per 15 g serving size]. **Results:** Six alternative mixes were formulated. The mixture of 3 g of liver, 7 g of fish, and 5 g of OSP (3:7:5) when processed by HH method, and the mixture of 4 g of liver, 8 g of fish, and 3 g of OSP (4:8:3) when processed by DD method, had significant advantage in preference scores in all attributes over the others. Nutritional values of these formulas were 37-55% RDA of protein, 146-194% RDA of vitamin A, and 30-40% RDA of iron for a serving size. **Conclusion:** This study demonstrated that household preparation, as well as preparation using drum drying could be used to process raw fish, cow liver, and OSP into a sprinkle mix. The FMM-S provided appropriate amounts of protein, vitamin A, and iron to supplement nutrient intake in pre-schoolers. However, a consumer test is needed to ensure acceptance by the target population.

Keywords: food multimix, nutritional formulation, sprinkle

INTRODUCTION

Nutritional deficiency during the first year of life significantly contributes to impaired growth and development of a child, which has long-term adverse effects. Suboptimal caregiver feeding practice is the most frequent factor inducing postnatal growth retardation (Branca & Ferrari, 2002). Inappropriate feeding of infants has been recognised as being practised in southern Thailand. Early introduction of complementary foods, feeding of sweetened condensed milk, late introduction of animal sourced foods, and inappropriate food choices are key issues (Mo-suwan & Sanguan-chua, 1980; Maisarow & Usaman, 1993; Bureau of Nutrition, 2006). In a preliminary survey of the dietary intake among children aged under 2 years in Pattani province (one of the three southernmost provinces of Thailand), it was found that children consumed a diet inadequate in some micronutrients,

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particularly iron, vitamin A, and vitamin B (Chaimongkol & Soison, 2012). Recently, it was found that 29.4% of children aged 6-59 months in the three southernmost provinces had low serum levels of vitamin A (Thammapalo *et al.*, 2020).

Caregivers are the key persons responsible for providing nutritionally qualified diets to children. In this geographical area, caregivers are mostly the mothers and sometimes the grandparents or relatives, or older siblings; and normally have no good grasp of an appropriate diet for this specific age group. Furthermore, they usually have no time because of busy daily schedule, including work. Most caregivers would rather buy ready-cooked foods or use the same food for their children as provided to adults in the family (household meals). Due to these constraints, it might be difficult to promote the cooking of foods that are specifically made to have appropriate nutrient proportions for young children (Chaimongkol & Soison, 2012). Therefore, food supplements might be an appropriate alternative strategy to enhance nutrient adequacy in this setting. There are various forms of food supplements, including food fortification, sprinkles, ready-to-eat foods, etc., which have their own advantages and disadvantages in terms of application to alleviate nutrient deficiencies. Choosing the right forms of food supplements for the context might determine the efficacy of this approach in solving the problem.

Food sprinkles are recognised as a means to improve the nutritional value of homemade baby foods. Sprinkles originated as a form of nutrient that can be easily mixed in any homemade foods. By sprinkling on the bulk food vehicles, these products provide dosing flexibility and convenience of administration, as well as cost-effectiveness (Lee *et al.*, 2020). As a home fortificant, sprinkles ensure that the food eaten contains adequate amounts of essential micronutrients. Furthermore, their use do not require any changes in other food practices; thus, they can be easily accepted in diverse cultural settings (Zlotkin *et al.*, 2005). Although food sprinkles have many advantages, there are some limitations to be considered in their application. Actually, nutrient sprinkles are not locally produced and are perceived as additive chemicals rather than as food, which might make them unacceptable, especially to low-income people. It was suggested that powdered local foods should be mixed to a sustainable super sprinkle (Nordin, 2005); hence, the idea of a food multimix (FMM) was introduced to cope with this issue. The FMM approach uses locally available, traditional and culturally adaptable complementary foods. Their review demonstrated that it is possible, in one composite mix, to provide a food recipe even in the midst of scarcity, to meet nutrient requirements through a food-based approach that is balanced, acceptable and appropriate within a cultural context. Products can be developed at a low cost, which can then be made easily available and affordable to most consumers (Zotor & Amuna, 2017). Thus, combining the concepts of sprinkle and FMM to formulate a “Food Multimix Sprinkle (FMM-S)” might be an approach to effectively enhance the adequacy of nutrients among pre-schoolers in southernmost Thailand.

In this study, we aimed to formulate FMM by using locally available ingredients, prepared in the form of sprinkles to provide additional nutrients, especially protein, vitamin A, and iron, to complement and complete the usual diet. Cow liver, fish, and orange fleshed sweet potato (OSP) were chosen as the FMM ingredients, since they are commonly consumed and available in the southernmost provinces. Cow liver

is a rich source of vitamin A and iron, while fish is a source of protein. OSP was included for its attractive colour and sweet flavour in the product. To make a suitable FMM-S, both locally applicable household method and drum drying method were used in processing to produce the ingredients mixed into formulas that were designed with proper nutritional proportions.

MATERIALS AND METHODS

Raw materials

The main raw materials used for sprinkles preparation were fish (Threadfin bream, *Nemipterus hexodon*), cow liver, and orange fleshed sweet potato (Khai variety, *Ipomoea batatas* L.). All were purchased from a local market in the Pattani province. Each material was freshly prepared according to the processing steps described below.

Raw material preparation

Fish fillets were obtained by removing the inedible parts, namely head, gut, scales and bones, and were steamed and later shredded into small pieces. Cow liver was cleaned, sliced into thin pieces, and soaked in 1.2% vinegar for two hours to reduce the liver flavour. After that, it was steamed for 30 minutes, set aside to cool, and then finely homogenised for one minute with a household grade food processor (Philips HR 1393/00, China). OSP was cleaned, peeled, sliced into 1-2 mm thin slices for frying in a household method, or chopped manually with a knife into small pieces for drum drying.

Production of sprinkles from main ingredients

Household (HH) method

Cooked fish and liver were roasted in a pan for approximately 20-30 minutes, or until dry and began to crumble. Sliced OSP was deep-fried in palm olein oil at 170±5°C until the pieces were cooked, then continued to dry in a hot air tray dryer (Kluay Num Thai Towop, Thailand) at 60°C for one hour. The moisture content of all ingredients was below 10% as determined by a moisture analyser (Sartorius MA150, Germany).

Drum drying (DD) method

Chopped OSP was steamed for 15 minutes and mashed before drying. All prepared raw materials were dried on a double drum dryer with settings of drum temperature at 130°C, gap between drums at 1 mm, and rotation speed at 1-2 rpm. The collected flakes were further dried in a tray dryer and their moisture content was controlled to be similar to the HH method. Dried fish, cow liver and OSP derived from both methods were milled and sieved through a 20 mesh screen for further use.

Formulation of FMM-S

Particle size of sprinkles

The dried ingredients were milled with a household blender and sieved to three fractions: (1) bigger than 8 mesh (over 2.36 mm), between 8 and 20 mesh (0.85 – 2.36 mm), and passing through 20 mesh (smaller than 0.85 mm). The optimum particle size for sprinkles was chosen by using a Just about right (JAR) approach. Finally, one particle size of each ingredient, prepared by each processing method was chosen.

Formulation of FMM-S

This product concept aimed to supplement nutrients that have a tendency to be deficit among pre-schoolers in the southernmost provinces, namely protein, vitamin A, and iron. The product should provide at least 30% of RDA per a typical serving size (15 g). Since the main target users of FMM-S were preschool children, the Thai Recommended Dietary Allowance (RDA) of protein, vitamin A, and iron used were those for children aged 1-3

years (Bureau of Nutrition, 2003). Three main components, namely fish, cow liver, and OSP sprinkles were mixed in various proportions to meet the nutrient targets. Thus, one serving contained 3 or 4 g of cow liver; 6, 7, or 8 g of fish; and 3, 4, 5, or 6 g of OSP to complete 15 g in total weight. By calculation using Microsoft Excel (Office Professional Plus 2016), six blends were formulated in this study. All formulas were subjected to colour measurement and sensory preference test. The most appropriate FMM-S formula was determined for its final quality characteristics of colour, moisture content, water activity, and TBA (thiobarbituric acid).

Colour and chemical property analyses

Colour

FMM-S formulas were measured by using Hunter Lab (Colour Quest XE, USA) for the colour coordinates L*, a* and b*.

Chemical analysis

Moisture, crude protein, fat and ash in the sprinkles were determined by official methods (AOAC, 2000). Water activity (a_w) was measured by using AQUALAB 4TE water activity meter (Meter Group, Inc., USA). Only cow liver was analysed for vitamin A and iron since it was recognised as an excellent source of these two nutrients. Vitamin A was determined by HPLC method (Kangsadalampai & Sungpuag, 1984), while iron content was analysed by Atomic Absorption Spectroscopy according to the methods of AOAC (Latimer, 2019). TBA was determined by the method of Egan, Kirk & Sawyer (1981).

Sensory evaluation

Just about right (JAR)

The 3-point JAR approach was used to determine the appropriate particle size for each sprinkle ingredient. Three sizes of each sample were served to 30 untrained panellists. They were asked to give their opinion on the particle fraction of sprinkles, whether it was too large, just right, or too small, by considering the overall size compared to the commercial rice seasoning sample (Proteilife, J.D. Food Products Co., Ltd.) as a control sample.

Preference test by hedonic scale

Samples of the alternative FMM-S were served with a bowl of porridge to 30 untrained panellists at the Food Science and Nutrition Department of Prince of Songkla University, Pattani campus. Sensory test of samples from each processing method was separately assessed. The panellists were asked to rate their preference scores on the attributes of the samples on a 9-point hedonic scale. The appearance, liver flavour, fish flavour, sweet potato flavour, overall flavour, and overall liking attributes of FMM-S formulas were evaluated. The Balanced Incomplete Block Design (BIB) was applied in the experiment by using plan 11.6, type II ($t = 6$, $k = 4$, $r = 10$, $b = 15$, and $\lambda = 6$) provided in a reference text (Cochran & Cox, 1992). Each formula was tested 40 times by some individuals among the panellists.

Ethics approval

The ethical committee of Prince of Songkla University, Pattani campus, approved the study protocols. All panellists were informed about the purpose of the study and procedures of sensory evaluation. Oral informed consent was obtained before performing product sensory evaluation.

Statistical analysis

All measurements were performed in triplicates. Statistical package R programme version 3.5.2 was used. Data were analysed using descriptive statistics and Analysis of Variance

(ANOVA). Post-hoc multiple comparison of means of different formulations was done by Duncan's multiple range test and considered statistically significant at $p \leq 0.05$.

RESULTS

Proximate composition of sprinkled ingredients

Fish, cow liver, and OSP were processed into coarse powder by household and drum drying methods. Protein contents were very high in fish and cow liver (70-90% and 58-68% dry basis, respectively). Only cow liver was analysed for vitamin A and iron. Vitamin A and iron contents in cow liver were 21,353 µg and 103 mg per 100 g dried ingredients, respectively (Table 1).

Effect of particle size fraction on properties of sprinkles

Moisture content in a food plays an important role in its spoilage. It is generally recommended that the moisture content in dried food should not exceed 15%. We found that the coarse powder of all three ingredients made by the two methods had a moisture range of 5.3 to 7.5% (Table 2). TBA is an indicator for the degree of lipid oxidation, as this liberates secondary oxidation products of oil and fat to the food. Most ingredients prepared by HH method had higher TBA than that prepared by DD method.

Formulation of FMM-S

Fish, cow liver, and OSP sprinkles were mixed in various proportions as shown in Table 3. The six FMM-S formulas were created from 3-4 g of cow liver, 6-8 g of fish, and 3-6 g of sweet potatoes. Protein, vitamin A, and iron contents per serving size (15 g) and %RDA were calculated for each formula (nutrient calculation based on dry weight). Estimated protein and iron in all formulas per one serving met one-third of the requirements, while the estimated vitamin A was 1.5 to 2 times the daily requirement. These amounts indicated that FMM-S has a high potential for being an effective food supplement of protein, vitamin A, and iron for young Thai children.

Colour

Appearance of FMM-S was evaluated from the L*, a* and b* colour coordinates. The results in Table 4 showed that each formula had a different colour, depending on proportions of the ingredients. Overall, the lightness (L*) of FMM-S was inversely related to the quantity of liver added, while the redness (a*) was directly related to the amount of OSP. When the proportion of cow liver increased, L* of FMM-S decreased. At the same time, if the proportion of OSP increased, a* significantly increased. When comparing between drying methods, DD resulted in the sample being more light-coloured,

Table 1. Selected nutrient contents (on dry basis) per 100 g in each of the three ingredients, when prepared by household (HH) and drum drying (DD) methods

Nutrient	*Fish*		*Cow liver*		*OSP*	
	HH	*DD*	*HH*	*DD*	*HH*	*DD*
Protein (g)	70.79±0.65	90.88±0.81	58.50±0.77	68.55±1.28	3.44±0.28	5.83±0.50
Fat (g)	1.54±0.09	2.69±0.22	6.95±0.24	14.81±0.15	11.13±0.32	0.18±0.03
Ash (g)	5.33±0.13	5.20±0.10	4.23±0.32	4.28±0.06	1.92±0.08	2.55±0.07
Vitamin A (µg)	na	na	21,353	na	na	na
Iron (mg)	na	na	103.09	na	na	na

na means 'not analysed'

Values are means of triplicate determination ± standard deviation

Table 2. Moisture content and TBA in fish, cow liver and OSP powder according to size fractions, prepared by using household (HH) and drum drying (DD) methods

Ingredient	*Size fraction (mesh)*	*Moisture (g/ 100g)*		*TBA (mg MDA/ kg)*	
		HH	*DD*	*HH*	*DD*
Fish	> 8	6.44±0.30	5.84±0.22	0.87±0.07	0.07±0.00
	8 to 20	5.48±0.21	5.78±0.24		
	< 20	5.30±0.24	5.93±0.34		
Cow liver	> 8	7.58±0.28	6.34±0.14	1.15±0.07	1.25±0.03
	8 to 20	7.58±0.35	6.52±0.30		
	< 20	5.78±0.10	6.08±0.32		
OSP	> 8	5.75±0.27	6.34±0.16	1.34±0.11	0.85±0.01
	8 to 20	5.36±0.11	6.32±0.13		
	< 20	6.81±0.02	6.74±0.25		

Values are means of triplicate determination ± standard deviation

redder, and less yellow than that of the HH method at the same mixture proportions.

Sensory Preference Test

Sensory evaluation results for the six FMM-S formulas prepared by HH method are shown in Table 5. The ingredient proportions in various FMM-S formulas significantly impacted the preference scores for the attributes of appearance, liver flavour, and fish flavour, but had no statistical effect on preference scores of sweet potato flavour, overall flavour, and overall liking (p>0.05). When considering all attributes, it was found that the formula with 4 g of liver, 7 g of fish, and 4 g of OSP (4:7:4) had significantly higher preference scores than the other formulas, but did not significantly

Table 3. Mixture proportions in FMM-S and calculated† amounts of protein, vitamin A and iron in each formula per one serving size

Proportions of ingredients (g/ 15g total) (liver:fish:OSP)	*Protein (g) (% RDA)‡*	*Vitamin A (µg) (% RDA)‡*	*Iron (mg) (% RDA)‡*
Formula 1 (3:6:6)	6.0 (33.4)	584.7 (146.2)	2.8 (30.3)
Formula 2 (3:7:5)	6.7 (37.3)	584.7 (146.2)	2.8 (30.3)
Formula 3 (3:8:4)	7.4 (41.2)	584.7 (146.2)	2.8 (30.3)
Formula 4 (4:6:5)	6.6 (36.6)	779.6 (194.9)	3.8 (40.3)
Formula 5 (4:7:4)	7.3 (40.5)	779.6 (194.9)	3.8 (40.3)
Formula 6 (4:8:3)	8.0 (44.5)	779.6 (194.9)	3.8 (40.3)

†Calculated nutrients by using the nutritional values of ingredients prepared by HH method
‡Values in bracket were calculated based on Thai RDA of preschool children aged 1-3 years, 2003 (Bureau of Nutrition, 2003)

Table 4. Colour coordinates of the tested FMM-S formulas

Method of processing	*Colour coordinate*	*Mixture proportions (liver:fish:OSP)*					
		3:6:6	*3:7:5*	*3:8:4*	*4:6:5*	*4:7:4*	*4:8:3*
Household (HH)	L*	43.3±0.6^{d}	45.8±0.7^{b}	48.0±1.4^{a}	44.7±0.8^{c}	42.8±0.6^{d}	44.9±1.6^{c}
	a*	11.1±0.6^{a}	10.4±0.5^{b}	9.5±0.8^{c}	10.1±0.9^{b}	8.5±0.5^{d}	7.1±0.7^{e}
	b*	32.5±0.6^{a}	31.5±0.9^{b}	31.2±1.5^{b}	28.8±0.6^{c}	27.5±0.9^{d}	26.7±1.0^{e}
Drum drying (DD)	L*	55.7±1.0^{a}	56.3±1.2^{a}	54.9±1.0^{b}	51.2±0.7^{c}	52.0±1.0^{c}	50.1±0.9^{d}
	a*	14.0±0.4^{a}	13.2±0.7^{b}	11.8±0.5^{c}	11.2±0.6^{c}	10.4±0.2^{d}	8.9±1.0^{e}
	b*	30.8±0.5^{a}	30.2±0.9^{a}	28.6±0.6^{b}	26.8±0.8^{c}	26.1±0.6^{c}	24.6±1.0^{d}

$^{a-e}$ Mean values ± standard deviation with different superscripts in the same row indicate statistically significant differences at $p \leq 0.05$

differ ($p>0.05$) from the 3:7:5 formula in all attributes. Since increasing liver powder use in the product would affect the commercial production cost, the formula of 3:7:5 was selected as a suitable FMM-S mix to be prepared by HH method. Nutritional values of this formula were 37% RDA of protein, 146% RDA of vitamin A, and 30% RDA of iron in 15 g of FMM-S.

Sensory results for the six FMM-S formulas prepared by the DD method are shown in Table 5. Again, the ingredient proportions significantly affected the preference scores for all attributes ($p \leq 0.05$). Increasing liver and fish portions caused the scores for liver and fish flavour to increase; and as expected, the score for sweet potato flavour decreased. Overall, the formula of 4:8:3 had a significantly higher score in all attributes than the others ($p \leq 0.05$). Therefore, this formula was considered suitable for preparing FMM-S by the DD method. Nutritionally, this formula provided 55% RDA of protein, 194%

Table 5. Preference scores of six FMM-S formulas from sensory evaluation

Attribute	*FMM-S formula (liver:fish:OSP)*					
	3:6:6	*3:7:5*	*3:8:4*	*4:6:5*	*4:7:4*	*4:8:3*
By household method						
Appearance	6.2±1.1^{cd}	7.3±0.9^{ab}	6.2±1.4^{cd}	6.8±1.0^{bc}	7.8±0.8^{a}	6.1±1.4^{d}
Liver flavour	6.1±1.5^{c}	6.4±1.2^{bc}	7.0±0.9^{a}	6.1±1.2^{c}	6.9±0.8^{ab}	5.9±1.4^{c}
Fish flavour	6.0±1.6^{c}	7.3±1.2^{a}	6.8±1.4^{ab}	6.3±1.5^{bc}	7.3±0.9^{a}	7.3±1.0^{a}
Sweet potato flavour	6.2±1.4^{a}	6.3±1.4^{a}	6.3±1.3^{a}	6.8±1.4^{a}	6.7±1.0^{a}	6.5±1.1^{a}
Overall flavour	6.6±1.0^{a}	6.5±1.3^{a}	6.2±1.3^{a}	6.2±1.6^{a}	6.5±1.0^{a}	6.4±1.3^{a}
Overall liking	6.5±0.9^{a}	6.5±1.4^{a}	6.3±1.4^{a}	6.3±1.5^{a}	6.9±1.1^{a}	6.3±1.3^{a}
By drum drying method						
Appearance	5.3±1.4^{c}	6.3±1.4^{b}	5.9±1.5^{bc}	6.5±1.5^{b}	6.5±1.4^{b}	7.3±1.3^{a}
Liver flavour	5.8±1.3^{b}	5.8±1.5^{b}	5.7±1.6^{b}	6.0±1.4^{ab}	6.3±1.5^{ab}	6.7±1.3^{a}
Fish flavour	5.8±1.0^{b}	6.2±1.2^{ab}	6.1±1.5^{ab}	5.9±1.2^{b}	6.4±1.5^{ab}	6.6±1.2^{a}
Sweet potato flavour	5.5±1.6^{b}	5.9±1.7^{ab}	6.2±1.3^{ab}	5.9±1.4^{ab}	6.3±1.3^{a}	6.4±1.5^{a}
Overall flavour	5.6±1.4^{b}	6.5±1.3^{a}	6.5±1.4^{a}	6.2±1.4^{ab}	6.9±1.5^{a}	6.7±1.2^{a}
Overall liking	5.7±1.2^{b}	6.2±1.3^{ab}	6.3±1.4^{ab}	6.3±1.5^{ab}	6.4±1.7^{ab}	6.5±1.4^{a}

$^{a-d}$ Mean values ± standard deviation with different superscripts in the same row indicate statistically significant differences at $p \leq 0.05$

RDA of vitamin A, and 40% RDA of iron per 15 g serving.

The total cost of producing a 15 g FMM-S was estimated, which included ingredients, instruments, personnel, and supplies for a batch processing. It was approximately 22 THB for the HH method and 23.5 THB for the DD method, respectively. The production cost might decrease when production capacity is increased. Lower cost will be preferable for the target population who has low income.

DISCUSSION

Household method and drum drying method were chosen to process raw materials to dried- ground powder since the application of FMM-S strategy might occur through two approaches, i.e., local or industrialised manufacturing. Local production is superior to industrialised production in terms of community involvement, which leads to the sustainability of the programme. Furthermore, it can facilitate income generation in a community as well.

The selected local materials for making FMM-S, i.e., fish (Threadfin bream) and cow liver contained significantly high amounts of protein. One gram of dried fish and cow liver had 0.7-0.9 g and 0.6-0.7 g of protein, respectively (Table 1). Protein and fat contents in cow liver and fish processed by the HH method were slightly lower than those processed by the DD method. The unexpected differences may have occurred due to differences of food sample matrix according to processing method. Raw materials prepared by HH method were exposed to high temperature for a long time during roasting, which was a more severe processing procedure in comparison to drum drying processing. This can result in the destruction, denaturation, or oxidation of molecule structures in food components. Consequently, this may lead to lower levels of protein and fat extraction of materials treated by HH method in comparison to DD method. In addition, different batches of raw materials were used in processing for the two processing methods.

Generally, liver is a good source of vitamin A and iron, but not fish. Vitamin A content of the local cow liver in this study (21,353 µg/100 g) was at the high range in comparison to prior studies that indicated the range of vitamin A content to be from 3,699 to 22,945 µg/100 g dry basis. Varied vitamin A content depends on the age and breed of cow, as well as the nutritional quality of its feed (Majchrzak, Fabian & Elmadfa, 2006). Thus, fish powder can serve as a source of protein, while cow liver as a source of vitamin A and iron, as well as of protein. OSP is known as a source of carotenoids. Bengtsson *et al.* (2008) found that the mean trans-β-carotene content in seven different OSP genotypes ranged between 108 and 315 µg/g dry basis. Therefore, OSP can be considered an excellent source of pro-vitamin A, and it can provide some additional vitamin A even if not as much as cow liver.

The two key factors to be controlled for prolonging the shelf-life of dried foods are moisture content and lipid oxidation. According to Table 2, there were similar moisture content among different particle sizes and processing methods, but TBA values of fish and OSP powder prepared by HH method were much higher than those prepared by DD method, probably because of the high temperature and long time in processing. In addition, the study of Lee & Yoon (2013) found that heating time and mean particle size of sample significantly affected its TBA. However, these ingredients still had lower TBA values, on top of lower moisture content levels compared to the standard dried product.

Size of particles has a direct effect on the acceptability of the sprinkles. Majority of panellists (70-89%) agreed that the particle size ranging from 8 to 20 mesh of each ground material was just the right size, comparable to the commercial rice seasoning sample (data not shown). Thus, this size was chosen to formulate FMM-S. The mixture of liver, fish, and OSP at 3:7:5 was selected as a suitable FMM-S mix to be prepared by HH method, while the formula of 4:8:3 was considered suitable for preparing FMM-S by the DD method. Nutritional analysis findings of these FMM-S formulas suggested that consumption of 2 servings (30 g) a day is able to fulfil the protein, iron, and vitamin A requirements of preschool children. Even though 30 g of FMM-S will provide about 300-400% RDA of vitamin A, which seems quite high, this level is far lower from the daily toxic dose of 1,212 µg/kg body weight for 6-15 months old children (WHO, 2021). However, product acceptance among target consumers need to be further investigated.

Water activity and moisture content of the selected FMM-S processed by HH method were 0.51±0.01 and 7.18±0.31%, respectively; while FMM-S processed by DD method had an a_w of 0.48±0.01 and moisture content of 6.77±0.25%. These values were in accordance with the quality standards of the Thai Community Product Standards: Seasoning fish powder (TCPS 1337/2549) (TISI, 2006) and with rice seasoning standard for a seasoning powder, which specified a range of 0.60 – 0.65 for a_w and moisture content not exceeding 13% (TCPS 494/2547) (TISI, 2004). Thus, these FFM-S formulas in dried forms with quite low a_w and moisture content do not require refrigeration. However, storage test should be performed to determine the optimal conditions of the product, particularly on how humidity, light, and climate change may affect product quality during storage.

CONCLUSION

This study demonstrated that household method or alternatively drum drying method can be used to process raw fish, cow liver, and OSP into dried ground sprinkles. The mixture of cow liver, fish, and OSP sprinkles at a ratio of 3:7:5 (prepared by HH method) or 4:8:3 (prepared by DD method) were the most acceptable in sensory evaluation. One serving of these formulas (15 g) is able to add protein and iron at approximately 30-55% RDA, and vitamin A at 150-200% RDA for preschool children. A consumer test would be needed to ascertain the acceptance by the target population.

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Authors' contributions

LC, designed the experiments, performed the statistical analysis and data visualisation, and wrote the manuscript; BS, designed and conducted all of the experiments and wrote the manuscript. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare that they hold no competing interests.

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Comparative amino acid composition and quality parameters of *Moringa oleifera* testa and cotyledon

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ABSTRACT

Introduction: *Moringa oleifera* is a drought-resistant plant, widely used in the tropical region. The leaves and stems have been extensively utilised in foods and neutraceuticals preparation, with less attention to the seeds. In this study, amino acid (AA) compositions of *M. oleifera* testa and cotyledon were examined comparatively. **Methods:** Samples were separately defatted, hydrolyed, and neutralised. The AA solution was purified by cation-exchange solid-phase extraction, derivatised and analysed by gas chromatography. **Results:** Glutamic (acidic amino acid) and phenylalanine (essential amino acid, EAA) were the most concentrated in both samples. Total EAA (g/100g crude protein, cp) was higher in cotyledon (51.0) than testa (41.9). Predicted protein efficiency ratios (P-PERs) were higher in testa (0.605-1.530) than cotyledon 0.286-1.460). EAA index ranged between 0.951-1.13 (soybean comparison) and 83.0-96.9 (egg comparison) with corresponding biological value of 78.7-93.9. The following AA had scores >1.0 in comparison to whole hen's egg, testa: glycine (Gly), glutamic acid (Glu), phenylalanine (Phe), histidine (His), and cysteine (Cys); cotyledon (Gly), proline (Pro), Glu, Phe, His, arginine (Arg) and Cys. In comparison with requirements of pre-school children, six AA (6/9 or 66.7%) had scores >1.0 in each sample. In provisional AA scoring pattern, isoleucine (Ile) (1.25) and Phe + tyrosine (Tyr) (1.68) had scores >1.0 in testa while methionine (Met) + Cys, Phe+Tyr, and tryptophan (Trp) in cotyledon. However, tryptophan and lysine were the limiting AAs in testa and cotyledon, respectively. **Conclusion:** The study showed that both anatomical parts would complement each other in terms of amino acid supply.

Keywords: amino acid scores, derivatisation, essential amino acid, hydrolysis, isoelectricpoint

INTRODUCTION

Moringa oleifera is a member of the Moringaceae family. It is a deciduous, draught-resistant, and fast growing tree with an average height of 12 metres at maturity (Olaofe, Adeyeye & Ojugbo, 2013). Moringa species has many varieties such as *Moringa ruspoliana, Moringa boziana, Moringa ovalifolia, Moringa longituba, Moringa oleifera* and *Moringa rivae.* Out of all these varieties, *Moringa oleifera* happens to be the

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most popular and widely planted in the tropical region (Jahn, 1988). *Moringa oleifera* has a lot of common names. It is fondly called drum stick tree due to the fact that the shape of its pod resembles the stick used for beating the drum (Olaofe *et al.*, 2013). Other common names are Marango, Mulangay, Sajna, Benz olive; and in Nigeria, it is popularly called Ewe Igbale (Gbile, 1984). Every part of the *Moringa oleifera* tree is used for one purpose or another; the seed is used for cooking and cosmetic oil, while the leaves, flowers and green pods are usually planted and useful as vegetable. The seeds may be eaten raw without any heat processing (Ramachandran, Peter & Gopalakrishnan, 1980). *M. oleifera* leaf is a very good source of omega-3 fatty acid (Olaofe *et al.*, 2013). The insulin-like protein observed in *M. oleifera* seed coat has antigenic epitopes similar to insulin and is known to display hypoglycemic activity on oral administration (Paula *et al.*, 2016). *Moringa oleifera* can be cultivated in tropical and subtropical areas. The temperature requirement for survival is between 25-35°C and it requires a net rainfall of between 250-3000mm.

Both the nutritional and medicinal importance of *Moringa oleifera* are well documented. *Moringa oleifera* contains a lot of nutritionally essential minerals such as calcium, iron and zinc. It was reported in the literature that *Moringa oleifera* contains more iron than spinach (Fuglie, 2005). Another report showed that *Moringa oleifera* meets the daily requirement of zinc as it contains around 25.5-31.0 mg of Zn/kg (Barminas, Charles & Emmanuel, 1998). The leaves also contain abundant essential vitamins such as vitamin A, folic acid, nicotinic acid and pyridoxine of vitamin B; with vitamins C, D and E also present (Mbikay, 2012). *Moringa oleifera* has been very popular in herbal medicine especially in countries like India and Nigeria due to the presence of phytochemicals. Research studies have revealed that aqueous extracts of *Moringa oleifera* can cure both streptozotocin-induced Type 1 diabetes and insulin-resistant Type 2 diabetes in rats (Divi, Bellamkonda & Dasireddy, 2012). Traditionally, *Moringa oleifera* has been used for the treatment of asthma, eye and ear infections, cholera, cough, headache, anaemia, killing of intestinal worms and glandular swelling in India, Puerto Rico and Malaysia (Barminas *et al.*, 1998).

The whole seed of *Moringa oleifera* consists of testa and cotyledon. The testa which develops from the integument of the ovule is the outermost covering of the seed. It protects the delicate inner part of the seed against external aggression from fungi, bacteria or insects. The cotyledon is the inner part of the seed that forms the first leaf when the plant germinates. It contains stored food that helps provide internal energy that the plant needs for the development of its embryo during germination.

There is no doubt that research studies have been published on *Moringa oleifera*, some of which include the nutritional values of *Moringa* leaves and pods (Barminas *et al.*, 1998), comparative study of proximate, amino acid and fatty acids of *Moringa oleifera* tree (Olaofe *et al.*, 2013) and synergy between *Moringa oleifera* seed powder and alum in the purification of domestic water (Dalen *et al.*, 2009). The present study is aimed at investigating the amino acid profiles and quality parameters of *Moringa oleifera* testa and cotyledon on comparative basis.

MATERIALS AND METHODS

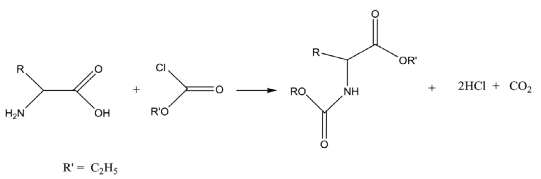
Sample collection

The *Moringa oleifera* seed samples were obtained from a farm in Odo-Ayedun Ekiti, Ikole Local Government area of

Ekiti State, Nigeria. The seeds were authenticated at the Plant Science and Biotechnology Department, Ekiti State University, Ado-Ekiti. They were sorted to remove defective ones, washed with distilled water to remove dirt and later soaked overnight. The testa of the soaked seeds was carefully separated from the cotyledon. Both testa and cotyledon were dried separately in an oven (Electric blast dry box, PEC Medical USA) set at 45°C. They were later dry-milled into fine powder using the Mallex electric blender and stored separately inside polythene containers prior to use for analysis.

Sample extraction and analysis

The extraction and amino acid analysis in this study were carried out following the procedures described by Danka *et al.* (2012). 10g of sample was accurately weighed into a 250ml conical flask. The sample was separately defatted using about 40ml petroleum ether (40-60°C) to extract fat content. The extraction was carried out three times inside a Soxhlet extractor equipped with thimble. The sample was hydrolysed three times to enhance amino acid recovery. The defatted sample was soaked with 30ml of 1M KOH (potassium hydroxide) solution and was incubated for two days at 110°C in a hermetically closed borosilicate glass vessel. The hydrolysate was later neutralised until pH was in the range of 2.5 – 5.0; the purification of the solution was achieved by cation-exchange solid-phase extraction. The amino acids in purified solution was derivatised with ethylchloroformate by the following established mechanism:



(equation 1)

After derivatisation, the reagent was removed by scavenging with nitrogen. The resulting amino acid was made up to 1ml in a vial for gas chromatography (GC) analysis. The GC conditions for the analysis were: GC: HP6890 powered with HP chemstation; injection temperature: split injection; split ratio: 20:1; carrier gas: hydrogen; flow rate: 1.0 ml/minute; oven programme: initial temperature at 110°C, first ramp at 27°C/min at 320°C, second ramp was constant for 5 mins at 320°C; inlet temp: 50°C; column type: EZ; column dimension: 10m by 0.2mm by 0.25 µm; compressed air: 35 psi; detector: PFPD; detector temperature: 320°C; hydrogen pressure: 20 psi.

Evaluation of quality parameters for the amino acids

Isoelectric point (pI)

The isoelectric point for a mixture of amino acids was determined by the equation of Olaofe & Akintayo (2000) as follows:

$$IPm = \sum_{i=1}^{n} IPiXi \qquad \textit{(equation 2)}$$

In the above equation, *I*Pm represents the isoelectric point for the mixture of amino acids, *I*Pi is the isoelectric point of the i^{th} amino acid in the mixture and Xi is the mole fraction of the i^{th} amino acid in the mixture.

Predicted protein efficiency ratio (P-PER)

Equations of Alsmeyer *et al.* (1973) were used to determine the protein efficiency ratios 1, 2, and 3 as given below:

$P–PER_1$ = -0.468 + 0.454 (Leu) - 0.105 (Tyr) *(equation 3)*

$P–PER_2$ = -0.684 + 0.456 (Leu) - 0.047 (Pro) *(equation 4)*

$P–PER_3$ = -1.816 + 0.435 (Met) - 0.78 (Leu) + 0.211 (His) - 0.944 (Tyr) *(equation 5)*

Essential amino acid index (EAAI)
The EAAI was calculated based on the ratios of essential amino acids (EAAs) in a protein relative to the respective amount in whole egg protein according to the following equation:

$$EAAI = \sqrt[n]{\frac{Lys_p}{Lys_s} x \frac{Trp_p}{Trp_s} x \ldots \frac{His_p}{His_s}} \quad \textit{(equation 6)}$$

where subscript 'p' represents food protein subscript s represents egg protein (standard) n represents the number of amino acids [Pairs like methionine (Met) + cysteine (Cys) and phenylalanine (Phe) + tyrosine (Tyr) are counted as one] (Nielsen, 2002).

The obtainable value in the above equation (equation 6) was taken to be $EAAI_1$. In another form, EAAI may be estimated from amino acid composition relative to total nitrogen in both the food and the standard. The estimation was computed logarithmically and used to calculate $EAAI_2$ (Albanese, 1959).

Biological value (BV)
Equation suggested by Albanese (1959) was used to compute the biological value. The equation is given below:
BV = 1.09 (EAAI) – 11.73 *(equation 7)*

Amino acid scores (AAS)
Computation of AAS was done using three different established procedures.
(i) Scores computed based on amino acid values of whole hen's egg (Paul, Southgate & Russel, 1976).
AAS = [g/100g of test protein (from results)]/g/100g of reference pattern

(ii) Scores based on EAA scoring pattern (FAO/WHO, 1973).
AAS = [mg/g of test protein (from results)]/mg/g of reference pattern

(iii) Scores based on EAA requirement for pre-school children (FAO/WHO/UNU, 1985).

Amino acid requirements for school boys (10-12years)
This requirement was based on the required EAA in mg/kg/day for 30kg body weight of school boys (age 10-12 years) computed using the following equation (FAO/WHO/UNU, 1985):

Amino acid requirement = EAA × 10 × protein (g/100g) *(equation 8)*

Parameters such as leucine/isoleucine ratio, (Leu/Ile), total essential amino acid (TEAA), total non-essential amino acid (TNEAA), total aromatic amino acid (TArAA), total essential aromatic amino acid (TEArAA), as well as their percentages were also calculated.

Statistical analysis
Results obtained from the amino acid composition in Table 1, AAS based on whole hen's egg amino acid (Table 3), AAS based on pre-school children standard (Table 4) as well as essential AAS based on provisional EAA standard requirements (Table 5) were subjected to both descriptive and inferential statistical analysis (Oloyo, 2011) using Microsoft Excel package in order to establish the existence of significant differences or otherwise among the samples.

RESULTS

The amino acid compositions (g/100g) of *Moringa oleifera* testa and cotyledon are depicted in Table 1. Among the amino acids investigated, glutamic acid (Glu) had the highest concentration in both samples: 17.3g/100g (testa), 18.0g/100g (cotyledon). However, tryptophan (Trp) had the least value in testa (0.283g/100g) and Met in cotyledon (1.44g/100g). Among the EAAs, Phe was the most concentrated in each sample: 7.59g/100g (testa),10.9g/100g (cotyledon). The following EAAs had

Table 1. Amino acid levels (g/100g) of *Moringa oleifera* testa and cotyledon

AA	*CID*	*Testa*	*Cotyledon*	*Mean±SD*	*CV%*	*Difference*	*%difference*
Gly	750	5.60	4.65	5.13±0.67	13.10	+0.95	+16.96
Ala	5950	3.46	3.61	3.54±0.11	2.99	-0.15	-4.34
Ser	5951	4.26	5.15	4.71±0.63	13.40	-0.89	-20.89
Pro	145742	3.69	4.49	4.09±0.57	13.80	-0.80	-21.68
Val	6287	3.94	4.29	4.12±0.25	6.00	-0.35	-8.88
Thr	6288	3.41	2.44	2.93±0.69	23.40	+0.97	+28.45
Ile	791	4.98	3.39	4.19±1.12	26.70	+1.59	+31.93
Leu	6106	4.99	5.00	5.00±0.01	0.14	-0.01	-0.20
Asp	5960	6.43	4.41	5.42±1.43	26.40	+2.02	+31.42
Lys	5962	3.51	1.99	2.75±1.07	38.90	+1.52	+43.30
Met	6137	0.85	1.44	1.15±0.42	36.30	-0.59	-69.41
Glu	33032	17.3	18.0	17.7±0.50	2.80	-0.70	-4.05
Phe	6925665	7.57	10.9	9.24±2.35	25.40	-3.33	-43.99
His	6274	2.46	2.87	2.67±0.29	10.90	-0.41	-16.67
Arg	6322	5.09	9.99	7.54±3.46	45.90	-4.90	-96.27
Tyr	6057	2.50	3.21	2.86±0.50	17.60	-0.71	-28.40
Trp	6305	0.28	1.47	0.88±0.84	95.70	-1.19	-425.00
Cys	67678	2.41	3.99	3.20±1.12	35.00	-1.58	-65.56
Total		83.20	91.30	87.30±5.73	6.56	-8.10	-9.74
CP		18.80	32.50	25.70±9.69	37.70	-13.70	-72.87

AA – amino acid, CID – chemical identification number, SD – standard deviation, CV% – coefficient of variation percent, CP – crude protein

their values greater in testa than in cotyledon: threonine (Thr) (28.4% more), isoleucine (Ile) (31.9% more), and lysine (Lys) (43.3% more). However, out of the eighteen amino acids investigated, thirteen (13) were more abundant in cotyledon than in testa.

The statistical analysis of the results in Table 1 are shown in Table 2. The values of mean, standard deviation (SD) and coefficient of variation (CV%) in testa (4.60, 3.65, and 79.3, respectively) were close to those in cotyledon (5.07, 4.09, and 80.7, respectively).

Table 2. Statistical description of amino acid composition results in Table 1 (testa/ cotyledon)

Statistics	*Testa*		*Cotyledon*
Total amino acid value	83.20		91.30
Mean value of amino acid	4.60		5.07
Standard deviation	3.65		4.09
Coefficient of variation (%)	79.30		80.70
Correlation coefficient (r_{xy})		0.91	
Variance (r_{xy}^2)		0.83	
Regression coefficient (R_{xy})		1.02	
Coefficient of alienation (C_A)		0.42	
Index of forecasting efficiency (IFE)		0.59	
Remark		*	

* - the results were significantly different at n-2=16 and $r_{=0.01}$ with critical value=0.590

The summary of amino acid compositions and quality parameters of Moringa testa and cotyledon are presented in Figures 1(a) and (b). Total essential amino acid (TEAA) levels were (g/100g crude protein, cp): testa (37.0) and cotyledon (43.8), with their corresponding percentages at 44.5% and 48.0%, respectively. This implies that both the moringa testa and cotyledon may adequately provide the required EAA for people of various age categories, including pre-school children (46.0), school children (24.1), and adults (12.7) (FAO/WHO/UNU, 1985). Total non-essential amino acids (TNEAA) were (g/100g cp): testa (46.2) and cotyledon (47.5), indicating lesser values of TEAA than TNEAA in both samples.

The AAS based on whole hen's egg amino acid, pre-school children standard, as well as the FAO/WHO (1973) standard are shown in Table 3. For whole hen's egg amino acid, glycine (Gly), glutamic acid (Glu), Phe, histamine (His), and Cys had values greater than one in testa. In the cotyledon, Gly, proline (Pro), Glu, Phe, His, arginine (Arg), and Cys had their AAS values greater than one. In the pre-school children standard, six amino acids had scores greater or equal to one in both samples. In the FAO/WHO standard, only Ile and Phe + Tyr had scores above 1.0 in testa, whereas Met + Cys, Phe + Tyr and tryptophan (Trp) had values greater than 1.0 in cotyledon.

Table 4 presents the summary of statistical analysis of the results obtained from Table 3. For egg scores (testa/ cotyledon in column 2), the correlation coefficient (r_{xy}) was high at 0.781. In pre-school children EAA scores, as well as in provisional EAA scoring pattern (FAO/WHO standard), the calculated correlation coefficient values were lower than the critical values, at 0.798 and 0.834, respectively.

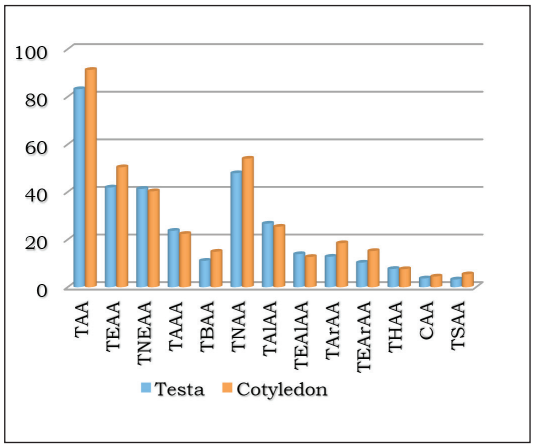


Figure 1(a). Summary of the amino acids class

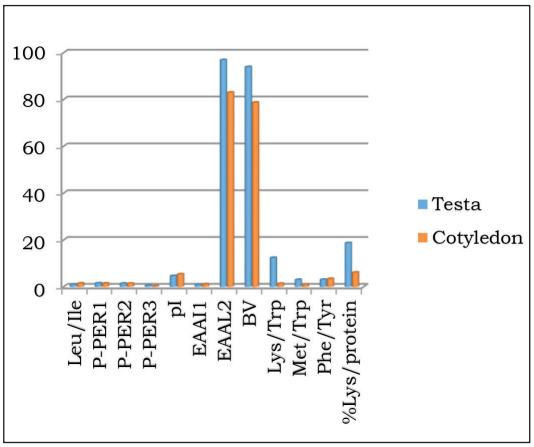


Figure 1(b). Amino acid quality parameters

TAA: Total amino acid; TEAA: total essential amino acid; TNEAA: total non-essential amino acid; TAAA: total acidic amino acid; TBAA: total basic amino acid; TNAA: total neutral amino acid; TAlAA: total aliphatic amino acid; TEAlAA: total essential aliphatic amino acid; TArAA: total aromatic amino acid; TEArAA: total essential aromatic amino acid; THAA: total hydroxylic amino acid; TCAA: cyclic amino acid; TSAA: total sulphur amino acid; P-PER1,2,3:predicted protein efficiency ratios; p*I* : isoelectric point; EAAI 1,2: essential amino acid index; BV: biological value

Table 3. Amino acid (AA) scores of *Moringa oleifera* testa and cotyledon using whole hen's egg amino acid (X), pre-school children standard (Y), and FAO/WHO (1973) standard (Z)

AA	*X*		*Y*		*Z*	
	Testa	*Cotyledon*	*Testa*	*Cotyledon*	*Testa*	*Cotyledon*
Gly	1.87	1.55				
Ala	0.64	0.67				
Ser	0.54	0.65				
Pro	0.97	1.18				
Val	0.53	0.57	1.13	1.23	0.79	0.86
Thr	0.67	0.48	1.00	0.72	0.85	0.61
Ile	0.89	0.61	1.78	1.21	1.25	0.85
Leu	0.60	0.60	0.76	0.76	0.71	0.71
Asp	0.60	0.41				
Lys	0.57	0.32	0.61	0.34	0.64	0.36
Met	0.27	0.45				
Glu	1.44	1.50				
Phe	1.48	2.14				
His	1.03	1.20	1.29	1.51		
Arg	0.83	1.64				
Tyr	0.63	0.80				
Trp	0.16	0.82	0.26	1.34	0.28	1.47
Cys	1.34	2.22				
Met+Cys			1.30	2.17	0.93	1.55
Phe+Tyr			1.60	2.24	1.68	2.35
Total	0.83	0.91	1.09	1.21	0.96	1.06

Table 4. Summary of statistical analysis of the score results in Table 3

Statistics	*Egg scores (Testa/ cotyledon)*	*Pre-school child scores (Testa/cotyledon)*	*FAO/WHO (1973) scores (Testa/cotyledon)*
r_{xy}	0.78	0.53	0.52
r_{xy}^2	0.61	0.28	0.27
R_{xy}	1.03	0.70	0.80
[a]Mean	0.86	1.08	0.89
[a]SD	0.45	0.48	0.42
[a]CV%	53.70	44.7	47.00
[b]Mean	0.99	1.28	1.10
[b]SD	0.59	0.64	0.65
[b]CV%	60.00	49.80	59.10
C_A	0.63	0.85	0.86
IFE	0.38	0.15	0.15
Remark	*	NS_1	NS_2

a- statistical results for testa, b- statistical results for cotyledon, *- results significantly different at n-2 = 16, $r_{=0.01}$ (critical value = 0.590), NS_1- results not significantly different at n-2 = 7, $r_{=0.01}$ (critical value = 0.798), NS_2- results not significantly different at n-2 = 6, $r_{=0.01}$ (critical value = 0.834)

DISCUSSION

In this research work, Glu was observed to have the highest level among all amino acids in both samples. Glu is the most abundant amino acid in most plant and animal samples, such as testa, dehulled, and whole seed of *Vigina subterranea* (Adeyeye & Olaleye, 2012), smooth loofa, Roselle, and sesame seeds flour (Adubiaro, Ogunbusola & Olaleye, 2017), as well as *Moringa oleifera* tree (Olaofe *et al.*, 2013). The difference between the total amino acid in testa and cotyledon was 8.10g/100g. In terms of percentage difference, cotyledon was just 9.74% higher than testa. This shows that both testa and cotyledon contained varied composition of amino acids, especially EAAs. Therefore, the testa could be seen as an important component of the seed, contributing immensely to its nutritive value, particularly as it contributes a higher percentage of the first limiting amino acid (Lys) to the seed protein. Although the difference in total amino acid between the testa and the cotyledon was small, the cp in cotyledon (32.5g/100g) was almost double than in testa (18.8g/100g). The implication of this is that the Moringa testa might have higher levels of solubilising protein and true protein than in the cotyledon. With the exception of Arg (45.9%) and Trp (95.7%), the coefficient of variation percent (CV%) of all amino acids were less than 40.0%, showing the closeness of the samples in all parameters.

In Table 2, there was a high level of correlation coefficient (0.910), showing significant difference of results at $r_{=0.01,}$ with a critical value of 0.590. The coefficient of alienation (C_A) was low at 0.4146, while a corresponding high index of forecasting efficiency (IFE) (0.5854) was noted. While C_A indicates the level at which there is lack of relationship between the samples, IFE is a measure of reduction in the level of error of prediction between the two samples. High IFE indicates low level of C_A. The high level of IFE in Table 2 show that there was high reduction in the error of prediction of relationship between the testa and the cotyledon. Therefore, it was easy to predict that the amino acids of the testa would be able to serve the functions of those in the colyledon and vice versa. The R_{xy} value of 1.02 showed that for each 1.00g/100g in the amino acid value of the testa, there was a corresponding increase of 1.02g/100g in the cotyledon; this attested to the fact that amino acid concentration in the testa was lower than in the cotyledon.

The TEAA (g/100g cp) values in this study as shown in Figure 1(a) were higher than those reported for leaves (35.4), stem (26.3), and root (28.4) of *Moringa oleifera* (Olaofe *et al.*, 2013). Percentage levels of total acidic amino acid (%TAAA): 28.5% (testa) and 24.5% (cotyledon) were higher than percentage levels of total basic amino acid (%TBAA): 13.3% (testa) and 16.3% (cotyledon). The following classes of amino acid were higher in testa than cotyledon (g/100g) - total acidic amino acid (TAAA): testa (23.6) and cotyledon (22.4); total aliphatic AA (TAIAA): testa (26.7) and cotyledon (25.4); total essential aliphatic AA (TEAIAA): testa (13.9) and cotyledon (12.7); total hydroxylic AA (THAA): testa (7.67) and cotyledon (7.59).

The total aromatic AA (TArAA) in this study, 12.8g/100g cp (testa) and 18.5g/100g cp (cotyledon) were higher than the recommended 6.8 - 11.8 g/100g cp for infants (FAO/WHO/UNU 1985). Total sulphur AA (TSAA) in the samples were 3.26 g/100g (testa) and 5.43 g/100g (cotyledon), and percent cysteine (Cys) in TSAA were 73.9% (testa) and 73.5% (colytedon). This shows that Cys was far more abundant than methionine in each sample. Higher Cys than Met in this study corroborates the literature reports on most proteins

of plant sources: coconut endosperm (62.9%) (Adeyeye, 2004), *Phoenix dactylifera* (56.1%) (Olaleye, 2013), and guinea corn samples (58.9 - 72.0%) (Adeyeye, 2008). Cys has been shown to have enhanced effects on Zn absorption (Mendoza, 2002).

The level of Leu/Ile ratio in testa (1.00) and cotyledon (1.47) [Figure 1(b)] showed that testa had almost equal levels of Leu and Ile, but Leu > Ile in cotyledon. Excess Leu in the diet could negatively affect Trp and niacin metabolism (Ghafoorunissa & Nasaringa Rao, 1973). The levels of predicted protein efficiency ratio (P-PER) for testa were PER_1 (1.53), PER_2 (1.42), and PER_3 (0.605); and for cotyledon: PER_1 (1.46), PER_2 (1.38), and PER_3 (0.286). In all three parameters used for calculating P-PER, values in the testa were higher than those in the cotyledon. This shows that protein in the testa would be more effectively utilised than in the cotyledon.

The isoelectric point (p*I*) levels in the samples were 4.65 for testa and 5.37 for cotyledon, showing the p*I* to be in the acidic medium of the pH. The samples may therefore be useful in preparing very low acid foods such as meat products. Olaofe *et al.* (2013) had earlier worked on *Moringa oleifera* tree parts; the p*I* results were: leaves (5.8), stem (5.5), and roots (5.4). The p*I* results in this study were close to the above literature values. The calculated isoelectric point is very useful for organic samples, whereby p*I* could be predicted without going through the rigorous process of obtaining the minimum pH value via protein solubility determination.

The levels of EAAI in the samples were $EAAI_1$: testa (0.951), cotyledon (1.13); $EAAI_2$ testa (96.9), cotyledon (83.0). $EAAI_1$ was computed based on comparison with soybean EAA index and $EAAI_2$ was computed based on comparison with whole egg protein EAA index. Biological value (BV), which was calculated from $EAAI_2$, ranged from 78.7 in cotyledon to 93.9 in testa. EAA index is important in the evaluation of food formulation for protein quality (Nielson, 2002). Also, BV is important in determining the percentage level of a given nutrient source that is utilised in the body (Mune, Mbome & Minka, 2013). High levels of EAAI and BV in this study showed that the samples' protein is of good quality and utilisation. The present study recorded the following ratios: Lys/Trp – testa (12.4), cotyledon (1.35); Met/Trp – testa (3.00), cotyledon (0.980); and Phe/Tyr - testa (3.03), cotyledon (3.40). Both the first two ratios were higher in testa than in cotyledon, whereas Phe/Tyr ratio was almost equal in both samples. The following ratio values are present in mammalian tissue patterns, Lys/Trp: plasma protein (6.2), viscera (5.3), muscle (6.3); Met/Trp: plasma (1.1), viscera (2.0), muscle (2.5) (Albanese, 1959). The optimum ratio of Phe/Tyr has been shown to be 1.5 (i.e., 60% Phe and 40% Tyr) (Pencharz, Hsu & Ball, 2007).

In the AAS based on whole hen's egg amino acid as shown in Table 3, out of the eighteen amino acids determined, five amino acids had their values greater than one in testa. This represents 5/18, with a percentage value of 27.8%. The remaining thirteen out of eighteen i.e., 13/18 (72.2%) had their scores less than 1. Also, two out of the five AAS with score values greater than 1 were EAAs - Phe (1.48) and His (1.03). In the cotyledon, 7/18 with a percentage value of 38.9% had their AASs greater than 1. Out of these, three were essential Phe (2.14), His (1.20), and Arg (1.64). Other amino acids (61.1%) had scores less than 1.0. The limiting amino acid (LAA) in testa was Trp, with a value of 0.157, and in cotyledon, it was Lys (0.321). Therefore, to make all AAs available for metabolism in the body, the LAAs have to be corrected for; 100/15.7 or 6.37 times

as much testa protein and 100/32.1 or 3.12 times as much cotyledon protein have to be eaten when they are served as the sole protein source in the diet.

For pre-school children standard, in each of the samples, six out of the nine parameters (66.7%) had scores greater or equal to one. Other amino acids Leu, Lys, and Trp in testa, and Leu, Lys, Thr in cotyledon had scores less than 1.0. The limiting essential amino acids (LEAAs) in testa and cotyledon were Trp (0.257) and Lys (0.343), respectively. The correction factor were 100/25.7 (3.89) times of testa and 100/34.3 (2.92) times of cotyledon, respectively, in order to make all EAAs available.

In the EAAS based on the FAO/ WHO (1973) standard, Trp (0.283) and Lys (0.362) were the LEAAs in testa and cotyledon, respectively. The correction factors for these two amino acids would be 100/28.3 (3.53) times of testa protein and 100/36.2 (2.76) times of cotyledon protein that must be consumed when the samples are the sole source of protein in the diet. Comparing the results of these two samples using the three scoring procedures, cotyledon was considered to have a higher number of scores greater than 100%.

In the statistical analysis of results summary obtained from Table 3, the correlation coefficient (r_{xy}) (0.781) for egg scores was higher than the critical value (0.590) at n -2 = 16 and $r_{= 0.01}$. The result of variance (r_{xy}^2) showed that 60.99% of variance in the cotyledon was associated with variance in the testa. The results were not significantly different at n -2 =7 and $r_{=0.01}$ (pre-school children), as well as n-2 = 6 and $r_{=0.01}$ (provisional). The coefficient of alienation in all the scoring patterns were high within the range of 0.62446 – 0.8552, with corresponding low levels of IFE ranging from 0.1448 – 0.3754. The high C_A implied that there was high level of lack of relationship between the two samples in terms of scores and low levels of IFE showed low reduction in the error of prediction of relationship between samples.

CONCLUSION

Findings of this study showed that *Moringa oleifera* contained appreciable amounts of amino acids, especially EAAs. The results of EAAI and BV clearly revealed that the proteins in the samples were of good quality. Though most of the AAS were close to or greater than 100% in both samples, the cotyledon was better in terms of score values. However, some amino acids, especially EAAs, were more concentrated in testa. Also, the protein efficiency ratio values showed that proteins in the testa would be more effectively utilised than those in the cotyledon. The testa should be seen as an important integral anatomical part of the seed; both the testa and the cotyledon would complement each other in terms of amino acid contribution and therefore should be consumed together in the diet. It will be an eye opener for those who are always fond of discarding the testa when preparing certain meals. This comparative study will provide new and additional information in the application of *M. oleifera* in food industry, especially in the production of animal feeds.

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Authors' contributions

EIA, the principal researcher, conceptualised and designed the research, wrote the original draft, and reviewed the manuscript; AAO, conducted the study, data analysis and interpretation, reviewed the manuscript; OTI, helped in writing the manuscript, data analysis and interpretation; HOA, conducted the study and reviewed the manuscript; KEA, conducted data analysis and interpretation, reviewed the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Combined effects of bee pollen supplementation and resistance training on aerobic capacity, muscular performance, antioxidant status, and bone metabolism markers in young men: A randomised controlled trial

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ABSTRACT

Introduction: This study investigated the combined effects of bee pollen and resistance training on aerobic capacity, muscular performance, antioxidant status, and bone metabolism markers among young men. **Methods:** Forty young men were randomly assigned into four groups: sedentary control (C), bee pollen supplementation (BP), resistance training (RT), and combined bee pollen supplementation and resistance training (BPRT) groups. Bee pollen was consumed by participants in BP and BPRT groups (1500 mg daily for eight weeks). Resistance training was performed thrice per week for eight weeks in RT and BPRT groups. Participants' anthropometry, aerobic capacity, isokinetic muscular peak torque (strength), and average power were measured. Concentrations of serum total antioxidant status (TAS), serum superoxide dismutase (SOD), serum alkaline phosphatase (ALP), and serum C-terminal telopeptide of type 1 collagen (1CTP) were determined. **Results:** After eight weeks of intervention, there was a significant decrease in 1CTP in BP group. In RT group, significant increases were observed in both muscular strength and power. In BPRT group, significant increases in both muscular strength and power, and a significant decrease in 1CTP were observed after 8 weeks. There were no significant changes in aerobic capacity, serum TAS, SOD, and ALP in all the study groups. **Conclusion:** Resistance training using dumbbells and elastic bands seemed to elicit beneficial effects on muscular strength and power, while bee pollen supplementation alone reduced the level of bone resorption marker. In addition, combining bee pollen with resistance training seemed to offer additive benefit in muscular strength and power.

Keywords: antioxidant status, bee pollen, bone metabolism markers, muscular performance, resistance training

INTRODUCTION

Bee pollen is naturally produced in pellet form with its own particular case and placed in the cells of honeycomb (Komosinska-Vassev *et al.*, 2015). It has been demonstrated that bee

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pollen contains phenolic compounds which have significant antioxidant activities, thereby making it a potential nutraceutical product (Mărgăoan *et al.*, 2019; Barbieri *et al.*, 2020). Antioxidants act to prevent cellular damage that can cause cancer, ageing, and other diseases. The interaction between antioxidants and free radicals can terminate the chain reaction before vital molecules are damaged (Damir *et al.*, 2014). It has been well-documented that some types of exercise or training and/or nutritional supplementation can enhance the antioxidant system, such as total antioxidant status (TAS) (Tartibian & Maleki, 2012; Tavafzadeh *et al.*, 2015) and superoxide dismutase (SOD) activity (Wadiah *et al.*, 2015).

Bee pollen has been reported to provide a source of energy and protein to humans (Campos *et al.*, 2010). Ivy *et al.* (2002) recommended the addition of protein to carbohydrate supplements as this can enhance muscle glycogen storage during heavy exercises. Therefore, it is speculated that the protein contained in bee pollen may give positive effects on certain physiological parameters when combined with resistance training. Regarding bee pollen and bone health, Kafadar *et al.* (2012) have demonstrated that bee pollen could decrease bone loss in ovariectomised rats. Bone loss may occur when the bones are not frequently used for movement (Burr, 1997). Smith & Gilligan (1991) mentioned that being physically active and consuming a proper diet can reduce bone loss to prevent osteoporosis during ageing. In terms of bone metabolism, changes in bone formation markers and bone resorption markers have been investigated in previous studies involving exercise or training and nutritional supplementation (Ooi, Ismail & Abdullah, 2011; Wadiah *et al.*, 2015; Rahim, Ooi & Hamid, 2016).

It is generally known that resistance training involves the contraction of particular muscles with resistance such as dumbbells, elastic bands or one's own body weight. Resistance training is believed to increase muscular strength, tone, mass, and endurance. Kwon *et al.* (2010) demonstrated that resistance training using elastic bands allows an individual to start using bands that are more elastic and gradually increasing the intensity by using bands with less elasticity as muscular strength increases. Previous studies have also indicated that muscular strength and power can be increased with physical training (Rahim *et al.*, 2016), as well as when training is combined with nutritional supplementation (Lau & Ooi, 2014; Chen *et al.*, 2016). A recent study demonstrated that two weeks of resistance training and bee pollen supplementation (10 g daily) resulted in a significant reduction in total cholesterol, triglycerides, and low-density proteins in young men (Abbass, Mahdi & Mohammad Javad, 2020).

To date, data on the effects of bee pollen supplementation and its combined effect with resistance training on aerobic capacity, muscular performance, antioxidant status, and bone metabolism markers are scarce. Thus, the primary objective of this study was to investigate the combined effects of bee pollen and resistance training on aerobic capacity and muscular performance in young men. In addition, the secondary objective of this study was to investigate the effects of bee pollen combined with resistance training on the antioxidant status and bone metabolism markers in young men. The hypothesis of this study was that significant differences will be observed between the combined bee pollen supplementation and resistance training group compared with the bee pollen supplementation alone, resistance training alone, and control groups in aerobic capacity, muscular strength and

average power, antioxidant status, and bone metabolism markers in young men after eight weeks of intervention.

MATERIALS AND METHODS

Forty physically healthy men aged between 19 to 26 years old from the Health Campus of Universiti Sains Malaysia in Kubang Kerian, Kelantan, were recruited in this study. Sample size was calculated by using G*power software version 3.0.10., based on a previous study by Wadiah *et al.* (2015) with mean difference of 0.2 and standard deviation (*SD*) of 0.64. All participants were age-matched and then randomly assigned via parallel randomisation into four groups, with ten participants per group. The participants were given detailed explanation about the objectives, procedures, benefits and risks, as well as possible discomforts and adverse effects that might be experienced during the study before signing the informed consent forms. This study was approved by the Human Research Ethics Committee, Universiti Sains Malaysia (Ref: USM/JEPeM/16020076).

The four groups of participants were: (i) sedentary without bee pollen supplementation and resistance training (C); (ii) bee pollen supplementation (BP); (iii) resistance training (RT), and (iv) combined bee pollen and resistance training (BPRT) groups. Participants in the C group did not perform any resistance training or consume any bee pollen supplements during the study period. Participants in the BP group consumed 1500 mg of bee pollen daily for eight weeks and did not perform any form of physical training. Participants in the RT group were required to perform threesessions per week of resistance training for eight weeks, while participants in the BPRT group were required to consume 1500 mg of bee pollen daily and perform three sessions per week of resistance training for eight weeks. All the participants were instructed not to consume any other types of supplements during the study period.

Anthropometric measurements were recorded at pre- and post-experimental period. Body weight, height, body mass index (BMI), and percentage body fat were measured using an electronic weighing machine (SECA 220, Germany) and a bioelectrical impedance analyser (Tanita® TBF-410, Japan). For the supplementation regimen in this study, Forever Bee Pollen® was used. This product is sold commercially, and it is registered with the National Pharmaceutical Regulatory Agency. The dosage of bee pollen consumed by the participants was 1500mg per day as prescribed by the manufacturer. The nutritional composition of bee pollen includes protein (13.8g/100g), total fat (6.3g/100g), total carbohydrate (68.0g/100g), energy (1613Kcal/100g), fructose (10.0g/100g), and glucose (8.4g/100g).

The resistance training programme was designed with ten stations of different exercises using either elastic bands or dumbbells based on a similar training method described by Chen *et al.* (2019). The types of exercises were biceps curl with dumbbells, leg curl with elastic band, front raise with dumbbells, knee extension with elastic band, standing chest fly with dumbbells, half squat with elastic band, triceps extension with dumbbell, leg abduction with elastic band, shoulder flexion with elastic band, and heel raise with dumbbells. The number of repetitions for exercises with dumbbells and elastic bands were 10 and 15, respectively. The weight of the dumbbells was between 3-10 kg, while the elastic bands were colour-coded in terms of their elasticity. For the first four weeks, the participants were instructed to use dumbbell weights

according to their initial strength level, while for elastic bands, they were told to use the colour-coded bands with lower resistance (more elastic). A three-minute rest was given to the participants before they performed a subsequent set of exercise. The participants were required to complete three sets of this circuit per session and this training programme was conducted three sessions per week for eight weeks. The intensity of the resistance training was increased after four weeks by using heavier dumbbells and elastic bands with less elasticity.

Participants' aerobic capacity was determined via a 20 m shuttle run test. The test was conducted before and after the eight weeks of intervention period. The equipment used to conduct the test included measuring tape, marker cones, CD player, and pre-recorded CD. The test started with ten minutes of warm-up and ended with five minutes of cooling down. After warm-up, the participants ran on a flat cement surface of 20 m distance that had been marked with cones until they were volitionally exhausted. The estimated maximal oxygen consumption (VO_{2max}) of the participants was calculated based on the number of shuttles completed by each participant (Paradisis *et al.*, 2014). An isokinetic dynamometer (Biodex Multi-Joint System 3 Pro, New York, USA) was used to measure the isokinetic peak torque (an indicator of muscular strength) and average power of the participants. Two different angular velocities ($60°.s^{-1}$ and $300°.s^{-1}$) were used to measure the participants' knee flexion and extension isokinetic peak torque and average power of the non-dominant leg, as well as shoulder flexion and extension isokinetic peak torque and average power of the non-dominant arm before and after eight weeks of intervention. The non-dominant leg and arm were used in this study as previous studies have shown that there was no difference between isokinetic strength between the dominant and non-dominant limbs (Abdelmohsen, 2019; Cengizel, 2019).

Six ml of blood sample was taken from each participant before and after the eight-week intervention period. Blood was taken from the antecubital vein of the participants after a 12-hour overnight fast by a laboratory technologist at the Sports Science Laboratory. The blood taken was used to determine the concentrations of blood antioxidant status – total antioxidant status (TAS) and superoxide dismutase (SOD), as well as bone metabolism markers, which were alkaline phosphatase (ALP) for bone formation and C-terminal telopeptide of type 1 collagen (1CTP) for bone resorption. Blood samples were centrifuged and serum was collected and stored at -80 °C for subsequent blood biochemical analysis. The serum was analysed for TAS by using the QuantiChromTM Antioxidant Assay Kit (BioAssay Systems, USA), SOD by using the EnzyChromTM Superoxide Dismutase Assay Kit (BioAssay Systems, USA), ALP by using commercially available reagent kit (Randox, UK), and 1CTP by using quantitative enzyme immunoassay kit (ELISA Kit 1CTP EZA, China). The intra-assay coefficients of variation for TAS, SOD, ALP, and 1CTP were 4.6%, 3.2%, 3.4%, and 6.0%, respectively.

Statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) version 23.0. All values were presented as mean±*SD*. Mixed analysis of variance (ANOVA) was performed to determine the differences between and within groups. Statistical significance was accepted at $p<0.05$.

RESULTS

Physical characteristics of the participants

The present study was completed with the participation of 40 sedentary young

Table 1. Baseline anthropometric characteristics and physiological parameters of all participants

Parameters	*Groups*			
	Control (C) (n=10)	*Bee pollen (BP) (n=10)*	*Resistance training (RT) (n=10)*	*Combined (BPRT) (n=10)*
Age (years)	21.8±2.1	22.0±1.9	22.0±2.7	20.4±0.8
Body weight (kg)	60.2±13.9	56.2±13.9	66.9±14.8	58.3±11.8
Body height (cm)	167.2±6.9	164.8±6.2	168.1±4.7	168.2±6.0
Body mass index (BMI) ($kg.m^{-2}$)	21.5±4.6	20.4±3.9	23.6±4.7	20.7±4.9
Percentage of body fat (%)	20.2±8.1	17.7±8.2	21.7±7.5	17.1±7.0
Fat free mass (FFM) (kg)	47.0±5.9	45.4±7.4	51.5±6.9	47.7±5.6

men with mean age of 21.6±2.1 years and mean body weight of 60.4±13.7 kg. All participants completed the study without any drop-out. Anthropometric data obtained from all participants (*N*=40) are summarised in Table 1.

There were no significant differences between groups in age, body height, body weight, and body fat percentage at the beginning and at the end of the experimental period (Table 1).

Compliance to bee pollen supplementation and resistance training programme

From the record of supplements consumed by the participants, the compliance of bee pollen supplementation was between 89.3 – 100%, while the attendance record for the resistance training programme indicated that the compliance of the participants was between 83.3 – 100% (minimum 20 training sessions out of 24).

Aerobic capacity

At pre-test, there were no significant differences in mean predicted VO_{2max} among all groups compared to the C group. There was no significant interaction effect of time and intervention

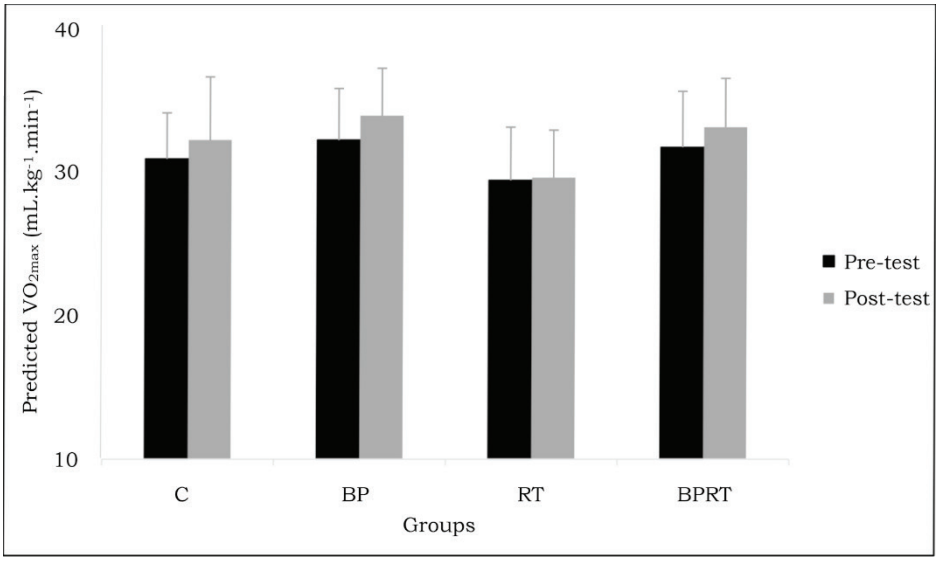

Figure 1. Mean predicted VO_{2max} at pre-test and post-test

Table 2. Mean isokinetic peak torque of knee and shoulder extension and flexion at pre-test and post-test (Mean±*SD*)

A) Mean isokinetic peak torque of knee extension and flexion at 60°.s^{-1} at pre-test and post-test

Groups	*Knee extension peak torque (N.m)*		*Knee flexion peak torque (N.m)*	
	Pre-test	*Post-test*	*Pre-test*	*Post-test*
C	127.4±32.7	137.2±25.9	62.8±19.6	68.7±17.4
BP	141.4±39.9	141.9±29.6	69.9±16.2	69.1±14.9
RT	151.6±21.2	180.7±35.4$^{***,++,‡‡}$	72.5±20.2	84.5±15.5$^{*,+,‡}$
BPRT	128.8±27.0	168.5±33.9$^{***,+}$	62.4±18.3	80.8±15.0***

B) Mean isokinetic average power of knee extension and flexion at 300°.s^{-1} at pre-test and post-test

Groups	*Knee extension average power (Watts)*		*Knee flexion average power (Watts)*	
	Pre-test	*Post-test*	*Pre-test*	*Post-test*
C	162.0±45.3	185.5±46.8	115.9±56.8	116.2±64.1
BP	181.9±56.1	219.5±52.9**	104.2±28.9	105.2±27.3
RT	192.4±60.3	274.7±68.3$^{***,+,‡}$	108.2±46.0	141.8±45.0**
BPRT	164.4±66.1	244.7±58.9$^{***,+}$	95.8±43.7	139.3±35.6***

C) Mean isokinetic peak torque of shoulder extension and flexion at 60°.s^{-1} at pre-test and post-test

Groups	*Shoulder extension peak torque (N.m)*		*Shoulder flexion peak torque (N.m)*	
	Pre-test	*Post-test*	*Pre-test*	*Post-test*
C	50.9±12.4	55.9±14.3	49.3±8.8	40.9±7.3
BP	43.0±9.7	43.0±11.9	39.5±6.6^{+}	47.9±9.8
RT	56.7±18.3‡	63.8±19.2‡	48.5±10.4‡	59.7±18.5$^{+,‡}$
BPRT	49.4±13.1	55.5±12.7	48.7±9.6‡	49.1±10.0

D) Mean isokinetic average power of shoulder extension and flexion at 300°.s^{-1} at pre-test and post-test

Groups	*Shoulder extension average power (Watts)*		*Shoulder flexion average power (Watts)*	
	Pre-test	*Post-test*	*Pre-test*	*Post-test*
C	54.5±29.1	71.6±20.6	59.2±20.1	64.1±18.9
BP	51.5±24.6	57.2±32.2	60.5±12.7	65.0±10.1
RT	74.1±34.4	106.7±58.6	65.8±22.0	84.5±23.8$^{***,+}$
BPRT	57.9±37.1	87.7±41.9	64.0±23.7	83.8±24.7$^{***,+}$

$^{*, **, ***}$significantly different from pre-test (p<0.05, p<0.01, p<0.001 respectively)
$^{+, ++}$significantly different from respective C group (p<0.05, p<0.01 respectively)
$^{‡, ‡‡}$significantly different from respective BP group (p<0.05, p<0.01 respectively)

in mean predicted VO_{2max} (df=3, F=0.763, p=0.522) among all groups (Figure 1).

Knee extension and flexion isokinetic peak torque at 60°.s^{-1}

There were no significant differences in mean isokinetic peak torque of knee extension at 60°.s^{-1} at pre-test between all groups (Table 2A). Mean isokinetic peak torque of knee extension at 60°.s^{-1} in RT (p<0.001) and BPRT (p<0.001) groups were significantly higher at post-test compared to their respective pre-test values. At post-test, isokinetic peak torque of knee extension at 60°.s^{-1} in RT and BPRT groups were significantly higher (p<0.01 and p<0.05, respectively) compared to C group, and significantly higher in RT group (p<0.01) compared to BP group.

There were no significant differences in mean isokinetic peak torque of knee flexion at 60°.s^{-1} at pre-test among all groups (Table 2A). At post-test, isokinetic peak torque of knee flexion at 60°.s^{-1} in RT group was significantly higher (p<0.05) compared to C and BP groups. Isokinetic peak torque of knee flexion at 60°.s^{-1} in RT and BPRT groups increased significantly (p<0.05 and p<0.001, respectively) compared to their respective pre-test values.

Isokinetic average power of knee extension and flexion at 300°.s^{-1}

There were no significant differences in mean isokinetic average power of knee extension at 300°.s^{-1} at pre-test among all groups (Table 2B). At post-test, isokinetic average power of knee extension at 300°.s^{-1} in RT and BPRT groups were significantly (p<0.05) higher compared to C group, and higher (p<0.05) in RT group compared to BP group. Isokinetic average power of knee extension at 300°.s^{-1} in BP, RT, and BPRT groups increased significantly (p<0.01, p<0.001, and p<0.001, respectively) compared to their respective pre-test values.

There were no significant differences in mean isokinetic average power of knee flexion at 300°.s^{-1} at pre-test among all groups (Table 2B). Isokinetic average power of knee flexion at 300°.s^{-1} in RT and BPRT groups increased significantly (p<0.01 and p<0.001, respectively) compared to their respective pre-test values.

Isokinetic peak torque of shoulder extension and flexion at 60°.s^{-1}

At pre-test, the isokinetic peak torque of shoulder extension at 60°.s^{-1} in RT group was significantly higher than BP group (p<0.05) (Table 2C). At post-test, isokinetic peak torque of shoulder extension in RT group was significantly higher than BP group (p<0.05). There were no significant changes in mean isokinetic peak torque of shoulder extension at 60°.s^{-1} among all groups compared to their respective resting values.

At pre-test, the isokinetic peak torque of shoulder flexion at 60°.s^{-1} in BP group was significantly lower than C, RT, and BPRT groups (p<0.05) (Table 2C). At post-test, the isokinetic peak torque of shoulder flexion at 60°.s^{-1} in RT group was significantly (p<0.05) higher than C and BP groups. There were no significant changes in mean isokinetic peak torque of shoulder flexion at 60°.s^{-1} among all groups compared to their respective resting values.

Isokinetic average power of shoulder extension and flexion at 300°.s^{-1}

There were no significant differences in mean isokinetic average power of shoulder extension at 300°.s^{-1} at pre-test among all groups (Table 2D). At post-test, isokinetic average power of shoulder extension at 300°.s^{-1} increased significantly in RT and BPRT groups (p<0.01) compared to their respective pre-test values. Isokinetic average power of shoulder extension at 300°.s^{-1} was

also significantly higher (p<0.05) in RT group compared to C and BP groups, and higher (p<0.05) in BPRT group compared to BP group.

There were no significant differences in mean isokinetic average power of shoulder flexion at 300°.s^{-1} at pre-test among all groups (Table 2D). At post-test, the isokinetic average power of shoulder flexion of 300°.s^{-1} in RT and BPRT groups were significantly (p<0.5) higher compared to C group. Isokinetic average power of shoulder flexion at 300°.s^{-1} in RT and BPRT groups increased significantly (p<0.001) compared to their respective pre-test values.

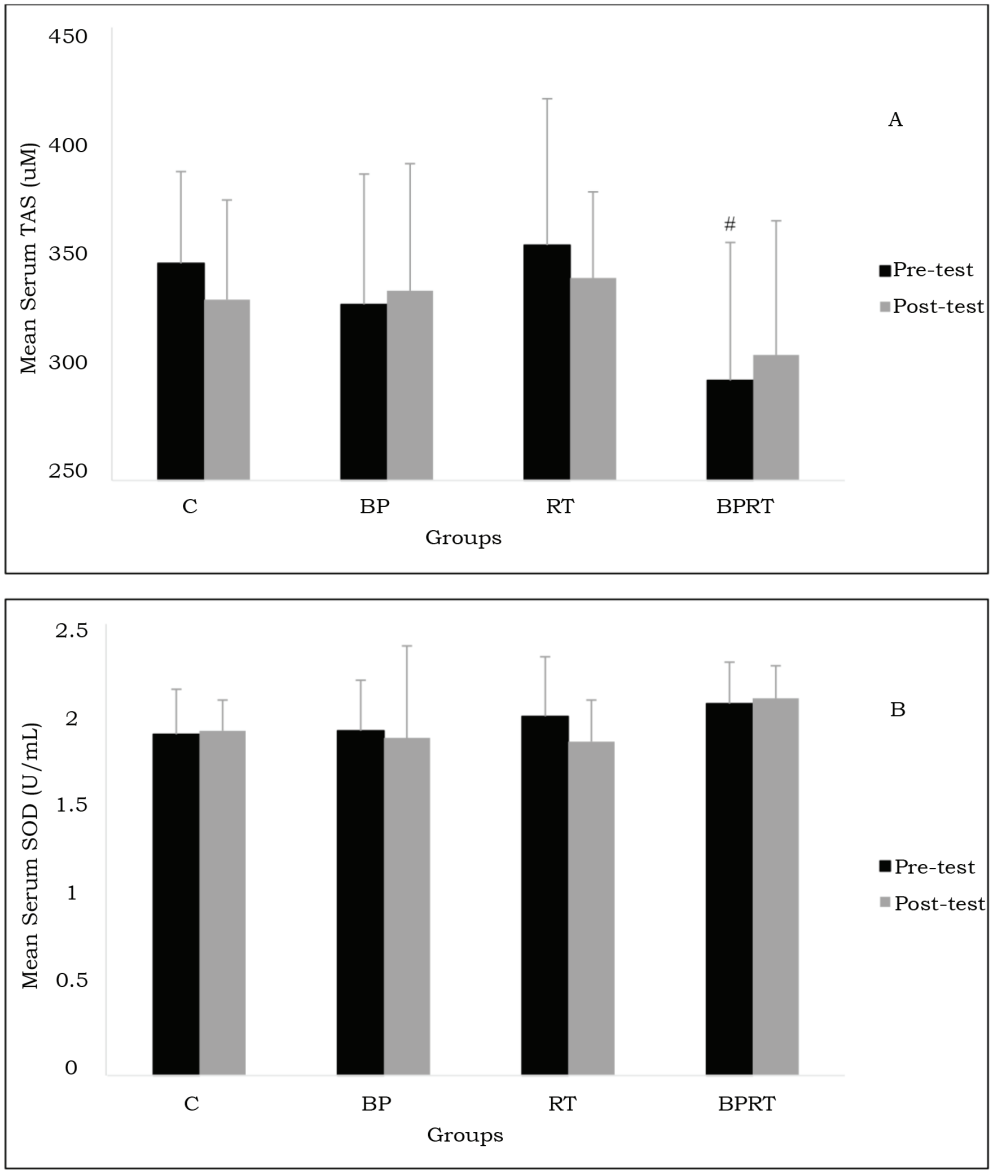

Figure 2. Mean serum total antioxidant status (TAS) (A) and mean serum superoxide dismutase (SOD) (B) at pre-test and post-test
#significantly different from RT group at pre-test (p<0.05)

Serum total antioxidant status (TAS) and superoxide dismutase (SOD)

At pre-test, mean serum TAS in BP group was significantly (p<0.05) lower than RT group (Figure 2A). There was no significant interaction effect of time and intervention in serum TAS (df=3, F=2.46, p=0.078). There was no significant effect of time on mean serum TAS between pre- and post-tests (df=1, F=1.004, p=0.323). Similarly, there was no significant effect of intervention in mean serum TAS among all groups at post-test. Regarding SOD, there was no significant difference in mean serum SOD among all groups at pre- and post-tests (Figure 2B). There was no significant interaction effect of time and intervention on serum SOD (df=3, F=0.425, p=0.736). There was no significant effect of time on mean serum SOD between pre- and post-tests (df=1, F=0.357, p=0.554). In addition, there was no significant effect of intervention on serum SOD among all groups at post-test.

Serum alkaline phosphatase (ALP) (bone formation marker) and C-terminal telopeptide of type 1 collagen (1CTP) (bone resorption marker)

There were no significant differences in mean serum ALP among all the experimental groups at pre- and post-tests (Table 3). There was no significant interaction effect of time and intervention in mean serum ALP (df=3, F=1.664, p=0.192) among all groups. There was also no significant effect of time on mean serum ALP between pre- and post-tests (df=3, F=1.082, p=0.369). Similarly, there was no significant effect of intervention in mean serum ALP (df=3, F=1.051, p=0.382) among all groups.

At pre-test, mean serum 1CTP in RT group was significantly (p<0.01) lower compared to BP group (Table 3). There was a significant interaction effect of time and intervention on mean serum 1CTP (df=3, F=4.119, p=0.013) among all groups. In addition, there was a significant effect of time on mean serum 1CTP between pre- and post-tests (df=1, F=10.069, p=0.003). However, there was no significant effect of intervention in mean serum 1CTP (df=3, F=0.839, p=0.481) among all groups.

DISCUSSION

In the present study, bee pollen supplementation alone, resistance training alone, and combined bee pollen supplementation and resistance training for eight weeks did not elicit any effects on estimated maximal oxygen consumption (VO_{2max}), which is an indicator of aerobic capacity (Figure 1). The results of no improvement in maximal oxygen consumption following bee pollen supplementation in this study was consistent with a previous study where bee pollen supplementation after six weeks did not show any significant

Table 3. Mean serum alkaline phosphatase (ALP) and mean serum C-terminal telopeptide of type 1 collagen (1CTP) at pre-test and post-test (Mean±*SD*)

Groups	*Serum ALP (U/L)*		*Serum 1CTP (ng/mL)*	
	Pre-test	*Post-test*	*Pre-test*	*Post-test*
C	73.6±26.6	76.8±25.8	6.1±2.3	4.8±1.1
BP	69.2±21.1	69.7±19.6	8.5±5.2	4.9±2.7**
RT	63.9±16.9	64.9±15.0	4.4±1.4§	5.5±4.2
BPRT	81.2±22.4	79.3±22.3	7.3±2.8	4.9±2.3*

*, **, significantly different from respective pre-test values (p<0.05, p<0.01 respectively)
§ significantly different from BP group at pre-test (p<0.01)

improvement in VO_{2max} among adolescent swimmers (Maughan & Evans, 1982). Thus, this demonstrated that the macronutrients contained in bee pollen did not offer beneficial effects on aerobic capacity.

However, the present study indicated that resistance training alone, and combined bee pollen supplementation and resistance training elicited some beneficial effects on both muscular strength and power of the lower and upper limbs despite the absence of statistical significance in some of the groups (Table 2 A, B, C, and D). Thus, the prescribed resistance training programme alone and when combined with bee pollen supplementation in this study seemed to be effective in improving muscular strength. However, the combination of bee pollen supplementation and resistance training did not provide additional effect in enhancing muscular strength. Similar findings of increased muscular strength have been reported in previous studies using different nutritional supplements. Circuit training alone, and combined *Eurycoma longifolia Jack* supplementation (400 mg per day for seven days per week) plus circuit training significantly increased the muscular strength of knee flexion and knee extension following eight weeks of intervention (Ooi *et al.*, 2015). In contrast with the present finding, 12 weeks of resistance training at two days per week did not show any significant increase in peak torque on knee flexion and extension in adolescent *taekwondo* athletes (Teng *et al.*, 2008). This difference could be attributed to the fitness levels of the participants, training frequency, and types of exercises carried out in these studies.

The current study findings also demonstrated that resistance training alone, and combined bee pollen supplementation and resistance training elicited beneficial effects on muscular power through increased isokinetic average power of knee extension and flexion, as well as shoulder flexion (Tables 2). Similar findings of increased muscular power have been reported in previous studies when resistance training was combined with other nutritional supplements (Ooi *et al.*, 2015; Chen *et al.*, 2016). Combined *Eurycoma longifolia Jack* supplementation and circuit training for eight weeks significantly increased the isokinetic muscular power of knee and shoulder extension (Ooi *et al.*, 2015). Another study also reported that combined *Lignosus rhinocerotis* supplementation (500 mg daily) with resistance training significantly increased muscular power among young males (Chen *et al.*, 2016). In the present study, there was no significant difference in average power between the resistance training group and the combined bee pollen supplementation and resistance training group, implying that bee pollen consumption did not offer additive effect in enhancing average power.

In the present study, all three interventions, i.e. bee pollen supplementation alone, resistance training alone, and combined bee pollen supplementation and resistance training did not affect serum TAS (Figure 2). These results implied that these interventions for eight weeks did not elicit significant effect on total antioxidant status in the blood. However, a previous study showed contradictory finding where honey supplementation during eight weeks of intensive cycling training increased serum total antioxidant status in non-professional male road cyclists (Tartibian & Maleki, 2012). The difference in the present finding with the previous study may be attributed to differences in the types of supplements and training protocols used.

The present study demonstrated that bee pollen supplementation alone, resistance training alone, and

combined bee pollen supplementation and resistance training did not affect serum SOD (Figure 2). Similar findings were also reported in other studies with different nutritional supplementation and different exercise modes (Tauler *et al.*, 2006; Wadiah *et al.*, 2015). For instance, SOD activity was not affected when chocolate malt drink consumption was combined with aerobic dance exercise (Wadiah *et al.*, 2015). Similarly, erythrocyte and lymphocyte SOD activity levels did not show any significant difference after the consumption of selenium supplement combined with intense exercise (Tauler *et al.*, 2006). In contrast, other studies reported that serum SOD activity was increased following a bout of training (Sahrir *et al.*, 2017; Wiecek *et al.*, 2018; Yan & Spaulding, 2020). Nevertheless, our data indicated that neither the prescribed resistance training programme or bee pollen supplementation alone, nor the combination of both affected SOD activity. Thus, it was shown that bee pollen supplement, which contains phenolic compounds and carotenoids that act as antioxidants, was not adequate to elicit any effect on the SOD activity in this study.

We also observed that there were no significant differences in serum ALP concentrations after eight weeks of experimental period in all four experimental groups (Table 3). The prescribed resistance training programme and the dosage of bee pollen given in this study did not affect serum ALP (a bone formation marker). Similar finding has been reported where aerobic dance exercise at three times a week for six weeks also did not affect serum ALP in young females (Ooi *et al.,* 2011). However, the present finding was in contrast with a previous study by Wadiah *et al.* (2015), where serum ALP increased with another nutritional supplementation and different exercise modes. In their study, it was observed that chocolate malt drink supplementation alone, aerobic dance exercise alone, and combined chocolate malt drink supplementation and aerobic dance exercise elicited increased serum ALP in their female participants. The difference between the present finding with this previous study is likely due to differences in types of supplements and exercises used.

Serum 1CTP was significantly lower in post-test compared to pre-test value in bee pollen supplementation alone, and combined bee pollen supplementation and resistance training groups (Table 3). The present data indicated that bee pollen supplementation alone and when combined with resistance training reduced the levels of serum 1CTP, a bone resorption marker. This finding was consistent with a previous study where the excretion of bone resorption markers (pyridinoline and deoxypyridinoline) decreased significantly after the consumption of soybean isoflavone supplement (Uesugi *et al.*, 2002). These data implied that nutritional supplementations may elicit beneficial effects on bone health by reducing bone resorption markers. Lau & Ooi (2014) have also shown that there was a significant reduction in 1CTP after six weeks of circuit training programme combined with consumption of chocolate milk supplement. These data indicated that bone metabolism markers are not only affected by nutritional supplements, but also by the combined effect of nutritional supplementation with certain types of training.

In summary, the consumption of bee pollen at a dosage of 1500mg daily for eight weeks did not provide any beneficial effect in some of the parameters measured in the present study. Hence, it is postulated that these observations could be attributed to inadequate dosage of bee pollen given to the participants and/or insufficient

duration of the intervention period. Thus, the limitations of the present study are as follow: i) no dose response of bee pollen supplementation was performed; ii) the intervention period of this study was only eight weeks; iii) only one biomarker was used to determine bone formation and another biomarker for bone resorption; and iv) the lack of micronutrient data on bee pollen supplements.

CONCLUSION

The prescribed resistance training programme using dumbbells and elastic bands at three times per week for eight weeks elicited increases in muscular strength and power. Bee pollen supplementation at a dosage of 1500 mg per day (as prescribed by the manufacturer of this product) for eight weeks seemed to reduce the bone resorption marker. However, when bee pollen supplementation was combined with resistance training programme, additional benefits were not observed in aerobic capacity, muscular performance, antioxidant status, and bone metabolism markers. Under the conditions set up in this study, it is concluded that resistance training using dumbbells and elastic bands conferred beneficial effects on muscular strength and power, while bee pollen supplementation alone reduced the level of bone resorption marker.

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Authors' contributions

NN, conducted the study, data analysis and interpretation, drafting of the manuscript; CCK, conceptualised and designed the study, reviewed the manuscript; OFK, conceptualised and designed the study, data analysis, reviewed the manuscript; MM, designed the study, reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Factors related to low birth weight in Indonesia

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ABSTRACT

Introduction: Previous studies have reported that low birth weight (LBW) correlates with neonatal death and 15 - 20% of all births worldwide are LBW. This research aimed to analyse the factors related to LBW in Indonesia. **Methods:** The authors collated secondary data from the 2017 Indonesian Demographic and Health Survey (IDHS). The sample consisted of 17,443 respondents. Besides LBW as the dependent variable, the independent variables consisted of maternal age, residence, wealth, education, employment, marital status, health insurance, antenatal care (ANC) visits, smoking behaviour, and gender of the baby. The final stage employed binary logistic regression. **Results:** Women aged 35-39 years were 0.688 times less likely than women aged 15-19 years to give birth to LBW babies. The wealthiest women were 0.712 times less likely than the poorest women to give birth to LBW babies. Women with higher education levels were 0.670 times less likely to have a LBW baby than women with no education level. Women who attended ≥4 ANC visits were 0.829 times less likely to have LBW babies than women who attended <4 ANC visits. Baby girls were 1.161 times more likely than baby boys to be born with LBW. **Conclusion:** The study concluded that the factors related to LBW in Indonesia were maternal age, wealth, education, ANC, and gender of the baby.

Keywords: antenatal care visit, education level, low birth weight, wealth status, women of reproductive age

INTRODUCTION

The term low birth weight (LBW), as defined by the World Health Organization (WHO), refers to babies born weighing less than 2500 grammes (g). Previous studies have reported that 15-20% of births worldwide are LBW. LBW has become a significant global public health problem associated with short-term and long-term complications. LBW babies need extra nursing care to survive and the risk is higher for smaller babies (Kusrini *et al.*, 2021; UNICEF & WHO, 2019).

Asian and African countries contribute most towards neonatal mortality rate globally, with Indonesia contributing 1-3% (Alifariki, Kusnan & Rangki, 2019). Of the 7000 stillbirths around the world daily, 185 are in

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Indonesia. The neonatal mortality rate in Indonesia is as high as 15/1000 live births. Three-quarters of Indonesia's neonatal deaths occur in the first week and as many as 40% died within the first 24 hours. Neonatal deaths are strongly correlated with the quality of labour and delivery, as well as non-optimal neonatal care (Achadi, 2019). LBW is also correlated with neonatal deaths (Vilanova *et al.,* 2019).

Usually, we find LBW babies being born with unfavourable conditions, unlike an average newborn baby. LBW babies are associated with asphyxia, recurring apnoea, hypothermia, hypoglycaemia, icterus, and a longer intensive care period at the hospital (Chidiebere *et al.,* 2018). A meta-analysis reported that children born with LBW tend to have more significant cognitive and motoric impairments than normal newborn babies (Upadhyay *et al.,* 2019). LBW has a complex impact on baby's growth and development, not only during delivery in terms of complications, but also in relation to long-term implications. LBW babies need optimal intensive care management, which includes oxygen maintenance, warm room temperature maintenance, minimising infection, breastfeeding, bonding, and relaxing (WHO, 2011).

A previous study found that there are several conditions that cause LBW, whereby ten conditions are associated with pregnancy and labour preparation. The first condition is the women's age during pregnancy (Siramaneerat, Agushybana & Meebunmak, 2018). The women's age is correlated to the reproduction system, which supports the foetus and its development. It is associated with the women's readiness to give birth and self-care during pregnancy. The second condition is the mother's place of residence. This relates to environmental air pollutant exposure which affects mother and foetal health. Third is wealth status that is associated with the mother's purchasing power, enabling her to fulfil her nutritional needs during pregnancy for the purpose of foetal health. Fourth is education level (Siramaneerat *et al.,* 2018), which correlates with the mother's knowledge concerning health care during pregnancy and foetal health. A knowledgeable mother is expected to have healthy habits. Fifth, the mother's employment status is associated with the mother's burden increment during pregnancy, which in turn will affect both pregnancy and foetal health (Laksono *et al.,* 2021). Sixth, marital status, which correlates to whether there is an unwanted pregnancy within or outside of the marriage. An unwanted pregnancy affects the mother's mental and foetal health (Tang *et al.,* 2017). Seventh, health insurance ownership correlates to the mother's opportunity to access good healthcare services. Eighth is ANC visit (Ngwira, 2019), which relates to the mother's chance of having good pregnancy health care and early detection of any pregnancy risk factors. Ninth is smoking behaviour, which is correlated to air pollutant exposure by cigarette smoke, affecting the mother's health as well as foetal health. Tenth, the gender of the foetus that is associated with the tendency of baby boys or baby girls being born with LBW (Ngwira, 2019). Based on the brief background above, this research aimed to analyse the factors related to LBW in Indonesia.

MATERIAL AND METHODS

The authors collated secondary data from the 2017 Indonesian Demographic and Health Survey (IDHS). The 2017 IDHS is a nationwide cross-sectional survey conducted by Statistics Indonesia, the Indonesian Agency of National Population and Family Planning, and the Indonesia Ministry of Health.

The study population included women of reproductive age (15-49 years old) who had given birth in Indonesia in the last five years and who had birth

weight reports available, either written records or maternal memories. The data for this research were collected using two-step stratified multistage random sampling. The sample consisted of 17,443 respondents.

The dependent variable in this research was LBW. Meanwhile, the independent variables were maternal/household characteristics (age, residence, wealth status, education level, employment status, marital status, health insurance ownership, ANC visits, and smoking behaviour) and individual baby's characteristics (gender).

The term LBW defined in this manuscript refers to the Statistics Indonesia's category of babies who were born weighing less than 2500 g. The maternal age group of 15-49 years old consisted of seven groups. The types of residence consisted of urban and rural groups, as determined by Statistics Indonesia.

The 2017 IDHS used the wealth index computation of the survey to assess wealth status. Wealth status consisted of five categories: the poorest, poorer, middle, richer, and the richest. The wealth index was a composite assessment of a household's whole level of life. The 2017 IDHS produced the wealth index using simple data on a household's possession of certain goods, such as television and bicycle; the building materials used in housing construction; and the type of water access and sanitation services (Wulandari *et al.,* 2019).

The study determined the respondents' education level by acknowledging the most recent diplomas. Education level consisted of four groups: no education, primary, secondary, and higher. On the other hand, employment status referred to the mother's current job. Employment status consisted of two groups, namely unemployed and employed. Marital status consisted of three groups: never in a union, married/living with a partner, and widowed/divorced. Health insurance ownership consisted of two groups: uninsured and insured.

On the other hand, ANC was the total ANC visit attendance. ANC consisted of <4 ANC visits and ≥4 ANC visits. Smoking behaviour of the mother consisted of two types: non-smoker and smoker. Finally, the gender of the baby consisted of two groups: boy and girl.

In this research, the researcher analysed the data using a two-step analysis. First, we conducted a chi-square bivariate test because all of the variables were in a state of dichotomy. Second, we performed the multivariate test using binary logistic regression to determine the LBW determinants in Indonesia.

The 2017 IDHS adhered to the Standard DHS survey procedure under The Demographic and Health Surveys (DHS) Programme (DHS-7) authorised by ICF International's Institutional Review Board. This had been evaluated initially and approved by the ORC Macro IRB 2002. The DHS-7 Programme supported the DHS surveys that adhered to the Standard, including the approval paperwork. ICF International's Institutional Review Board follows the US Department of Health and Human Services criteria for "Protection of Human Subjects" (45 CFR 46).

RESULTS

Table 1 presents an overview of LBW in Indonesia. The results show that the national average for newborns with low birth weight is 13.6%. The teenage group (15-19 years) has the highest proportion of babies born with low birth weight. Moreover, women in rural residences were more likely to birth LBW babies than those in urban areas.

The lowest wealth status women gave birth to the most LBW babies, and women with no education gave birth to the most LBW babies as well. More unemployed women had LBW babies than employed women. Meanwhile,

Table 1. Descriptive statistics of LBW in Indonesia (*n*=17,443)

Characteristics	*All* *n (%)*	*Low birth weight, n (%)*		*p-value*
		No	*Yes*	
Maternal age				
15-19 years	424 (2.4)	344 (81.1)	80 (18.9)	0.003**
20-24 years	2740 (15.7)	2334 (85.2)	406 (14.8)	
25-29 years	4484 (25.7)	3873 (86.4)	611 (13.6)	
30-34 years	4618 (26.5)	4031 (87.3)	587 (12.7)	
35-39 years	3425 (19.6)	2990 (87.3)	435 (12.7)	
40-44 years	1446 (8.3)	1238 (85.6)	208 (14.4)	
45-49 years	306 (1.8)	260 (85.0)	46 (15.0)	
Place of residence				
Urban	8671 (49.7)	7528 (86.8)	1143 (13.2)	0.055
Rural	8772 (50.3)	7542 (86.0)	1230 (14.0)	
Wealth status				
Poorest	4690 (26.9)	3921 (83.6)	769 (16.4)	<0.001***
Poorer	3433 (19.7)	2949 (85.9)	484 (14.1)	
Middle	3221 (18.5)	2795 (86.8)	426 (13.2)	
Richer	3109 (17.8)	2724 (87.6)	385 (12.4)	
Richest	2990 (17.1)	2681 (89.7)	309 (10.3)	
Education level				
No education	244 (1.4)	199 (81.6)	45 (18.4)	<0.001***
Primary	4268 (24.5)	3591 (84.1)	677 (15.9)	
Secondary	9753 (55.9)	8431 (86.4)	1322 (13.6)	
Higher	3178 (18.2)	2849 (89.6)	329 (10.4)	
Employment status				
Unemployed	9281 (53.2)	7966 (85.8)	1315 (14.2)	0.020*
Employed	8162 (46.8)	7104 (87.0)	1058 (13.0)	
Marital status				
Never in a union	28 (0.1)	22 (78.6)	6 (21.4)	0.104
Married/living with partner	16896 (96.9)	14613 (86.5)	2283 (13.5)	
Widowed/divorced	519 (3.0)	435 (83.8)	84 (16.2)	
Health insurance				
Uninsured	6553 (37.6)	5683 (86.7)	870 (13.3)	0.327
Insured	10890 (62.4)	9387 (86.2)	1503 (13.8)	
ANC visits				
<4 times	3972 (22.8)	3339 (84.1)	633 (15.9)	<0.001***
≥4 times	13471 (77.2)	11731 (87.1)	1740 (12.9)	
Smoking behaviour				
No	17144 (98.3)	14812 (86.4)	2332 (13.6)	0.956
Yes	299 (1.7)	258 (86.3)	41 (13.7)	
Baby's gender				
Male	8993 (51.6)	7845 (87.2)	1148 (12.8)	0.001**
Female	8450 (48.4)	7225 (85.5)	1225 (14.5)	

Note: Chi-square test; *p<0.05; **p<0.01; ***p<0.001

women who were never in a union were also found to be prevalent in giving birth to LBW babies. However, women with insurance ownership had higher prevalence of giving birth to LBW babies than women who did not have insurance. The percentage of LBW was higher for women who attended <4 ANC visits. The proportion of LBW babies born from a smoker and non-smoker mother seemed almost equal; whereas there were more baby girls who were born with LBW than baby boys. Age, wealth status, education level, employment status, ANC visits, and gender were statistically significantly correlated with LBW occurrence in Indonesia.

Table 2 shows the logistic binary regression test results for LBW occurrence. The reference in this research was the non-occurrence of LBW. The analysis results showed that women in the 15-19 years age group had the highest chance of giving birth to LBW in Indonesia. As shown in Table 2, women in the 25-29 years age group

Table 2. Binary logistic regression for low birth weight in Indonesia (n=17,443)

Determinants	*Low birth weight*			*p-value*
	OR	*95% CI*		
		Lower bound	*Upper bound*	
Maternal age				
15-19 years	-	-	-	-
20-24 years	0.788	0.604	1.029	0.080
25-29 years	0.754	0.581	0.978	0.033*
30-34 years	0.699	0.539	0.908	0.007**
35-39 years	0.688	0.527	0.898	0.006**
40-44 years	0.780	0.585	1.041	0.091
45-49 years	0.805	0.539	1.203	0.290
Wealth status				
Poorest	-	-	-	-
Poorer	0.889	0.784	1.008	0.067
Middle	0.848	0.743	0.968	0.015*
Richer	0.821	0.713	0.944	0.006**
Richest	0.712	0.609	0.833	<0.001***
Education				
No education	-	-	-	-
Primary	0.915	0.652	1.283	0.606
Secondary	0.814	0.580	1.142	0.233
Higher	0.670	0.469	0.958	0.028*
Employment				
Unemployed	-	-	-	-
Employed	0.973	0.888	1.065	0.552
ANC visits				
<4 times	-	-	-	-
≥4 times	0.829	0.749	0.917	<0.001***
Baby's gender				
Girl	1.161	1.065	1.267	0.001**
Boy	-	-	-	-

Note: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$

were 0.754 times less likely than those in the 15-19 years age group to give birth to LBW babies (OR 0.754; 95% CI 0.581-0.978). Moreover, the 35-39 years age group women were 0.688 less likely than those in the 15-19 years age group to give birth to LBW babies (OR 0.688; 95% CI 0.527-0.898).

Table 2 presents that wealth status was one of the predictors of LBW occurrence. Women of the middle wealth status were 0.848 times less likely than the most deficient women to give birth to LBW babies (OR=0.848; 95% CI 0.743-0.968). The wealthiest women were 0.712 times less likely than the most impoverished women to give birth to LBW babies (OR=0.712; 95% CI 0.609-0.833). The analysis results found that the poorer the women, the more extraordinary the LBW occurrence.

Based on education level, women with higher education were 0.670 times less likely than women with no education to give birth to LBW babies (OR=0.670; 95% CI 0.469-0.958). This analysis result showed that women with the highest education were the least likely to give birth to LBW babies (OR=0.670; 95% CI 0.469-0.958).

Based on ANC visit attendance, women who attended ≥4 ANC visits were 0.829 times less likely than those who attended <4 ANC visits to give birth to LBW babies (OR=0.829; 95% CI 0.749-0.917). Furthermore, baby girls were 1.161 times more likely than baby boys to be born with LBW (OR=1.161; 95% CI 1.065-1.267).

DISCUSSION

This research has confirmed that age is an important factor that could lead to LBW occurrence in Indonesia. Women in the 15-19 years age group were the most likely to give birth to LBW in Indonesia; indicating that women who are too young (i.e. teenagers) during pregnancy, are more likely to have LBW babies. This finding is consistent with the data obtained in a previous study that found that age influences Indonesia's LBW occurrence (Siramaneerat *et al.,* 2018). Previous research in China also found that a young maternal age influences LBW occurrence (Tang *et al.,* 2017).

This current research found that the wealth status variable was correlated to LBW occurrence in Indonesia. Previous studies also found that women with the most deficient wealth status were correlated with LBW babies (Mahumud, Sultana & Sarker, 2017). Wealth status is associated with food purchasing power and nutritional fulfilment during pregnancy. Pregnant women who cannot afford nutritional dignity are at risk of malnutrition (He *et al.,* 2018). Poverty, an insufficiency when purchasing food, and a lack of high dietary foods during pregnancy influences foetal development, resulting in LBW babies.

Another important finding in this research was that education level was correlated to LBW occurrence in Indonesia. This finding is similar to a previous study that found that lower educational status is associated with LBW babies. A previous study showed that illiteracy predicts LBW occurrence in developing countries (Siramaneerat *et al.,* 2018). Moreover, a better level of education is often reported as a positive determinant to achieve better health outcomes (Megatsari *et al.,* 2020; Wulandari & Laksono, 2020); whereas a low level of education is a barrier to good performance (Laksono & Wulandari, 2022; Masruroh *et al.*, 2021; Rohmah *et al.,* 2020).

This research also found that women with fewer ANC visits were more likely to have an LBW occurrence. These results are similar to a previous study finding which showed that antenatal care visits are correlated with LBW (Tang *et al.,* 2017). Ngwira in Malawi in South Africa has also shown that the lack of ANC visits correlates with LBW frequency. ANC visits are connected to pregnancy

care and early detection of pregnancy complications (Ngwira, 2019), thus beneficial in reducing LBW occurrence.

According to this study, baby girls were more likely than baby boys to be born with LBW. This finding contradicts previous studies conducted in Pakistan and Japan, which suggested that baby boys were more likely than baby girls to be born with LBW (Ghouse & Zaid, 2016; Arima *et al.,* 2017).

This study has shown that poverty and a lack of ANC visits had a suggestive role in promoting LBW occurrence. However, ANC visits were found to be more beneficial than wealth status at preventing LBW in Indonesia. Women of reproductive age in the wealthiest group but attending <4 ANC visits were more likely to have LBW babies than those attending ≥4 ANC visits. This is because ANC visits provide an insight into maternal health, advice, medical treatment, and nutritional supplements (Denny *et al.,* 2022; Wulandari e*t al.,* 2021).

In this research, education level was also found to be more beneficial than employment status when controlling LBW occurrence. The study found that unemployed women with higher education levels were less likely to have LBW babies. Education is beneficial to health because teaching is a motivational opportunity to live healthily. Education can increase the women's skills and give them a chance to live a healthy life (Pakaya *et al.*, 2022). Women with a higher education level are more likely to have a healthier pregnancy and therefore less likely to encounter the risk of having LBW babies (Laksono *et al.,* 2021; Rohmah *et al.,* 2020).

This study was conducted using a quantitative approach, thus results obtained were superficial. This study cannot explain the phenomena obtained from previous studies such as the value of children and family values, including dietary restrictions for pregnant and lactating women (Kusrini, Ipa & Laksono, 2019; Laksono *et al.,* 2020).

CONCLUSION

Five factors related to LBW in Indonesia were determined in this study: maternal age, wealth status, education level, ANC visits, and gender of the baby. This study implies that it can provide specific targets for policy makers if they want to accelerate the reduction of LBW in Indonesia. These targets are young women, prospective brides, and pregnant women who are poor and have a low education level. On the other hand, policy makers need to encourage a policy for complete ANC visits among pregnant women because it has been proven to reduce the risk of LBW.

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Authors' contributions

NR, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; MM, prepared the draft of the manuscript and reviewed the manuscript; NBM, led the data collection, advised on the data analysis and interpretation, and reviewed the manuscript; NP, led the data collection, advised on the data analysis and interpretation, and reviewed the manuscript; JP, conducted the study, data analysis and interpretation; SW, conducted the study, data analysis and interpretation; ADL, assisted in drafting of the manuscript and reviewed the manuscript.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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The effect of goat's milk consumption on the clinical health of middle-aged adults with lactose intolerance

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ABSTRACT

Introduction: People with lactose intolerance are suggested to consume dietary items containing less lactose, such as goat's milk. This study aimed to investigate the effects of goat's milk powder on the health of lactose intolerant middle-aged adults. **Methods:** A total of 60 subjects were recruited into this randomised controlled trial. They were divided into four groups and received different dietary interventions (goat's milk, goat's milk with curcumin, goat's milk with coffee, lactose-free milk) for five weeks. Health effects were compared between pre- and post-intervention. Anthropometric and biochemical parameters (blood glucose, insulin, lipid profile, C-reactive protein, and lactoferrin) were evaluated. Dietary intake was recorded using a food record. **Results:** Fifty-one lactose intolerant subjects completed the study. After ingestion of goat's milk, there were significant reductions in body fat (p=0.033) and a significant increase in the percentage of muscle (p=0.021). Waist circumference (WC) decreased in both the goat's milk with curcumin and goat's milk with coffee groups (p<0.05 for all). Unfortunately, high-density lipoprotein cholesterol (HDL-C) dropped after the five-week intervention in the goat's milk group (p=0.002). Lactoferrin level of the goat's milk group was higher than other groups at post-intervention (p<0.001). Besides, the goat's milk with coffee group seemed to consume more carbohydrates after completing the intervention (p=0.034). **Conclusion:** A five-week intake of goat's milk reduced the risk of abdominal obesity among middle-aged adults. In addition, it resulted in improved lactoferrin levels.

Keywords: goat's milk, lactoferrin, lactose-free, lactose intolerance, middle-aged adults

INTRODUCTION

Lactose is a disaccharide which consists of glucose and galactose, and appears in mammalian milk (Deng *et al.*, 2015). To absorb lactose, the body needs the digestive enzyme, lactase, to break down lactose's glycosidic bond. Generally, lactase can be found on the mucosal surface of the small intestine from eight week gestation onwards (Deng *et al.*, 2015). When lactase is deficient, digestion of lactose in the small intestine is disrupted, and lactose will be delivered to the large intestine. Colonic bacteria within the large intestine will then ferment this undigested lactose, eventually causing digestive symptoms

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such as nausea, bloating, flatulence, diarrhoea, and abdominal cramps. This disordered condition is called lactose intolerance (Law, Conklin & Pimentel, 2010).

Impaired lactose digestion is usually found in Asian adults due to age-related lactase reduction. Lactose intolerant people are recommended to avoid foods or beverages that contain lactose, such as cow's milk and dairy products. However, this probably leaves lactose intolerant people susceptible to nutrient deficiencies, especially nutrients found in milk and dairy products, such as highly-bioavailable calcium (National Institute of Diabetes and Digestive and Kidney Disease, 2020). To prevent nutrient deficiency, lactose-free milk or alternative milk products containing less lactose are suggested.

Goat's milk and its products are nutrient-rich alternatives, with a small lactose content. Additionally, with smaller fat globules and softer casein curd, goat's milk can be digested easier than cow's milk (Banjare *et al.*, 2017). Goat's milk has been studied for centuries. However, previous studies frequently focused on infants. A double-blind study among 72-hour-old infants was conducted to investigate the efficiency of goat's milk and cow's milk. Infants were randomly given 150-200 ml goat's milk or cow's milk per kilogram (kg) body weight per day until 168 days of age. At the end of the study, the frequency of intestinal movements in infants who received goat's milk infant formula was significantly better than those who received cow's milk infant formula (Grant *et al.*, 2005). This may have been a result of the differences in digestibility between goat's milk and cow's milk. In addition, goat's milk infant formula has been reported to contain a comparable level of prebiotics compared with human milk. The prebiotics found in goat's milk promote the growth of beneficial gut bacteria, as well as reduce the growth of pathogenic bacteria (Leong *et al.*, 2019).

Besides, to promote gut health, curcumin, a polyphenol contained in turmeric, has also been widely utilised. Curcumin considerably increases the beneficial bacteria and reduces the pathogenic bacteria in mice. It has also been found to induce weight loss in ovariectomised rats (Zam, 2018). Consistently, coffee has been reviewed for its effect on the gut. Due to its prebiotic constituents, consumption of coffee has been found to increase *Bifidobacterium* in both animals and humans (Cowan *et al.*, 2014).

Regarding the health benefits of goat's milk, current data is limited to younger age groups. Studies on the effect of goat's milk consumption among middle-age adults with lactose intolerance are insufficient. Therefore, this study was conducted to investigate whether consumption of goat's milk, goat's milk with curcumin, goat's milk with coffee or lactose-free milk promotes the health of lactose intolerant middle-aged adults.

MATERIALS AND METHODS

Study subjects

Subjects were recruited using a purposive sampling technique. Sixty lactose intolerant adults (40-60 years old) living in Bangkok, Thailand participated in this single-blind randomised controlled trial study, of which 51 completed the study. The subjects were asked to present their last 6-12 months medical check-up report. Those who were diagnosed with chronic diseases such as diabetes, chronic kidney disease and cancer, had any infection or inflammation six months prior to the study, were currently taking medication or nutritional supplements, smoking, or regularly drinking alcohol were excluded. Pregnant or lactating

women were also excluded. Subjects were individually informed of the risks, discomforts, and benefits associated with the study before providing their signed informed consent. The study procedure was approved by the Ethics Committee (Certificate of Approval No. MUPH 2019-129) and the trial was registered with the Thai Clinical Trials Registry (TCTR20210219001). Subjects were initially screened for the symptoms of lactose intolerance using an online questionnaire. Based on the questionnaire responses, those who had symptoms of lactose intolerance were asked to fast for eight hours, then blood samples were collected at baseline and 120 minutes after drinking 50 g of lactose. Subjects with a blood glucose increase of <1.1 mmol/L were defined as lactose intolerant.

Study intervention

All subjects were allocated to four study groups using stratified and blocked randomisation techniques. Each group was requested to consume isocaloric (approximately 211 kcal/day) milk (either goat's milk or lactose-free milk) contained in a sealed aluminium foil bag. The goat's milk provided to each group was similar and originated from the same source. The duration of the dietary intervention was five weeks. The experimental products which were given to each group were as follows: 1) goat's milk group: 40.0 g goat's milk powder (contains 31.9% fat, 28.0% protein, 32.3% lactose); 2) goat's milk + curcumin group: 40.0 g goat's milk powder and 0.025 g extracted curcumin powder (contains 31.9% fat, 28% protein, 32.3% lactose); 3) goat's milk + coffee group: 40.0 g goat's milk powder and 0.025 g coffee powder (contains 31.9% fat, 28.0% protein, 32.3% lactose); and 4) lactose-free group: 46.5 g lactose-free milk powder (contains 30.0% fat, 16.0% protein, lactose-free).

Dietary and anthropometry assessments

Subjects were requested to take photos of their dietary items prior to and after consuming them, and report their dietary intake three times a week (two weekdays and one weekend) using the food record provided. To estimate energy and macronutrient intakes, the NutriSurvey program (Copyright© 2007, SEAMEOTROPMED RCCN-University of Indonesia) was conducted.

At baseline and after the five-week intervention, anthropometric assessment and biochemical evaluation were performed. Regarding anthropometry, subjects' weight, percentage body fat, percentage visceral fat, and percentage muscle were assessed by using a body composition monitor (HBF-375, Omron Healthcare, Japan). To reduce errors, waist circumference (WC) and height were measured three times. WC at umbilical level was measured using a measuring tape to define abdominal obesity. After measuring height by a stadiometer, body mass index was calculated by dividing weight (kg) with height squared (m^2).

Biochemical parameters assessment

Blood pressure was taken using an automatic blood pressure monitor (HEM 7120, Omron) before blood sample withdrawal. Blood sampling was performed after an eight-hour fast. Fasting blood glucose (FBG) and lipid profile (total cholesterol: TC; high-density lipoprotein cholesterol: HDL-C; low-density lipoprotein cholesterol: LDL-C; triglyceride: TG) were evaluated using a Cobas® 6000 analyser (Roche Diagnostics Ltd., Switzerland). Fasting insulin was examined using a human insulin Elisa kit (ab200011, Abcam). FBG and fasting insulin were then used to assess insulin resistance by applying the homeostatic model assessment of insulin resistance (HOMA-IR) equation as follows: HOMA-IR = [Fasting insulin (μU/

ml) × FBG (mmol/L)] / 22.5 (Matthews *et al.*, 1985). High-sensitivity C-reactive protein (hs-CRP) was determined using the nephelometry method. Lactoferrin concentration was measured using the enzyme-linked immunosorbent assay technique.

Statistical analysis

Sample size was computed using G*Power programme. The minimum sample in each group for detecting the difference of -3.7±2.7 cm in WC and -12.4±15.2 nmol/L in hs-CRP between pre- and post-intervention, with 80% power and α=0.05 was 11. Statistical analysis was performed using the Statistical Package for Social Science (SPSS) software for Windows version 18 (IBM Corp., United States). Differences between the four study groups before and after the intervention were determined by Kruskal-Wallis test and post-hoc Mann-Whitney U test. The Wilcoxon signed-rank test was utilised to evaluate differences between pre- and post-intervention within each study group. Data were expressed as median [interquartile range (IQR)]. A p<0.05 was considered to be statistically significant.

RESULTS

Baseline characteristics of study subjects

After screening, 60 subjects participated in this study. Nine subjects declined to participate in this study during the intervention period, thus there were 51 subjects (47 women and 4 men) at the end of the study (Figure 1). The median [IQR] age of subjects was 50 [45.0-54.0] years old. Both systolic and diastolic blood pressures of each study group were comparable (Table 1). There were no significant differences in anthropometric parameters when compared between study groups (Table 1). Regarding biochemical parameters, the goat's milk group had significantly higher median hs-CRP than the goat's milk with coffee group (p=0.031) and the lactose-free group (p=0.001) as shown in Table 2. Other biochemical parameters and dietary intake at baseline were similar among the four study groups (Tables 2 and 3).

Effects of goat's milk on blood pressure, anthropometric parameters, biochemical parameters, and dietary intake

The effect of experimental milks on study parameters after the five-week intervention are presented in Tables 1 to 3. Comparing between study groups, noteworthy differences were found in lactoferrin (p<0.001). The goat's milk group had significantly higher lactoferrin concentration relative to the other groups (goat's milk group vs. goat's milk with curcumin group: 1,029.1 [1,021.6-1,038.3] µg/L vs. 702.4 [381.3-945.3] µg/L, p=0.001; goat's milk group vs. goat's milk with coffee group: 1,029.1 [1,021.6-1,038.3] µg/L vs. 908.4 [832.4-986.1] µg/L, p=0.001; goat's milk group vs. lactose-free group: 1,029.1 [1,021.6-1,038.3] µg/L vs. 926.2 [682.5-996.1] µg/L, p<0.001). Additionally, the lactoferrin level of the goat's milk with coffee group was remarkably higher than that of the goat's milk with curcumin group (908.4 [832.4-986.1] µg/L vs. 702.4 [381.3-945.3] µg/L, p=0.041).

Comparing within each study group, notable changes appeared in blood pressure, anthropometric parameters, and biochemical indices as shown in Tables 1 and 2, respectively. The lactose-free group experienced a significant decrease in systolic blood pressure after the five-week intervention (week 0: 122.5 [110.0-140.5] mmHg vs. week 5: 112.5 [105.3-143.8] mmHg, p=0.037). Regarding anthropometry, the 5th-week body mass index of the lactose-free group was significantly increased compared to

baseline (week 0: 24.4 [20.0-27.9] kg/m^2 vs. week 5: 24.6 [20.2-28.4] kg/m^2, p=0.040). The subjects who consumed goat's milk with curcumin or with coffee had significantly reduced WC at post-intervention (week 0: 82.5 [76.6-85.8] cm vs. week 5: 78.5 [73.9-85.0] cm, p=0.038 and week 0: 83.0 [75.5-92.5] cm vs. week 5: 80.0 [76.0-88.0] cm, p=0.008, respectively). Body fat percentage of the goat's milk group also significantly dropped upon completion of the study (week 0: 34.9 [33.4-37.6] vs. week 5: 33.5 [32.2-37.7], p=0.033). Simultaneously, muscle mass percentage of the goat's milk group substantially increased after the five-week intervention (week 0: 22.6 [22.1-23.4] vs. week 5: 22.9 [22.3-24.1], p=0.021).

No significant differences were observed in FBG, fasting insulin, and HOMA-IR between pre- and post-intervention for all study groups (Table 2). Regarding lipid profile, subjects who consumed lactose-free milk experienced a remarkable decline in TC after the intervention (week 0: 5.5 [4.8-6.5] mmol/L vs. week 5: 5.3 [4.5-5.7] mmol/L, p=0.006). HDL-C of the goat's milk group and the lactose-free group had notably decreased after the five-week intervention (week 0: 1.6 [1.4-2.0] mmol/L vs. week 5: 1.5 [1.4-1.8] mmol/L, p=0.002 and week 0: 2.2 [1.5-

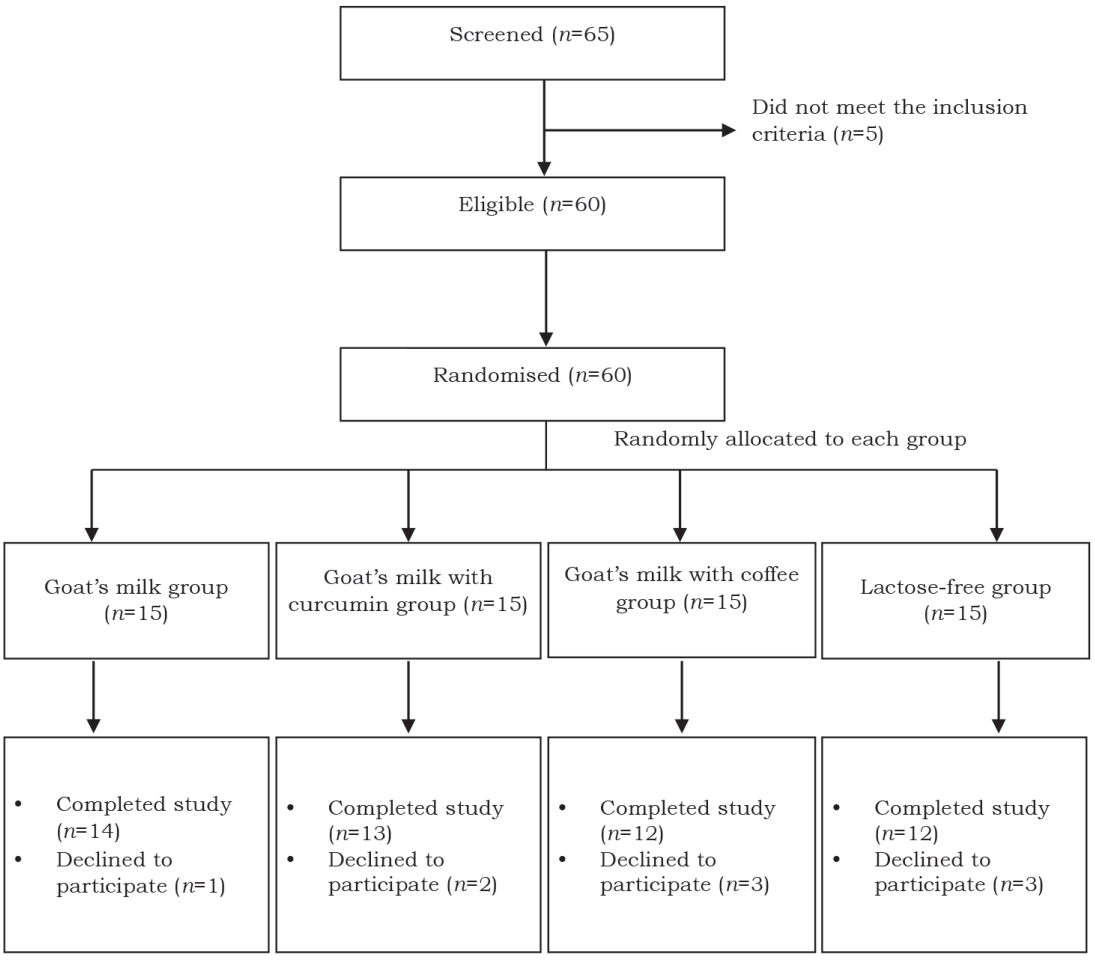

Figure 1. Flow diagram of the study

Table 1. Comparison of subject's blood pressure and anthropometric parameters between pre- and post-intervention

Variables	*Goat's milk*	*Goat's milk with curcumin*	*Goat's milk with coffee*	*Lactose-free*	*p*†
	Median [IQR]	*Median [IQR]*	*Median [IQR]*	*Median [IQR]*	
n (female/male)	14 (12/2)	13 (12/1)	12 (12/0)	12 (11/1)	0.609‡
Age (years)	50.0 [43.5-53.0]	52.0 [45.5-55.0]	48 [41.3-54.5]	48.0 [41.3-54.5]	0.619
Systolic blood pressure (mmHg)					
Week 0	121.5 [113.5-128.0]	128.0 [110.5-135.5]	122.0 [108.0-125.0]	122.5 [110.0-140.5]	0.569
Week 5	116.5 [110.0-123.0]	117.0 [110.5-128.0]	118.0 [107.0-120.0]	112.5 [105.3-143.8]	0.924
p§	0.157	0.074	0.286	0.037*	
Diastolic blood pressure (mmHg)					
Week 0	80.0 [71.8-86.3]	76.0 [69.0-85.5]	74.0 [70.0-78.0]	74.0 [67.0-93.3]	0.662
Week 5	77.0 [70.5-81.5]	75.0 [66.8-83.3]	71.0 [67.5-77.3]	75.0 [61.5-92.8]	0.585
p§	0.068	0.540	0.371	0.134	
Weight (kg)					
Week 0	58.9 [55.2-62.7]	51.6 [50.5-52.4]	55.6 [51.3-65.9]	56.0 [48.9-68.8]	0.177
Week 5	58.6 [54.4-62.7]	51.6 [50.0-52.0]	54.8 [51.0-66.0]	55.8 [49.3-70.0]	0.186
p§	0.056	0.944	0.212	0.247	
Body mass index (kg/m^2)					
Week 0	23.4 [22.4-26.8]	21.6 [19.6-22.8]	23.9 [19.9-25.6]	24.4 [20.0-27.9]	0.213
Week 5	23.3 [22.4-26.9]	21.6 [19.3-23.1]	23.6 [20.3-25.8]	24.6 [20.2-28.4]	0.268
p§	0.194	0.786	0.478	0.040*	
Waist circumference (cm)					
Week 0	84.7 [79.5-95.1]	82.5 [76.6-85.8]	83.0 [75.5-92.5]	87.3 [77.9-102.5]	0.381
Week 5	86.8 [78.6-91.4]	78.5 [73.9-85.0]	80.0 [76.0-88.0]	87.9 [80.5-99.4]	0.137
p§	0.055	0.038*	0.008**	0.286	
Body fat (%)					
Week 0	34.9 [33.4-37.6]	31.9 [27.8-33.2]	32.9 [29.8-37.9]	32.1 [29.0-37.8]	0.071
Week 5	33.5 [32.2-37.7]	31.3 [27.7-32.9]	32.2 [29.9-37.7]	30.8 [28.8-36.9]	0.120
p§	0.033*	0.230	0.307	0.084	
Visceral fat (%)					
Week 0	6.5 [5.0-9.6]	5.0 [3.0-6.8]	5.8 [2.9-6.9]	4.0 [3.3-8.5]	0.139
Week 5	6.3 [5.0-9.5]	4.8 [3.1-7.3]	5.8 [3.0-6.9]	4.0 [3.3-8.5]	0.200
p§	0.083	0.257	0.655	0.157	
Muscle (%)					
Week 0	22.6 [22.1-23.4]	23.9 [22.7-25.4]	24.9 [23.2-25.5]	23.7 [21.5-24.5]	0.092
Week 5	22.9 [22.3-24.1]	24.0 [22.9-25.5]	24.6 [23.6-25.1]	23.8 [20.7-24.5]	0.081
p§	0.021*	0.210	0.766	0.953	

Data are presented as median [interquartile range (IQR)].

†Significant difference between study groups before and after intervention based on Kruskal-Wallis test'

‡Significant difference between study groups based on Chi-square test

§Significant difference between pre- and post-intervention based on Wilcoxon signed-rank test, *p<0.05, **p<0.01

Table 2. The effect of goat's milk consumption on blood parameters

Variables	*Goat's milk*	*Goat's milk with curcumin*	*Goat's milk with coffee*	*Lactose-free*	$p^{\dagger}$
	Median [IQR]	*Median [IQR]*	*Median [IQR]*	*Median [IQR]*	
Fasting blood glucose (mmol/L)					
Week 0	4.9 [4.6-5.1]	4.8 [4.5-5.0]	5.1 [4.7-5.3]	4.8 [4.6-5.2]	0.666
Week 5	4.9 [4.8-5.0]	4.7 [4.6-4.9]	5.2 [4.7-5.3]	4.8 [4.7-5.2]	0.078
$p^{\ddagger}$	0.284	0. 580	0.091	1.000	
Fasting insulin (pmol/L)					
Week 0	20.8 [15.5-26-9]	17.6 [15.1-33.1]	14.3 [13.1-15.5]	20.6 [15.4-37.1]	0.099
Week 5	18.7 [14.7-18.7]	15.9 [11.1-32.6]	24.7 [13.0-30.7]	17.2 [14.0-31.1]	0.839
$p^{\ddagger}$	0.139	0.515	0.116	0.953	
HOMA-IR					
Week 0	0.5 [0.4-0.7]	0.7 [0.5-1.3]	0.5 [0.4-0.6]	0.6 [0.4-1.2]	0.352
Week 5	0.7 [0.5-1.1]	0.7 [0.4-1.5]	0.7 [0.4-0.9]	0.7 [0.5-1.6]	0.991
$p^{\ddagger}$	0.594	0.844	0.575	0.432	
Total cholesterol (mmol/L)					
Week 0	5.2 [4.7-5.7]	5.3 [5.1-6.0]	5.3 [4.9-5.9]	5.5 [4.8-6.5]	0.494
Week 5	4.9 [4.4-5.6]	5.5 [5.2-6.5]	5.6 [4.9-6.3]	5.3 [4.5-5.7]	0.226
$p^{\ddagger}$	0.084	0.695	0.272	0.006**	
Triglyceride (mmol/L)					
Week 0	1.0 [0.7-1.2]	0.9 [0.6-1.0]	1.0 [0.9-1.2]	0.8 [0.8-1.2]	0.648
Week 5	1.0 [0.9-1.2][a,b]	0.9 [0.6-1.1][a]	1.1 [1.0-1.4][b]	0.9 [0.8-1.1][a]	0.034*
$p^{\ddagger}$	0.925	0.953	0.066	0.824	
HDL-C (mmol/L)					
Week 0	1.6 [1.4-2.0]	1.9 [1.6-2.0]	1.7 [1.6-2.1]	2.2 [1.5-2.7]	0.203
Week 5	1.5 [1.4-1.8]	1.7 [1.4-2.0]	1.8 [1.5-1.9]	1.9 [1.5-2.4]	0.199
$p^{\ddagger}$	0.002**	0.152	0.075	0.006**	
LDL-C (mmol/L)					
Week 0	3.4 [2.4-3.9]	3.1 [2.7-3.5]	3.5 [3.0-3.7]	3.0 [2.7-4.1]	0.840
Week 5	3.2 [2.5-3.6]	3.1 [2.5-4.0]	3.7 [3.3-4.2]	2.9 [2.5-3.7]	0.200
$p^{\ddagger}$	0.600	0.789	0.114	0.091	
hs-CRP (nmol/L)					
Week 0	9.5 [6.4-22.8][a]	11.2 [3.8-30.2][a,b]	5.2 [3.0-7.9][b]	4.6 [3.9-5.5][b]	0.036*
Week 5	9.1 [5.4-14.0]	6.3 [4.0-32.0]	5.7 [3.8-6.3]	4.7 [3.3-6.2]	0.093
$p^{\ddagger}$	0.136	0.937	0.446	0.624	
Lactoferrin (µg/L)					
Week 0	966.7 [841.1-1033.7]	924.6 [873.1-1039.1]	1001.6 [954.9 -1039.5]	967.4 [848.1-1018.0]	0.541
Week 5	1,029.1 [1,021.6-1,038.3][a]	702.4 [381.3-945.3][b]	908.4 [832.4-986.1][c]	926.2 [682.5-996.1][b,c]	<0.001**
$p^{\ddagger}$	0.066	0.004**	0.013*	0.110	

Abbreviations: HOMA-IR, the homeostatic model assessment of insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein

Data are presented as median [interquartile range (IQR)]

[†]Significant difference between study groups before and after intervention based on Kruskal-Wallis test'

[‡]Significant difference between pre- and post-intervention based on Wilcoxon signed-rank test, *p<0.05, **p<0.01

[a,b,c]Different alphabets denote significant differences between the study groups using Kruskal-Wallis test and post-hoc Mann-Whitney U test

Table 3. Comparison of the subject's dietary intake at baseline and week 5

Variables	*Goat's milk*	*Goat's milk with curcumin*	*Goat's milk with coffee*	*Lactose-free*	*p*†
	Median [IQR]	*Median [IQR]*	*Median [IQR]*	*Median [IQR]*	
Energy intake (kcal/d)					
Week 0	1419 [1103-1769]	1375 [1045-1774]	1525 [1301-1819]	1242 [1079-1862]	0.801
Week 5	1566 [1281-2134]	1333 [1134-1755]	1949 [1331-2096]	1170 [935-1801]	0.264
p‡	0.069	0.477	0.139	0.374	
Carbohydrate (g/d)					
Week 0	180 [140-212]	161 [121-211]	180 [155-241]	229 [139-245]	0.527
Week 5	199 [175-308]	194 [146-238]	244 [194-299]	205 [125-249]	0.427
p‡	0.086	0.139	0.034*	0.374	
Protein (g/d)					
Week 0	55 [50-64]	59 [41-73]	66 [55-77]	57 [45-81]	0.622
Week 5	59 [46-69]	56 [46-62]	68 [60-78]	53 [47-63]	0.061
p‡	0.445	0.767	0.260	0.612	
Fat (g/d)					
Week 0	48 [43-80]	58 [52-72]	65 [51-78]	61 [49-75]	0.845
Week 5	68 [47-89]	61 [33-73]	70 [50-85]	42 [35-74]	0.246
p‡	0.260	0.508	0.646	0.013*	

Data are presented as median [interquartile range (IQR)]
†Significant difference between study groups before and after intervention based on Kruskal-Wallis test'
‡Significant difference between pre- and post-intervention based on Wilcoxon signed-rank test, $^{*}p<0.05$, $^{**}p<0.01$

2.7] mmol/L vs. week 5: 1.9 [1.5-2.4] mmol/L, *p*=0.006, respectively). Significant changes were also found in lactoferrin concentrations of the goat's milk with curcumin group and goat's milk with coffee group. At the end of the study, lactoferrin concentration was likely to be higher than its level at baseline in the goat's milk group, but not significant; while the goat's milk with curcumin group and goat's milk with coffee group expressed significantly lower levels (week 0: 924.6 [873.1-1,039.1] μg/L vs. week 5: 702.4 [381.3-945.3] μg/L, *p*=0.004 and week 0: 1001.6 [954.9-1,039.5] μg/L vs. week 5: 908.4 [832.4-986.1] μg/L, *p*=0.013, respectively).

Regarding dietary intake as presented in Table 3, no significant differences were observed in energy and protein intakes between pre- and post-intervention for all study groups. However, the goat's milk with coffee group consumed more carbohydrates at the end of the study (week 0: 180 [155-241] g/d vs. week 5: 244 [194-299] g/d, *p*=0.034). On contrary, compared to baseline, the median fat intake in the lactose-free group decreased at the end of the study (week 0: 61 [49-75] g/d vs. week 5: 42 [35-74] g/d, *p*=0.013).

DISCUSSION

Lactose malabsorption is a condition in which people

typically secrete low concentrations of β-galactosidase from the small bowel mucosa, thus the body cannot properly digest and absorb lactose in the small intestine. Most adults worldwide (approximately 70%), particularly in Asian countries, have been reported to have hypolactasia (Di Costanzo & Berni Canani, 2018). Restriction of lactose consumption is recommended for lactose intolerant people. Goat's milk is an alternative food, which comprises of approximately 4.2 g lactose/100 g, and is commonly used to replace higher lactose dairy products due to its digestibility, nutritional components, and hypoallergenic property (Szilagyi & Ishayek, 2018; Stergiadis *et al.*, 2019). Globally, especially in Asia, consumption of goat's milk has risen year by year, with increased goat's milk production (Skapetas & Bampidis, 2016). However, evidence in support of the effects of goat's milk consumption in lactose intolerant adults is not well understood, therefore this study aimed to clarify its effects.

The study results revealed significant alterations in body composition, lipid profile, and lactoferrin level after intake of goat's milk for five weeks. At baseline, the subjects were above the abdominal obesity cut-off point (WC >80 cm for women and >90 cm for men) (WHO, 2008). However, after the five-week goat's milk intervention, body fat remarkably decreased. Likewise, after intake of either goat's milk with curcumin or goat's milk with coffee, waist circumference significantly dropped. Reasonably, the muscle percentage among subjects who consumed goat's milk significantly elevated. The subjects reported no changes in their daily physical activities. When dietary intake was observed to define whether changes in body composition was a result from dietary pattern modification, the results showed a similar dietary pattern between baseline and at the end of the study. Actually, the lactose-free group tended to reduce food intake as there were negative changes in energy, carbohydrate, protein, and fat intakes; whereas the macronutrients intake of subjects who consumed goat's milk, goat's milk with curcumin, and goat's milk with coffee increased. These changes were probably a result of the higher protein content in goat's milk relative to that of lactose-free milk.

Consumption of goat's milk and lactose-free milk did not affect insulin resistance parameters including FBG, fasting insulin, and HOMA-IR, except those who consumed goat's milk fortified with coffee powder that were likely to exhibit improved fasting plasma insulin concentration. This might have been a result of the fortified coffee contained in the experimental milk. A study conducted in China revealed that subjects had greater insulin levels after consumption of coffee (Gao *et al.*, 2018). Similarly, Alperet *et al.* (2016) conducted a study and found an improvement in insulin sensitivity after daily consumption of coffee for 24 weeks. Likewise, when diabetic subjects received 5 mg caffeine/kg body weight, insulin concentrations significantly increased (Robinson *et al.*, 2004).

Regarding lipid profile, subjects who ingested lactose-free milk showed a reduction in TC. Surprisingly, an undesirable change was observed in anti-atherogenic blood cholesterol; HDL-C concentrations of the subjects who consumed goat's milk and lactose-free milk dropped following the intervention. Shin *et al.* (2017) also reported a reduction in HDL-C concentrations, whereby consuming >1 serving of milk per day (for men) and >2 servings daily (for women) was associated with lower HDL-C. Unfortunately, the authors did not collect data regarding the sub-types of milk. Likewise, Villalpando *et al.* (2015) conducted a study to determine the effects of consuming cow's milk on

blood cardiovascular indicators and found that consumers of defatted milk had significantly reduced TC, LDL-C, and HDL-C after a four-month intervention. In contrast, a three-week randomised cross-over study among healthy adults revealed a noteworthy increase in HDL-C after the ingestion of whole milk (Engel *et al.*, 2018). Differences in the response of HDL-C might be explained by differences in the proportion of fatty acids contained in the experimental milks. As a previous study reported, increments in LDL-C and HDL-C were observed after increasing intakes of saturated fatty acids (Lichtenstein, 2006).

Lactoferrin is an iron-binding glycoprotein which is found in mammalian milk, such as human milk, goat's milk, and sheep's milk. This protein plays an antimicrobial role, regulating the inflammatory responses and enhancing gastrointestinal tract health (Park & Nam, 2015). When lactoferrin is ingested, a derivative of lactoferrin named lactoferricin is created. This derivative peptide can potentially act against both gram-positive and gram-negative pathogenic bacteria (Newburg & Walker, 2007). Lactoferrin exerts anti-inflammatory action by promoting the activity of natural killer cells, phagocytic cells, and macrophages. Modulation of macrophages in response to inflammation results in the secretion of cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and nitric oxide (NO) (Hanson, 2007; Actor, Hwang & Kruzel, 2009). A previous study (Sorimachi *et al.*, 1997) reported that cytokines, such as TNF-α and NO, are secreted when macrophages are cultured with lactoferrin. Moreover, lactoferrin is capable of promoting gut health by stimulating the proliferation and differentiation of the intestinal cells (Playford, MacDonald & Johnson, 2000). In this study, an increased trend of lactoferrin level was observed after goat's milk ingestion. Consistently, Ochoa *et al.* (2013) conducted a randomised double-blind placebo-controlled trial to investigate the effect of lactoferrin on diarrhoea prevention among children. They reported a decrease in longitudinal prevalence and severity of diarrhoea after receiving 0.5 g of lactoferrin diluted in 25 ml of water twice daily.

CONCLUSION

In conclusion, this trial revealed positive health effects related to goat's milk consumption among middle-aged adults. Ingestion of goat's milk for five weeks could significantly reduce the risk of non-communicable diseases as found in the reductions of WC and body fat. Furthermore, the intake of goat's milk promotes gastrointestinal health by increasing blood lactoferrin concentration.

The current study still contains limitations. Firstly, the intervention period was short because it was interrupted by the pandemic of COVID-19. Secondly, the data observed in this study were mostly obtained from female subjects. Thus, further studies should investigate the long-term effect of goat's milk consumption and include men in order to define the different effects between men and women.

List of abbreviations

WC: Waist circumference; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; HOMA-IR: the homeostatic model assessment of insulin resistance; hs-CRP: high-sensitivity C-reactive protein.

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Authors' contributions

CP, principal investigator, conceptualised and designed the study, supervised data collection, advised on data analysis and interpretation, prepared the draft of the manuscript, and reviewed the manuscript; CP, led the data collection, data analysis and interpretation, assisted in drafting of the manuscript, and reviewed the manuscript; CH, conceptualised and designed the study, advised on data analysis and interpretation, and reviewed the manuscript; PPP, led the data collection and reviewed the manuscript; KK, advised on data analysis and interpretation, and reviewed the manuscript.

Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Validation of a Sustainable Diet Index among young Malaysian adults

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ABSTRACT

Introduction: A sustainable diet which is healthy and environmentally friendly provides the means of climate change mitigation in addition to promoting health of the population. There is an urgent need to have an indicator to measure if one's diet is sustainable. This paper aimed to validate a newly developed Sustainable Diet Index (SDI) among young Malaysian adults. The SDI was developed based on the dietary guidelines of a sustainable diet. **Methods**: Five indicators (rice, animal-based food, plant-based food, food waste, and packaging) were included in the SDI. The index was validated via content validity, exploratory factor analysis (EFA), and confirmatory factor analysis (CFA) among young Malaysian adults. The dietary assessment tool used was an Android application named Sustainable Food Record. **Results**: Content validity showed fair to moderate correlations (0.331 - 0.816) between the indicators in the SDI. EFA produced five final factors with eight indicators in the index as follows: 1) fruits and vegetables; 2) dairy, eggs, and meat; 3) rice, cereals, and grain products; 4) food packaging; and 5) food waste management with strong factor loadings (0.760 – 0.984). All five factors with eight indicators were retained and proceeded with CFA. The fit indices from CFA demonstrated that the model was an absolutely fit. **Conclusion**: The validated SDI can be used as a tool to measure the sustainability of an individual's diet in Malaysia, incorporating both health and environment considerations.

Keywords: environment, health, sustainable diet index, validation

INTRODUCTION

Sustainable diets are protective and respectful of biodiversity and eco-systems, culturally acceptable, accessible, economically fair and affordable, nutritionally adequate, safe and healthy, while optimising natural and human resources (FAO, 2010). Previous studies and existing guidelines suggest to reduce meat intake and substitute them with plant-based protein, increase the consumption of vegetables and fruits, choose seasonal and local products, choose organic foods, and opt for drinks in recyclable packaging (NHMRC, 2013; Fischer & Garnett, 2016) to achieve a sustainable healthy diet (Lagerberg, 2013).

National dietary guidelines are the main sources of reference for recommendations on healthy diets for the population. Food Climate Research Network (FCRN) (2016) reported that out of 83 available dietary guidelines

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globally, only four countries (Brazil, Germany, Sweden, and Qatar) considered sustainability factors or ecological concerns in their main messages (Fischer & Garnett, 2016). Diet has a direct effect on human health and well-being; while its indirect effects on the environment was newly introduced via the concept of sustainable diet (FAO, 2010).

Globally, there are few studies that focus on environmental sustainability, food system, assessment of sustainable diet and healthy diet. The Environment Performance Index (EPI) was developed as a performance indicator on environmental areas of a country (Hsu & Zomer, 2014). Besides, the Food Sustainability Index (FSI) was also developed to assess the sustainability of a food system (The Economist Intelligence Unit & Barilla Center for Food & Nutrition, 2017). On the other hand, the Diet-related Greenhouse Gas Index was developed to assess the environmental impact of Danish diet (Lund *et al.*, 2017). As for the assessment of healthy and sustainable diet at an individual level, two Sustainable Dietary Indices (SDI)s were developed for the French and Australian population, respectively (Harray *et al.*, 2015; Seconda *et al.*, 2019).

The general Malaysian diet was found to be low in carbon footprint, where some ethnic groups contributed more carbon footprint emission than others (Moy *et al.*, 2020). Adaptation of a sustainable diet can improve the qualities of diet and environment in the long term. The roles of individuals in making changes to environmental issues like climate change are under-estimated as it is hard for individuals to appreciate the significance of the cumulative impact from their small actions (Dahl, 2012). An assessment of the population's diet in terms of its contribution to health and environment will provide explanation and increase the awareness on sustainable diets. However, there is no available indicator assessing the sustainability of our country's diet. To fill this gap, an SDI was developed to measure the sustainability of the Malaysian diet. This paper described the validation of the SDI using exploratory and confirmatory factor analyses among young Malaysian adults.

MATERIALS AND METHODS

The SDI was developed based on the Malaysian Dietary Guidelines (MDG) 2010 and other countries' dietary guidelines that included sustainability or environmental aspects in their recommendations (Sustainable Development Commission, 2009; Health Council of the Netherlands, 2011; Williamson, 2011; Fogelholm, 2013; Lagerberg, 2013; NHMRC, 2013; Oberritter *et al.*, 2013; Constante Jaime *et al.*, 2014; Garnett *et al.*, 2014; Seed, 2014; Montagnese *et al.*, 2015; Ruini *et al.*, 2015). This index is intended to measure the level of healthy and sustainable diet behaviours among Malaysian adults.

Under this health proxy, the scoring method used in the Malaysian Diet Quality Index study was adapted (Fokeena, Jamaluddin & Khaza'ai, 2016). A maximum score of five was allocated when the recommended intake of servings per day was met. As for the environment proxy, the same scoring method in the France SDI was adapted, where median of the food carbon footprint was used as the cut-off value (Seconda *et al.*, 2019). The Comstock 6-point scale method was used to evaluate food waste percentage (Comstock, St Pierre & Mackiernan, 1981). Meanwhile, the score for the management of food waste and packaging were allocated according to the waste management hierarchy in the Waste Hierarchy Guidance using the Waste Management Pyramid (DEFRA,

Table 1. Scoring methods for health indicators, food waste and management of packaging used

Environmental indicators	*Score*					
	5	*4*	*3*	*2*	*1*	*0*
Percentage	0–10%	25%	50%	75%	90%	100%
Food waste management	0 waste	re-use/ give to others	animal feed	composting	other recovery	disposal
Management of packaging used	0 waste	re-use	give to other	recycling	other recovery	disposal

2011). Table 1 presents the scoring system for all indicators.

The sum of all indicators in both proxies were then summed up. The scores for health and environment proxies contributed to 50% each. Both scores were summed up and the final SDI score that ranged from 0 to 100 was derived. Higher SDI score indicated better compliance towards the practice of a healthy and sustainable diet. The development of SDI was published elsewhere (Zulkefli & Moy, 2021).

This study was of cross-sectional design among students from a public university in Malaysia. Hair *et al.* (2014) proposed that a sample size of 100 was adequate for the calculation of correlations between variables. Participants' recruitment was carried out by email invitations to all students via their official university emails. Promotion posters were also distributed within the campus. Their participation was voluntary. The inclusion criteria were students from that particular university, Malaysian citizen, and using a smartphone with Android operating system. Meanwhile, individuals who were pregnant or breastfeeding and following a restrictive diet were excluded.

Ethics approval from the University Malaya Ethics Committee (Reference Number: UM.TNC2/UMREC - 478) was obtained before the study was conducted. Permission from the university's Student Affair Division was also obtained. Written informed consent was obtained from all participants. The participants were randomly divided into two groups. Content validity and exploratory factor analysis (EFA) were carried out among participants in Group 1 (n=100), while confirmatory factor analysis (CFA) was carried out in Group 2 (n= 51).

An android application named Sustainable Food Record (SFR) was developed to upload food images (foods or dishes and drinks in a meal) taken using smart phones to be used as a dietary assessment tool for the index, similarly as reported by Harray *et al.* (2015). Participants were required to capture images of foods and drinks taken before and after eating occasions. The images were captured and uploaded in the SFR application from two different angles (45° and 90°) on all meals taken for three days. The food image recognition and quantification executed by SFR were conducted manually by the researchers. The food images demonstrated acceptable relative validation and reliability for the macro- and micronutrients intakes when tested against the traditional 24-hour diet recall (Ho *et al.*, 2021).

Content and construct validation were conducted to validate the SDI. Content validity is defined as the extent to which the items selected represent a summated scale and its conceptual definition (Joseph *et al.,* 2014). Content

validation was carried out by assessing the correlation between the individual indicators and the index. Construct validation was assessed using EFA and CFA (Hurley *et al.*, 1997). Construct validation using factor analysis was conducted to confirm the indicators belonged to the same group as allocated. Principal component analysis (PCA) with Promax rotation was used in EFA. CFA maximum likelihood (ML) was assessed via AMOS (Analysis of Moment Structures) version 20 to confirm the underlying factor structure and model fit of the data. The study sample used for CFA was mutually exclusive from samples used in EFA analysis. Descriptive statistics were presented as frequencies (*n*) and percentages (%) or means and standard deviations (*SD*). Results with *p*-values of <0.05 were considered statistically significant. All statistical analyses were conducted using the Statistical Package for Social Sciences version 17.0 software (SPSS, Inc. Chicago, IL, USA) and Microsoft Excel spreadsheet.

RESULTS

A total of 185 students participated in the study where 151 students were included in the analysis as 34 were excluded due to non-eligibility, withdrawal from the study, and missing data. Most participants were females (70.9%), half were of Chinese ethnic origin, slightly more than half were from Science background and in undergraduate study. Their mean±*SD* age was 24.7±5.2 years old (Table 2).

In content validation, all indicators, with the exception of indicators E1, E2, and E5, showed fair to strong correlations with the SDI (*R*=0.331-0.721). Negative correlation was found between E1 (*R*=-0.250) with the SDI. In addition, health proxy (*R*=0.816) correlated better with the SDI as compared to the environment proxy (*R*=0.408) (Table 3).

Table 2. Demographic characteristics of participants

Characteristics	*Total*	*EFA (n=100)*	*CFA (n=51)*
	n (%)	*n (%)*	*n (%)*
Gender			
Male	44 (29.1)	27 (27.0)	17 (33.3)
Female	107 (70.9)	73 (73.0)	34 (66.7)
Ethnicity			
Malay	54 (35.8)	39 (39.0)	15 (29.4)
Chinese	88 (58.3)	54 (54.0)	34 (66.6)
Indian	7 (4.6)	6 (6.0)	1 (2.0)
Other	2 (1.3)	1 (1.0)	1 (2.0)
Students' background			
Art-based	61 (40.4)	44 (44.0)	17 (33.3)
Science-based	90 (59.6)	56 (56.0)	34 (66.7)
Level of study			
Undergraduate	85 (56.3)	53 (53.0)	32 (62.7)
Master	51 (33.8)	34 (34.0)	17 (33.3)
PhD	15 (9.9)	13 (13.0)	2 (4.0)
Accommodation			
University's hostel	109 (72.2)	66 (66.0)	43 (84.3)
Out of campus	42 (27.8)	34 (34.0)	8 (15.7)
Age in years (Mean±*SD*)	24.7±5.2	24.8±5.1	24.6±5.3

Table 3. Correlation between all indicators with total SDI

	Mean±SD	*Pearson correlation coefficient*	*p-value*
Total SDI	68.41±7.83		
Health proxy	28.25±7.29	0.816	<0.001
H1	8.11±3.55	0.331	0.001
H2	6.51±2.62	0.351	<0.001
H3	13.64±5.34	0.721	<0.001
Environment proxy	41.16±4.61	0.408	<0.001
E1	8.03±1.86	-0.250	0.012
E2	8.42±1.31	-0.047	0.645
E3	1.55±0.50	0.462	<0.001
E4	7.28±2.95	0.399	<0.001
E5	9.68±0.69	0.105	0.297

H1= Fruits and vegetables (portion size); H2= Meat (portion size); H3= Rice (portion size); E1= Fruits and vegetables (carbon footprint); E2= Meat (carbon footprint); E3= Rice (carbon footprint); E4= Food packaging; E5= Food waste

The following results: KMO=0.501; Bartlett test: χ^2=166.35, df=28 (p<0.001) indicated that the data were suitable for factor analysis. PCA with Promax rotation produced a five-factor solution with eigenvalues above 1.0, accounting for 82.7% of total variance (Table 4). The total variance of 82.7% suggested satisfactory results to ensure practical significance for the derived factors.

After EFA, there were five factors with high factor loadings (0.760–0.984) retained (Table 5). However, there was some modification in the grouping of indicators as suggested by EFA. H1 and E1 were grouped as F1, while H2 and E2 were combined into one factor, F2. Similarly, H3 and E3 were placed into the same factor as F3. Meanwhile, E4 and E5 both remained within their individual groups, F4 and F5, respectively. Two of the indicators, E1 (-0.948) and E2 (-0.788) produced negative factor loadings.

Table 4. Results of factor extraction using principal component analysis with promax rotation

Factor	*Initial eigenvalues*			*Extraction sums of squared loadings*			*Rotation sums of squared loadings*†
	Total	*% of variance*	*Cumulative %*	*Total*	*% of variance*	*Cumulative %*	*Total*
1	1.99	24.93	23.93	1.99	24.93	24.93	1.93
2	1.41	17.58	42.50	1.41	17.58	42.50	1.37
3	1.13	14.16	56.66	1.13	14.16	56.66	1.21
4	1.08	13.51	70.17	1.08	13.51	70.17	1.10
5	1.00	12.54	82.70	1.00	12.54	82.70	1.05

†When factors are correlated, sums of squared loadings cannot be added to obtain a total variance

Table 5. Rotated factor matrix for all indicators

Indicators	*Factors*†				
	F1	*F2*	*F3*	*F4*	*F5*
F1: Fruits and vegetables					
H1 Serving size	0.958				
E1 Carbon footprint	-0.948				
F2: Dairy, meat, chicken, and fish					
H2 Serving size		0.849			
E2 Carbon footprint		-0.788			
F3: Rice, cereals, and grain products					
H3 Serving size			0.779		
E3 Carbon footprint			0.760		
F4: Food packaging					
E4 Waste management				0.925	
F5: Food waste					
E5 Percentage and waste management					0.984

†Factors: F1:Fruits and vegetables; F2:Dairy, meat, chicken, and fish; F3:Rice, cereals, and grain products; F4:Food packaging; F5:Food waste (*N*=100)

The final five factors and their distribution of indicators previously extracted from EFA were tested for CFA. The fit indices demonstrated that the model with five factors and eight indicators was an absolute fit (χ^2=5.844, df=12, χ^2/df=0.487, GFI=0.972, NFI=0.955, CFI=1.000, AGFI=0.916, RMSEA=0.000, TLI=1.142).

DISCUSSION

Majority of the participants were young adults, females, and undergraduate students. The higher response rate among females was expected as the Malaysia Higher Education Institutes (2018) reported that the ratio of male to female was 1:1.6 in public institutions (Ministry of Education Malaysia, 2018).

The content validation showed that all indicators exerted substantial contribution on the participants' scoring and ranking in the index (Seconda *et al.*, 2019). The results showed fair to moderate correlations between the indicators and the SDI. There were negative correlations between the environmental indicators for plant-based and meat-based food in terms of their carbon footprint with total SDI. Higher score on the food's carbon footprint reduced the overall SDI score, which indicated less sustainable diet practice.

Our results showed that the indicators under the health proxy were highly correlated with the SDI. However, Seconda *et al.* (2019) found environment proxy to be better correlated with their SDI. The difference might be due to different indicators used in each index, different dietary patterns among the populations, and the different sources of carbon footprint data used in the calculation (Garnett *et al.,* 2014; Seed, 2014; Ho *et al.,* 2021).

EFA produced a five-factor structure which explained 82.7% of the variance. All eight original indicators were retained as they had high factor loadings. Indicators were grouped

into their own respective food groups, regardless of their representativeness in health or environment proxies. Portion size and carbon footprint of each food group were combined into one factor, instead of separately by health and environmental factors. For example: H1 (fruits and vegetables, portion size) was grouped with E1 (fruits and vegetables, carbon footprint) into one factor, F1. This applied to F1, F2, and F3. These results might be due to the use of serving size in calculating both health and environmental impacts from the indicators.

Two of the indicators, E1 (fruits and vegetables) and E2 (meat) showed negative correlations or negative direction of the correlation, but only E1 was statistically significant. This does not affect the interpretation of the magnitude of the factor loading or the number of factors to retain (Yong & Pearce, 2013).

The final factors generated from EFA to be included in the SDI were: 1) fruits and vegetables; 2) meat, chicken, dairy, and fish; 3) rice, cereals, and grain products; 4) food waste; and 5) food packaging management. These final factors confirmed through CFA had good model fit. These factors are representative of the Malaysian diet where rice is the main staple of the country. The internal validation of the SDI is now complete and can be used for further assessment on the practice of sustainable diet among the population.

Our developed SDI differed from the other existing sustainable diet indices globally. It was developed based on a few dietary guidelines on sustainability and the MDG. The different categories incorporated in the index provided information to determine the barriers to practising a sustainable diet. Most of the other national SDIs were based on the definition of sustainable diet itself with focus on food groups, without incorporating environmental aspects, such as food waste and management, of food packaging. This may be the strength of our index as our SDI has included these two components, which contribute significantly to the environment (DEFRA, 2011).

A few limitations of this study need to be considered while interpreting the results. Firstly, the study sample was limited to university students and almost 60% were from the Chinese ethnic group. Thus, this population may not represent the general population as they were young adults with higher education level and there was low representation from the Malay and Indian respondents. Besides, since the MDG was used as a reference, the developed SDI is therefore only exclusive for the Malaysian population and not for people of other countries. In addition, the MDG 2010 was used in the current study, while MDG 2020 was just launched recently. Hence, the current SDI may need to incorporate the MDG 2020 in the future. Its validity, however, may be affected and should be re-established as there are variations in the serving sizes for food groups in the MDG 2020. Nevertheless, the validated SDI can be a pioneer in setting an easy and measurable indicator in the field of sustainability, incorporating both health and environmental aspects.

CONCLUSION

In summary, the validated SDI provides a novel and feasible method to measure the sustainability of eating practices at an individual level among the young Malaysian adult population. Future studies could further validate the SDI in more diverse adult population across Malaysia in terms of age and occupation groups.

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Authors' contributions

NFZ & FMM, conceptualised and designed the study; NFZ, prepared the draft of the manuscript and reviewed the manuscript; FMM, advised on data analysis and interpretation, and reviewed the manuscript.

Conflict of interest

None.

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Household income, frequency of purchasing outside meals, eating behaviour and body mass index status among undergraduate students during first phase of COVID-19 lockdown

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ABSTRACT

Introduction: COVID-19 lockdown has changed the eating behaviours of people, which could affect their body mass index (BMI). These changes affected meal purchasing habits of university students, depending on their household income. Thus, the current study aimed to investigate the association between eating behaviour, household income, frequency of purchasing outside meals with BMI among undergraduate students. **Methods:** This was a retrospective cross-sectional study conducted among 112 undergraduate students. Subjects recalled information during the first phase of COVID-19 lockdown, which was from March 2020 till July 2020. Questionnaire consisted of socio-demography, anthropometry, frequency of purchasing outside meals, and eating behaviour using the Malay version Dutch Eating Behaviour Questionnaire (DEBQ). **Results:** About 64.3% of subjects reported purchasing outside meals 1-2 times per week. Higher restrained eating behaviour score was correlated with purchasing outside meals about 3-4 times and >4 times a week. Normal weight students had significantly higher restrained eating behaviour score [3.0(1.1)] than those in the obese group [2.9(1.1)]. Household income had no association with frequency of purchasing outside meals. **Conclusion:** Eating behaviour affected BMI and the frequency of purchasing outside meals during COVID-19 lockdown. COVID-19 lockdown has resulted in tremendous changes in the eating behaviour and physical activity pattern of university students. Future studies should focus on increasing the nutrition knowledge of university students, especially on the aspect of eating out.

Keywords: body mass index, COVID-19, eating behaviour, household income, purchasing meals

INTRODUCTION

The worldwide pandemic of COVID-19 has caused a significant burden on public health and disruption in daily life. In Malaysia, the government introduced the enforcement of the Movement Control Order (MCO) effective from 18th March 2020, with the main aim of isolating the source of COVID-19 outbreak and as a strategy to flatten the pandemic curve in the country (Heikal Ismail *et al.*, 2020). During the restriction order, people had to stay at home, and all working activities were shuttered temporarily and turned

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into working from home (Pellegrini *et al.,* 2020).

The adverse mental health burden during the COVID-19 pandemic has been evaluated by a few studies and was greatly associated with increased weight gain (Pellegrini *et al.*, 2020). Social isolation has impacted people's lifestyle behaviours with increased sedentarism and reduced outdoor time, ultimately causing increased weight gain (Pellegrini *et al.*, 2020). Higher body mass index (BMI) was also shown to correlate with lower levels of physical activity, poor diet quality, and a greater frequency of overeating during the crisis (Tan, He & MacGregor, 2020). A study in Italy involving 41 children and adolescents with obesity found that participants reported less time exercising and increased consumption of junk foods than before the lockdown period (Pietrobelli *et al.,* 2020).

Malaysia is ranked as having the highest obesity rate in Southeast Asia. A study conducted among five public universities in Malaysia demonstrated that the prevalence of overweight and obesity among undergraduate students are 23.0% and 17.6%, respectively (Wan Mohamed Radzi *et al.*, 2019). The major factors that lead to obesity are inadequate physical activity, overeating, and other environmental factors such as stress and exposure to unhealthy foods (Wan Mohamed Radzi *et al.*, 2019). The COVID-19 pandemic may worsen and increase the prevalence of obesity in Malaysia as the crisis has impacted normal lifestyle behaviours.

University students' eating behaviour is influenced by various factors such as peer influence, adjusting to campus lifestyle, exam pressure, cost of living, nutrition knowledge, and cooking skills (Kabir, Miah & Islam, 2018). Before the COVID-19 pandemic, university students often purchased foods and beverages for lunch and snacks on campus. During the lockdown period, people had to stay at home; some might cook at home, while others might go out and buy food through an online ordering system or application. Food purchasing via online applications was not common before the pandemic due to cost (Tam *et al.*, 2016). However, during the pandemic lockdown period, food purchasing using online food applications increased, as it helped to avoid going to crowded places (Candra, Ayudina & Arashi, 2021; Hassen *et al.,* 2021).

Outside foods, or out-of-home foods, have become increasingly popular recently with online ordering applications and e-hailing deliveries such as Grabfood, Foodpanda, Dahmakan, Bungkusit, and many more. Due to easy accessibility to foods, they are thought to be one of the key contributory factors in the increasing number of overweight and obese individuals. In addition, most of the meals served in restaurants are unfavourable in terms of their nutritional content (Janssen *et al.,* 2018).

Food purchasing behaviour among consumers, especially university students, is affected by several factors such as purchasing intention, social pressure, socioeconomic status, food availability, and convenience (Whatnall *et al.,* 2021). Frequent out-of-home food consumption has been associated with a higher BMI, enhanced cardio-metabolic risk, and various negative health outcomes (Janssen *et al.,* 2018). Therefore, food type, quantity, and frequency of eating food outside of home may significantly impact nutrient intake levels and BMI status (Choi *et al.,* 2019). With that, this study aimed to determine the association between household income, frequency of purchasing outside meals, eating behaviour, and BMI status among Universiti Sains Malaysia (USM) undergraduate students during the COVID-19 lockdown period.

MATERIALS AND METHODS

This study was a retrospective cross-sectional study conducted among undergraduate students between the ages of 19 and 30 years old, who were enrolled in the Medical, Dental, and Health Sciences courses at USM Health Campus. The exclusion criteria were postgraduate students. Subjects were required to recall information during the first phase of the COVID-19 lockdown in Malaysia from March 2020 to July 2020. A questionnaire in using the Google Form platform was distributed via WhatsApp, Telegram, and email. Only subjects who provided consent on the first page of the form were recruited in this study. Subjects were chosen via convenience sampling method. This study obtained ethical approval from the USM Human Research Ethics Committee (USM/JEPeM/21010069).

In this study, the prevalence value was obtained from the frequency of changes in weight and eating behaviour in 150 randomly selected young adults with obesity during the COVID-19 lockdown period (Pellegrini *et al.*, 2020). Sample size was calculated using the single proportion formula (Ahmad *et al.*, 2012). With a drop-out rate of 20%, a sample size of 112 was calculated with a prevalence value of 93.5%, a z-score of 1.96, and a precision value of 0.05. An additional 20% of research subjects were used to overcome non-response bias or when the study obtained incomplete data from participants that would affect the outcome of this study.

The questionnaire in this study consisted of four sections: socio-demography, anthropometry, frequency of purchasing outside meals, and eating behaviour. Socio-demography consisted of age, location during COVID-19 lockdown (either in the hostel or at home), and household income. Household income classification was adapted from the study by Ibrahim *et al.* (2019). Anthropometry information were self-reported, with weight in kilograms (kg), height in metres (m), and BMI in kg/m^2. BMI was calculated by dividing weight over height squared, then further categorised into underweight (BMI <18.5 kg/m^2), normal (BMI 18.5 – 24.9 kg/m^2), overweight (BMI 25.0 – 29.9 kg/m^2), and obesity (BMI ≥30.0 kg/m^2) (WHO Expert Consultation, 2004).

For the section on purchasing outside meals, students were asked, "How often in a week do you purchase meals from outside during the first phase COVID-19 lockdown?" It had four answer options: no purchase of outside food in a week, purchase 1-2 times per week, 3-4 times per week, and >4 times per week. Purchasing outside meals included online purchasing via food delivery applications.

Lastly, eating behaviour was assessed using the Malay version of Dutch Eating Behaviour Questionnaire (DEBQ) (Subramaniam *et al.*, 2017). The Malay DEBQ had 30 items in the questionnaire as opposed to 33 items in the English version. Three items had to be removed due to low reliability. The Malay DEBQ measured three domains of unhealthy eating behaviours. The domains were restrained eating, emotional eating, and external eating. Restrained eating refers to restricting food intake to control body weight. Emotional eating refers to coping with negative emotions, such as anxiety or irritability, using food. Lastly, external eating refers to external triggers that influence eating behaviour, such as the presentation or aroma of food. Each item has five answer options, namely 1 = never, 2 = seldom, 3 = sometimes, 4 = often, and 5 = very often. The total score for each domain was calculated by adding the scores for each item in the respective domains. Higher scores indicated a greater tendency for poor eating behaviours. The results for each

domain were presented as numerical values. After removing items 21, 14, and 27 from the questionnaire, the internal consistency values for emotional, external, and restrained eating were 0.914, 0.819, and 0.856, respectively.

Statistical analysis

The SPSS software version 27.0 (IBM Corp, Armonk, New York) was used to analyse the data. The statistical significance level was set at $p<0.05$ (two-tailed) at a 95% confidence interval. Descriptive statistics were used to summarise the sociodemographic characteristics of subjects. Numerical data were presented as mean (standard deviation, *SD*) for normally distributed data or median (interquartile range, IQR) for non-normally distributed data. Categorical data were presented as frequency (percentage). Chi-square test of independence was used to assess the association between the frequency of purchasing outside meals, BMI, and household income. Comparison had been done between each individual eating behaviour domains (numerical data), with frequency of purchasing outside meals (categorical data), and BMI (categorical data) using the one-way between-group analysis of variance (ANOVA) (if normally distributed) or Kruskal-Wallis test (if not normally distributed).

Table 1. Socio-demography, frequency of purchasing outside meals and anthropometric parameters of subjects during COVID-19 lockdown [data expressed as mean (*SD*) or *n* (%)]

Characteristic	*Mean (SD)*	*n (%)*
Age, years	22.5 (1.3)	
Monthly household income		
≤MYR 1000		26 (23.2)
MYR 1001-3000		28 (25.0)
MYR 3001-5000		17 (15.2)
≥MYR 5001		41 (36.6)
Location		
Home		92 (82.1)
Campus hostel		20 (17.9)
Frequency of purchasing outside meals		
No purchase of outside food in a week		14 (12.5)
1 – 2 times per week		72 (64.3)
3 – 4 times per week		15 (13.4)
>4 times per week		11 (9.8)
Weight (kg)	57. 2 (14.3)	
Height (cm)	156.2 (6.8)	
BMI (kg/m^2)	24.3 (5.3)	
BMI category		
Underweight		19 (17.0)
Normal		61 (54.5)
Overweight		16 (14.3)
Obese		16 (14.3)
Eating behaviour score		
External eating domain	3.5 (0.6)	
Restrained eating domain	2.7 (0.8)	
Emotional eating domain	2.6 (0.7)	

BMI=body mass index, MYR=Malaysian Ringgit, m=metre, kg=kilograms, cm=centimetre, *SD*=standard deviation

RESULTS

There were 112 subjects with mean age of 22.5 (SD=1.3) years. The majority of subjects (82.1%) resided in their respective homes during the study period. About 36.6% of the subjects had a high monthly household income of MYR 5001 and above. A total of 64.3% of students reported purchasing meals from outside 1-2 times per week during the lockdown period. On the other hand, mean weight and height of subjects were 57.2 (14.3) kg and 156.2 (6.8) cm, respectively. About 54.5% of subjects had normal BMI. The worst domain of eating behaviour was external eating domain, reported at a score of 3.5 (0.6) (Table 1).

Table 2 shows the associations between household income and BMI with the frequency of purchasing outside meals. Both parameters had no statistically significant associations (p>0.05) and were tested using Pearson's chi-square test (Table 2).

A higher restrained eating score was reported among those who had higher frequency of purchasing meals outside of home. Students who purchased 3-4 times per week and >4 times per week had the highest scores in the retrained eating domain as compared to other categories. Besides, significant comparison was observed between BMI categories with restrained eating behaviour between BMI status and restrained eating (p<0.001), tested using the One-way ANOVA test. Subjects with normal BMI had the highest score in the restrained eating domain [3.0(1.1)] as compared to other BMI categories (Table 3).

DISCUSSION

In the current study, the majority of the subjects (64.3%) reported purchasing outside meals about 1-2 times a week during the COVID-19 lockdown period. A cross-sectional online survey involving 1,071 adults in three European countries found that most respondents had never shopped online for foods or groceries before the pandemic struck. However, most respondents stopped eating out during the pandemic and preferred home

Table 2. Associations between frequency of purchasing outside meals with household income and BMI status among subjects

	Frequency of purchasing outside meals			
	None in a week	*1-2 times/ week*	*3-4 times/ week*	*>4 times/ week*
Monthly household income, *n* (%)				
≤MYR 1000	6 (33.3)	17 (23.6)	2 (13.3)	1 (14.3)
MYR 1001-RM3000	5(27.8)	17 (23.6)	4 (26.7)	2 (28.6)
MYR 3001-RM5000	3 (16.7)	10 (13.9)	3 (20.0)	1 (14.3)
≥MYR 5001	4(22.2)	28 (38.9)	6 (40.0)	3 (42.9)
p-value	0.710			
BMI, *n* (%)				
Underweight	5 (27.8)	11 (15.3)	2 (13.3)	1 (14.3)
Normal	6 (33.3)	46 (63.9)	6 (40.0)	3 (42.9)
Overweight	3 (16.7)	8 (11.1)	4 (26.7)	1 (14.3)
Obese	4 (22.2)	7 (9.7)	3 (20.0)	2 (28.6)
p-value	0.185			

*Significant difference at p<0.05 using Chi-Square test
BMI=body mass index

Table 3. Comparisons between eating behaviour domains with frequency of purchasing outside meals and BMI status among subjects [presented as median *(IQR*)]

	Eating behaviour domain scores		
	Emotional [a]	*External* [a]	*Restrained* [b]
Frequency of purchasing outside meals			
None in a week	2.4 (0.7)	3.5 (0.7)	2.5 (1.8)
1-2 times/week	2.6 (0.7)	3.5 (0.6)	2.8 (1.1)
3-4 times/week	2.8 (0.6)	3.6 (0.7)	3.1 (1.7)
>4 times/week	2.9 (1.0)	3.7 (0.3)	3.1 (1.3)
p-value	0.333	0.821	<0.001*
BMI			
Underweight	2.5 (0.7)	3.7 (0.6)	1.7 (0.9)
Normal	2.7 (0.7)	3.5 (0.6)	3.0 (1.1)
Overweight	2.5 (0.8)	3.5 (0.7)	2.8 (0.9)
Obese	2.8 (0.8)	3.5 (0.5)	2.9 (1.1)
p-value	0.512	0.720	<0.001*

[a]One-way ANOVA
*[b]Significant difference at $p<0.001$ using Kruskal-Wallis test
BMI=body mass index

delivery (Skotnicka *et al.*, 2021). A study by Seguin *et al.* (2016) conducted among adults showed that 34.0% consumed food from outside 2-4 times per week, but the current study only reported 13.4% who purchased outside meals 3-4 times per week. Another recent study by Whatnall *et al.* (2021) showed that most students reported purchasing foods and/or beverages more than once a week. Students who frequently purchased outside meals had a higher preference for energy-dense foods (Whatnall *et al.*, 2021). A survey conducted in Spain during the COVID-19 lockdown reported that Spanish consumers purchased grocery items, such as flour, bread, fresh vegetables, fruits, milk, and chicken, instead of complete meals for weight control. Lockdown has made people more conscious about their health, driving them to watch health-related videos for a lifestyle change. Thus, Spanish consumers were motivated to change their lifestyle by consuming more home-cooked foods (Laguna *et al.,* 2020).

This study demonstrated that the worst eating behaviour domain was the external domain, rather than restrained or emotional eating behaviours. One of the possible reasons could be the influence of environmental cues such as social media and mass media (Huang & Su, 2018). Instagram or other social media has become a great concern among youths as it has been reported to inflict negative outcomes on their food consumption due to numerous food advertisements. External eating behaviour can be defined as eating in response to food-related external cues such as the visual and palatability of foods (Subramaniam *et al.,* 2017). For example, food and plating play vital roles in attracting people's attention and interest (Paakki *et al.,* 2019). Similarly, food advertisements are external stimuli that influence an individual's food choice, contributing to an increase in excessive energy intake, obesity, and cardiovascular disease (Qutteina *et al.*, 2019). Food advertisements also stimulate the viewers through food content, which influences them to buy and consume the advertised food regardless of its price and nutrient

content. Thus, exposure to external cues from the environment may be one of the reasons why most students exhibited external eating behaviour. On the other hand, another study done by Norazman & Wan Mahmood (2020) among undergraduate students showed that most students manifested restrained eating behaviour, followed by external eating behaviour. However, they did not exhibit emotional eating behaviour.

In addition, the current study demonstrated that there was no significant association between household income and the frequency of purchasing outside meals. During the first pandemic lockdown, only one person from a family was allowed to go out to purchase groceries at nearby shops. Due to this movement restriction, the frequency of purchasing outside meals online had increased across all categories of household income. People purchased meals from preferred or affordable restaurants far away from their homes. This finding was inconsistent with a previous study done by French, Wall & Mitchell (2010) that showed a significant association between household income, food sources, and food purchases among a community-based sample of 90 households Minneapolis, Minnesota, USA. The study hypothesised that higher-income households spent more on eating out per person than lower-income households (French *et al.*, 2010). Ho *et al.* (2021) stated that students who came from higher-income families might have received more pocket money from their parents and thus had a higher possibility of eating out, such as at fast-food outlets, coffee shops, hawker stalls, buying takeaway or delivery.

According to Yau & Potenza (2013), restrained eating is defined as the voluntary cognitive control to restrict food intake, typically for weight loss or weight maintenance purposes. The current study revealed no significant association between the frequency of purchasing outside meals with emotional and external eating behaviours. However, there was a significant association with restrained eating behaviour among the students. Students who purchased outside meals 3-4 times a week and >4 times a week had restrained eating behaviour. Students with restrained eating behaviour who frequently purchased outside meals may lack healthy food options in their respective homes or hostels. Thus, they had to purchase healthy meals, such as vegetable soup or noodle soup, from outside. However, there are no data available for comparison. There are many controversial issues and inconsistencies across findings in this area of research (Adams *et al.,* 2015). Whatnall *et al.* (2021) demonstrated contradicting findings of an association between greater frequency of purchasing outside meals and a higher intake of energy-dense, nutrient-poor foods. However, there was no significant association with diet quality score using the Australian Recommended Food Score (ARFS). Additionally, a study by Roy *et al.* (2017) conducted among 103 university students found that students who frequently purchased outside meals for >5 days compared with <2 days had lower diet quality scores. Although there are conflicting findings in most previous research studies, including the current ones, it is important to note that this could be due to differences in assessment tools and varying sample sizes (Roy *et al.*, 2017).

Based on the current study results, there was no significant association between BMI status with emotional and external eating behaviours. However, there was a significant association between BMI status and restrained eating behaviour among the students. Subjects with normal BMI had higher restrained eating behaviour scores than

those in the overweight and obese groups. Restrained eaters in this study had normal BMI, maybe due to consuming smaller portions of less energy-dense foods and beverages. However, they must be aware of not restricting food intake too severely to avoid drastic or excessive weight loss. In addition, restrained eating may prevent weight gain if practised correctly (Olea Lopez & Johnson, 2016). Most people practising restrained eating think they eat a limited amount of food, but the food consumed may still be considered excessive (Muharrani, Achmad & Sudiarti, 2018).

The COVID-19 lockdown has changed the eating behaviours and lifestyles of most individuals. People became more sedentary, and their sense of hunger increased during the lockdown period. Increased consumption of foods, especially snacks, has contributed to weight gain during the lockdown period (Di Renzo *et al.*, 2020). In the current study, overweight and obese subjects also had restrained eating scores closer to those in the normal BMI category. This may be due to the wrong way of practising restrained eating by frequently consuming foods high in sugar and fat, although in smaller amounts, which can still contribute to excessive calorie intake.

This study has limitations. We used a cross-sectional design, which makes determining causality impossible, limiting the validity of the data. Besides, subjects could have under- or overestimated their self-reported weight and height values.

CONCLUSION

In conclusion, this study revealed that most undergraduate students were from higher-income families. Most of the students reported having purchased meals 1-2 times per week using food delivery applications such as Food Panda or Grab Food during COVID-19 lockdown. Those with normal weight had higher restrained eating scores than overweight and obese students due to food restrictions. Meanwhile, those who purchased outside meals at least three times per week also had more restrained eating behaviour.

It is hoped that this study will increase the awareness of healthy eating and a healthy lifestyle among young adults, especially university students, thereby reducing the risks of obesity and other chronic diseases. Future studies should focus on increasing university students' nutrition knowledge and skills, especially on the effects of eating out. Moreover, future studies can focus on developing healthy meal preparation modules to motivate university students to prepare healthy home-cooked meals themselves.

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Authors' contributions

DV, principal investigator, conceptualised and designed the study, advised on data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript; NHAR, led the data collection, and prepared the draft of the manuscript and reviewed the manuscript.

Conflict of interest

All authors declare no conflict of interest.

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Effect of adherence to follow-up on recovery from moderate acute malnutrition among under-fives in a supplementary feeding programme

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ABSTRACT

Introduction: Supplementary feeding programme is a strategy for managing under-fives with moderate acute malnutrition (MAM). This study aimed to determine the effect of adherence to follow-up on recovery from MAM among under-fives. **Methods:** A clinical trial to evaluate the effectiveness of daily supplementary rations of a standardised milk-based formulation (SMBF), standardised non-milk-based formulation (SNMBF), and hospital-based formulation (HBF) on recovery from MAM over a four months period was conducted among eligible children aged 6 – 59 months. Recovery from MAM among participants was determined based on their status of adherence to follow-up at week 16. It was deemed statistically significant if *p*-value was <0.05. **Results:** Of the 157 children evaluated, 41/54 (75.9%) who received the SMBF, 32/57 (56.1%) who received the SNMBF, and 22/46 (47.8%) who received the HBF had good adherence. Adherence to follow-up was significantly higher with SMBF than SNMBF and HBF (χ^2=8.923; *p*=0.012). In all, 95/157 (60.5%) had good adherence to follow-up with 73/95 (76.8%) recovery from MAM against 42/62 (67.7%) recovery in those with poor adherence (*p*=0.208). **Conclusion:** The status of adherence to scheduled follow-up was not significantly associated with recovery from MAM among under-fives enrolled in the supplementary feeding programme. Nevertheless, efforts at promoting adherence to scheduled follow-up visits should be sustained.

Key words: adherence, clinical, follow-up, malnutrition, nutrition, trial

INTRODUCTION

Malnutrition is presently a leading cause of childhood morbidity and mortality globally. Micha *et al.* (2020) gave an estimate of about 144 million stunted and 47 million wasted under-fives globally. Of the 47 million under-fives with wasting, 14.3 million have severe acute malnutrition, while 32.7 million have moderate acute malnutrition (MAM). Most malnourished children reside in Asia and sub-Saharan Africa (Micha *et al.,* 2020).

Supplementary feeding programme is one of the strategies recommended by the World Health Organization (WHO)

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for managing childhood MAM. The programme entails provision of additional foods beside the child's regular diet that is aimed at reducing the gap between the actual dietary intake and requirement of the child. A Cochrane systematic review by Sguassero *et al.* (2012) showed that supplementary feeding optimised growth and facilitated recovery from MAM in under-fives. An overview of systematic reviews by Visser *et al.* (2018) showed that ready-to-use therapeutic foods or nutrient-dense formulations prepared from locally available food stuffs can be used over a period of time at rations to help meet the full or partial daily caloric requirements of malnourished population. The findings of Kekalih *et al.* (2019) in a clinical trial among wasted children showed that adherence to follow-up in a supplementary feeding programme impacted the frequency of clinic visits, use of foods by participants, and influenced recovery from malnutrition. Poor adherence to study protocol or dietary regimen among malnourished under-five children enrolled in a supplementary feeding programme has been reported by Pietraville *et al.* (2021) to be associated with poor clinical outcomes and failure in community management of acute malnutrition.

There is paucity of information on the relationship between the extent of adherence to follow-up schedules and recovery from MAM among under-fives enrolled in a supplementary feeding programme. Therefore, this study aimed to evaluate the effect of adherence to follow-up on recovery from MAM among children aged 6 – 59 months enrolled in a supplementary feeding programme in Nigeria.

MATERIALS AND METHODS

Study location and duration

This study was conducted in Primary Health Centres located at Mbak Etoi, Adadiah, and Okopedi Use in the Uyo Senatorial District of Akwa State, Nigeria from May 2016 to April 2017.

Sample size calculation

The sample size was calculated based on 80% certainty that the lower limit of a 95% two-sided confidence interval will be above -0.3, assuming a standard deviation of 0.55 units (Martha 1993). The sample size for each of the study arm was 48 children. To accommodate a 10% attrition, the minimum sample size was increased to 53 children per study arm.

Eligibility criteria

Children aged 6-59 months with MAM defined as weight-for-height/length z-score between -2 and -3 standard deviation (*SD*) or mid-upper arm circumference (MUAC) of 11.5 cm to 12.5 cm, in the absence of oedema or a concomitant medical or surgical illness, and with the issuance of parental consent.

Randomisation

Balloting technique was used to randomise the children to receive one of the three formulations – standardised milk-based formulation (SMBF), standardised non-milk-based formulation (SNMBF), and hospital-based formulation (HBF). Participants enrolled in PHC Adadiah received the SMBF, those enrolled in the PHC Okopedi Use received the SNMBF, while those enrolled in PHC Mbak Etoi received the HBF.

Administration of supplementary feeds

A daily supplementary ration of the SMBF, SNMBF, and HBF were given to meet 50% of the caloric requirements of children in addition to their regular family diet for four months. Table 1 shows nutrient composition of the formulations.

Table 1. Comparison of nutrient composition of different formulations

Average nutrient composition (unit)	*Cereal-based formulation (Standardised milk-based formulation)*		*Soya-cereal based formulation (Standardised non-milk based formulation)*		*Hospital-based formulation*	
	Per meal (50g=200ml)	*% RDA*	*Per meal (50g=200ml)*	*% RDA*	*Per meal (200ml)*	*% RDA*
Energy (kcal)	205	29	199	29	215	31
Fat (g)	5.0	17	4.5	15	10.7	36
Linoleic acid (g)	0.8	16	1.7	37	1.9	41
Protein (g)	7.5	68	7.5	68	7.2	65
Carbohydrate (g)	32.5	34	32.1	34	22.1	23
Dietary fibre (mg)	2.2	43	3.5	70	0.2	3
Vitamin A (IU)	650	130	750	150	555	111
Vitamin D (IU)	100	50	100	50	40	20
Vitamin E (IU)	3.4	68	3.4	68	2.0	39
Vitamin C (mg)	25	50	25	50	43.6	87
Vitamin B1 (mg)	0.3	100	0.4	133	0.4	140
Vitamin B2 (mg)	0.4	94			0.6	153
Niacin (mg)	1.5	38	2.0	50	4.8	123
Vitamin B6 (mg)	0.5	50	0.2	50	0.1	3
Folic Acid (µg)	20.0	25	40.0	50	41.0	51
Vitamin B12 (µg)	0.6	110	0.4	80	0.3	64
Calcium (mg)	300	111	195	72	129	48
Sodium (mg)	72.5	36	105.0	53	48.7	25
Iron (mg)	3.8	35	5.0	45	3.2	30
Zinc (mg)	3.0	100	3.0	100	0.4	13

Children aged 6–24 months received 100 kcal/kg/day of the formulation assigned to them, while those aged 25–59 months received 90 kcal/kg/day. The caregivers were trained by a dietitian on the preparation of the formulations, feeding of the children, and were instructed not to share the formulations with other members of the household. They were also counselled on the preparation of age-appropriate complementary foods using locally available food stuffs, infant and young child feeding practices, hand hygiene, and food hygiene. A flip chart with appropriate pictures was used to reinforce the key messages of the nutrition counselling. Mothers were encouraged to continue breastfeeding children aged 6-24 months.

Follow-up

Children were followed-up on a biweekly basis from the commencement of the study. Clinical assessment, anthropometric measurements, and nutrition counselling were performed during the follow-up visits. The supply of supplementary food for the next two weeks was given to those who kept their scheduled follow-up visits; while those who failed to keep their visits missed out on their ration of food. Adherence to follow-up visits was considered good if a child kept the entire follow-up visits (eight visits) and poor if a child defaulted on one or more follow-up visits.

Statistical analysis

Data were entered into Excel 2016

(Microsoft Corporation, USA). The software was also used for analyses. Analyses were done per protocol for children who completed the study only. The characteristics of the children were described using frequencies and percentages. Likewise, the proportion of recovery from MAM based on adherence status of the participants was presented in percentages. The test of association between the status of adherence (good adherence versus poor adherence) and recovery from MAM was assessed at week 16 among evaluable children using chi-square test. A *p*-value of <0.05 was considered as statistically significant.

Ethical approval

Approval for the conduct of the study was obtained from the Health Research Ethics Committee of the University of Uyo Teaching Hospital, Uyo, with the approval registry number UUTH/AD/S/96/VOL. XXI/341. Parental consent was obtained before enrolment of participants into the study. The primary study from which this work was derived (effectiveness and tolerability of standardised milk-

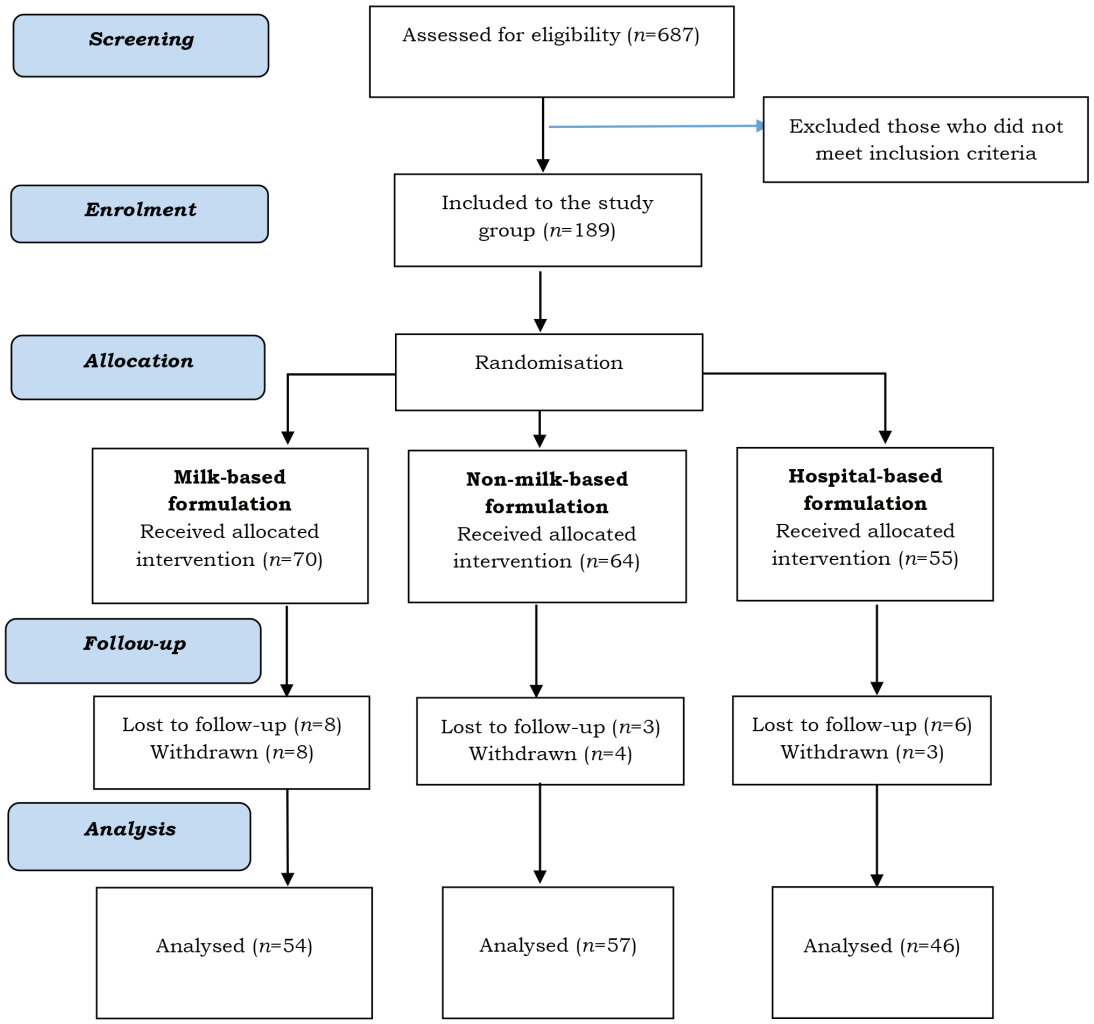

Figure 1: Flow diagram of study

Table 2: Recovery from MAM based on formulation of supplementary feed

Outcome	*SMBF n (%)*	*SNMBF n (%)*	*HBF n (%)*
Recovery from MAM	43 (79.6)	40 (70.2)	32 (69.6)
No improvement or worse	11 (20.4)	17 (29.8)	14 (30.4)
Total	54 (100)	57 (100)	46 (100)

based, standardised non-milk-based, and hospital-based formulations in the management of moderate acute malnutrition in under-five children: a randomised clinical trial) was registered with the Pan African Clinical Trial Registry with a trial registration number PACTR201704002119141.

RESULTS

Figure 1 shows the flow diagram of the study highlighting screening of participants for eligibility into the study, enrolment and randomisation of eligible participants, administration of the investigational products, follow-up of participants, and analysis of evaluable participants.

Recovery from MAM following supplementary feeding

Of the 157 evaluable children, 115/157 (73.2%) recovered from MAM. The highest proportion of recovery was noted among those that received the SMBF, 43/54 (79.6%), as seen in Table 2.

Adherence of study participants to follow-up schedules

As represented in Table 3, adherence to scheduled follow-up was good in 41/54 (75.9%) of those enrolled in the SMBF group, 32/57 (56.1%) of those enrolled in the SNMBF group, and 22/46 (47.8%) of those enrolled in the HBF group. There was a statistically significant association between the type of formulation used in the supplementary feeding programme and adherence to scheduled follow-up visits (χ^2=8.923; p=0.012). Participants in the SMBF group were more likely to adhere to scheduled follow-up visits than those in the SNMBF or HBF groups.

Overall effect of adherence to follow-up on recovery from MAM

Of the 157 evaluable children, 95 (60.5%) had good adherence, while 62 (39.5%) had poor adherence to follow-ups. Among those with good adherence, 73/95 (76.8%) recovered from MAM against 42/62 (67.7%) among those with poor adherence. The difference in proportion of recovery based on status of adherence was not statistically significant (χ^2=1.585; p=0.208) as shown in Table 4.

Effect of adherence to follow-up on recovery among participants in various groups

The effect of adherence to follow-up on recovery among participants in different study arms is displayed in Table 4. Of the 41 children with good adherence in the SMBF group, 33/41 (80.5%) recovered from MAM against 10/13 (76.9%) of those with poor adherence. There was no difference in recovery based

Table 3: Adherence status of participants to scheduled follow-up visits

Status of adherence	*SMBF n (%)*	*SNMBF n (%)*	*HBF n (%)*	*Statistical indices*
Good adherence	41 (75.9)	32 (56.1)	22 (47.8)	χ^2=8.923
Poor adherence	13 (24.1)	25 (43.9)	24 (52.2)	p=0.012
Total	54 (100)	57 (100)	46 (100)	

Table 4: Effect of adherence on recovery from MAM in all study participants and by supplementary feed grouping

Status of adherence to follow up	*Recovered n (%)*	*Not recovered n (%)*	*Total n (%)*	χ^2 *value*	*p-value*
Adherence on recovery from MAM in all study participants				1.585	0.208
Good adherence	73 (76.8)	22 (23.2)	95 (100)		
Poor adherence	42 (67.7)	20 (32.3)	62 (100)		
Adherence on recovery by supplementary feed grouping					
SMBF Group					
Good	33 (80.5)	8 (19.5)	41 (100)		0.715[a]
Poor	10 (76.9)	3 (23.1)	13 (100)		
SNMBF Group					
Good	24 (75.0)	8 (25.0)	40 (100)	0.811	0.368
Poor	16 (64)	9 (36.0)	17 (100)		
HBF Group					
Good	16 (72.7)	6 (27.3)	22 (100)	0.199	0.655
Poor	16 (66.7)	8 (33.3)	24 (100)		
Total	115 (73.2)	42 (26.8)	157 (100)		

[a]Fisher's exact test

on status of adherence in the group (p=0.715). In the SNMBF group, 24/32 (75.0%) children with good adherence recovered from MAM against 16/25 (64.0%) of those with poor adherence to follow-up. The difference in recovery based on the status of adherence in the group was not statistically significant (χ^2=0.811; p=0.368). Of the 22 children with good adherence to follow-up in the HBF group, 16 (72.7%) recovered from MAM against 16/24 (66.7%) of those with poor adherence to follow-up. The effect of status of adherence to follow-up on recovery in this group was also not statistically significant (p=0.655; χ^2=0.199).

DISCUSSION

There was 73.2% recovery from MAM among the evaluable children enrolled in the supplementary feeding programme at the end of the study. The highest recovery was noted among those who received the SMBF, while the proportion of recovery in those who received the SNMBF and HBF was similar. The recovery from MAM with the formulations evaluated in this study was less than 85% as observed by Medoua *et al.* (2016) among children treated with a lipid-based nutrient supplement (LNS), but similar to the 73% recovery among those who received a corn soy blend in the same study conducted in Cameroon. Karakochuk *et al.* (2012) in Ethiopia reported 73.0% recovery among under-fives with MAM treated with LNS. These observations highlight the influence of variation in the composition of nutritional formulations and possibly the effect of variation in nutrient concentration of formulations with similar composition on the recovery of childhood MAM.

The extent of adherence to scheduled follow-up visits was significantly higher in those who were treated with the SMBF compared to those who received either the SNMBF or the HBF. The observed differences in the adherence to follow-up based on the nature of the supplementary

food could be attributed to variations in the composition, packaging, preparation, and palatability of the formulations. The SMBF was a pre-packaged formulation contained in sachets that was relatively easy to prepare and more palatable when compared to the SNMBF or HBF. Kebede & Haidar (2014) in Ethiopia identified dislike of taste as a reason for poor adherence to supplementary feeding among HIV positive patients. On the other hand, the SNMBF was contained in tins, thereby requiring a high level of accuracy in the number and volume of scoops to be taken by the caregivers when preparing the formulation as compared to the SMBF that had a predetermined number of sachets of formulation to be used for a particular child. The HBF was the most difficult to prepare because the constituents were packed differently and needed to be introduced in an orderly sequence during preparation and heated for a specified period for optimum nutrient bioavailability. The variations in packaging and complexities in preparation, especially for the HBF, might have contributed to the differences in adherence of the participants to scheduled follow-up visits.

The overall result of this study showed that recovery from MAM was slightly better among those with good adherence to follow-up compared to those with poor adherence. This was also true among participants in different study arms even though the differences between them were not statistically significant. Recovery from MAM among those with good adherence was highest in the SMBF group, followed by the SNMBF group, and least in the HBF group. This apparent gradation in recovery appeared to be related to the extent of adherence to follow-up by participants in various study arms, as the level of adherence to follow-up was highest in those that received the SMBF and lowest in those that received the HBF.

There is no fixed cut-off value for good adherence to follow-up in clinical trials. Probstfield (1989) indicated that arbitrary values are often used based on study outcomes, condition of interest, effectiveness of the intervention, and the duration of follow-up. The inability to detect a statistically significant difference in the proportion of recovery based on adherence status among the participants in this study might be due to the fact that we used 100% adherence to follow-up to connote good adherence. In a study among children with HIV, Odeny *et al.* (2012) used a cut-off value of 90% adherence to follow-up as indicative of good adherence, while Masaya *et al.* (2017) in a study that assessed the effect of inhaled steroid for bronchial asthma used ≥80%. The lower the percentage used as the cut-off value for good adherence, the more the likelihood of appreciating the effect that status of adherence to follow-up has on outcome measures. This is particularly important in supplementary feeding programme where a reasonable length of time is needed to observe appreciable changes in outcome measures.

Poor adherence has been reported by Dunbar-Jacob *et al.* (2000) to attenuate optimum clinical benefits of treatment interventions. Poor adherence to scheduled follow-up visits had a negative impact on recovery from MAM in this study. Besides militating against recovery from MAM, poor adherence to scheduled follow-up visits among malnourished children in a supplementary feeding programme could also increase their vulnerability to other childhood co-morbidities as observed by Schaible & Kaufmann (2007).

Most cases of childhood malnutrition in developing countries like Nigeria are mainly due to dietary inadequacy arising from poverty, household food insecurity or lack of awareness in feeding practices among caregivers of young children

as noted by Bain *et al.* (2013) and Babatunde *et al.* (2011). It was therefore expected that the caregivers of children in this study would take full advantage of the free feeding programme by adhering strictly to the follow-up schedules. This was not the case as a high rate of default to scheduled follow-up visits was noted among participants in the programme.

The relatively low recovery from MAM that was associated with poor adherence to scheduled follow-ups in this study is of immense clinical importance to child survival in resource limited countries considering the health consequences of child malnutrition as reported by Black *et al.* (2008). In view of the high mortality attributable to childhood malnutrition and its adverse impacts on physical growth, cognitive and immunologic functions, it is imperative that children with MAM enrolled in supplementary feeding programmes adhere strictly to their scheduled follow-up visits.

Studies aimed at determining the factors associated with adherence to follow-up in childhood nutrition clinical trials are needed in order to develop strategies for improving adherence to scheduled follow-up visits. This will go a long way to improve recovery among under-fives with MAM enrolled in supplementary feeding programmes.

CONCLUSION

The extent of adherence to scheduled follow-up visits was not significantly associated with recovery from MAM in the participants. Nevertheless, efforts at promoting adherence to scheduled follow-up visits are still necessary in evaluating the effect of supplementary feeding programmes among under-fives with MAM.

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Authors' contributions

EEU, conceived the study, conducted the work, analysed the data, interpreted the results and drafted the manuscript; RAU, participated in the data collection, interpretation and preparation of the manuscript; KBE, participated in the data collection, interpretation and preparation of the manuscript; FSO, made critical inputs to the manuscript; ENU, participated in the data collection, interpretation and preparation of the manuscript; BNN, participated in the study design, data collection, analysis and preparation of the manuscript; OOM, participated in the study design, data collection, analysis and preparation of the manuscript. All authors read and approved the final version of the work.

Conflict of interest

EEU received support from Nestle Nutrition Institute Africa to conduct the study. The other authors have no competing interest to declare.

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