MALAYSIAN JOURNAL OF NUTRITION

VOL. 30 NO. 2 AUGUST 2024

PP18053/02/2013 (033331)

ISSN 1394-035X

9 771394 035008

Official Publication of the
PERSATUAN PEMAKANAN MALAYSIA
NUTRITION SOCIETY OF MALAYSIA

Malaysian Journal of Nutrition is abstracted/indexed by Google Scholar, the WHO Western Pacific Region Index Medicus, Elsevier databases of the Scopus, EBiology and Ecare, ASEAN Citation Index (ACI), CABI Global Health database and Asian Digital Library (ADL)

MALAYSIAN JOURNAL OF NUTRITION

Peer-reviewed Journal of the Nutrition Society of Malaysia

(http//www.nutriweb.org.my)

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Malaysian Journal of Nutrition
Vol. 30 No. 2, 2024

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Mal J Nutr 30(2): 129-142, 2024

Factors affecting stunting in children aged 6-23 months in South Sumatra Province, Indonesia

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ABSTRACT

Introduction: South Sumatra Province in Indonesia has a relatively high prevalence of stunting and there is limited research about this phenomenon in this area. The study aimed to identify what factors affected stunting in children aged 6-23 months in the province. **Methods:** Using a comparative cross-sectional design involving 139 mothers with children aged 6-23 months, the study collected data from September to December 2022 via anthropometric measurements and interviews using a questionnaire. Data on parental, child, socioeconomic, and environmental factors were analysed using chi-square test and logistic regression analysis. **Results:** Overall, there were significant relationships among the following variables: child's age (p=0.031), birth length (p=0.017), and weight-for-age (WAZ) status (p<0.001) with stunting. Children in the underweight and severely underweight categories were 28.7 times at risk of stunting compared to those in the normal category of WAZ status; children aged 12-23 months had a 2.8 times risk of stunting compared to children aged 6-11 months, while stunted birth length showed a 4.6 times risk of stunting compared to that of normal birth length. **Conclusion:** This research found that child age, birth length, and WAZ status were significant factors affecting stunting in the South Sumatra Province. Given these results, this study offers recommendations for the provincial government to focus on intervention programmes that provide additional food for pregnant women with chronic energy deficiency, monitor the growth of toddlers, and manage toddlers with nutritional problems in this province.

Keywords: birth length, child age, stunting, WAZ status

INTRODUCTION

Stunting is a nutritional problem that has impacted the world, especially in poor and developing countries. Indonesia, as a country in the Southeast Asia region, has a fairly high stunting rate when compared to other Southeast Asian countries, namely 30.5% in 2018 (UNICEF 2019). The results of the Indonesian Nutrition Status Study (INSS) in 2021 still showed a prevalence of 24.4%, while the World Health Organization (WHO) targets a reduction in the prevalence of stunting to below

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doi: https://doi.org/10.31246/mjn-2023-0101

20% in 2025 (Kemenkes RI, 2021). The INSS data also show that the prevalence of stunting increased 1.6 times from the age of 6-11 months to the age of 12-23 months (Sumiwi, 2023).

Stunting is defined as growth and development failure experienced by children that is caused by prolonged insufficient nutritional intake, recurrent infectious diseases, and inadequate psychosocial stimulation (WHO, 2014). Stunting is of concern as it can lead to disturbances in immunological, cognitive, and physical developments that cannot be completely repaired or become irreversible, thus increasing the risk of diseases later in life – i.e., obesity, diabetes, hypertension, and mental retardation (Mayneris-Perxachs & Swann, 2018).

The WHO summarised the various factors behind the occurrence of stunting, but each country and each region certainly has different causes. This is influenced by socio-cultural differences, as well as differences in lifestyle, food consumption patterns, weather/climate, and others. Systematic reviews using the WHO's conceptual framework on stunting in children have been conducted in Indonesia (Beal *et al.*, 2018) and Bangladesh (Islam *et al.*, 2020). Evidence shows that the determining factors for stunting in children are short birth length, non-exclusive breastfeeding, low socioeconomic status (characterised by food insecurity and lack of access to nutrition), low maternal education level, birth prematurity, low maternal height, low maternal weight and childbirth weight, and diarrhoea caused by pathogens (Beal *et al.*, 2018; Islam *et al.*, 2020). Other studies have found that growth disorders in terms of children's body length become more common as children get older. The prevalence of stunting is higher, and the risk of stunting is also greater in children aged 12–23 months compared to children aged 0–11 months (Afsana *et al.*, 2019).

We conducted this study in South Sumatra Province, the sixth most populous province in Indonesia, reaching 8,497,196 people in 2020 (BPS Sumatera Selatan Province, 2022). With such a large population, this province is faced with an extremely serious risk of overpopulation, including a high risk of child health problems (Kemenkes RI, 2021). South Sumatra Province is also home to a significant number of young people – as many as 2.8 million individuals (35% of the total population) in the province are children. In 2015, almost 500,000 children (17%) lived below the provincial poverty line. However, more households are in a vulnerable position and live on incomes just above the poverty line. The prevalence of malnutrition is relatively high, including among children from the wealthiest households (BAPPENAS-UNICEF, 2017). The prevalence of stunting in 2021 was 24.8%, including in one of the 34 provinces in the chronic-acute category (stunted >20% and wasted >5%) (Kemenkes RI, 2021).

The Indonesian government, through the Ministry of Health, has implemented various policies and programmes to reduce stunting, including specific nutrition intervention programmes related to the health sector and sensitive nutrition interventions related to the non-health sector, which are carried out in an integrated and comprehensive manner. In this nutrition intervention programme, 11 specific interventions were designed to accelerate the reduction of stunting, namely anaemia screening, consumption of blood supplement tablets for young women, pregnancy checks (antenatal care), consumption of blood supplement tablets for pregnant women, the provision of additional food for pregnant women lacking energy, monitoring the growth of toddlers, exclusive breastfeeding, providing complementary foods rich in animal protein for toddlers, managing toddlers with nutritional problems, increasing coverage and expanding immunisation, and educating teenagers, pregnant women, and their families, including triggering for behaviour change towards open defecation (Sumiwi, 2023).

Most of the previous researches in this province were carried out within a limited area or small community with simple variable pieces, so they could not describe the condition of stunting in South Sumatra (Khairani, Tjahjono & Rosidi, 2022; Louis, Mirania & Yuniarti, 2022). Therefore, this study aimed to identify what factors influence stunting in children in South Sumatra Province, particularly in those aged 6-23 months, thereby providing recommendations for the provincial government to focus on stunting intervention programmes.

MATERIALS AND METHODS

Study design

The research design was a comparative cross-sectional study. Subjects included children with normal body length-for-age *z*-score and children with body length-for-age *z*-score <-2 standard deviation (*SD*) (stunting). Respondents were mothers who had children aged 6-23 months when the study was conducted. Sample calculation used the two-proportion difference hypothesis test formula (with an estimated proportion of 0.62 in the stunting group) and the result obtained was a minimum total sample of 134 people. The sampling technique was carried out in two stages: the first stage was the selection of regions representing districts or municipalities (three regions were obtained in the province of South Sumatra, namely Palembang City, Muara Enim Regency, and Ogan Komering Ulu Regency); the second stage was selecting the sub-district with the highest prevalence (five sub-districts were obtained); and finally, the respondents were selected randomly based on data recorded at the local health office. Data collection was carried out in September-December 2022.

The inclusion criteria were mother-child pairs (children aged 6-23 months, registered at a community health centre, and had a health card), with mothers willing to sign the informed consent forms. Children with congenital diseases or syndromes that affected anthropometry and who suddenly became ill or who were undergoing certain medications when the research was conducted were excluded as research subjects. The study involved 139 mother-child pairs; 72 children were assigned to the normal group and 67 to the stunting group.

Anthropometric measurements

Anthropometric measurements were carried out by researchers, accompanied by trained health workers at the community health centres. The measurement of children's body length was carried out by one researcher using a baby length board (Kenko), with an accuracy of up to 0.1 cm. The child was measured in a lying position; the trained health workers assisted by stabilising the child on the length board and the measurement was recorded in centimetres (cm). Children's body weight was measured using a digital baby weight scale; children were weighed with minimal or no clothing in a lying or sitting position in kilograms. The instruments for anthropometric measurements had been standardised and calibrated by the district health office.

Children were grouped into the stunting and normal categories based on results of the anthropometric measurements, namely body length-for-age *z*-scores. If measurement results showed that body length-for-age was below -2*SD* (WHO median growth

Table 1. Description of study variables

Variable	*Operational definition*
Parental factors	
Mother's age	Mother's age at the time of the study. Categorised: <20 years; 20-35 years; >35 years
Mother's height	Mother's height Categorised: normal (≥150.1 cm), stunted/short (<150.1 cm)
Father's height	Father's height Categorised: normal (≥161.9 cm), stunted/short (<161.9 cm)
Maternal body mass index	Maternal body mass index by dividing body weight (kg) with quadrat of height (m^2) Categorised: underweight (BMI<18.5kg/m^2), normal (BMI 18.5 – 24.9 kg/m^2), overweight (BMI 25 – 29.9 kg/m^2), obese (BMI ≥30 kg/m^2)
Child factors	
Age	Child's age at the time of study Categorised: 6-11 months, 12-23 months
Gender	Child's gender Categorised: boy, girl
Gestational age	Age (period) when the child was in the womb (intrauterine) or the gestational age of the mother when the child was born Categorised: preterm (<37 weeks), Term (37-41 weeks), post term (≥42 weeks)
Birth weight	Child's weight at birth Categorised: low birth weight (<2.5 kg), normal birth weight (2.5-4.0 kg), high birth weight (>4.0 kg)
Birth length	The child's body length at birth with the following provisions (normal): - Male: >46.1 cm - Female: >45.4 cm Categorised: normal, stunted/short
Exclusive breastfeeding	Child's history receiving only breast milk (exclusively) at age ≤ 6 months Categorised: yes, no
Minimum dietary diversity (MDD) for children aged 6–23 months	The minimum variety of foods consumed by children aged 6–23 months, namely consuming food and drinks from at least five of the eight food groups determined on the previous day Categorised: fulfilled, not fulfilled
Minimum milk feeding frequency (MMFF) for non-breastfed children aged 6–23 months	Non-breastfed children aged 6-23 months who had at least two servings of milk on the previous day Categorised: fulfilled, not fulfilled
Minimum meal frequency (MMF) for children aged 6–23 months	Minimum meal frequency for children aged 6–23 months who eat solid, semi-solid, or soft foods (but also including dairy foods for children who are not breastfed) at least several times on the previous day Categorised: fulfilled, not fulfilled

Table 1. Description of study variables *(continued)*

Variable	*Operational definition*
Minimum acceptable diet (MAD) for children aged 6-23 months	The minimum acceptable diet for children aged 6–23 months is by assessing the minimum acceptable food during the previous day Categorised: achieved, not achieved
WAZ status	Nutritional status of children based on body weight for age Categorised: severely underweight (<-3*SD*) and underweight (-3*SD* - <-2*SD*), normal weight (-2*SD* - > +1*SD*)
Socioeconomic factors	
Domicile location	The residence of the mother and child Categorised: urban, rural
Mother's education	Formal education completed by the mother Categorised: low (elementary school, junior high school), medium (high school), high (diploma, bachelor, master, doctor)
Family income	Household income received by family every month in line with the determination of the provincial minimum wage in 2022 Categorised: high, low
Environmental factors	
Clean water source	Availability of clean water sources for drinking, cooking, and other purposes Categorised: yes, no
Toilet availability	Availability of standardised toilets at home Categorised: yes, no
Exposure to nicotine/ cigarette smoke	Whether the mother is exposed to nicotine or cigarette smoke Categorised: yes, no

standard), then the child was declared stunted, whereas if the measurement results were above -2*SD*, the child was declared normal.

Variables

Interviews were performed with the children's mothers using a questionnaire. The interview was conducted to assess the independent variables, and the validity and reliability of the questionnaire were tested. The validity of the instrument was assessed using the Pearson's correlation test (r=0.754, p<0.001) and overall, it had an acceptable reliability, with Cronbach's alpha=0.68. The independent variables studied were parental factors (including mother's age, mother's height, father's height, and mother's body mass index), child factors [including age, sex, gestational age, birth weight, birth length, exclusive breastfeeding status, minimum dietary diversity (MDD) status of children aged 6-23 months, minimum milk feeding frequency (MMFF) for non-breastfed children aged 6-23 months, minimum meal frequency (MMF) for children aged 6-23 months, minimum acceptable diet (MAD) for children aged 6-23 months, and WAZ (weight-for-age z-score) status], socioeconomic factors (including mother's education level and family's income level), and environmental factors (including domicile location, clean water sources, availability of toilets, and exposure to nicotine/cigarette smoke).

Table 2. Characteristics of stunted children and normal children in South Sumatra (*N*=139)

Variable	*n (%)*	*Normal, n (%)*	*Stunting, n (%)*	*p-value*†
Child's age				0.007*
6-11 months	35 (25.2)	25 (71.4)	10 (28.6)	
12-23 months	104 (74.8)	47 (45.2)	57 (54.8)	
Gender				0.030*
Girl	63 (45.3)	39 (61.9)	24 (38.1)	
Boy	76 (54.7)	33 (43.4)	43 (56.6)	
Gestational age				0.428
Term	114 (82.0)	62 (54.4)	52 (45.6)	
Preterm	20 (14.4)	8 (40.0)	12 (60.0)	
Post-term	5 (3.6)	2 (40.0)	3 (60.0)	
Birth weight				0.107
Normal	119 (85.6)	66 (55.5)	53 (44.5)	
Low	17 (12.2)	5 (29.4)	12 (70.6)	
High	3 (2.2)	1 (33.3)	2 (66.7)	
Birth length				0.003*
Normal	117 (84.2)	67 (57.3)	50 (42.7)	
Stunted	22 (15.8)	5 (22.7)	17 (77.3)	
Exclusive breastfeeding				0.516
Yes	97 (69.8)	52 (53.6)	45 (46.4)	
No	42 (30.2)	20 (47.6)	22 (52.4)	
MDD status				0.450
Fulfilled	68 (48.9)	33 (48.5)	35 (51.5)	
Not fulfilled	71 (51.1)	39 (54.9)	32 (45.1)	
MMFF status				0.621
Fulfilled	80 (57.6)	40 (50.0)	40 (50.0)	
Not fulfilled	59 (42.4)	32 (54.2)	27 (45.8)	
MMF status				0.298
Fulfilled	138 (99.3)	72 (52.2)	66 (47.8)	
Not fulfilled	1 (0.7)	0 (0.0)	1 (100.0)	
MAD status				0.115
Fulfilled	41 (29.5)	17 (41.5)	24 (58.5)	
Not fulfilled	98 (70.5)	55 (56.1)	43 (43.9)	
WAZ status				<0.001*
Normal	88 (63.3)	67 (76.1)	21 (23.9)	
Underweight & severely underweight	51 (36.7)	5 (9.8)	46 (90.2)	
Mother's age				0.942
20-35 years	96 (69.1)	49 (51.0)	47 (49.0)	
<20 years	8 (5.8)	4 (50.0)	4 (50.0)	
>35 years	35 (25.2)	19 (54.3)	16 (45.7)	
Mother's height				0.056*
Normal	88 (63.3)	51 (58.0)	37 (42.0)	
Stunted	51 (36.7)	21 (41.2)	30 (58.8)	
Father's height				0.470
Normal	77 (55.4)	42 (54.5)	35 (45.5)	
Stunted	62 (44.6)	30 (48.4)	32 (51.6)	
Mother's body mass index				0.755
Normal	77 (55.4)	37 (48.1)	40 (51.9)	
Underweight	23 (16.5)	13 (56.5)	10 (43.5)	
Overweight	29 (20.9)	17 (58.6)	12 (41.4)	
Obese	10 (7.2)	5 (50.0)	5 (50.0)	

Table 2. Characteristics of stunted children and normal children in South Sumatra (*N*=139) *(continued)*

Variable	*n (%)*	*Normal, n (%)*	*Stunting, n (%)*	*p-value*†
Domicile location				0.589
Urban	53 (38.1)	29 (54.7)	24 (45.3)	
Rural	86 (61.9)	43 (50.0)	43 (50.0)	
Mother's education				0.454
High	5 (3.6)	3 (60.0)	2 (40.0)	
Medium	69 (49.6)	39 (56.5)	30 (43.5)	
Low	65 (46.8)	30 (46.2)	35 (53.8)	
Family Income				0.392
High	40 (28.8)	23 (57.5)	17 (42.5)	
Low	99 (71.2)	49 (49.5)	50 (50.5)	
Clean water source				0.732
Yes	121 (87.1)	62 (51.2)	59 (48.8)	
No	18 (12.9)	10 (55.6)	8 (44.4)	
Toilet availability				0.480
Yes	125 (89.9)	66 (52.8)	59 (47.2)	
No	14 (10.1)	6 (42.9)	8 (57.1)	
Exposure to nicotine				0.503
No	18 (12.9)	8 (44.4)	10 (55.6)	
Yes	121 (87.1)	64 (52.9)	57 (47.1)	

MDD: Minimum dietary diversity; MMFF: Minimum milk feeding frequency; MMF: Minimum meal frequency; MAD: Minimum acceptable diet; WAZ: Weight-for-age z-score
†Chi-square test
Univariate and bivariate analyses; *$p<0.05$

Statistical analysis
We compared the factors between normal children and stunted children. Chi-square test was used to test the significance of relationship between independent variables and incidence of stunting. In addition, a calculation of the odd ratio for stunting was also carried out. Multivariate logistic regression analysis was performed to estimate how much influence the independent variables had on the dependent variable. All variables resulting from the bivariate analysis with a cut-off *p*-value <0.25 were included in the final model of the multivariate logistic regression, with a 95% confidence interval. All analyses were conducted using IBM SPSS Statistics for Windows, version 20.0 (IBM Corporation, Armonk, NY, USA), whereby a *p*-value <0.05 indicated statistical significance.

Ethics approval
This research received ethical approval from the Ethics Committee of the Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital (No.KET-878/UN2.F1/ETIK/PPM.00.02/2022). In addition, permission was obtained from the South Sumatra Provincial Health Office (No.074/3198/Kes/XI/2022), the local district/municipal health service, and related institutions, as well as written consent from the children's parents.

RESULTS

Table 2 shows the factors affecting stunted children and normal children in South Sumatra Province. Most respondents lived in rural areas (61.9%). Stunting prevalence as a whole was 48.2%, more common in boys (56.6%

Table 3. Factors influencing stunting in children in South Sumatra (*N*=139)

Variable	*Adjusted*	
	OR (95% CI)	*p-value* †
Child's age		0.228
6-11 months	1	
12-23 months	2.045 (0.639-6.546)	
Gender		0.240
Girl	1	
Boy	1.755 (0.687-4.483)	
Birth weight		
Normal	1	0.305
Low	1.097 (0.185-6.504)	0.919
High	7.528 (0.569-99.596)	0.126
Birth length		0.332
Normal	1	
Stunted	2.242 (0.438-11.477)	
MAD status		0.677
Fulfilled	1	
Not fulfilled	0.803 (0.286-2.255)	
WAZ status		
Normal	1	<0.001*
Underweight & severely underweight	28.720 (9.430-87.465)	
Mother's height		0.140
Normal	1	
Stunted	2.150 (0.778-5.939)	

MAD: Minimum acceptable diet; WAZ: Weight-for-age z-score
†Logistic regression multivariate analysis
*$p<0.05$

of the study population) than in girls and the largest proportion occurred in the age range of 12-23 months (54.8%). Gender and child's age showed significant differences (p=0.030 and p=0.007, respectively). In addition, the variables of birth length and WAZ status showed significant differences (p=0.003 and p<0.001, respectively) between normal and stunted children.

Multivariate analysis of the logistic regression model shown in Table 3 revealed that the most significant influencing factor of stunting was child's WAZ status (p<0.001). Children with underweight and severely underweight nutritional statuses were 28.7 times at risk of stunting (95% CI: 9.430-87.465).

Table 4 shows the multivariate analysis results when WAZ status was not included in the analysis, given its significance as a determining factor for stunting diagnosis. The variables that significantly influenced stunting were child's age (p=0.031) and birth length (p=0.017). Children aged 12-23 months had a 2.8 times risk of stunting (95% CI: 1.098-6.900) compared to children aged 6-11 months, while stunted birth length showed a 4.6 times risk (95% CI: 1.318-16.062) of stunting compared to normal birth length.

DISCUSSION

This study was the first to be carried out in three regions of South Sumatra

Table 4. Factors influencing stunting apart from WAZ status in children in South Sumatra (*N*=139)

Variable	*Adjusted*	
	OR (95% CI)	*p-value*[†]
Child's age		0.031*
6-11 months	1	
12-23 months	2.752 (1.098-6.900)	
Gender		0.154
Girl	1	
Boy	1.704 (0.819-3.544)	
Birth weight		
Normal	1	0.798
Low	1.172 (0.290-4.732)	0.823
High	2.242 (0.184-27.244)	0.526
Birth length		0.017*
Normal	1	
Stunted	4.600 (1.318-16.062)	
MAD status		0.493
Fulfilled	1	
Not fulfilled	0.753 (0.334-1.695)	
Mother's height		0.220
Normal	1	
Stunted	1.633 (0.745-3.576)	

MAD: Minimum acceptable diet; WAZ: Weight-for-age z-score
[†]Logistic regression multivariate analysis
*$p<0.05$

simultaneously. It identified the factors that determine the incidence of stunting in children aged 6-23 months in South Sumatra Province.

According to this study, the child's age factor had a significant relationship with the occurrence of stunting (p=0.031); namely, children aged 12-23 months had a 2.8 times risk of stunting (95% CI: 1.098-6.900) compared to children aged 6-11 months (Table 4). This result is consistent with those of other studies that demonstrated an increased likelihood of stunting as a child's age advances (Chowdhury *et al.*, 2020; Farah *et al.*, 2021). Similar results were shown by a study conducted in Northeast Ethiopia, wherein stunting was more common in children aged 12–23, 24–35, and 36–39 months compared to children aged 6–11 months (Gebreayohanes & Dessie, 2022).

This condition may be caused by motor skills development in different age groups, wherein children aged 6-11 months tend to be in their mother's arms more often and played in limited environments (e.g., on the bed, in a room at home) because children aged under 1 year must be kept safe and free from potential hazards so as to lessen the chances of them getting infected or getting sick from exposure to the outside environment (Herzberg *et al.*, 2022). Meanwhile, children aged over 1 year (≥12 months) prefer to move freely and explore the environment, so they are more likely to be in large playrooms and open play areas, such as parks or yards, to provide opportunities for them to run, jump, or move actively. On the other hand, this opens up opportunities for infection if the outside environment turns out to be dirty and polluted or if the children played outside of parental supervision (Kwong *et al.*, 2020).

Food intake is also an influential factor, especially at the beginning of the complementary food period, when such intake is still insufficient among young children. This is compounded by the family's poor economic conditions, along with requirements that grow with age. As a result, stunting occurs more frequently at older ages because of chronic malnutrition (Gewa & Yandell, 2012). One study result showed that the nutritional intakes of stunted children (energy, protein, calcium, and phosphorus) are on average below the recommended dietary allowance (RDA). Inadequate nutritional intake, especially from total energy, can lead to physical growth deficits in children (Ismawati *et al.*, 2020).

Limited food consumption and food diversity are additional determining factors. Children aged 6-11 months tend to consume more breast milk and foods prepared by their mothers (Nicklas, O'Neil & Fulgoni, 2020) in contrast to children aged 12-23 months, who can access various foods outside the home, but do not follow/practise overtly diverse diets given the potential to consume contaminated foods and/ or foods with low nutritional content and micronutrient deficiencies (Damtie, Tefera & Haile, 2020). While one study on children's oral activity revealed that children aged <12 months had a significantly higher frequency and average duration of object-to-mouth/ hand-to-mouth contact than children aged >12 months, this measurement was carried out indoors (Tsou *et al.*, 2015). For parents, improving the quality of food at home and supervising foods obtained outside the home are necessary actions.

In terms of gender disparity, the incidence of stunting as a whole was more common in boys (66.1%) than in girls (p=0.030). Boys were 1.8 times more at risk of stunting than girls (95% *CI* 0.687-4.483). This showed that the condition in South Sumatra was not much different from those in other regions of the world (Chowdhury *et al.*, 2020; Farah *et al.*, 2021). While stunting can affect boys and girls similarly, various studies have shown differences in the prevalence of stunting based on gender. For instance, research conducted in India from 2006 to 2016 revealed that boys were more susceptible to stunting than girls and that they also faced a higher likelihood of mortality at the age of 1 year. This indicates notable sex differences in the prevalence of stunting and mortality (Alderman *et al.*, 2021). A study conducted in Bangladesh also yielded similar results. The proportion of boys (35.7%) with stunting was higher than girls (31%) in the same age range of less than 24 months (Chowdhury *et al.*, 2020). A possible explanation for these findings is that boys and girls may exhibit differences in their preferences and styles of physical activity, although these differences are general and do not apply to every individual. However, this variation can impact the amount of energy expended, as well as the balance between food intake received and energy expenditure.

Some differences that may be seen between boys and girls in terms of physical activity include the following: (1) the type of activity chosen (boys tend to be more interested in strength- and speed-oriented physical activities, while girls are more interested in physical activities which involve more subtle movements); (2) the style of play (boys tend to play more vigorously and energetically, while girls tend to play in a more organised way, involving imaginative play and social roles); (3) and the level of activity (boys generally have a higher level of physical activity than girls; they have a greater urge to move, explore, and be intense) (Rosselli *et al.,* 2020). Therefore, for boys who have more physical activity than girls, insufficient food intake can lead to an energy imbalance, making boys more prone to malnutrition and eventual stunting.

The results of a review by Thurstans *et al.* revealed that despite having larger body sizes at birth and during infancy, boys are more susceptible to malnutrition than girls under conditions of food shortages. Additionally, boys exhibit more vulnerability to infectious diseases because of differences in their immune and endocrine systems. Another significant finding is that while the incidence of low birth weight (LBW) is higher in girls, LBW boys experienced a higher mortality rate than LBW girls (Thurstans *et al.*, 2020).

The results of the analysis also showed a significant relationship between birth length and stunting (p=0.017), and the risk of stunting was 4.6 higher in children with short birth length than children born with normal birth length (95% CI: 1.318-16.062). This finding aligns with those of previous studies. An examination of the results from the 2016 Ethiopian Demographic and Health Survey showed that one of the determinants of stunting was small stature at birth (Farah *et al.*, 2021). Short birth length indicates that there has been a restriction on the child's growth while in the uterus, likely caused by the mother's health during pregnancy (e.g., infectious diseases, chronic diseases, and other health problems) or nutritional factors during pregnancy that were not adequately fulfilled (Thahir *et al.*, 2023). This is in line with the results of this study, which showed that children born with LBW were 1.1 times at risk for stunting. In addition to welfare factors in the womb, genetic factors can also affect the child's body length at birth; when parents have shorter body sizes, it is most likely that the child will have a shorter birth length (Li *et al.*, 2020). According to the results of this study, stunted mothers had a higher prevalence of stunted children (58.8%) compared to mothers with normal height (42.0%); however, this difference did not reach statistical significance (p=0.056).

It is well known that a child's height is influenced by a combination of genetic and environmental factors. Although genetic factors generally play a significant role in determining a person's height, a child's height is also influenced by environmental factors, such as good nutrition, adequate health care, and generally healthy living conditions (Thahir *et al.*, 2023). Adequate nutrition during childhood growth and an environment that supports healthy growth can help children reach their optimal height potential. Although short birth length does not directly determine stunting in children, it can serve as a predictive factor for the risk of stunting at later stages of child development.

The WAZ status of children, as indicated by the results of both bivariate (Table 2) and multivariate analyses (Table 3), revealed a significant relationship with and exerted a substantial influence on stunting. Children with WAZs in the underweight and severely underweight categories had higher odds of stunting (*OR* = 28.7, 95% *CI*: 9.430-87.465; p<0.001) than children in the normal category. In another study in Northwestern Nigeria, children with wasting were more likely to be stunted, and those who were thin and short were predominantly in the age group of 12-23 months (52.4%) (Imam *et al.*, 2021).

Weight-for-age is considered very important to ensure optimal growth in children; adequate nutrition not only supports overall growth, but also fulfils the nutritional requirements for building and repairing tissues, including bones and muscles. Conversely, if a child is underweight or has poor nutrition, his or her body may not have sufficient resources to support optimal growth. Inadequate food intake can cause weight loss and if it is sustainable, it has the potential to inhibit child growth/height, followed by decreased immunity and mucosal damage, increasing the risk of disease (Thurstans *et al.*, 2022). However, being overweight or obese can also have a negative impact; obesity can interfere with the performance of growth hormones, which can then limit a child's height (Zhang *et al.*, 2021).

In cases of malnutrition, a child's nutritional status is also determined from the time of the mother's pregnancy and can be predicted from the infant's birth weight. Maternal nutrition during pregnancy plays an important role in the regulation of foetal-placental development. When the mother is malnourished, the structure of the placenta changes, so the flow of nutrients to the foetus also changes, ultimately affecting the growth and development of the foetus and pregnancy outcomes (Thurstans *et al.*, 2022). Regarding nutrition during pregnancy, this can be well prepared if the mother is still a teenager. It is known that adolescents easily accept lifestyle changes that may determine the course of their lives. They can be protected from diseases, malnutrition, and the immediate and long-term impacts on their own and their children's health by receiving health education interventions on nutrition-related knowledge, attitudes, and practices. This knowledge includes details of food groups, diversity, micronutrient-rich food sources, sanitation, hygiene, and the impact of malnutrition (Shapu *et al.*, 2020).

Referring to the research results that identified the factors influencing stunting in South Sumatra Province such as child's age, birth length, and WAZ status, it is recommended that the provincial government implements a targeted nutrition intervention programme. This programme should prioritise providing additional food for pregnant women with chronic energy deficiency, monitoring the growth and development of toddlers, and managing toddlers with nutritional problems. Through these measures, it is hoped that the province can accelerate the reduction in stunting rates.

This study has several strengths. The researchers deliberately selected locations with diverse characteristics, encompassing both districts and cities in South Sumatra Province. This careful selection aimed to ensure that the findings were representative of the entire province. Additionally, the study examined a substantial number of variables. The limitation of this study was that for this large number of variables, the sample size may not be adequate. Therefore, it is suggested that further research can add more subjects and reach other provinces. Another limitation was that we were unable to match the subjects investigated. We have made matching efforts at the district-level subject selection stage; however, at the health service centre level, when data collection was to be carried out, the research subjects present did not match the designated subjects. Because of time, resource, and technical limitations, matching was not feasible; hence, the children present who fulfilled the study criteria were recruited as research subjects. To mitigate the impact of selection bias, we performed multivariate analysis using a logistic regression test adjusted for age and gender.

CONCLUSION

Factors that affect stunting in children aged less than 24 months vary widely. This study found evidence that a number of factors had a significant influence on the prevalence of stunting, namely child's age, birth length, and WAZ status. In alignment with the specific nutrition intervention programme outlined by the Indonesian government, it is recommended that the South Sumatra provincial government, in accelerating the reduction of stunting in its region, focuses on interventions providing additional food for pregnant women with chronic energy deficiency, monitoring the growth of toddlers, and managing toddlers with nutritional problems. Efforts to increase parents' knowledge about child nutrition need to be carried out on an on-going basis to reduce the incidence of malnutrition and to prevent stunting.

Acknowledgements

Special thanks to the Doctoral Programme in Biomedical Sciences, heads of community health centres, village midwives, nutrition staffs, cadres, and parents of children who have supported this research. This research was funded by the Directorate of Research and Development, University of Indonesia under the PUTI Grant 2022 (Grant No. NKB-202/UN2.RST/HKP.05.00/2022).

Authors' contributions

Andreinie R, principal investigator, conceptualised and designed the study, led the data collection in the South Sumatra Province, conducted the study, data analysis and interpretation, drafted the manuscript, prepared the draft of the manuscript and reviewed the manuscript; Sekartini R, advised on the concept of study and data collection, reviewed the manuscript; Chandra DN, advised on data analysis and interpretation, assisted in drafting the manuscript, reviewed the manuscript; Mudjihartini N, advised on the examination of subject samples and reviewed the manuscript.

Conflict interests

The authors declare that they have no conflict of interest.

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Mal J Nutr 30(2): 143-151, 2024

Plasma amino acid profiles in thalassaemia major with iron overload

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ABSTRACT

Introduction: Iron overload in thalassaemia major patients mainly occurs due to periodic transfusions. When iron exceeds transferrin capacity, non-transferrin bound iron accumulates and causes tissue damage, including in the gastrointestinal tract, resulting in impaired enterocyte function and amino acid absorption. The aim of this study was to evaluate amino acid profiles in patients with thalassaemia major after repeated transfusions and chelation. **Methods:** Whole blood amino acids were analysed from dried blood spots using liquid chromatography tandem mass spectrometry. This study consisted of two parts: a cross-sectional and a cohort study in thalassaemia-β-major patients. In the cross-sectional study, amino acid profiles were analysed in 219 thalassaemia patients who received routine transfusion and chelation therapy, and 60 healthy control subjects. The cohort study included 21 subjects, from whom blood samples were taken at pre-transfusion, 1-day post-transfusion, one and three months post-chelation to evaluate changes in amino acid levels. **Results**: There were significant differences between amino acid levels in thalassaemia subjects and controls. Positive correlations were found between serum iron and transferrin with age, also between transferrin with proline, valine, phenylalanine, aspartic acid, and glutamic. Phenylalanine and aspartic acid were significantly lower in subjects with transferrin lower than 180 μg/dL. Significant correlations were found between haemoglobin with essential and non-essential amino acid groups. From the cohort study, significant changes were observed in glycine, alanine, leucine, and aspartic acid. **Conclusion:** Amino acid profiles in thalassaemia patients differed compared to healthy controls, even after transfusion and chelation. Phenylalanine and aspartic acid were significantly lower in subjects with low transferrin levels.

Keywords: amino acid, chelation, iron overload, thalassaemia, transfusion

INTRODUCTION

Thalassaemia is a congenital disorder characterised by impaired synthesis of one or more globin chains of the haemoglobin protein. It is the most common genetic disorder in the world,

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doi: https://doi.org/10.31246/mjn-2023-0085

including in Indonesia, with a global prevalence of 7%. Thalassaemia is prevalent in the thalassaemia belt, which stretches from the Eastern Mediterranean through the Middle East, India to Southeast Asia, and North to South Africa (Mehta & Keohane, 2012). The prevalence of carriers in India, Thailand, and Indonesia are among the highest in Asia and Southeast Asia (up to 15%). Thalassaemia's clinical manifestation is associated with its genetic mutations (Mehta & Keohane, 2012).

Data obtained from the Thalassaemia Center in Cipto Mangunkusumo Hospital (RSCM) showed 1,570 registered thalassaemia patients and the number of new patients per year was approximately 49-75 (Thalassaemia Center, 2016). Thalassaemia major patients may suffer from iron overload due to ineffective erythropoiesis and periodic transfusions, which may be accompanied by the dysfunction of multiple organs (Mariani, *et al.*, 2009; Patel & Ramavataram, 2012). The amount of plasma iron could exceed the binding capacity of transferrin and as a result, not all iron is bound because it has been fully saturated, generating toxic-free iron or non-transferrin bound iron (NTBI) (Brittenham, 2011; Gkouvatsos, Papanikolaou & Pantopoulos, 2012). Free iron stimulates reactions in body cells, generating oxygen radicals, which can affect tissues in the gastrointestinal tract, liver, pancreas, and small intestine; this can lead to the organ's dysfunction and damage (Mariani *et al.*, 2009; Patel & Ramavataram, 2012; Brittenham, 2011). Less transferrin availability will result in more NTBI and more damage.

Abnormalities in the enterocytes due to iron overload can also cause diminished protein digestion and amino acid absorption (Wu, 2013). Children with thalassaemia often have short stature and are undernourished. Iron overload in thalassaemia major patients can be reduced by chelation therapy and the many iron binding proteins in the body. Reductions in protein levels, such as transferrin, will cause increases in NTBI. Therefore, the level of amino acids in a person's body needs to be sufficient to sustain the level of proteins, including transferrin (Brittenham, 2011; Poggiali *et al.*, 2012).

Amino acids are organic substances containing amino and acid groups (Wu, 2013). The breakdown of dietary and endogenous proteins is a source of amino acids for protein synthesis. Dietary protein is digested by proteases in the gastrointestinal tract, which produces amino acids. Amino acids that cannot be synthesised *de novo* are called essential amino acids and these are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. These amino acids are obtained from dietary intakes of meat, milk, eggs, and fish. Amino acids that can be synthesised by the body are called non-essential amino acids, which include aspartic acid, asparagine, glutamic acid, cysteine, glycine, serine, tyrosine, citrulline, ornithine, arginine, proline, and alanine (Wu, 2013). Absorption of free amino acids and small peptides occurs in the enterocytes through several mechanisms: passive diffusion, the Na-independent system, and the Na-dependent system.

The aim of this study was to describe the plasma amino acid profile of thalassaemia major patients and to determine the profile changes after transfusion and chelation therapy. Differences in the amino acid levels of thalassaemia patients compared to healthy subjects may provide insights into the nutritional requirements for improving their quality of life.

MATERIALS AND METHODS

This study consisted of two parts: a cross-sectional study and a cohort study of thalassaemia-β major and thalassaemia-β/Hb E patients, as well as healthy controls. Control subjects were healthy patients with normal haematology results and normal liver and kidney function tests. The cross-sectional study included 219 patients who had received repeated transfusions and iron chelation therapy, and 60 healthy controls. The sample size for thalassaemia subjects was calculated based on the rule of thumb parameters, using a ratio of 1:10 and the samples were taken consecutively. The study was conducted at the Thalassaemia Center and the Central Laboratory of Cipto Mangunkusumo Hospital and Indonesian Medical Education and Research Institute (IMERI) Faculty of Medicine, University of Indonesia, from March 2018 to October 2019. The participants were children with thalassaemia major who visited the polyclinic, routinely received transfusion and iron chelation therapies, and were willing to participate in the study. Their parents signed a form of informed consent. The controls were all healthy subjects with no history of thalassaemia and they were unmatched.

For this study, haemoglobin, serum iron (SI), transferrin, and ferritin were extracted from the hospital medical records. Samples were collected on the first day of the polyclinic routine control schedule before transfusion (pre-transfusion). Haemoglobin was analysed using the Sysmex blood cell counter (XE 5000, Japan) and iron was analysed using the Roche autoanalyser (Cobas-311, USA). Transferrin was measured as total iron binding capacity (TIBC). Blood samples for amino acid analysis were collected using dried blood spot (DBS) samples and kept at –20°C until analysis.

The cohort study was performed with 21 thalassaemia subjects. Blood samples were taken at pre-transfusion; one day post-transfusion; one month post-transfusion; and three months post-transfusion. The purpose was to identify if their amino acid profiles would change during and after chelation and transfusion therapies. This study was approved by the Research Ethics Board of the Faculty of Medicine, University of Indonesia.

Amino acid concentrations from DBS samples were examined using the Waters Xevo TQD – liquid chromatography tandem mass spectrometry (LCMSMS, UK), with the Chrom-system kit for 13 amino acids (Chromsystem, 2019). To evaluate amino acid profile, the concentration of each amino acid was calculated using pre-transfusion data as baseline.

Statistical analyses of changes in the amino acid concentration after transfusion and after chelation were performed using IBM SPSS Statistics for Windows version 20.0 (IBM Corp, Armonk, New York, USA). Results with $p<0.05$ were considered statistically significant. Data with normal distribution data are shown as mean±standard deviation (*SD)* and data with abnormal distribution are shown as median (percentile 5-95).

RESULTS

In the cross-sectional study, there were 219 thalassaemia-β major and thalassaemia-β/Hb E subjects, consisting of 105 females and 114 males, who had their amino acid profiles analysed (Table 1). The results analyses in this study were not differentiated by gender, type of thalassaemia, or chelation received. There was no significant difference in the haemoglobin, SI, TIBC, transferrin

Table 1. Characteristics of thalassaemia subjects (*n*=219)

Characteristic of subject	*n* (%)	*Mean/Median*
Gender		
Female	105 (47.9)	
Male	114 (52.1)	
Age (year)		11.9 (0.3 – 32)
Haemoglobin (g/dL)†		8.7 (5.0 – 11.3)
SI (μg/mL)		158 (59 – 258)
TIBC (μg/dL)		183 (127–294)
Transferrin Saturation (%)		96 (32 – 100)
Ferritin (ng/mL)		1038 (434 – 11751)

SI: Serum iron; TIBC: Total iron binding capacity
Data are shown in median (min-max), except haemoglobin (g/dL)† in mean±*SD*

saturation, and ferritin levels between males and females ($p>0.05$). The median transferrin saturation and ferritin levels were very high, but transferrin measured as TIBC was mostly low. The analysis was compared to control subjects, comprising 30 healthy males and 30 healthy females aged 8-35 years old with no history of thalassaemia.

Haemoglobin levels in thalassaemia major subjects were between 5.6 and 11.3 g/dL, respectively, with 27.4% subjects having a haemoglobin level of below 8 g/dL. Significant, positive correlations were found between total essential and non-essential amino acids group levels and haemoglobin levels ($p<0.050$).

There were significant differences between the medians of each amino acid level in thalassaemia subjects and control subjects ($p<0.05$), except for aspartic acid ($p=0.160$), as shown in Table 2. Different patterns of amino acids can be observed, especially between the lower and upper boundaries. In subjects with thalassaemia, amino acid levels were mostly higher compared to the control group. The levels of amino acids in control subjects were compared to other countries, as shown in Table 3 (Tan & Gajra, 2006; Mayo Clinic

Table 2. Profile of amino acids in thalassaemia subjects compared to control group

Amino acids (μmol/L)	*Thalassaemia (Median; n=219)*	*Control subjects (Median; n=60)*	*p*
Glycine	349 (96-583)	194 (130-308)	0.001
Alanine	268 (89-446)	95 (74-159)	0.001
Proline	189 (97-312)	124 (62-191)	0.004
Valine	121 (56-196)	111 (53-285)	0.014
Leucine	155 (66-290)	117 ((57-277)	0.025
Ornithine	321 (78-627)	50 (30-93)	0.001
Methionine	244 (11-555)	15 (5-19.8)	0.007
Phenylalanine	77 (23-325)	36 (10-68)	0.002
Arginine	16 (2-34)	23 (7-40)	0.028
Citrulline	58 (13-164)	27 (17-46)	0.006
Tyrosine	31 (1-92)	31(28-65)	0.012
Aspartic acid	31 (2-182)	33 (6-138)	0.160
Glutamic acid	102 (39-266)	23 (6-42)	0.004

Table 3. Range of amino acids compared to other populations

Amino acids (µmol/L)	*Control subjects (n=60)*	*Reference Singapore*†	*Reference USA*‡
Glycine	130-308	135-342	149-417
Alanine	74-159	260-585	144-557
Proline	62-191	82-301	80-357
Valine	53-285	169-354	106-320
Leucine	57-277	96-203	51-196
Ornithine	30-93	36-92	22-97
Methionine	5-19.8	26-48	11-37
Phenylalanine	10-68	48-73	30-95
Arginine	7-40	61-132	31-132
Citrulline	17-46	14-61	11-45
Tyrosine	28-65	48-96	31-106
Aspartic acid	6-138	2-18	<11
Glutamic acid	6-42	4-68	22-131

† Tan IK & Gajra B (2006).
‡ Mayo Clinic Laboratories (2020).

Laboratories, 2020). Some were lower compared to reference values from Singapore and USA, except for aspartic acid. The references are included here as a note that it is common to see differing reference levels by country as they can be reflective of many factors, including nutritional status.

There were positive, significant correlations between age and serum iron (r=0.189, p=0.005), transferrin as TIBC (r=0.149, p=0.027), and ferritin (r=0.461, p=0.001), but no correlations were found between age and transferrin saturation (Table 4). Transferrin was fully or nearly saturated (90-100%) in 54% of subjects and only 16% of subjects had a transferrin saturation below 55%. Total iron binding capacity (TIBC), which represents transferrin, was between 117-420.5, with 46.1% of subjects having low TIBC (below 180 µg/dL). Significant correlations were present between transferrin, expressed as TIBC, and proline, valine, phenylalanine, aspartic acid, and glutamic acid levels.

Using multivariate analysis, it was shown that TIBC was most influenced by the amino acids arginine and tyrosine (TIBC= 130.008 + 2.299 Arginine + 0.948 Tyrosine). Phenylalanine and aspartic acid were significantly lower in thalassaemia subjects, with a TIBC of less than 180 µg/mL compared to those with a TIBC of more than 180 µg/mL. Subjects with ferritin levels of more than 8000 ng/mL had significantly lower levels of aspartic acid (p=0.032) and glutamic acid (p=0.011) compared to subjects with lower ferritin levels.

Table 4. Correlations for TIBC to amino acids

Amino acids	*Correlation coefficient (r)*	*p*
Glycine	-0.007	0.923
Alanine	0.104	0.126
Proline	0.151	0.026
Valine	0.219	0.001
Leucine	0.129	0.570
Ornithine	-0.038	0.567
Methionine	-0.085	0.210
Phenylalanine	0.270	0.001
Arginine	0.092	0.175
Citrulline	0.046	0.502
Tyrosine	0.095	0.160
Aspartic acid	0.271	0.001
Glutamic acid	0.152	0.025

TIBC: Total iron binding capacity

Table 5. Cohort study results: changes in amino acid profiles during transfusion and chelation therapies

Amino acids (µmol/L)	*Pre-transfusion (n=21)*	*D-1 post-transfusion (n=21)*	*D-30 post-transfusion (n=21)*	*D-90 post-transfusion (n=21)*	*p*
Glycine	142 (97-206)	144 (115-225)	233 (161-308)	242 (161-326)	0.001*
Alanine	324 (160-142)	325 (214-478)	280 (168-394)	298 (164-486)	0.021*
Proline	218 (163-283)	234 (123-408)	226 (114-419)	248 (155-395)	0.813
Valine	161 (119-215)	173 (111-275)	174 (116-240)	176 (123-263)	0.306
Leucine	182 (123-247)	201 (125-334)	133 (100-235)	139 (100-235)	0.001*
Ornithine	246 (172-380)	246 (176-363)	220 (166-331)	218 (138-414)	0.488
Methionine	21 (14-43)	25 (14-42)	20 (11-28)	19 (13-42)	0.261
Phenylalanine	70 (40-181)	93 (50-264)	75 (42-148)	78 (50-251)	0.553
Arginine	9.8 (2.2-30)	13.3 (4-25)	8.6 (2.4-27)	11.0 (2.6-27)	0.261
Citrulline	27 (20-39)	29 (19-54)	33 (24-43)	31 (24-52)	0.261
Tyrosine	52 (32-89)	64 (35-92)	49 (33-87)	52 (46-118)	0.137
Aspartic acid	77 (47-99)	81 (50-112)	91 (56-145)	94 (69-144)	0.001*
Glutamic acid	189 (132-395)	164 (119-261)	160 (112-250)	153 (121-515)	0.634

Cohort study data of pre-transfusion; day 1 post-transfusion (D-1); 1 month post-transfusion (D-30); 3 months post-transfusion (D-90)
Data are shown in median (percentiles 5 & 95)
*$p<0.05$

In the cohort study, there were 21 thalassaemia major subjects (10 females and 11 males; aged 4-18 years). In this study, we also did not differentiate data by gender, type of thalassaemia, or chelation therapy. Data showed that following transfusion and chelation therapies, there were mostly no significant changes in amino acid levels, except for increases in glycine and aspartic acid, and decreases in alanine and leucine compared to pre-transfusion levels (Table 5). Transfusion of packed red cells still contained 20%-30% plasma, which can increase or decrease post-transfusion amino acid concentrations depending on the concentration of amino acids in the donor's plasma. These changes could also be caused by the food intake of the subjects. Thus, it is important to review all of the amino acid profiles, regardless of transfusion and chelation therapies, in order to determine the patient's nutritional needs.

DISCUSSION

Thalassaemia major is a congenital disorder that causes anaemia, which requires repeated transfusions and iron chelation therapy to prevent iron overload. In a state of iron overload, NTBI can stimulate reactions that produce oxygen radicals, which can damage tissues, including those in the gastrointestinal tract, consequently impairing nutrient digestion and absorption (Wu, 2013).

The mean haemoglobin levels of thalassaemia subjects in this cross-sectional study was 8.7 (range 5.0-11.3) g/dL, and 27.4% of subjects had a haemoglobin level of below 8 g/dL. Low haemoglobin causes lower metabolic body capacity, although some studies in sickle cell haemoglobinopathy have shown a hypermetabolic state requiring more micro- and macronutrients such as arginine for body metabolism (Umeakunne & Hibbert, 2019). In this study, both essential and non-essential amino acid groups were positively correlated with haemoglobin, showing that more amino acids might improve the haemoglobin levels in thalassaemia patients. A study by Malluvalasa among thalassaemia children in India reported lower haemoglobin values than those reported in our study. In their study, mean haemoglobin was 7.73±1.10 g/dl; 52% had a pre-transfusion haemoglobin of less than 8 g/dL and there was no difference between genders. The mean ferritin level in those children was more than 3400 ng/mL, higher than our study (Malluvalasa, Sahoo & Kuppilli, 2018).

The analyses of age and iron status in thalassaemia subjects showed that body iron concentration, serum iron, and transferrin (measured as TIBC and ferritin) increased with age. Increased serum iron due to multiple transfusions can be a result of insufficient chelation therapy in these subjects, causing body iron to increase with age. Higher levels of iron increase the risk of oxidative stress caused by free iron not bound to transferrin. Transferrin, measured as TIBC, ranged from 127 to 294 mg/dL and showed a positive correlation, but 46.1% subjects had low transferrin (below 180 µg/dL). Impaired body metabolism and insufficient amino acid intake can result in low body production of transferrin. Less transferrin availability causes an increase in non-transferrin bound iron in the circulation and results in more damage to the organs.

Transferrin was fully or nearly saturated (90-100%) in 54% of subjects. Higher transferrin level decreases the saturation and also reduces the chance of producing NTBI. Table 4 shows significant, positive correlations between transferrin concentration (expressed as TIBC) and proline, valine, phenylalanine, aspartic acid, and glutamic acid; suggesting that the availability of these amino acids may be helpful in increasing the concentration of transferrin. Transferrin levels were most influenced by arginine and tyrosine. Arginine and tyrosine have a high influence on TIBC and haemoglobin production, although other amino acids also have a role (Papassotiriou *et al.*, 2018). Therefore, higher levels of arginine and other amino acids may be beneficial in increasing transferrin level, decreasing NTBI, and improving oxidative stress in high metabolic states (Malluvalasa *et al.*, 2018). Lower phenylalanine and aspartic acid levels may be caused by low intake, increased consumption by body metabolism, or gut microbiota metabolism (Umeakunne & Hibbert, 2019; Dodd *et al.*, 2017).

No correlation was found between age and transferrin saturation. This was due to the reaction principle of the reagents that were used. Transferrin as TIBC was measured indirectly by calculation of the unsaturated iron binding capacity and a high NTBI interferes with the reaction, causing error in the TIBC calculation (Roche, 2018).

The profiles of amino acids are important to be determined among thalassaemia patients as their levels were significantly different from controls. Most amino acids were significantly higher in thalassaemia patients, except for arginine and tyrosine. These differences in amino acid levels showed that thalassaemia subjects have a distinctly different metabolism and a need for more amino acids to improve their impaired body metabolism due to iron overload. Arginine has a role in cell division, stimulation of protein synthesis, hormone release, and cytokine production. Arginine is a conditionally essential amino acid in children and can become an essential amino acid under stressful states, such as haemolytic anaemia and thalassaemia (Umeakunne & Hibbert, 2019; Morris *et al.*, 2017). Tyrosine can be synthesised from phenylalanine; it is a precursor of some hormones and has a role in metabolism in the adrenal, pituitary, and thyroid glands. These glands are also usually affected and damaged by the condition of iron overload. Different profiles of amino acids in thalassaemia subjects versus the control group may also reflect the higher body turnover of cell catabolism, increased need for amino acids as building blocks for protein, and increased absorption of some amino acids among the former.

Amino acid levels in the control group were somewhat different in comparison to the Asian population (Singapore) and in the USA. This could be due to different nutritional intakes, mostly proteins, in each country. Our hospital is the national referral hospital in Indonesia. Patients treated in this hospital come from different provinces in Indonesia, with different food habits. In the Thalassaemia Center in Bandung City, a study of 80 thalassaemia subjects aged 10-14 years showed that 81.2% had a short stature (Elizabeth *et al.*, 2018); improving their amino acid intakes may help to improve their body condition. In this study, very high ferritin levels were found in 35.6% subjects and levels of more than 10,000 ng/mL were also found in 10.5% subjects. A negative correlation between height-for- age *z-score* of <–2 and serum ferritin had also been reported by other studies (Moiz *et al.*, 2018; Abdulrazzaq *et al.*, 2005).

In the cohort study, 21 thalassaemia major subjects (10 females and 11 males; aged 4–18 years) were observed for three months. The fluctuations observed in amino acids before and three months after transfusion and chelation in this study mostly did not change significantly except for glycine, aspartic acid, alanine, and leucine. This showed that these amino acids were required for body metabolism. The results also indicated that the enterocyte function had remained relatively unchanged during the three-month period of transfusion and chelation. These changes could be caused by daily diet and the content of plasma in the transfused blood.

CONCLUSION

Not many research studies on the metabolomic analysis of amino acid levels are available that include patients with thalassaemia suffering from iron overload while receiving multiple transfusion and chelation therapies. This study showed that there was a different pattern of amino acid levels in the thalassaemia and control groups. Most of the amino acid levels were significantly higher in thalassaemia subjects. The differences were caused by high turnover of cells as the body requires more amino acids for its metabolism and regeneration of new cells. Nearly half of the thalassaemia subjects had low transferrin levels and it can cause more toxic NTBI. Half of the thalassaemia subjects also had very high ferritin levels, which showed that their chelation therapy was not adequate. Transferrin as TIBC was most influenced by the levels of arginine and tyrosine. Further study is required to determine if supplementation of these amino acids will improve the availability of transferrin. In the cohort study, it was found that some amino acid levels were significantly higher after three months of observation, which showed that these amino acids may be required for body metabolism.

Acknowledgments

We would like to thank The Human Genetic and Research Center Indonesian Medical Education and Research Institute (IMERI) – Faculty of Medicine; the University of Indonesia through PITTA for facilitating and funding this study (ID: NKB-1700/UN2.R3.1/HKP.05.00/2019). We would also to thank all the subjects for participating in this study.

Authors' contributions

Timan IS, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; Wahidiyat PA, supervised the data collection of patients and reviewed the manuscript; Sjarif DR, led the data interpretation and reviewed the manuscript; Pasaribu MM, co-designed the study and supervised the data analysis; Putri F, collected the blood samples and analysed the samples; Widjaja L, reviewed the manuscript.

Conflict of interest

The authors declare that they have no potential conflict of interest.

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Mal J Nutr 30(2): 153-165, 2024

Effect of vitamin D supplementation on serum 25(OH) D levels and blood pressure among the elderly in a nursing house: A double-blind, randomised placebo-controlled trial

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ABSTRACT

Introduction: Hypertension is the most common cardiovascular disease, especially in the elderly. Previous studies have reported that vitamin D plays a role in blood pressure. This study aimed to analyse the effect of vitamin D supplementation on serum 25(OH)D levels and blood pressure. **Methods:** This was a double-blind, randomised placebo-controlled trial (RCT) on elderly subjects. Serum 25(OH)D levels were examined using chemiluminescent immunoassay (CLIA) method, while blood pressure was checked using a digital sphygmomanometer. Supplementation was given once per day for eight weeks; the control group was given a placebo, while the treatment group was given 2,000 IU vitamin D_3 for subjects with insufficiency and 4,000 IU for subjects with deficiency. **Results:** A total of 62 subjects aged 60-89 years participated and were randomised into 30 control and 32 treatment group subjects. Data analysis showed that vitamin D supplementation significantly increased 25(OH)D levels in treatment group (Δ=18.2±5.2 ng/mL) compared to control group (Δ=4.2±2.5 ng/mL) (p<0.001). However, vitamin D supplementation did not cause significant reduction in systolic blood pressure (Δ=-4.6(-25 - -0.5) mmHg for control group and Δ=-9.2(-20 - -27) mmHg for treatment group; p=0.109), and diastolic blood pressure (Δ=-7.2(-16 - -2) mmHg for control group and Δ=-8.4(-14.5 - -8.5) mmHg for treatment group; p=0.559). **Conclusion:** Vitamin D supplementation significantly increased serum 25(OH)D levels, but did not significantly reduce systolic and diastolic blood pressures in the elderly. Elderly people need to regularly check their vitamin D levels so that the provision of supplementation can be timely and their quality of life can be improved.

Keywords: blood pressure, elderly, serum 25(OH)D level, vitamin D supplementation

INTRODUCTION

Hypertension is the most common cardiovascular disease and it affects most people. Data from the World Health Organization (WHO) in 2015 showed that 1.13 billion people worldwide suffer from

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doi: https://doi.org/10.31246/mjn-2023-0136

hypertension, which increased yearly (Bloch, 2016). Approximately 9.4 million people die yearly from hypertension and its complications (Whelton *et al.*, 2017). The incidence of hypertension is higher in people of developing countries compared to developed countries, with around 75% of people with hypertension living in developing countries (Hamdan *et al.*, 2012). According to data from the 2018 Basic Health Research (Riskesdas), the prevalence of hypertension in Indonesia was 34.1%, with a mortality rate of 42,7218 deaths. Hypertension mainly affects the age groups of 55-64 years (55.2%), 65-74 years (63.2%), and >75 years (69.5%) (Ministry of Health Indonesia, 2018).

Indonesia is currently entering a period of an ageing population with an increase in life expectancy, followed by an increase in the number of elderly. The increase in the number of elderly in Indonesia from 2010-2019 amounted to 7.9 million (2.1%) and will continue to increase to 48.2 million by 2035. As such, everyone will need to start paying attention to the needs and health of the elderly so that they can remain healthy, independent, active, and productive (Ministry of Health Indonesia, 2019). Based on Riskesdas's 2018 data, the most common diseases suffered by the elderly are non-communicable diseases such as hypertension, dental problems, joint disease, mouth problems, diabetes mellitus, heart disease, and stroke. In the elderly, there are also functional changes in the body that can affect nutrient bioavailability, including vitamin D (Ministry of Health Indonesia, 2018).

Several studies have shown that vitamin D is associated with cardiovascular disease, especially hypertension. Vitamin D plays a role in suppressing renin production so that the renin-angiotensin-aldosterone system is not activated and blood pressure does not increase (Zhang *et al.*, 2020). Vitamin D deficiency can be defined by low serum levels of 25-hydroxy vitamin D (25(OH)D) and can be prevented by increasing vitamin D synthesis through sun exposure, consuming foods rich in vitamin D, and supplementation (Whelton *et al.*, 2017).

Previous studies have also shown that vitamin D supplementation significantly increased serum 25(OH)D levels. Still, there are inconsistent results in blood pressure reduction among the elderly when the administration of vitamin D supplementation was the same for all subjects regardless of their previous serum 25(OH)D level status. This study was conducted to better analyse the effect of vitamin D supplementation on serum 25(OH)D levels and blood pressure in elderly people with hypertension compared to placebo based on their serum 25(OH)D level status.

MATERIAL AND METHODS

Ethics statement

This study was approved by the Ethics Committee of Faculty of Medicine, Universitas Indonesia – Dr. Cipto Mangunkusumo Hospital, Jakarta on March 13, 2023, with certificate number KET-314/UN2.F1/ETIK/PPM.00.02/2023. All subjects had signed an informed consent before starting the study and all data collected in this study were kept confidential. Presentation of study results in scientific meetings/conferences and publications in scientific journals will not include subjects' name.

Study design and population

This was an experimental, randomised, double-blind, placebo-controlled trial of elderly with vitamin D insufficiency or deficiency and hypertension. Subjects were the elderly living in the Budi Mulia I Nursing Home between

April 2023 and June 2023. Inclusion criteria included: age of ≥60 years; a resident in Panti Sosial Tresna Werdha Budi Mulia I at recruitment and for an eight-week study period; systolic blood pressure >120 mmHg and/or diastolic >80 mmHg; serum 25(OH)D levels <30 ng/mL; and ability to give written informed consent. Exclusion criteria included: bedridden, malnourished (Mini Nutrition Assessment-Short Form (MNA-SF) score <8), hypercalcemia (calcium level >10.4 mg/dL), suffering from cancer / autoimmune / kidney or liver diseases, and consumed vitamin D supplementation >400 IU for four weeks.

Following informed consent, a screening evaluation of demographic and laboratory assessments were performed. A total of 69 subjects attended the baseline interview. The interview included collecting information on demographic status (age, sex, education level, co-morbidities, intake of drugs/vitamins, sun exposure, MNA-SF questionnaire). Blood pressure was measured after resting with a digital sphygmomanometer, with an average result from two -time measurements. Serum 25(OH)D level was measured using blood samples from the subject's cubital fossa after sterilising the area with alcohol cotton. As much as 5 ml of blood specimen was taken and stored in a vacutainer ethylenediamine tetraacetic acid (EDTA), labelled with the subject's identity. 25(OH)D level was examined using the chemiluminescent immunoassay (CLIA) method. Blood pressure and laboratory tests were performed prior to initiating therapy and after eight weeks.

Randomisation and treatment allocation

Subjects were randomly assigned to receive either 2,000 IU vitamin D_3 for subjects with insufficiency [25(OH) D level 20-30 ng/mL] and 4,000 IU for subjects with deficiency [25(OH) D level <20 ng/mL] or an identical placebo supplement containing Saccharum lactis (a lactose powder with no pharmacological activity). One individual (pharmacist) was assigned to randomise the subjects; this individual did not participate in recruitment, data collection or analysis. Subjects, nursing home staffs, investigators, and analysts were also blinded to group assignment. Nursing home staffs made sure all subjects took their vitamin D_3 or placebo supplementation every day. There was no cross-over and all subjects were analysed in per-protocol manner.

An eight-week vitamin D supplementation was chosen because of pharmacokinetic evidence indicating that the loading dose of vitamin D is 8-10 weeks (Michael *et al.*, 2011). Vitamin D_3 or placebo oral capsules were distributed to subjects. Capsules for the first four weeks were distributed one day after randomisation, followed by capsules of vitamin D_3 (or placebo) for the next four weeks distributed on the fourth week. Evaluation of side effects were checked every two weeks.

Outcomes

The primary outcome measures were systolic blood pressure, diastolic blood pressure, and serum 25(OH)D level. All assessment were collected in the morning after breakfast at baseline and 8 weeks after.

Statistical analysis and sample size

All analyses were conducted using the IBM SPSS Statistics for Windows version 20.0 (IBM Corp, Armonk, New York). Normality of data was tested using the Kolmogorov-Smirnov test; normal distribution data ($p>0.05$) were presented in mean±standard deviation (*SD*), while abnormal distribution data ($p<0.05$) were shown in median. Equality of baseline characteristics were performed using

Chi-square test for categorical scale, independent *t*-test or Mann-Whitney U test for numeric scale. Within group comparisons were performed using the paired *t*-test or Wilcoxon signed-rank test. Between group comparisons were performed using independent *t*-test. A $p<0.05$ was considered statistically significant. Comparison of two mean calculation estimated that a minimum of 31 subjects in each arm would enable us to detect a change in blood pressure and serum 25(OH)D level. The sample size was sufficient to detect approximately 10% reduction.

RESULTS

Six of the 69 research subjects who attended the initial screening session did not meet the inclusion criteria; only 63 subjects met the requirements of the study. Furthermore, the subjects were randomly divided into two groups – the control group and the treatment group. The 63 selected subjects followed the entire study procedure for eight weeks, but one subject dropped out due to death because of tuberculosis in the seventh week. Thus, the subjects with complete data included in the analysis were 62 (Figure 1).

Baseline characteristics

Baseline characteristics of research subjects can be seen in Table 1. The average age of subjects was 68 years, with the majority aged 60-74 years. Majority of subjects were females (42 subjects). The highest levels of education were primary education and secondary education. The most common co-morbidity was hypertension in 22 subjects. The drug consumed by subjects was anti-hypertensive in 12 people.

Sun exposure score was assessed using a questionnaire asking how long subjects were exposed to the sun per day and which parts of the body were exposed to the sun. The mean exposure score was 23, with 36 subjects exposed to the sun for 5-30 minutes per day. For

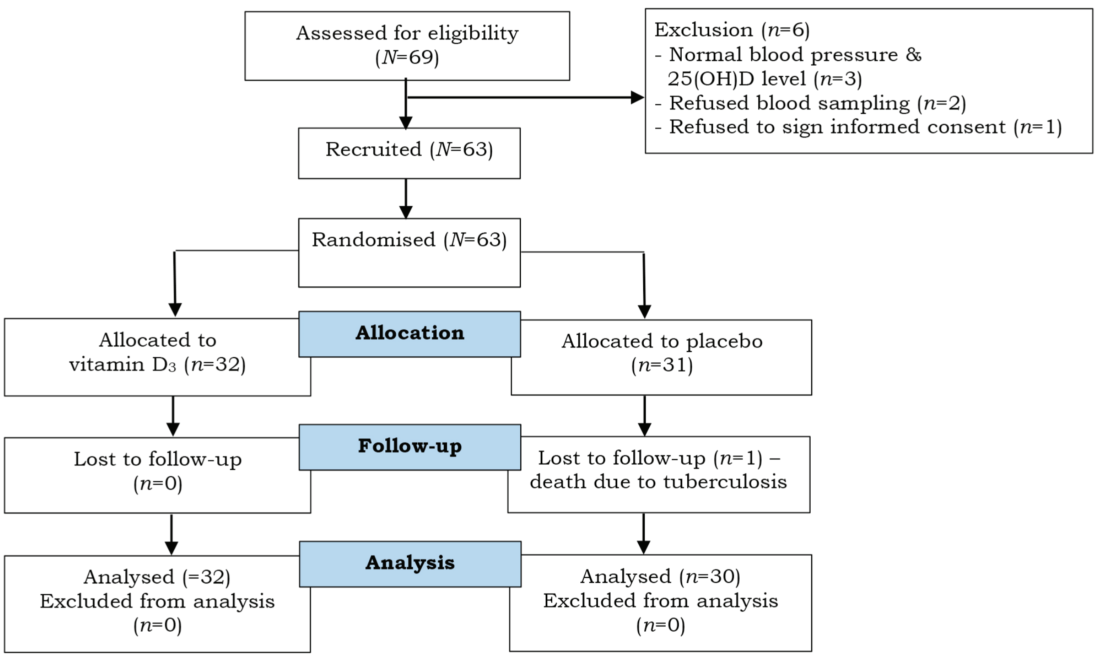

Figure 1. Flow chart of subject selection

Table 1. Baseline characteristics of research subjects

Characteristics	*Total (N=62)*		*Control (n=30)*		*Treatment (n=32)*		*p*
	n	*%*	*n*	*%*	*n*	*%*	
Gender							0.005†
Male	20	32.0	14	46.7	6	18.7	
Female	42	68.0	16	53.3	26	81.2	
Age							0.446†*
60-74 years	50	80.6	25	83.3	25	78.1	
75-90 years	12	19.3	5	16.7	7	21.9	
Education							0.268†*
No education	12	19.3	4	13.3	8	25.0	
Primary school	24	38.7	12	40.0	12	37.5	
Middle school	24	38.7	14	46.7	10	31.2	
High school	2	3.2	0	0.0	2	6.2	
Co-morbidity							0.689†*
Hypertension	22	35.5	10	33.4	12	37.5	
Diabetes mellitus	3	4.8	1	3.3	2	6.2	
Gout	9	14.5	3	10.0	6	18.7	
Osteo-arthritis	8	12.9	4	13.3	4	12.5	
Etc.	20	32.3	12	40.0	8	25.0	
Drug consumed							<0.001†
Antihypertension	12	19.3	8	26.7	4	12.5	
Sun exposure							0.454†*
Duration per day							
<5 minutes	4	6.4	2	6.7	2	6.2	
5 – 30 minutes	36	58.1	17	56.6	19	59.4	
>30 minutes	22	35.5	11	36.7	11	34.4	
Exposure area							
Face, hand	17	27.4	10	13.3	7	40.6	
Face, hand, arm	45	72.6	20	86.7	25	59.8	
Body mass index (kg/m^2)							0.213†*
Underweight (<18.5)	10	16.1	6	20.0	4	12.5	
Normal (18.5 – 22.9)	29	46.8	13	43.3	16	50.0	
Overweight (23 – 24.9)	10	16.1	7	23.3	3	9.4	
Obese I (25 – 29.9)	7	11.3	1	3.4	6	18.7	
Obese II (≥30)	6	9.7	3	10.0	3	9.4	
MNA-SF score							0.518‡*
Normal nutritional status (12-14)	16	25.8	8	26.7	8	25.0	
At risk of malnutrition (8-11)	46	74.2	22	73.3	24	75.0	
25(OH)D level (ng/mL)	20.4 (8.7 – 29.4)		23.5 (8.7 – 29.4)		16.7 (8.7 – 26.2)		<0.001‡
Calcium level (mg/dL)	9.5 (8.8 – 10.4)		9.5 (8.8 – 10.4)		9.5 (8.9 – 10)		0.681‡*
Blood pressure (mmHg)							
Systolic	134.6 (121-180)		133.9 (121-159.5)		135.3 (121-180)		0.787§*
Diastolic	89.4 (80–105)		89.6 (80-105)		89.2 (81.5–98.5)		0.594§*

MNA-SF score: Mini Nutrition Assessment-Short Form
†Chi-square test; ‡Independent *t*-test, §Mann-Whitney U test
**p*>0.05 indicates equality

exposed body areas, 45 subjects reported face, hands, and arms. Calculation of body mass index found 29 subjects with normal weight. The results of malnutrition screening using the MNA-SF questionnaire found that 46 subjects were at risk of malnutrition.

For 25(OH)D levels before supplementation, 34 subjects experienced vitamin D insufficiency and 28 subjects experienced vitamin D deficiency, with an average 25(OH)D of 23.2 ng/mL before supplementation. Examination of blood calcium levels before supplementation showed normal results in all subjects, with an average calcium value of 9.5 mg/dL. Blood pressure was checked twice and the results were averaged. The results obtained were: 46 subjects had pre-hypertension, 14 subjects had grade I hypertension, and two subjects had grade II hypertension, with an average systolic blood pressure of 134.7 mmHg and diastolic blood pressure of 89.4 mmHg.

In this study, an equivalence test was carried out on the characteristics of subjects between the control and treatment groups. The results of the equivalence test on the characteristics of gender, age, education, co-morbidities, drugs consumed, sun exposure, BMI, MNA-SF score, 25(OH)D levels, calcium levels, and blood pressure can be seen in Table 1. The *p*-values for age (*p*=0.446), education (*p*=0.268), co-morbidities (*p*=0.689), sun exposure (*p*=0.454), BMI (*p*=0.213). MNA-SF score (*p*=0.518), calcium levels (*p*=0.618), and blood pressure (*p*=0.787 for systolic and *p*=0.594 for diastolic) were >0.05, meaning there was equality of characteristics between the control and treatment groups. Meanwhile, the *p*-values for gender, drugs consumed, and 25(OH)D levels were <0.05, so there was no equality in these characteristics of the subjects.

Distribution of subjects based on 25(OH)D level and blood pressure

The distribution of research subjects based on 25(OH)D levels before and after being given supplementation in the control and treatment groups can be seen in Table 2. The average level of 25(OH)D in 62 subjects was 20.4 ng/mL. The average systolic blood pressure in 62 research subjects was 134.7 mmHg and the average diastolic blood pressure was 89.4 mmHg.

In the control group, 25 subjects experienced vitamin D insufficiency and five subjects experienced vitamin D deficiency, with an average 25(OH)D level of 23.1 ng/mL. Subjects were then given placebo supplementation for eight weeks. The average 25(OH)D level after placebo was 27.3 ng/mL, with 11 subjects experiencing sufficiency, 16 subjects experiencing insufficiency, and three subjects experiencing deficiency. Whereas in the treatment group before supplementation, nine subjects experienced vitamin D insufficiency and 23 subjects experienced vitamin D deficiency, with an average 25(OH)D level of 17.9 ng/mL. After supplementation, the average level of 25(OH)D increased to 36.1 ng/mL, with 27 subjects experiencing sufficiency, three subjects experiencing insufficiency, and two subjects experiencing deficiency.

For blood pressure, in the control group, 22 subjects had pre-hypertension and 8 subjects had grade I hypertension. The average systolic blood pressure was 133.9 mmHg and diastolic blood pressure was 89.6 mmHg. After receiving placebo, the average systolic blood pressure became 129.3 mmHg and diastolic blood pressure 82.4 mmHg, with five normotension subjects, 21 subjects with pre-hypertension, and four subjects with grade I hypertension. Whereas in the treatment group before supplementation, 24 subjects had pre-

Table 2. Distribution of subjects based on 25(OH)D level and blood pressure, before and after supplementation

Variables	*Control group (n=30)*		*Treatment group (n=32)*		*p*
	n	*%*	*n*	*%*	
25(OH)D level					
Before supplementation					<0.001†
Insufficiency	25	83.3	9	28.1	
Deficiency	5	16.7	23	71.9	
After supplementation					<0.001†
Sufficiency	11	36.7	27	84.4	
Insufficiency	16	53.3	3	9.4	
Deficiency	3	10.0	2	6.2	
Blood pressure					
Before supplementation					0.315†*
Pre-hypertension	22	73.3	24	75.0	
Hypertension grade I	8	26.7	6	18.8	
Hypertension grade II	-	-	2	6.2	
After supplementation					0.680†*
Normotension	5	16.7	8	25.0	
Pre-hypertension	21	70.0	21	65.6	
Hypertension grade I	4	13.3	3	9.4	
Hypertension grade II	-	-	-	-	

†Chi-square test
*p>0.05 indicates equality

hypertension, six subjects had grade I hypertension, and two subjects with grade II hypertension. The average systolic blood pressure was 135.3 mmHg and diastolic blood pressure was 89.2 mmHg. After supplementation, the average systolic blood pressure was 126 mmHg and diastolic blood pressure was 80.8 mmHg, with eight normotension subjects, 21 subjects with pre-hypertension, and three subjects with grade I hypertension.

Changes in 25(OH)D levels and blood pressure

The normality of data distribution was tested using the Kolmogorov-Smirnov test. Data on 25(OH)D levels were normally distributed and analysed using paired *t*-test. As for systolic and diastolic blood pressures, the data were not normally distributed, so they were analysed using the Wilcoxon signed-rank test. Table 3 shows that in the control group, the level of 25(OH)D increased from 23.1±4.9 ng/mL to 27.3±7.3 ng/mL. After being analysed using paired *t*-test, the result showed a significant change (p<0.001). Likewise, in the treatment group, the level of 25(OH)D increased from 17.9±4.4 ng/mL to 36.1±9.8 ng/mL. The analysis using paired *t*-test also obtained a significant result (p<0.001).

The change in systolic blood pressure for the control group was 133.9(121-159.5) mmHg to 129.3(96-159) mmHg; the Wilcoxon signed-rank test showed a significant change (p<0.05). For the treatment group, the change was 135.3(121-180) mmHg to 126(101-153) mmHg, with a p<0.001 from the Wilcoxon signed-rank test, indicating a significant change. Similar results were also found for diastolic blood pressure, where the change in the control group was 89.6(80-105) mmHg to 82.4(64-103) mmHg, with a $p<0.001$ from the Wilcoxon signed-rank test. In the treatment group, diastolic blood pressure reduced from 89.2(81.5-98.5) mmHg to 80.8(67-90) mmHg; the Wilcoxon signed-rank test also obtained a significant result ($p<0.001$).

Mean changes in 25(OH)D levels for the control and treatment groups are shown in Table 4. The mean change in 25(OH)D level in the control group was 4.2±2.5 ng/mL and 18.2±5.5 ng/mL for the treatment group. Independent *t*-test showed a significant change ($p<0.001$) in 25(OH)D levels between groups. For systolic blood pressure, the mean change in the control group was -4.6(-25 - -0.5) mmHg and -9.2(-20 - -27) mmHg in the treatment group. Independent *t*-test obtained no significant differences in systolic blood pressure change between groups ($p=0.109$). Likewise, with diastolic blood pressure, the mean change in the control group was -7.2(-16 - -2) mmHg and the mean change in the treatment group was -8.4(-14.5 - -8.5) mmHg; results using the independent *t*-test also obtained no significant differences in diastolic blood pressure change between groups ($p=0.559$).

Adverse event

In this study, no side effects were reported in each group. However, one research subject could not continue until the end of the study due to death (tuberculosis) in the seventh week. The subjects of this study had an initial screening examination with a serum 25(OH)D level of 26 ng/mL, calcium level of 9.5 mg/dL, blood pressure of 130/90 mmHg, normal nutritional status, and no co-morbidities. Subjects included in the control group were given placebo supplementation, so any adverse events could be considered unrelated to the administration of intervention in this study.

Table 3. Mean changes in 25(OH)D level, systolic and diastolic blood pressures

Variable	*Control group (n=30)*			*Treatment group (n=32)*		
	Before supplementation	*After supplementation*	*p*	*Before supplementation*	*After supplementation*	*p*
25(OH)D level	23.1±4.9	27.3±7.3	<0.001†*	17.9±4.4	36.1±9.8	<0.001†*
Systolic blood pressure	133.9 (121-159.5)	129.3 (96-159)	0.027‡*	135.3 (121-180)	126 (101-153)	<0.001‡*
Diastolic blood pressure	89.6 (80-105)	82.4 (64-103)	<0.001‡*	89.2 (81.5-98.5)	80.8 (67-90)	<0.001‡*

Value in mean±standard deviation (*SD*) or median (IQR, 25th-75th percentile)
†Paired *t*-test, ‡Wilcoxon signed-rank test
*$p<0.05$ indicates statistically significant

Table 4. Changes of 25(OH)D level, systolic and diastolic blood pressures between groups

Variable	*Control (n=30)*	*Treatment (n=32)*	*p*
Change in 25(OH)D level	4.2±2.5	18.2±5.5	<0.001[†]*
Change in systolic blood pressure	-4.6 (-25 - -0.5)	-9.2 (-20 - -27)	0.109[†]
Change in diastolic blood pressure	-7.2 (-16 - -2)	-8.4 (-14.5 - -8.5)	0.559[†]

Value in mean±standard deviation (*SD*) or median (IQR, 25th-75th percentile)
[†]Independent *t*-test
*p<0.05 indicates statistically significant

DISCUSSION

25(OH)D is a marker to determine human vitamin D status. Vitamin D enters the blood, is assisted by vitamin D binding protein to the liver and is hydroxylated to 25(OH)D, which has the highest serum concentration and longer half-life (Hamdan *et al.*, 2012). The Institute of Endocrinology defines 25(OH)D levels below 20 ng/mL as vitamin D deficiency and 25(OH)D levels 20 - <30 ng/mL as vitamin D insufficiency (Larsen *et al.*, 2012). Various factors like age can cause vitamin D deficiency. One of the causes of vitamin D deficiency in the elderly is a decrease in the production of pre-vitamin D in the skin. Around 80-90% of vitamin D comes from sunlight. Therefore, outdoor activities will expose them to more sunlight. In this nursing home, the elderly routinely did morning exercise every day between 7 – 9 o'clock; this activity causes the elderly to be frequently exposed to sunlight.

Supplementation is one of the most effective ways to overcome vitamin D deficiency (Pfotenhauer & Shubrook, 2017). Because the subjects of this study were elderly, the safest dose of vitamin D_3 was used - a dose of 4,000 IU for subjects with deficiency and 2,000 IU for subjects with insufficiency for a supplementation duration of eight weeks, which was proven to provide a significant increase in 25(OH)D levels. In this study, there was a change in the average level of 25(OH)D in the control and treatment groups. The mean change in 25(OH)D level in the treatment group compared to the control group was also statistically significant. This is consistent with previous studies where there was a significant increase in 25(OH)D levels after vitamin D supplementation. In a study by Wood *et al.* (2012) where vitamin D supplementation was given to women aged 64±2 years at a dose of 1,000 IU per day for 48 weeks, there was a significant change in serum 25(OH) D level from 13±5.5 ng/mL to 55.9±5.5 ng/mL. A study by Pilz *et al.* (2015) also showed that vitamin D supplementation at a dose of 2,800 IU per day for 32 weeks in subjects aged 60.1±11.3 years who suffered from hypertension and 25(OH)D levels <30 ng/mL resulted in a significant change in 25(OH)D level from 22±5.5 ng/mL to 36.2±7.3 ng/mL. This is also consistent with the study of Husna *et al.* (2021) where a positive correlation was found between serum 25(OH)D level with a median duration of 40 minutes of sun exposure in elderly subjects (r=0.425, p=0.006). Farapti *et al.* (2020) said that vitamin D deficiency is common in the elderly due to lack of sun exposure and low intake of vitamin D.

Food intake and decreased nutrient bioavailability in the elderly can also inhibit vitamin D metabolism, which results in decreased levels of 25(OH) D (Russell, 2018). In this study, food weighing could not be analysed, so the subjects' intake data were only obtained based on the food menu list from the nursing home. From the food menu list, food sources of vitamin D consumed by subjects were tempeh/tofu every day and gourami/mackerel/cob/catfish every two days without any additional vitamin D fortified foods. Tempeh and fish consumed by subjects had low vitamin D content, indicating that subjects had a low tendency to consume food sources of vitamin D. Based on the low consumption of vitamin D food sources, it can be concluded that the significant change in 25(OH)D level of the control group was because subjects routinely received sun exposure.

Vitamin D also has a vasoprotective effect, including slowing atherosclerosis, increasing endothelial function, and suppressing the renin-angiotensin-aldosterone system (Pilz *et al.*, 2015). The increased risk of cardiovascular disease, especially hypertension, is associated with vitamin D deficiency (Vaidya & Williams, 2012). Basic science research showed that vitamin D plays a role in hypertension by suppressing the renin-angiotensin-aldosterone system, the primary regulator of blood pressure, electrolytes, and volume homeostasis (Park *et al.*, 2014). Vitamin D can suppress renin production by suppressing gene transcription renin so that the renin-angiotensin-aldosterone system is not activated. In addition, vitamin D can also inhibit the expression of cyclooxygenase-2 (COX-2), so arachidonic acid is not converted into prostaglandins. As a result of the inactivated renin-angiotensin-aldosterone system, blood pressure does not increase (Hamdan *et al.*, 2012). In addition, vitamin D can affect endothelial function by increasing the production of nitric oxide, which plays a role in relaxing blood vessels and reducing blood pressure, as well as reducing inflammation in the endothelium so that it can repair the disturbed endothelial structure.

In this study, systolic blood pressure in the control group and treatment group decreased significantly. However, the change in systolic blood pressure in the treatment group compared to the control group was not statistically significant, meaning that vitamin D supplementation did not affect a significant reduction in systolic blood pressure. The same results were also obtained with diastolic blood pressure in the control and treatment groups, which experienced a significant decrease. Changes in diastolic blood pressure in the treatment group compared to the control group also did not yield statistically significant results, meaning that vitamin D supplementation had no significant effect on diastolic blood pressure reduction. The results of this study are similar to those of Wood *et al.* (2012), Gepner *et al.* (2012), Pilz *et al.* (2015), and Tomson *et al.* (2017), where there were no significant decreases in systolic or diastolic blood pressures. A study from Wood *et al.* (2012) conducted on 265 postmenopausal women aged 64±2 years who were given vitamin D at a dose of 1,000 IU/day for 48 weeks and divided into two seasons found no significant decrease in systolic and diastolic blood pressures. A study from Gepner *et al.* (2012) reported that 62 in 114 menopausal women in Wisconsin, USA, who were given 2,500 IU/day of vitamin D for 16 weeks also found a non-significant decrease in systolic and diastolic blood pressures. A study by Pilz *et al.* (2015) stated that the insignificant difference in blood pressure might be caused by a significant increase in triglyceride lipid profile due to vitamin supplementation, but these results still require validation and more examination on lipid profile. Conflicting results were found in several studies, with a significant decrease in systolic and diastolic blood pressures. A study from Larsen *et al.* (2012), which was conducted on 112 elderly subjects at a dose of 3,000 IU/day for 80 weeks showed a significant decrease in systolic blood pressure from 132±10 mmHg to 128±0 mmHg (Δ=-4) and diastolic blood pressure from 77±6 mmHg to 76±6 mmHg (Δ=-3). A study by Chen *et al.* (2014), which was conducted on 126 elderly subjects with grades I and II hypertension at a dose of 2,000 IU/day for 24 weeks also found a significant decrease in systolic blood pressure from 132.1±9.4 mmHg to 125.9±9.4 mmHg (Δ=-6.2) and diastolic blood pressure from 75.1±9.1 mmHg to 70.9±9.1 mmHg (Δ=-4.2). Likewise, in the study of Hermawan & Andoko (2017), there was a significant decrease in systolic blood pressure from 172 mmHg to 153 mmHg (Δ=-19) and diastolic blood pressure from 93 mmHg to 83 mmHg (Δ=-10).

According to the research by Vaidya & Forman (2010), the inconsistent results of several RCT studies regarding the effect of vitamin D on reducing blood pressure could be due to the variability of study population, sample size, vitamin D dosage, and duration of supplementation. In previous studies, a duration of more than eight weeks of supplementation is required to obtain a significant reduction in blood pressure, whereas in this study the duration of supplementation followed the loading dose of vitamin D to increase serum 25(OH)D levels. Therefore, the duration of supplementation may have caused a non-significant decrease in systolic and diastolic blood pressures in this study.

An equivalence test on subject characteristics (baseline) of anti-hypertensive drugs consumed (p<0.001) was found. Out of a total of 12 subjects taking anti-hypertensives, eight subjects were in the control group. Inequality in the randomisation of subjects on these characteristics can affect the analysis results on reducing blood pressure, which was not significant in the treatment group compared to the control group. Study results by Jorde *et al.* (2010) also found no significant reduction in blood pressure because 21% of the study population took anti-hypertensive drugs. In this study, eight subjects in the control group were taking anti-hypertensives; which likely caused the decrease in blood pressure for the treatment group to be insignificant compared to the control group.

Limitations

This study had some limitations needed to be considered: (i) body composition was not measured, especially fat mass, so it did not consider the loading dose duration of vitamin D for subjects with obesity or sarcopenia; (ii) sun exposure score was only assessed at the beginning of the examination, so it was not known whether there was a change in the subjects' behaviour towards sun exposure and this could be a confounding factor in the increase of serum 25(OH)D levels in the control group; (iii) subjects who consumed anti-hypertensive drugs were only assessed at the beginning of the examination, so it was not known whether there were any additional subjects who consumed anti-hypertensive drugs during supplementation and this could be a confounding factor in the decrease of blood pressure in the control group; (iv) access to obtain food intake data by food weighing cannot be carried out, so no analysis was done in terms of food intake, especially food sources of vitamin D and its effect on serum 25(OH)D levels and blood pressure; (v) other factors that can cause hypertension, such as family history, smoking habit, salt consumption, and lipid profile (cholesterol, triglycerides) were not examined in this study.

CONCLUSION

Vitamin D supplementation significantly increased serum 25(OH)D levels but did not significantly reduce systolic and diastolic blood pressures in the elderly. Researchers and medical educationists are encouraged to continue to assess a more varied elderly population, a larger sample size (to eliminate confounding factors that may affect serum 25(OH) D levels and blood pressure), a longer duration of supplementation, food intake especially food sources of vitamin D on its effect on 25(OH)D levels, and other factors that can cause hypertension in the elderly. Also, the elderlies need to regularly have their health, nutritional status, and vitamin D levels checked so that the provision of therapy can be timely and their quality of life can be increased.

Acknowledgement
The authors would like to thank all participants of this study and Universitas Indonesia for the grant (PUTI Pascasarjana 2022) that had been given.

Authors' contributions
Ferawaty, principal investigator, conceptualised and designed the study, led the data collection and analysis, prepared the draft of the manuscript; Diana S, advised on conceptualisation, study design, data analysis, interpretation, assisted in the drafting of the manuscript, and reviewed the manuscript; Noto D, advised on conceptualisation, study design, data analysis, interpretation, assisted in the drafting of the manuscript, and reviewed the manuscript; Dian NC, advised on conceptualisation, study design, data analysis, interpretation, assisted in the drafting of the manuscript, and reviewed the manuscript; Ninik M, advised on conceptualisation, study design, data analysis, interpretation, assisted in the drafting of the manuscript, and reviewed the manuscript; Nurul RMM, advised on conceptualisation, study design, data analysis, interpretation, assisted in the drafting of the manuscript, and reviewed the manuscript.

Conflict of interest
There is no conflict of interest in this research.

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Mal J Nutr 30(2): 167-179, 2024

Infant feeding practices and associated factors during the COVID-19 pandemic: Findings from an online cross-sectional study in Indonesia

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ABSTRACT

Introduction: COVID-19 pandemic presents a challenge to ensuring optimal infant feeding practices. This study aimed to assess infant feeding practices and investigate potential factors associated with exclusive and continued breastfeeding practices during the COVID-19 pandemic in Indonesia. **Methods:** An online survey was conducted during December 2020 – August 2021. A total of 817 Indonesian mothers aged ≥18 years old with infants aged <18 months were obtained through convenience sampling. Exclusive breastfeeding was the practice at the time of the survey among infants <6 months old; continued breastfeeding was considered when infants aged ≥6 months received any type of breastfeeding in the previous day. **Results:** Most subjects were aged 25-34 years old (81.3%), had a high education level (85.7%), with middle household income level (40.5%), and lived in Java Island (81.6%). Infants' age and sex were comparable between younger vs. older infants and boys vs. girls, respectively. Exclusive breastfeeding was 81.3%. Continued breastfeeding was 93.4%, with 74.3% meeting the minimum acceptable diet. Breastfeeding intention (92.9%) and husband's support for infant feeding (67.2%) were reported during the COVID-19 pandemic. Multivariate analyses showed that breastfeeding intention was one of the factors associated with exclusive breastfeeding [a*OR*=12.6; 95%CI (4.1-39.1)] and continued breastfeeding [10.9 (4.4-27.0)]. **Conclusion:** The study suggested that mothers' intention to breastfeed during the COVID-19 pandemic provided affirmation of good breastfeeding experiences by allowing mothers to have more time for childcare activities and more opportunities to develop meaningful co-parenting practices while staying at home.

Keywords: breastfeeding intention, COVID-19 pandemic, continued breastfeeding, exclusive breastfeeding, minimum acceptable diet

INTRODUCTION

Proper infant feeding practices are important to ensure adequate nutrition for the survival, growth, and development of children. The Government of Indonesia recommends the following infant feeding practices i.e., exclusive breastfeeding for the first six months

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doi: https://doi.org/10.31246/mjn-2023-0097

of life, introduction of complementary foods after 6 months of age, adequate complementary foods in terms of amount, frequency, and variations, as well as continued breastfeeding (Kemenkes, 2020). However, even before the COVID-19 pandemic, adherence to these recommendations was suboptimal in Indonesia. The national data showed that the proportion of exclusive breastfeeding was 37.3% (Agency of Health Research and Development, 2018). Infants aged 6-11 months who met the minimum acceptable diet (MAD) were 26%. Continued breastfeeding at 1 year old was considerably better, i.e., 77%, but declined to 55% at 2 years old (National Population and Family Planning Board (BKKBN) *et al.*, 2018).

Exclusive breastfeeding practice is known to be associated with multilayer factors from maternal attributes to family characteristics and societal support (Februhartanty *et al.*, 2020), as well as exposure to information on exclusive breastfeeding way before getting pregnant and provision of home support from husbands (Februhartanty *et al.*, 2012). Furthermore, maternal knowledge, workplace environment, attributes of the society, culture and economy were identified as contributory factors to continued breastfeeding practice. In addition, aside from the factors related to support received by mothers, complementary feeding practices are also complicated with the feeding skills of caregivers and the capacity of families to access proper foods (Blaney, Februhartanty & Sukotjo, 2015).

COVID-19 pandemic can present new challenges in infant feeding practices in terms of accessing support and reduced access to foods. The implementation of movement control orders (MCOs) with various levels of restriction may contribute to limited access and utilisation of health services and support, specifically for maternal and newborn health (MNH), nutrition services and counselling for infant and young child feeding (Busch-Hallen *et al.*, 2020). In addition, COVID-19 pandemic also has negative psychological impacts on mothers. In the UK, 11% of mothers reported an impact of the lockdown measures on their mental health, mentioning anxiety, depression, loneliness, and isolation (Vazquez-Vazquez *et al.*, 2020). The restriction of movement and separation from loved ones or other family members, loss of freedom, fear of disease, and changes in income and job, can lead to negative psychological effects, especially for vulnerable groups such as pregnant mothers and new mothers (Brooks *et al.*, 2020). Mother's caregiving desire can be low due to maternal psychological distress. Thus, maternal mental health has an impact on breastfeeding practices, including the cessation of exclusive and partial breastfeeding duration and the introduction of formula (Sha *et al.*, 2019).

Based on these conditions, a study is needed to assess infant feeding practices and investigate the potential factors associated with exclusive and continued breastfeeding practices during the COVID-19 pandemic in Indonesia.

MATERIALS AND METHODS

The study was a cross-sectional study administered online from December 2020 to August 2021. Subjects were mothers aged 18 years or older, had an infant aged <18 months, and were Indonesian citizens. Mothers who were pregnant and stayed outside Indonesia were excluded from the study.

The study used an online structured questionnaire adapted from the UK COVID-19 New Mums study and the Malaysia COVID-19 Mom-Baby study. The questionnaire of this study consisted of 6 parts, namely: 1) Information of the survey; 2) Informed consent and screening questions; 3) Background and socio-demographic characteristics; 4) The impact of COVID-19 on household, work, and finances; 5) Infant feeding and behaviour; and 6) Impact of COVID-19 on mother's activities. The socio-demographic characteristics questionnaire collected data on maternal age, maternal education, household income, household/family composition, and living location. Infant feeding and behaviour questionnaire collected data on infant feeding practices, infant feeding plans (including intention to breastfeed), support on infant feeding, and feeding-related infant behaviours. Additional questions on infant and young child feeding practices, such as exclusive breastfeeding at time of survey, complementary feeding practices, i.e., time of complementary foods introduced, complementary food frequency and diversity, including the indicators for minimum meal frequency (MMF), minimum dietary diversity (MDD), and MAD [adopted from the 2018 Indonesia Basic Health Research (Agency of Health Research and Development, 2018) and the 2017 Indonesian Demographic Health Survey (BKKBN *et al.*, 2018)] were also included. Infants currently breastfeeding in the present study were defined as those who received any type of breastfeeding in the previous day, including exclusive breastfeeding, predominant breastfeeding, and mixed feeding (breast milk + formula + solid). Breastfeeding intention was identified from a single question on the plan (during pregnancy) of the mode of infant feeding practices. The options to this question included breastfeeding, formula feeding, mixed feeding, or no preference yet. The impact of COVID-19 on mother's activities questionnaire comprised of how maternal mood was affected by the COVID-19 pandemic. Mothers' mood condition was assessed based on the mood experienced in the last 7 days, including feeling connected with family, friends, and local community; available time to do exercise, personal interest, or hobbies; coping with situations; relaxation; appetite; and loneliness. Total maternal mood score was calculated by summing the scores of all 17 question items; the higher the score, the better the maternal mood condition.

The questionnaire was translated into Bahasa Indonesia by the authors and back translated by a certified translator. To ensure the questionnaire's validity and reliability, content review and pre-testing of 43 mothers with similar characteristics with study subjects were conducted. This study used convenience sampling to obtain the samples. The link to the questionnaire was posted on websites and further distributed through various links and networks via social media. Invitation to the online survey was also communicated through various mother support or community groups and personal contacts. Duplicate and incomplete survey responses were excluded from the analysis.

Data were processed and analysed using IBM Statistical Package for Social Science (SPSS) Version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to display distribution of key indicators concerned in this study. For feeding practices, data were divided into practices related to exclusive breastfeeding among respondents with infants aged <6 months (n=347) and complementary feeding among infants aged $\geq$6 months (n=470). Multivariate analyses using logistic regression with the Enter method were performed to assess factors associated with exclusive breastfeeding among respondents with infants <6 months old and continued breastfeeding among those with infants $\geq$6 months old, with p<0.05 for significant association. Factors included

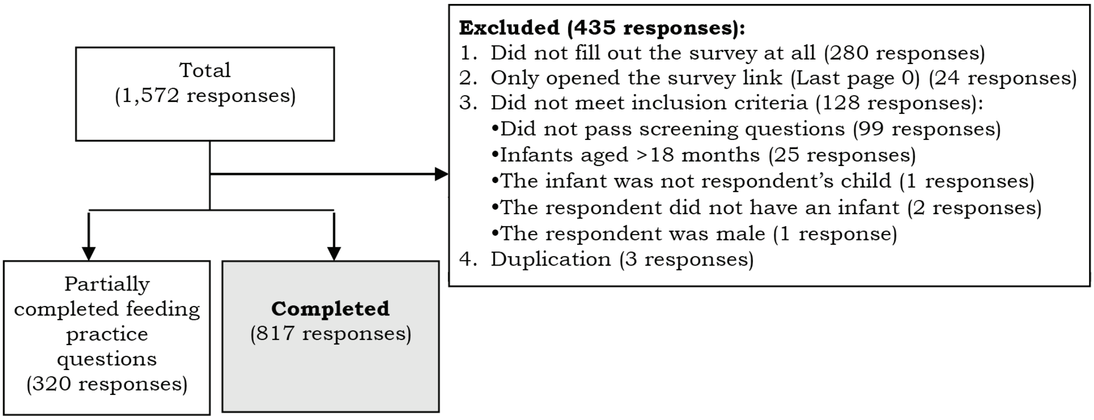

Figure 1. Total responses used in the analysis

in the multivariate analyses were decided based on their potential associations shown in the bivariate analyses (*p*<0.2) and prior literature.

The study was carried out in accordance with the Declaration of Helsinki. An ethical approval for conducting this study was obtained from the Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo General Hospital with the number KET-1357/UN2.F1/ETIK/PPM.00.02/2020.

RESULTS

As many as 1,572 survey responses were collected. However, only 817 responses were included in the analysis due to the reasons explained in Figure 1. Mothers' and infants' characteristics are shown in Table 1. Most mothers were between 25-34 years old (81.3%), with a median age of 29 years old, had an undergraduate degree (72.7%), and lived in Java Island (81.6%). Almost half of the mothers were housewives (49.2%) and had a middle household income level (40.5%), with a range of IDR. 3,000,000 – IDR. 7,199,999 (equivalent to USD 192.76 – 462.63). The mean age of infants was 6 months, with 57.5% of them aged 6-18 months, 53.2% males, and 53.4% being the first child. Almost all infants had a normal birth weight (93.3%).

Table 2 shows the self-reported impacts of the COVID-19 pandemic regarding risks of COVID-19 infection, abilities in household matters, feeding support, and breastfeeding practices. A few mothers reported having COVID-19 symptoms (11.9%) and positively tested for COVID-19 (5.9%). Almost half of the mothers reported having experienced major to moderate impacts on their ability to pay rent (44.2%), food (42.2%), and other necessities (46.9%). Even though almost all reported having breastfeeding intentions (92.9%), a few mothers reported a change in feeding their children during the pandemic. Husband was identified as the most influential source of feeding support by mothers (67.2%). In addition, the maternal mood score was generally good.

Among infants <6 months old, 81.3% were breastfed exclusively. Among older infants, continued breastfeeding and meeting the MAD were 93.4% and 74.3%, respectively (Table 3).

Table 4 shows the multivariate analyses to assess which among the maternal attributes and risks of COVID-19, infant's characteristics, and impact of the COVID-19 pandemic on household financial security, support, and breastfeeding changes were associated with exclusive breastfeeding (among younger infants) and continued breastfeeding (among older infants), assessed 24-hour prior to the survey. It revealed that exclusive breastfeeding was likely to be practised among mothers with higher education level (a*OR*; 95% *CI*=2.8; 1.2-6.4), not working (2.1; 1.1-3.9), and

Table 1. Characteristics of mothers and infants (*n*=817)

Characteristics	*n (%)*	*Median (Min-Max)*
Mother's age		29 (18-44)
18-24 years old	67 (8.2)	
25-34 years old	664 (81.3)	
35-49 years old	86 (10.5)	
Mother's education level		
Basic to secondary school	117 (14.3)	
Undergraduate	594 (72.7)	
Postgraduate	106 (13.0)	
Mother's occupation		
Civil servant/Army/Police	118 (14.4)	
Private employee	170 (20.8)	
Self-employed	58 (7.1)	
Others†	69 (8.4)	
Housewife	402 (49.2)	
Household income		
≤IDR 2,999,999	194 (23.7)	
IDR 3,000,000 – IDR 7,199,999	331 (40.5)	
≥IDR 7,200,000	292 (35.7)	
Geographical region		
Java	667 (81.6)	
Sumatra	77 (9.4)	
Others‡	73 (8.9)	
Infant's age		6 (0-18)
<6 months	347 (42.5)	
6-18 months	470 (57.5)	
Infant's sex		
Male	435 (53.2)	
Female	382 (46.8)	
Infant's birth order		1 (1-7)
First	436 (53.4)	
Second or above	381 (46.6)	
Infant's birth weight		3,100 (1,000-4,500)
Low (<2,500 g)	41 (5.0)	
Normal (2,500-4,000g)	762 (93.3)	
Large (>4,000g)	14 (1.7)	

1 USD = IDR 15,563.39

†Health care workers (doctor, dentist, nurse, midwife, pharmacist), teacher (honorary teacher, private tutor, early childhood teacher), contract employee, honorary employee, non-civil servant government employee, researcher, finance administrator, student

‡Bali, Nusa Tenggara, Kalimantan, Sulawesi, Maluku, Papua

with intention to breastfeed during the pregnancy period (12.6; 4.1-39.1), but not among first-time mothers (0.4; 0.2-0.8) as compared to their counterparts. Meanwhile, among mothers with older infants, mothers who had the intention to breastfeed at the last pregnancy had 10.1 higher odds (95% *CI*: 4.4-27.0) to continuously breastfeed than those who did not. In addition, mood scores

Table 2. Reported impacts of COVID-19 pandemic (*n*=817)

Relevant impact	*n (%)*	*Median (Min-Max)*
Mother's related risks of COVID-19[†]		
Had COVID-19 symptoms	97 (11.9)	
Tested positive for COVID-19	48 (5.9)	
Recommended to stay at home	344 (42.1)	
Household members had COVID-19 symptom	145 (17.7)	
Impact of COVID-19 pandemic on household matters		
Ability to pay rent		
Major to moderate impact	361 (44.2)	
Minor impact	128 (15.7)	
No impact	240 (29.4)	
Too soon to tell	88 (10.8)	
Ability to pay for food		
Major to moderate impact	345 (42.2)	
Minor impact	198 (24.2)	
No impact	226 (27.7)	
Too soon to tell	48 (5.9)	
Ability to pay for other necessities		
Major impact to moderate impact	383 (46.9)	
Minor impact	202 (24.7)	
No impact	182 (22.3)	
Too soon to tell	50 (6.1)	
Change in breastfeeding related practices		
Frequency of feeding		
Decrease	17 (2.1)	
Same	660 (80.8)	
Increase	127 (15.5)	
Irrelevant	13 (1.6)	
Duration of feeding		
Decrease	17 (2.1)	
Same	669 (81.9)	
Increase	118 (14.4)	
Irrelevant	13 (1.6)	
Most influential source of feeding support perceived by mothers		
Husband	549 (67.2)	
Parents/in-law	128 (15.7)	
Health worker	51 (6.2)	
Friends/relatives/nanny/support group	89 (10.9)	
Maternal mood scores		48 (21-66)
Breastfeeding intention	759 (92.9)	

[†]Multiple response

also showed a significant association with continued breastfeeding, but due to the low effect, this association was disregarded.

DISCUSSION

The present study involved 81.3% of exclusively breastfed infants at the time of the survey, which is higher than the national exclusive breastfeeding prevalence in 2018 (Agency of Health Research and Development, 2018). According to a previous online survey in Belgium during the COVID-19 outbreak, most breastfeeding women had no plans or intention of discontinuing breastfeeding (Burgess *et al.*, 2021). It can be inferred that the pandemic provided several opportunities for mothers to maintain breastfeeding practices that were not available prior to the pandemic, such as increased home support, more time at home to feed their child, fewer visitors, greater partner support, a longer time for returning to work, less pressure, and more time for family bonding (Brown & Shenker, 2020; Sakalidis *et al.*, 2021). In contrast, a study in Thailand found that exclusive breastfeeding rates declined during the COVID-19 pandemic. These inconsistent results could be attributed to the fact that the Thai study was conducted during the peak of COVID-19 infection where an adverse effect on mothers' breastfeeding was experienced due to limited access to breastfeeding support (Nuampa *et al.*, 2022). The present study also found that exclusive breastfeeding was more likely to be performed by higher educated and non-working mothers, but less likely by first-time mothers. Practising exclusive breastfeeding requires an understanding of appropriate information to be used for improving the confidence to breastfeed and attaining higher education may

Table 3. Infant and young child feeding practices (*n*=817)

Feeding practices	*n (%)*
Feeding practices among <6 months (*n*=347)	
Exclusive breastfeeding at the time of the survey	282 (81.3)
Predominant breastfeeding	37 (10.7)
Formula feeding	7 (2.0)
Mixed feeding (breast milk + formula + solid)	21 (6.0)
Feeding practices among ≥6 months (*n*=470)	
Continued breastfeeding practice	439 (93.4)
Timely introduction of solid food/semi solid/soft foods† (*n*=237)	233 (98.3)
Meeting minimum meal frequency (MMF)‡	434 (92.3)
Meeting minimum dietary diversity (MDD)§	371 (78.9)
Meeting minimum acceptable diet (MAD)¶	349 (74.3)

†Infants aged 6-8 months who were fed with solid/semisolid/soft food in the previous day
‡Breastfed infants aged 6-8 months and 9-11 months who were given solid, semi-solid, or soft foods minimum 2 and 3 times in the previous day. Non-breastfed children aged 6-11 months who were given solid, semi-solid, or soft foods a minimum 4 times (including milk feeds), with at least 1 meal must be semi-solid or soft feed in the previous day (World Health Organization & UNICEF, 2021).
§Infants aged 6-11 months who were given at least 5 from 8 food groups in the previous day (World Health Organization & UNICEF, 2021).
¶Infants aged 6-11 months who met minimum meal frequency and minimum dietary diversity for breastfed children and for non-breastfed children met minimum meal frequency and dietary diversity, as well as at least 2 milk feeds (World Health Organization & UNICEF, 2021).

Table 4. Factors associated with exclusive and continued breastfeeding practices

Variables	Exclusive breastfeeding (n=347)		Continued breastfeeding (n=470)	
	p-value†	*aOR (CI 95%)*	*p-value*†	*aOR (CI 95%)*
Maternal attributes and risks of COVID-19				
Education level				
Low-Middle	0.017*	1	0.781	1
High		2.775 (1.198 – 6.425)		1.157 (0.415 – 3.225)
Occupation				
Working	0.026*	1	0.546	1
Not working		2.078 (1.094 – 3.946)		1.295 (0.559 – 2.998)
Mood score	0.109	1.029 (0.994 – 1.067)	0.044*	1.048 (1.001 – 1.098)
Related risks of COVID-19				
At risk	0.750	1	0.985	1
Not at risk		1.129 (0.171 – 3.172)		1.009 (0.382 – 2.665)
Infants' characteristics				
Birthweight				
Low	0.682	1	0.505	1
Normal		0.737 (0.171 – 3.172)		0.621 (0.153 – 2.520)
Birth order				
>1	0.005*	1	0.110	1
1st		0.413 (0.222 – 0.768)		0.110 (0.853 – 4.753)
Related impacts during COVID-19 pandemic				
COVID-19 impact on the ability to pay for food, for rent, and other necessity				
Impacted	0.359	1	0.132	1
Not impacted		0.726 (0.366 – 1.439)		2.755 (0.738 – 10.289)
Most influential source of support				
Others	0.895	1	0.233	1
Husband		0.958 (0.506 – 1.813)		1.661 (0.722 – 3.820)
Breastfeeding intention				
No	<0.001*	1	<0.001*	1
Yes		12.586 (4.050 – 39.111)		10.880 (4.379 – 27.033)

CI: Confidence interval, aOR: Adjusted odds ratio
†Tested using logistic regression (method: Enter), *p-value<0.05

give benefits to this process. As for non-working moms, they are known to be equipped with time that can be used to give full attention for practising exclusive breastfeeding (Februhartanty, 2011). On the other hand, "just" being a first-time mother is already challenging, let alone facing the complexities of factors surrounding infant feeding practices. A previous study in Jakarta revealed a similar finding that first-time mothers not receiving enough support would be less likely to practise exclusive breastfeeding (Februhartanty *et al.*, 2020).

The present study also found that the proportion of continued breastfeeding practice was high during the COVID-19 pandemic. This finding is similar to studies in Indonesia with only 11.2% of young children below 24 months (Muslimatun, 2021) and Malaysia with 1.6% below 18 months (Mohd Shukri *et al.*, 2022) who were reported to have completely stopped breastfeeding during the pandemic. As compared to before the pandemic, the breastfeeding rates of 105-110 countries in 2019 were 88.7% at 6 months old and 81.1% at 1 year old (Neves *et al.*, 2021). This suggests that generally the COVID-19 pandemic did not diminish breastfeeding practice.

The current study also indicated that 98.3% of the children had a timely introduction to complementary foods and 74.3% met MAD by meeting the necessary frequency (92.3%) and diversity (78.9%). These figures are higher than those of the Health Surveillance System in Indonesia (BKKBN *et al.*, 2018) and Asia Pacific (Neves *et al.*, 2021). A higher proportion of young children meeting MAD during the COVID-19 pandemic may be contributed by staying-at-home orders where parents had more time to cook, cared for their children, received support from other family members, and focused on child nutrition to prevent infection (Bahatheg, 2021; Brown & Shenker, 2020). Another reason may be due to the online survey method used, which is known to be more accessible to respondents with higher education and income, better internet access, and high interest in the survey topic (Andrade, 2020). This phenomenon is also reflected in a recent online survey in Indonesia, revealing that mothers with higher education and more regular income were more likely to have children meeting the MAD (Fadlina, Februhartanty & Bardosono, 2021).

In this study, 92.9% of the mothers chose breastfeeding as their feeding intention and it became the determinant factor of exclusive and continued breastfeeding practices. Mothers who intended to breastfeed were 12.6 times more likely to exclusively breastfeed and 10.1 times more likely to continue breastfeeding. Similar findings are noted from a 5-country study involving Thailand, United Kingdom, South Korea, Taiwan, and Brazil during COVID-19 pandemic (Chien *et al.*, 2022). This study validated conventional knowledge and demonstrated that intention to breastfeed is important even during the COVID-19 pandemic. Breastfeeding intention is a primary driver of breastfeeding practices regardless of other factors (Nazirah *et al.*, 2020). This may be explained through Theory of Planned Behaviour (TPB), in which intention is considered a direct component that shapes behaviour. According to TPB, one of the elements that influence intention is attitude towards behaviour, which refers to a person's subjective likelihood of performing the behaviour of interest to produce a particular outcome or provide a particular experience (Ajzen, 2020). During the COVID-19 pandemic, studies highlighted favourable perceptions of the impact of the COVID-19 pandemic on breastfeeding benefits and experiences, such as protecting the baby from virus infection and more time for motherhood, which could improve mothers' intention to continue breastfeeding (Busch-Hallen *et al.*, 2020; Pacheco *et al.*, 2021).

No significant associations were found between mother's mood condition and both breastfeeding practices in the current study. A possible reason for this discovery is that the COVID-19 pandemic may have both positive and negative effects on breastfeeding expectations, altering mothers' psychological mood and mental health status (Pacheco *et al.*, 2021). A study on maternal mental health during the COVID-19 lockdown in the UK discovered that while there was a significant number of mothers with symptoms of low mood, anxiety, and loneliness, there was also a high proportion of mothers who were able to cope with the circumstances (Dib *et al.*, 2020). Meaningful parenting experiences due to increased time at home during the COVID-19 pandemic may explain better mental health outcomes of mothers (Pacheco *et al.*, 2021). Furthermore, the current study was conducted several months after the onset of the COVID-19 pandemic, when slightly relaxed MCOs were applied.

In general, fathers have specific roles in supporting mothers' breastfeeding practices, including facilitating psychological support for breastfeeding, childcare involvement, engagement in decision about infant feeding mode, and enthusiasm for fatherhood (Februhartanty *at al.*, 2020). The present study found husbands as the most influential source of feeding support during the pandemic as perceived by mothers, followed by parents/in-law, friends/relatives/nanny/support group, and health workers. However, this study found no association between husband's support and both breastfeeding practices.

A similar result was found from another study in Indonesia (Agustin, Februhartanty & Bardosono, 2021). Some mothers who received their husband's support may still face challenges to breastfeed because a mother's decision to breastfeed is influenced by a variety of circumstances including personal experience, social and professional support, changes in family dynamic, and others (Asiodu *et al.*, 2017). In contrast, other studies have shown that husband's support increases the likelihood of longer breastfeeding duration during the COVID-19 pandemic. This is because mothers during the pandemic have limited access to support from other family members or friends, thus husband's support comes first (Vazquez-Vazquez *et al.*, 2021). Husbands spend more time at home during the pandemic, which increases mental and physical support through their involvement in domestic tasks and childcare (Brown & Shenker, 2020).

Ajzen (2020) suggests that time availability and cooperation by other people or other resources are important influencing factors of intention. These key elements observed in the present study (i.e., being at home during the pandemic and the presence of supportive husbands/family members) should be used as a guide to improving the implementation of breastfeeding promotion programmes by creating or providing mothers with opportunities or sources that are commonly found during the pandemic period to increase mothers' intention to breastfeed after the pandemic ends. For example, providing time available to care for their newborn, particularly for working mothers, through flexible working arrangements or remote working (working from home), as well as providing informational and action support through various distant services or media, such as webinars, online lactation classes, telehealth services, and social media marketing, that can reach not only mothers, but also husbands and families. In addition, the timing for information exposure is key as found in previous studies that breastfeeding intention was frequently made before or during pregnancy (Febrianingtyas, Februhartanty & Hadihardjono, 2019; Februhartanty *et al.*, 2012).

As with other online surveys, our current study purposely sampled subjects with better internet literacy, characterised dominantly by those living in Java Island and having higher levels of education. Furthermore, the survey recruited subjects with less heterogeneity in terms of their current breastfeeding practices. This can be explained in two ways, i.e., the survey was done online in which any potential subjects with the survey link could participate, thus random sampling was not feasible; the recruited subjects truly represented the current population who were breastfeeding due to better opportunities during lockdowns. Nevertheless, the present study was able to provide insights into the magnitude of some key infant feeding practices during the COVID-19 pandemic among these socio-economically better-off subjects to be a basis for the estimation of a contrary situation among the socio-economically disadvantaged population in Indonesia.

CONCLUSION

The present study found that exclusive breastfeeding at the time of the survey among infants <6 months old was 81.3%, whilst among older infants, continued breastfeeding was 93.4%, with 74.3% of infants meeting MAD. Breastfeeding intention was found to be associated with both exclusive and continued breastfeeding practices. Mothers who had breastfeeding intentions planned during pregnancy were 12.6 and 10.9 times more likely to perform exclusive and continued breastfeeding practices, respectively. Further studies need to investigate factors that are likely to shape breastfeeding intention. Understanding these factors may help refine current breastfeeding promotion to more effectively address the intention to breastfeed.

Acknowledgement

This study and the publication of this article were supported by the Southeast Asian Ministers of Education Organization - Regional Centre for Food and Nutrition (SEAMEO RECFON). We are also grateful to Dr. Nurul Husna (Universiti Putra Malaysia) for introducing us to the research group.

Authors' contributions

Februhartanty J, conceptualised and designed the study; Agustin CA & Fadlina A, collected the data. All authors analysed and interpreted the data, drafted the initial manuscript, and approved the manuscript for submission.

Conflict of interest

The authors declare that they have no competing interests.

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Mal J Nutr 30(2): 181-191, 2024

Predictors for 25-hydroxyvitamin D concentration in early pregnancy

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ABSTRACT

Introduction: There are very few studies of vitamin D deficiency in Indonesia. Since vitamin D deficiency is indicated by the level of 25-hydroxyvitamin D (25(OH) D) in blood, the present study aimed to analyse the predictive factors of 25(OH) D concentration in early pregnancy. **Methods:** The present study was a cross-sectional observational study. The sample consisted of 67 pregnant women in their 1st trimester of pregnancy. Parameters assessed included levels of 25(OH)D, glucose, and haemoglobin in the blood. Demographic information such as parity, family history of diabetes mellitus (DM), exposure to cigarette smoke, physical activity level, dietary intake, and anthropometric measurements were recorded. Linear regression analysis was employed. **Results:** Mean concentration of 25(OH)D was 16.5 (6.6-34.1) ng/ml. Majority of the participants (77.6%) were deficient in vitamin D (25(OH)D <20ng/mL); only 1.5% had normal vitamin D levels (25(OH)D >30 ng/mL). Bivariate analysis performed revealed that vitamin D intake (p=0.002) and family history of DM (p=0.043) played a significant role in determining 25(OH)D concentration. Additionally, dietary vitamin D intake, blood glucose level, exposure to cigarette smoke, and parental DM history served as predictors of 25(OH)D concentration in 54.5% of cases. **Conclusion:** The findings indicate that vitamin D deficiency was high in the target population. Thus, it is critical to ensure that for pregnant women to take vitamin D supplements, since nearly 80% of pregnant mothers are vit D deficient. Vitamin D supplementation can be included in national pregnancy programmes

Keywords: cigarette, diabetes mellitus, pregnant women, vitamin D concentration, vitamin D intake.

INTRODUCTION

While vitamin D deficiency affects people in developed countries (Pérez-López, Pilz & Chedraui, 2020), it remains a significant public health issue in the developing world as well. Vitamin D is a vital micronutrient that enhances glucose and insulin metabolism, and plays an important role in preventing fat accumulation (Cândido & Bressan, 2014; Li *et al.*, 2020; Mathieu, 2015). Vitamin D deficiency can lead to an array of problems, most notably rickets in children and osteoporosis in

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doi: https://doi.org/10.31246/mjn-2023-0015

adults. Numerous studies have found a strong association between vitamin D deficiency and chronic diseases such as obesity, diabetes mellitus, and renal failure. Moreover, the large amount of observational data currently available indicate pathophysiological associations between vitamin D and energy homeostasis, as well as regulation of the immune and endocrine systems (Amrein *et al.*, 2020; Kulie *et al.*, 2009).

Recent epidemiological data indicated that vitamin D deficiency is widespread and pregnant women are at greater risk of developing vitamin D deficiency. The data suggest that pregnant women with vitamin D deficiencies are more likely to experience pre-eclampsia (Aghajafari *et al.*, 2013; Pena *et al.*, 2015), gestational diabetes mellitus (Aghajafari *et al.*, 2013; Arnold *et al.*, 2015; Bennett *et al.*, 2013; Chen *et al.*, 2015; Larqué *et al.*, 2018; Skowrońska-Jóźwiak *et al.*, 2014), spontaneous abortion, and to have small-for-gestational age and low birth weight infants (Aghajafari *et al.*, 2013; Chen, Chen & Xu, 2021). However, there is presently very little scientific evidence about the risk factors involved in vitamin D deficiency, for example, sun exposure, vitamin D supplementation, body weight, ethnicity, and age (AlFaris *et al.*, 2019; Ekwaru *et al.*, 2014; Li *et al.*, 2020; Yun *et al.*, 2017). The extent to which other factors, such as food intake, impact vitamin D deficiency is largely unknown.

A study has shown that although the frequency of vitamin D deficiency is higher in pregnant women who are obese, vitamin D level in blood is not significantly influenced by the incidence of obesity (Pena *et al.*, 2015). Some studies indicated that exposure to the sun and cold climates can impact vitamin D deficiency during pregnancy, but findings from different studies have been mixed. As the city of Luwuk on the island of Sulawesi, Indonesia is located on the Equator, it receives more sunlight than other places, thus facilitating the measurement of vitamin D status and its associated factors, other than exposure to sunlight, in pregnant women.

MATERIALS AND METHODS

The present study was a cross-sectional observational study, which served as a baseline study investigating the impact of vitamin D concentrations on blood glucose levels in pregnant women during the first trimester. Characteristics and vitamin D levels were measured at week 11 or 12 of pregnancy. This research was approved by the Research and Community Engagement Ethical Committee Faculty of Public Health, University of Indonesia, as indicated by letter No. 699/UN2.F10.D11/PPM.00.02/2019.

Research time and location

This study was conducted in the city of Luwuk, Central Sulawesi Province, Indonesia. Luwuk is the capital city of the Banggai Regency. The Biak, Kampung Baru, Nambo, and Simpong public health centres in Luwuk City were used as research bases. The main reason for choosing Luwuk was the access to laboratories for analysing vitamin D levels. Data were collected over an eight-month period, from January to August 2020.

Research subject

A total of 67 pregnant women participated in this study. They were recruited from the lists of pregnant women provided by the Biak, Kampung Baru, Nambo, and Simpong public health centres. Inclusion criteria were: age 18-40 years, gestational age <12 weeks, maximum two parities, and willingness to follow research procedures. Exclusion criteria were:

multiple pregnancies, severe anaemia, hyperglycaemia (DM) (as determined by a blood glucose examination > 200 mg/dl), kidney failure, hypertension, high cholesterol level, heart disease, and tuberculosis.

Research variables

The variables in the present study included: 25(OH)D concentration during the first trimester of pregnancy, age, education background, physical activity level, occupation, history of GDM, family history of diabetes, exposure to cigarette smoke, dietary intake, and anthropometric measurements (i.e., body weight, height, mid-upper arm circumference (MUAC), and waist circumference).

Research instruments

25(OH)D levels in blood samples were measured in the Prodia Laboratory using Chemiluminescent Microparticle Immunoassay (CMIA) Method. Research assistants with training in nutritional sciences conducted a semi-quantitative FFQ (food frequency questionnaire) to evaluate and measure the participants' food intakes. The FFQ was developed independently by the research team, based on a preliminary survey of the diets of local pregnant women. Physical activity levels were measured using the Global Physical Activity Questionnaire (GPAQ) (Global Physical Activity Questionnaire (GPAQ) WHO STEPwise Approach to NCD Risk Factor Surveillance, 2016). Anthropometric measurements (height, weight, waist circumference, and mid-upper arm circumference) were taken by assistants trained to use tools such as MUAC tape, digital scale, Microtoise Stature Meter, and waist ruler.

Data analysis

Vitamin D concentration was considered to be deficient when <20ng/mL, insufficient at 20-30 ng/mL, and sufficient at > 30 ng/mL (Holick *et al.*, 2011); by quartiles: Q1 at <13.9 ng/mL; Q2 in the range > 13.9 ng/mL - <15.6 ng/mL; Q3 > 15.6 ng/mL - < 19.7 ng/mL; and Q4 ng/mL > 19.7 ng/mL.

Before the analysis was carried out, a normality distribution test was performed on all continuous variables. The findings of this test indicated that the data distributions for age and waist circumference were abnormal, although the other variables were all found to be normally distributed. Median value (range) was used to describe age and waist circumference values. Mean (standard deviation, *SD*) was taken for all other continuous variables. When performing a descriptive analysis, mean (*SD*) and number of participants in each group (*n* %) were used.

In order to evaluate the relationships between 25(OH)D concentration and different variables, bivariate analysis was performed. Independent *t*-test and one-way analysis of variance (ANOVA) test were carried out to identify differences in vitamin D levels between groups. Pearson's and Spearman's correlation tests were also carried out using continuous data. Meanwhile, linear regression was performed at the multivariate stage, while statistical analysis results and general theory were used to evaluate the interactions among variables ($p<0.05$). Non-significant variables (i.e., those with $p>0.05$) were excluded one at a time. To determine confounding variable(s), the difference in coefficient B was calculated before and after removal of a variable from the regression model (i.e., if the coefficient difference was >10%, then it was considered a confounding variable). IBM SPSS Statistics for Windows, version 28.0 (IBM Corp., Armonk, New York, USA) was used to carry out all statistical analyses.

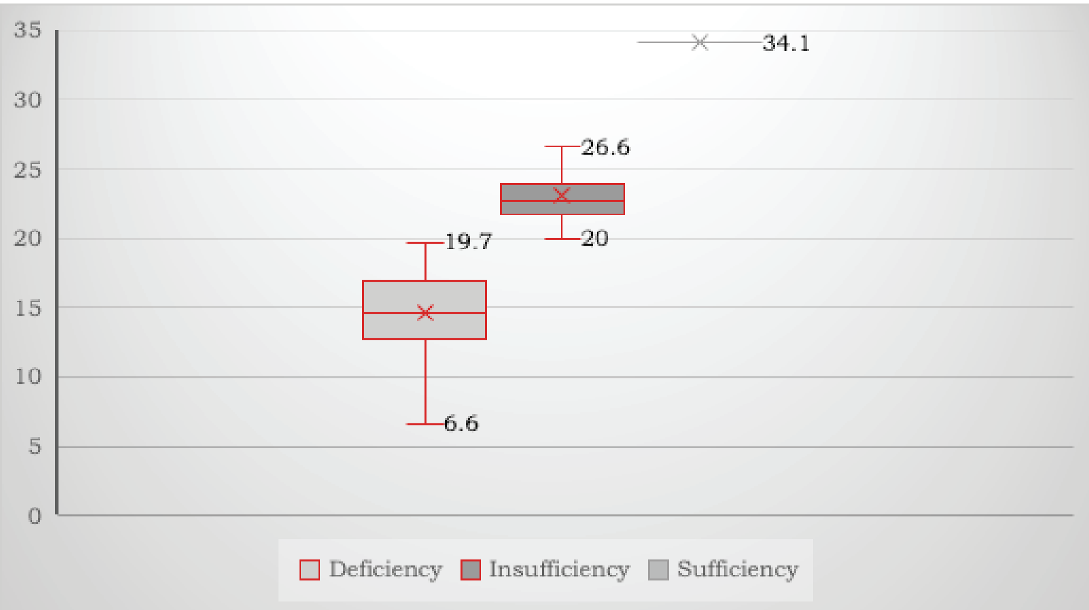
Table 1. Levels and categories of early 25(OH)D concentration in pregnant women

Level and categories of vitamin D	*Mean (min-max) or n (%)*
Vitamin D levels 25 (OH) D (ng/mL)†	16.4 (6.6-34.1)‡
Vitamin D deficiency	52 (77.6)
Vitamin D insufficiency	14 (20.9)
Vitamin D sufficiency	1 (1.5)
Q-1st	16 (23.9)
Q-2nd	18 (26.9)
Q-3rd	17 (25.4)
Q-4th	16 (23.9)

Q-1st until Q-4th are the quartile of 25(OH)D concentration: Q-1st at <13.9 ng/mL; Q-2nd in the range >13.9 ng/mL - <15.6 ng/mL; Q-3rd >15.6 ng/mL - <19.7 ng/mL; and Q-4th ng/mL >19.7 ng/mL)
†Normal distribution; $p>0.05$
‡Minimum and maximum

RESULTS

In Table 1, the average 25(OH)D level in pregnant women (67 respondents) was low, namely 16.4 ng/mL, with a minimum level of 6.6 ng/mL and a maximum level of 34.1 ng/mL. Vitamin D deficiency was a common issue for a majority of pregnant women (77.6%) in the study. In Figure 1, it can be seen that participants in the deficient group had 25(OH)D concentrations that neared the upper limit (maximum value), but vitamin D levels in the insufficient group were closer to the lower limit (minimum value), of their respective groups. There



Number of respondents (mean): Deficiency = 52(14.3); Insufficiency = 14(23.0); Sufficiency =1 (34.1)

Figure 1. Minimum and maximum 25(OH)D concentrations in pregnant women according to different categories

was only one participant in the sufficient category.

Findings from the bivariate analysis presented in Tables 2 and 3 showed that vitamin D intake and family history of diabetes mellitus had statistically significant relationships with 25(OH) D concentration. Thus, these variables

Table 2. Characteristics of pregnant women and their level of vitamin D at recruitment

Characteristics	*Categories of vitamin D, n (%)*		*Mean±SD*	*p*†
	Deficiency	*Insufficiency*		
Mother's occupation¹				
Government officer	7 (13.5)	2 (14.3)	15.9±5.7	0.251
Private company	7 (15.)	1 (7.1)	15.6±3.4	
Entrepreneur/Freelancer	3 (5.7)	3 (21.4)	20.5±2.5	
Housewives	35 (66.0)	8 (57.1)	15.8±4.8	
Education¹				
No school	1 (1.9)	0 (0.0)	18.5	0.903
Primary school	3 (5.8)	1 (7.1)	17.7±2.8	
Junior high school	3 (5.8)	1 (7.1)	15.8±5.3	
Senior high school	20 (38.4)	6 (42.9)	16.5±5.3	
Diploma/Bachelor/Undergraduate	25 (48.1)	6 (42.9)	15.8±4.6	
Family history of diabetes mellitus²				
Yes	16 (30.2)	1 (7.1)	14.8±3.7	0.043*
No	37 (69.8)	13 (92.9)	17.0±5.5	
Cigarette smoke exposure²				
Yes	29 (54.7)	8 (57.1)	16.7±4.2	0.151
No	24 (45.3)	6 (42.9)	15.3±5.4	
Physical activity²				
Low (MET≤ 3000)	28 (53.8)	6 (42.9)	15.4±5.0	0.069
High (MET>3000)	24 (46.2)	8 (57.1)	17.1±4.4	
Vitamin D supplement consumption²				
No	36 (69.2)	9 (64.3)	16.1±4.6	0.360
Yes	16 (30.8)	5 (35.7)	16.6±5.1	
Parity				
Multiparous	13 (25.0)	6 (42.9)	15.9±6.0	0.940
Primiparous	11 (21.2)	3 (21.4)	16.1±3.7	
Nulliparous	28 (53.8)	5 (35.7)	16.4±4.4	
Categories of body mass index (BMI)				
Obese	13 (25.0)	2 (14.3)	15.9±4.3	0.762
Normal	39 (75.0)	12 (85.7)	16.3±4.9	
Categories of waist circumference				
Central obesity	38 (71.7)	11 (78.6)	16.2±5.0	0.961
Normal	15 (28.3)	3 (21.4)	16.3±4.0	

The number of respondents for each variable varied: occupation= 66, education= 66, parental history of diabetes mellitus= 66, cigarette smoke exposure= 66, physical activity= 66, Vitamin D supplement consumption= 66, parity= 66, BMI= 66, categories of BMI=66, categories of waist circumference= 66

†results of one-way ANOVA test¹ or *t*-test²

*$p<0.05$

Table 3. Correlation between continuous variables and early 25(OH)D concentration

Characteristics	*Mean±SD of each vitamin D categories*		*Total groups*	
	Deficiency	*Insufficiency*	*Mean±SD*	*p*†
Age (years)[1]	29.0±5.3	29.0±5.7	29.0±19.0	0.892
Dietary vitamin D intake[1]	16.2±11.5	38.6±25.5	18.1±4.8	0.002*
Body mass index (kg/m^2)[1]	24.7±4.1	24.3±2.6	24.0±17.0	0.305
Waist circumference (cm)[2]	88.5±8.6	86.5±8.1	85.0±10.4	0.668
Mid-upper arm circumference (cm)[1]	27.7±3.5	26.9±3.0	27.3±3.2	0.314
Haemoglobin concentration (mg%)[1]	11.5±1.3	11.7±1.9	12.1±1.5	0.268
Glucose level 1st trimester (mg/dl)[1]	82.0±22.6	76.5±8.0	89.0±11.2	0.264

The number of respondents for each variable varied: age=66, waist circumference=66, mid-upper arm circumference=66, haemoglobin concentration=66, glucose level 1st trimester=66
†Correlation: [1]Pearson's; [2]Spearman's
*p<0.01

were used in the multivariate analysis to evaluate their impact on 25(OH)D concentration. In addition, exposure to cigarette smoke and physical activity levels showed a significance value of <0.25, which also met the requirements for incorporation into the multivariate analysis. Finally, considering the significance of body mass index (BMI) and blood glucose levels, these variables were also included in the multivariate analysis.

Interaction tests were performed between vitamin D intake and a number of covariates before confounding factors were evaluated. The analysis findings showed that there were no significant relationships between vitamin D intake, covariates, and blood vitamin D levels (no interactions). Since physical activity and BMI were not found to be statistically significant confounders (change in coefficient B <10%), they were not included in the regression model. Meanwhile, parental history of diabetes and exposure to cigarette smoke were found to be significant confounding factors (change in B coefficient >10%), so they were both included in the regression model.

In Table 4, the parsimony linear regression model used to predict blood

Table 4. Predictor model of early vitamin D concentration in pregnant women

First Model				*Final Model*			
Variable	*Coeff. B*	*p*	*R square*	*Variable*	*Coeff. B*	*p*	*Adjusted R square*
Constant	22.529	0.003	0.498	Constant	24.102	<0.001	0.545
Vitamin D intake	0.259	<0.001		Vitamin D intake	0.260	<0.001	
Parental history of DM	-2.256	0.187		Blood glucose	-0.155	0.004	
Exposure of Smoke	2.298	0.153		Parental history of DM	-2.325	0.147	
Blood glucose	-0.153	0.007		Exposure of Smoke	2.388	0.122	
Physical activity	0.178	0.916					
BMI	0.054	0.778					

*Using multivariate linear regression
DM: diabetes mellitus

25(OH)D concentrations is presented. The final model included the following factors: vitamin D consumption, blood glucose, family history of DM, and smoking exposure, all of which served as predictors of 25(OH)D concentration during the 11th and 12th weeks of pregnancy in 54.5% of participants (*p*=0.001). This was a significant increase from the initial model, of which the percentage was 49.8%.

DISCUSSION

It is important to note that vitamin D can be both produced endogenously and consumed as a micronutrient in food. Key food sources of vitamin D include fish oil (salmon, sardines, mackerel), milk, juice, and egg yolks (AlFaris *et al.*, 2019). The World Health Organization (WHO) and the Food and Agricultural Organization (FAO) recommend three strategies to increase micronutrient intake: (1) increase the diversity of foods consumed, (2) food fortification, and (3) supplementation. Each of these strategies, together with sensible sunlight exposure (when and if available), can reduce the widespread prevalence of inadequate vitamin D intake. Dietary supply (including supplementation) is particularly important for those of whom sunlight exposure is limited (Allen *et al.*, 2006).

In the present study, 77.6% of the participants experienced vitamin D deficiency, with 20.9% clinically insufficient in vitamin D and just 1.5% sufficient. These findings are consistent with those of other studies that indicated very few pregnant women have sufficient levels of vitamin D (Yun *et al.,* 2017). A study reported that 90% of pregnant Chinese women were found to be vitamin D deficient at 23-28 weeks of gestation (<20 ng/ml) (Zhao *et al.,* 2017), while another research revealed that 31.8% of women were severely deficient in vitamin D, 40.7% were deficient, 25.1% were insufficient, and just 2.4% had normal vitamin D levels (Yang *et al.,* 2021). Thus, it is critical that vitamin D deficiency is detected at an early stage during pregnancy because it has significant impact on the progression of pregnancy and childbirth.

The present study revealed that several factors had significant relationships with vitamin D levels, including dietary intake of vitamin D and family history of diabetes mellitus. The findings showed that pregnant women with vitamin D deficiency consumed less vitamin D than those in the insufficient group. AlFaris *et. al* (2019) showed that women who did not take vitamin D supplements had more than three times (3.14) risk of experiencing vitamin D deficiency than those who took vitamin D supplements. 25(OH)D concentrations also seemed to fluctuate depending on whether or not there is a parental history of diabetes. The results indicated that 25(OH)D concentrations in pregnant women with diabetes were lower than those who did not have diabetes, which is in line with the findings from other studies (AlFaris *et al.*, 2019; Al-Timimi & Ali, 2013; Iqbal *et al.*, 2016).

Other studies have revealed that vitamin D levels are impacted by factors such as exposure to cigarette smoke. Pregnant women who have been exposed to cigarette smoke are more likely to be vitamin D deficient or have insufficient levels of vitamin D than those who have not been exposed to cigarette smoke (Yun *et al.*, 2017). Although this study did not find significant differences in Vitamin D levels between parity groups, Yang *et al.* (2021) found that multiparous women had lower mean 25(OH)D concentrations and were more likely to be obese or centrally obese, as well as low in physical activity levels (Yang *et al.*, 2021). Thus, it is critical that multiparous women are provided more care and attention during pregnancy.

The findings in this study showed that there was no statistically significant difference in 25(OH)D concentrations between participants who took oral vitamin D supplements and those who did not. However, it appeared that pregnant women who took vitamin D supplements orally had higher average 25(OH)D concentrations than women who did not take vitamin D supplements. Of the participants who did not consume vitamin D supplements, there was a higher percentage of vitamin D deficiency than vitamin D insufficiency. Conversely, in the group of women who consumed vitamin D supplements, there were more women who were vitamin D insufficient compared to vitamin D deficient. The statistically insignificant results may be due to the higher number of participants who took vitamin D supplements than those who did not. Yosephin *et al.*'s study (2015) showed that vitamin D levels increased after participants took vitamin D supplements (Yosephin *et al.*, 2015). This difference, which was not statistically significant, could have been caused by the small number of participants in the study.

Prior studies have also revealed that overweight or obese individuals should consume 1.5 to 2-3 times more vitamin D per day than those of normal weight (Bouillon, 2017; Ekwaru *et al.,* 2014). Despite these findings, vitamin D supplementation is not yet a regular part of any nationwide maternal health programmes in Indonesia. Thus, it is important that vitamin D supplementation be incorporated in national pregnancy programmes and that the vitamin D levels of pregnant women are carefully monitored.

The parsimony model developed during the multivariate analysis process predicted 54.4% of vitamin D levels in the blood. This model is applicable to the participants in the present study. In terms of other variables, dietary vitamin D intake was found to be positively related to 25(OH)D concentration. To be more precise, the findings indicated that concentration of vitamin D in the blood increased when more vitamin D was consumed.

However, this study found that variables with a negative relationship with 25(OH)D concentration in the blood included parental history of diabetes and blood glucose levels. This is consistent with the findings of other studies, which revealed low serum vitamin D levels in those with poorly controlled type 2 diabetes mellitus (Al-Timimi & Ali, 2013; Iqbal *et al.*, 2016; Zoppini *et al.*, 2013); while statistically significant, we question its clinical relevance. The current research solely establishes statistical proof of this correlation, lacking clinical investigation. Existing studies focus on the link between parental diabetes history and offspring diabetes incidence, suggesting a potential mechanism for the observed correlation. Further research must provide clinical validation of this mechanism.

All variables included in the multivariate model had a simultaneous influence on the dependent variable. Nonetheless, the impact of each variable in this multivariate model differed from those in the bivariate model.

This study had several limitations. Firstly, as a cross-sectional design was used for data collection, it was impossible to determine causality between vitamin D levels and the parameters measured in the research subjects. Secondly, there may be information bias in this study, given the lack of existing data pertaining to vitamin D intake from food sources. Moreover, the sample size was not calculated in relation to the research objective. This is because the study is the first part of a cohort study involving a number of dependent variables, which may impact the statistical significance of the data. These limitations should be carefully considered by researchers examining this topic in the future.

It is evident from this study that more efforts should be made to prevent vitamin D deficiency in women during pregnancy. For example, if a family history of diabetes mellitus is present, appropriate measures should be taken from the start of the pregnancy. The effectiveness of health services for pregnant women such as nutritional management or counselling will increase if they are based on knowledge about health conditions before pregnancy. Moreover, vitamin D and blood glucose levels, as well as other parameters indicative of a syndrome or disease, should be monitored carefully to prevent problems during pregnancy. Pregnant women should be advised on how to manage their food intake and avoid risky behaviours. Maternal health programmes should recommend vitamin D supplementation during the preconception period. Currently, existing antenatal care policies in most regions do not pay attention to medical history or health conditions before pregnancy (preconception period), which impacts antenatal services, especially for pregnant women with poor health histories.

CONCLUSION

The prevalence of vitamin D deficiency in the pregnant women studied was high (77.6%). The average 25(OH)D concentration was only 16.4 (6.6-34.1) ng/ml. Blood glucose level, vitamin D dietary intake, parental history of diabetes, and exposure to smoke were strong predictors of vitamin D deficiency in 54.5% of the cases (adjusted *R* square). Thus, it is critical to ensure that for pregnant women to take vitamin D supplements, since nearly 80% of pregnant mothers are vitamin D deficient. Vitamin D supplementation and monitoring can be included in national pregnancy programmes. However, further investigations are needed regarding the impact of vitamin D intake from food sources and exposure to sunlight on vitamin D levels in the blood.

Acknowledgement

The authors would like to express their deepest appreciation to the Chancellor of Universitas Indonesia for providing funding for this study through the "Hibah Research UI" programme. They also express their deep gratitude to the Dean of the Faculty of Public Health, University of Tompotika, Luwuk, who facilitated the research assistants, research sites, and use of research equipment. They are also most thankful to the Heads of Kampung Baru, Luwuk, Nambo, and Biak Public Health Centres for the assistance they provided to facilitate this study.

Authors' contributions

Lalusu EY, carried out this research; Hatma RD, involved in the implementation of research starting from the initial planning of research and various scientific considerations in conducting research; Sudaryo MK, participated in article review and provided various suggestions for improvement during the preparation of the article, contributed greatly to data processing and analysis; Ocviyanti D, participated in article review and provided various suggestions for improvement during the preparation of the article, contributed more to the discussion of the findings of this study; Rimbawan R, participated in article review and provided various suggestions for improvement during the preparation of the article, contributed more to the discussion of the findings of this study. All authors listed above contributed to preparing, drafting, and revising the manuscript, giving final approval of the published version, and agreeing to be responsible for all aspects of the work.

Conflict of interest

The authors declare that they have no conflict of interest. This article has received approval from all relevant parties to be published.

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Mal J Nutr 30(2): 193-202, 2024

Effect of apple peel flour addition on physicochemical characteristics and fatty acid profile of reduced-fat mayonnaise

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ABSTRACT

Introduction: Mayonnaise is popular throughout the world and contains various types of processed products. While reduced-fat mayonnaise (RFM) contains less than 50% oil, the drawback is the potential to reduce the physical quality of mayonnaise. The use of apple peel in the form of flour added to mayonnaise is predicted to enhance its physicochemical quality. **Methods:** The research comprised laboratory experiments using randomised design with four treatments and six repetitions. Apple peel flour was added to the treatments in the following proportions: 0% (RFA0), 1% (RFA1), 2% (RFA2), and 3% (RFA3). Analysis of variance (ANOVA) was used to obtain average values and standard deviations. **Results:** Addition of 3% apple peel flour to RFM showed highly significant effect ($p \leq 0.01$) on droplet size (2.15–9.49μm), viscosity (3965.00cP), colours L (71.93), a* (15.75), and b* (50.65), protein content (1.44%), and fat content (50.93%). It also produced an organoleptic quality that was acceptable to semi-trained panellists and fatty acid profiles containing various types of fatty acids. **Conclusion:** The use of 3% apple peel flour in RFM represented the best treatment, with potential for further improvement.

Keywords: apple peel flour, reduced-fat mayonnaise, stabiliser

INTRODUCTION

Mayonnaise is popular throughout the world and encompasses several types of processed products. One very popular version is the traditional type of mayonnaise or full-fat mayonnaise (Elsebaie *et al.*, 2022). However, various low-fat mayonnaise products are currently being developed. The reduction of fat levels aligns with the trend of promoting foods with little oil content for maintaining a healthy body. The health benefits associated with such a move include reducing both the fat content and the number of calories (Kumar, 2021). Oil and water are ingredients that greatly influence the final mayonnaise product. The role of oil is as the dispersed phase and water as the dispersing phase, both contributing significantly to the stability of the product. Reducing the amount of oil used can be a first step in achieving fat reduction; however, this would result in an unstable emulsion during the emulsifying process, hence a stabiliser would be needed to unite the oil and water.

Flour is used in almost all processed foods derived from livestock products. In the food industry, special types of flour

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doi: https://doi.org/10.31246/mjn-2023-0020

have a beneficial effect on products, meaning many manufacturers prefer to use flour modified from real flour. Modified wheat flour, for example, has functional uses as a food ingredient, namely as a thickener, stabiliser, binder, and emulsifier (Kumar *et al.*, 2021). In its modified form, flour maintains the texture of food, in addition to acting as a gelling agent in emulsions to provide stability and prevent the components from separating, which has a beneficial effect on the overall physical appearance and taste of the product. Researchers have widely examined the use of flour derived from seeds, leaves, tubers, fruits, and plant waste. Some of these commonly used flours include pumpkin flour (Nidhal, Evanuarini & Thohari, 2022), banana peel flour (Evanuarini & Susilo, 2020), and watermelon peel flour (Evanuarini, Amertaningtyas & Utama, 2021). This research examined the potential use and development of apple peel waste as flour.

Apple industrial waste in the form of peels comes from the cider industry, canned apples, and puree. Batu City is known as "the city of apples" due to its many apple plantations, where some of the apples produced are processed into apple chips. To date, significant volumes of apple peel generated as industrial waste have not been handled or processed. Apple peel is generally used as a ruminant feed in various farms in Batu City and Malang Regency, where it acts as a source of fibre.

Apple peels that are processed into flour can be applied to food products. They are rich in dietary fibre, flavonoids, lipids, and ash, as well as polyphenols, unsaturated galactolipids and phospholipids (Wolfe & Liu, 2003). Dietary fibre will increase water holding capacity, emulsion, gel formation (change in texture, aroma, colour), stabilise the product, and increase shelf life (Rabetafika *et al.*, 2014). Apple peel flour contains 40.7% pectin (Subagyo & Ahmad, 2010), 9.96% crude fat, and 2.80% crude protein (Romelle, Rani & Manohar, 2016). Pectin will absorb water and reduce the water content of the product. Apple peel flour has a brownish tint and will naturally absorb water and air, meaning it must be used directly within a product. The use of apple peel flour in low-fat mayonnaise is expected to produce a unique colour, an increase in viscosity, and a different fatty acid profile. Our study aimed to determine the optimum percentage of apple peel powder to be added to reduced-fat mayonnaise (RFM) to improve its quality.

MATERIALS AND METHODS

Materials

The research material comprised RFM created using canola oil, egg yolk, and vinegar, with the addition of apple peel flour. The research methods comprised laboratory experiments with a completely randomised design, with four treatments and six repetitions. Four different apple peel flour treatments were used, with different percentages of apple peel flour and code reduced fat mayonnaise with apple peel flour (RFA): RFA0 (0%), RFA1 (1%), RFA2 (2%), and RFA3 (3%). Table 1 shows the materials used in the RFM formulation.

Apple peel flour preparation

Apple peels were obtained from apple chip manufacturers in the tourist town of Batu, Malang. Production began with the cleaning of the apple peels, which were then placed in an oven at a temperature of 60°C and dried for 46 hours. Any apple peel that was too dry was discarded. The dried peels were then mashed using a dry mill and filtered using a 100-mesh sieve.

Reduced-fat mayonnaise preparation

RFM was created based on the

Table 1. Reduced-fat mayonnaise formulation

Ingredient (%)	*RFA0*	*RFA1*	*RFA2*	*RFA3*
Canola oil	70.0	50.0	50.0	50.0
Egg yolk	20.0	20.0	20.0	20.0
Apple peel flour	0.0	1.0	2.0	3.0
Vinegar	5.0	5.0	5.0	5.0
Salt	1.5	1.5	1.5	1.5
Sugar	2.5	2.5	2.5	2.5
Mustard	0.5	0.5	0.5	0.5
White pepper	0.5	0.5	0.5	0.5
Water	0.0	20.0	20.0	20.0

RFA0: without apple peel flour; RFA1: 1% apple peel flour addition; RFA2: 2% apple peel flour addition; RFA3: 3% apple peel flour addition

formulation by Evanuarini *et al.* (2016), with some modifications. A hand mixer was first used to mix optional ingredients such as white pepper, mustard, sugar, and salt for 1 minute. Egg yolk, canola oil, and vinegar were then added. Finally, apple peel flour was added to the mixed ingredients.

Statistical analysis

Research data were processed using Microsoft Excel (Microsoft Corp., Redmond, Washington, USA). Average and standard deviation values were obtained by analysis of variance (ANOVA). All statistical analyses were carried out using IBM SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, New York, USA). Duncan's multiple range test (DMRT) was used where research data yielded different results at a significant level of 1%.

RESULTS

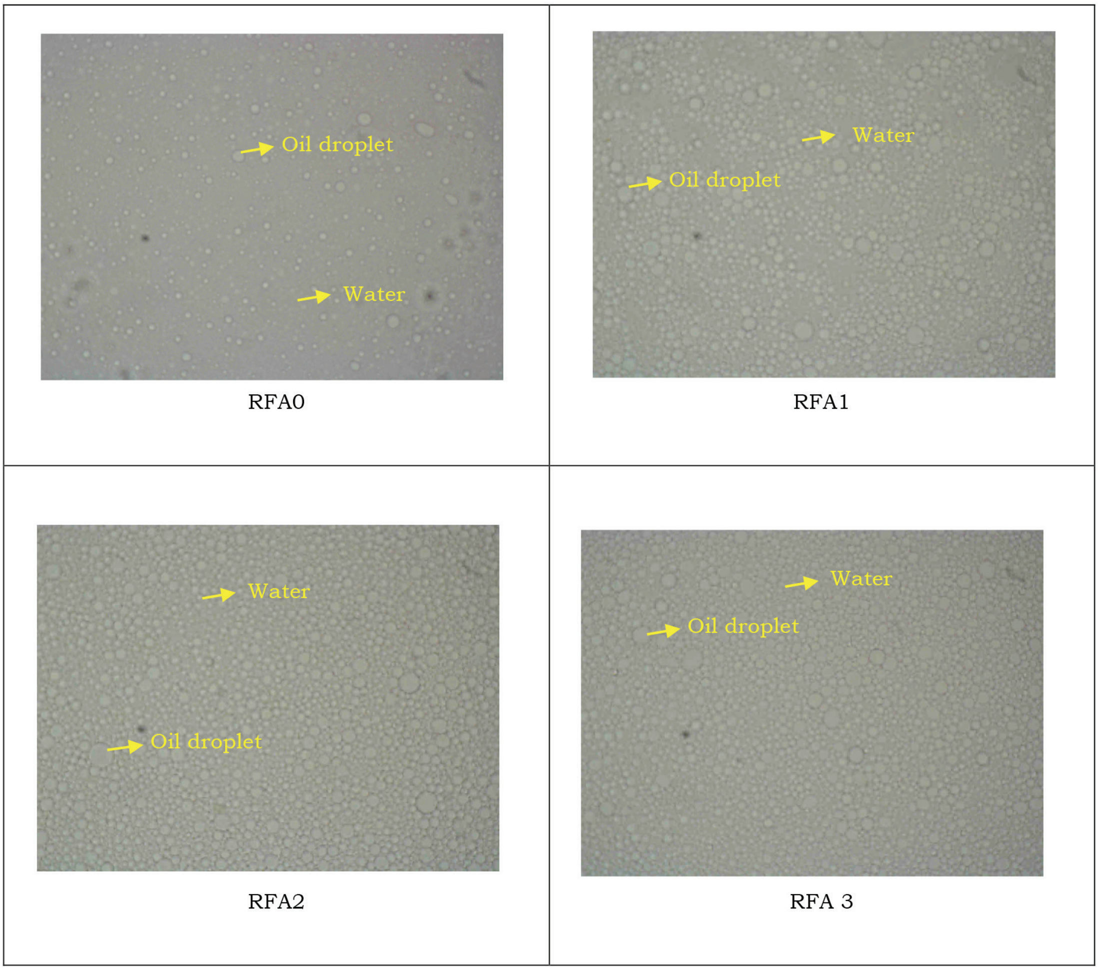
Table 2 contains the results of the emulsion droplet measurements in RFM with apple peel flour. The droplet sizes displayed for the different percentages of apple peel flour ranged from 2.15–18.15 μm. The smallest average emulsion droplet size was obtained for RFA3. The control (RFA0) mayonnaise, which received no treatment, had an emulsion droplet size of 5.32–18.15 μm. The RFM emulsion droplet results for the different percentages of apple peel flour are presented in Figure 1.

Table 2 contains the average RFM viscosity values with apple peel flour. There were signs of increased RFM viscosity with the addition of apple peel flour. The average values of RFM with apple peel flour were 3010.00–3965.00 cP. The highest viscosity was found in the mayonnaise with 3% apple peel flour (RFA3). This showed that the more apple peel flour added to the RFM, the higher the average viscosity. Based on the ANOVA results, apple peel flour had a very significant effect ($p \leq 0.01$) on the viscosity of RFM.

The average colours of L (lightness), a* (red/green), and b* (yellow/blue) in RFMs with apple peel flour are presented in Table 2. There was decreasing colour data for L in RFM with apple peel flour. The average values of the L colour in mayonnaise were 71.93–90.05, with the highest L colour found in full-fat mayonnaise (control) and the lowest in RFA3. In contrast, the colour a* in RFM with apple peel flour increased, with average colour values of 0.61–15.75. The highest a* colour was found in RFA3 and the lowest in full-fat mayonnaise. The b* colour in RFM with apple peel flour was found to decrease, with average b* colour values of 50.65–59.65. The highest b* colour was identified in control with no apple peel flour and the lowest in RFA3. ANOVA results showed that apple peel flour had a very significant effect on colours L, a*, and b* in RFM ($p \leq 0.01$).

The average protein contents of RFM with apple peel flour are presented in Table 2. The protein content in RFM increased with the addition of different percentages of apple peel flour. The average values for the protein content of mayonnaise were 1.16–1.44%. The highest protein content was found with the addition of 3% apple peel flour (RFA3) and the lowest for control mayonnaise. ANOVA results showed that apple peel flour had a very significant effect ($p \leq 0.01$) on the protein content of RFM.

Table 2 contains the average fat contents of RFM with apple peel flour. It showed that the fat content of RFM decreased with the addition of different percentages of apple peel flour. The average fat content values of mayonnaise



RFA0: without apple peel flour; RFA1: 1% apple peel flour addition; RFA2: 2% apple peel flour addition; RFA3: 3% apple peel flour addition

Figure 1. Emulsion droplets of flour in reduced-fat mayonnaise with apple peel flour at 400x magnification

Table 2. Physicochemical composition and organoleptic quality of reduced-fat mayonnaise with apple peel flour

Physicochemical	*RFA0*	*RFA1*	*RFA2*	*RFA3*
Emulsion droplets (μm)	5.32-18.15	4.48-14.76	2.99-13.02	2.15-9.49
Viscosity (cP)	3010.00±47.61[a]	3290.00±34.64[b]	3600.00±51.64[c]	3965.00±77.24[d]
L	90.05±0.62[d]	83.09±1.40[c]	74.59±1.13[b]	71.93±0.61[a]
a*	0.61±0.15[a]	7.94±0.30[b]	11.57±0.39[c]	15.75±0.64[d]
b*	59.65±0.54[d]	57.67±0.48[c]	54.74±0.68[b]	50.65±0.52[a]
Protein content (%)	1.16±0.03[d]	1.25±0.03[c]	1.33±0.02[b]	1.44±0.04[a]
Fat content (%)	68.09±0.36[d]	55.04±0.75[c]	51.48±0.42[b]	50.93±0.66[a]
Organoleptic				
Texture	3.60±0.68	3.75±0.64	4.10±0.79	4.25±0.79
Aroma	3.65±0.88	3.75±0.79	3.95±0.76	4.10±0.55
Colour	4.25±0.72	4.00±0.79	3.85±0.75	3.55±0.83
Flavour	3.75±0.72	3.95±0.76	4.00±0.73	4.20±0.77

RFA0: without apple peel flour; RFA1: 1% apple peel flour addition; RFA2: 2% apple peel flour addition; RFA3: 3% apple peel flour addition

L: lightness; a*: red/green; b*: yellow/blue

[a,b,c,d] Indicate very significant difference; $p \leq 0.01$

were 68.93–78.09%. The lowest fat content was found with the addition of 3% apple peel flour (RFA3), while the control mayonnaise had the highest fat content. The results of various analyses showed that apple peel flour had a very significant effect on the fat content of RFM ($p \leq 0.01$).

The average organoleptic values can be seen in Table 2. The organoleptic data and analysis showed no significant effect ($p \geq 0.05$) of the addition of apple peel flour on the texture, aroma, taste, and colour of RFM. In the spider graph (Figure 2), the larger the rectangle that points outward, the higher the organoleptic value. The largest rectangle shown denotes RFM with the addition of 3% apple peel flour, while the smallest is the control (RFM without treatment). Figure 2 showed that the average texture organoleptic values ranged from 3.60 to 4.25. ANOVA results showed that the addition of apple peel flour had no significant effect ($p \geq 0.05$) on the texture of RFM. The average aroma organoleptic value ranged from 3.65 to 4.10. RFA3 had the highest RFM aroma value, while control had the lowest value. As for colour, the average colour organoleptic value ranged from 3.55 to 4.25; control had the highest value, while RFA3 had the lowest value. The average taste organoleptic value ranged from 3.75 to 4.20. RFA3 had the highest score for RFM flavour, while RFA0 had the lowest.

Table 3 contains the fatty acid profile data for RFM with the addition of 3% apple peel flour (RFA3). It showed that the amount of SFA was 11.10% and the highest type of SFA was palmitic acid, as much as 7.26%. The types of SFA comprised of palmitic acid, stearic acid, arachidic acid, myristic acid, heptadecanoic acid, and heneicosanoic acid. Heneicosanoic acid had the lowest percentage among the SFA in this mayonnaise, at 0.04%. MUFA had the highest value in the types of fatty acid profile at 30.88%. MUFA comprised of unsaturated fatty acids, oleic acid, omega-9 fatty acids, eicosanoic acid, and palmitoleic acid. Palmitoleic acid had the lowest value among the different types of MUFA in this mayonnaise. Table 3 showed a value of 16.09% for

PUFA. The following types of PUFA were detected in RFM: omega-6 fatty acids, linoleic acid, omega-3 fatty acids, linolenic acid/w3, arachidonic acid, w6 acid, eicosapentaenoic acid (EPA), eicosadienoic acid, and docosahexaenoic acid (DHA).

Table 3. Fatty acid profile of reduced-fat mayonnaise with apple peel flour

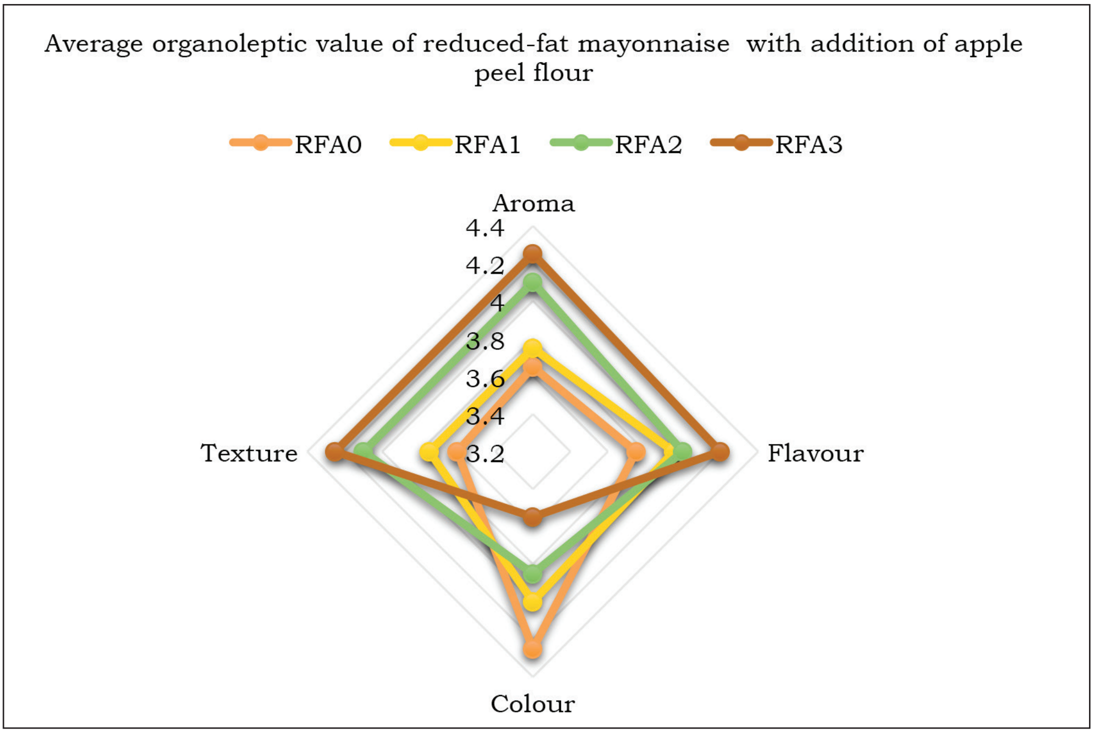
Types of fatty acids	%
Saturated fat	11.10
C 16:0 (Palmitic acid)	7.26
C 18:0 (Stearic acid)	3.45
C 20:0 (Arachidic acid)	0.23
C 14:0 (Myristic acid)	0.06
C 17:0 (Heptadecanoic acid)	0.04
C 21:0 (Heneikosan acid)	0.04
Monounsaturated fat	30.88
Unsaturated fats	46.97
C 18:1 W9C (c-oleic acid)	30.11
C 20:1 (Eicosanoic acid)	0.23
C 16:1 (Palmitoleic acid)	0.33
Polyunsaturated fat	16.09
Omega 6 fatty acids	12.20
C 18:2 W6 (Linoleic acid/w6)	10.87
Omega 3 fatty acids	3.80
C 18:3 (Linolenic acid/w3)	3.64
C 20:4 (Arachidonic acid)	1.01
C 18:3 (Linoleic acid/w6)	0.33
C 20:5 w3 (Eicosapentaenoic acid)	0.09
C 20:2 (Eicosadienoic acid)	0.08
C 22:6 w3 (Docosahexaenoic acid)	0.07

DISCUSSION

Figure 1 showed that during the emulsification process, a small proportion of pectin will absorb oil and some will also absorb water, thus protecting the droplets in the mayonnaise. Heating and shaking too quickly and excessively can cause the emulsion to split and form globules, which will be covered by the continuous phase (O'Brien, 2009). Oil is thus mixed slowly with the other ingredients to facilitate the tight formation of oil droplets. Conducting the dispersed and dispersing phases simultaneously will produce an emulsion in the form of water in oil, which will reduce the resulting viscosity. According to Golchoobi *et al.* (2016), a smaller particle size will change the colour of the emulsion from grey to white. Mun *et al.* (2009) revealed that creaming is prevented during the manufacture of low-fat mayonnaise products with the addition of a thickening agent, such as starch, to the aqueous phase to slow down the movement of the droplets.

The total pectin content in apple peel flour can produce an increase in viscosity. Based on the results of an introductory study, apple peel flour contains 15.35% pectin. Pectin is a type of crude fibre that can form a gel (Izzati & Salsabila, 2018). The pectin content of apple skin flour can increase the gelling of mayonnaise, thereby increasing its thickness. Pectin can also bind water or be hygroscopic, thereby reducing the water content of the product and increasing its viscosity. Amelia, Astuti & Zulferiyenni (2016) stated that gel formation can be caused by the presence of pectin; this can form fine fibres and clumps that hold liquid in the product. Furthermore, pectin is useful as a stabiliser to maintain emulsion stability and increase viscosity, thus preventing emulsion damage (Hutapea, Rusmarilin & Nurminah. 2016). Studies have shown that mayonnaise thickens with peanut powder to create a thick and stable consistency (Rudra *et al.*, 2020), while vegan mayonnaise with durian seed flour added has a viscosity value of 3160 cps (Cornelia, Siratrantri & Prawita, 2015).

Table 2 showed that the more apple peel flour added to RFM, the lower the L colour value of the mayonnaise. This is because when heated, the flour changes colour to brownish yellow, so the more apple peel flour is added, the browner the RFM. This is a positive aspect because mayonnaise will have a



RFA0: without apple peel flour; RFA1: 1% apple peel flour addition; RFA2: 2% apple peel flour addition; RFA3: 3% apple peel flour addition

Figure 2. Spider graph on the organoleptic average values of RFM with apple peel flour

different colour and can thus be used as a natural dye. Apple peel flour also contains anthocyanins, which helps in promoting skin health. The stability and effectiveness of anthocyanins render them suitable for use in products with low pH, in addition to functioning as fillers and economical natural dyes. Food colours are caused by caramelisation reactions, Maillard reactions, oxidation, and the addition of natural or artificial colouring agents (Winarno, 2004). A tendency for the colour a* to increase in RFM was observed due to the brownish colour of the apple peel flour. Rubbing it into mayonnaise produces a darker and reddish colour. The presence of hydrocolloids in mayonnaise produces a yellowish colour (Johary *et al.*, 2015). The greater the amount of apple peel flour added to RFM, the further the average b* colour value of the mayonnaise will decrease; the apple peel flour grows darker and will thus produce a bluish colour. This finding aligns with Johary *et al.* (2015), who stated that mayonnaise with added hydrocolloids would become darker and the b* value would decrease.

Protein content was mainly derived from egg yolk, which functions as an emulsifier. Apple peel flour has a protein content of only 2.36%. The greater the amount of apple peel flour added to RFM, the higher the protein content. The addition of acid, emulsifier, or other ingredients can increase the protein content of a food ingredient. Pectin is a compound that is predominantly hydrophilic since the hydrophobic part of the protein is related to arabinogalactan.

While the arabinogalactan protein structure and glycoprotein structure can increase protein levels, their contribution is limited (Hutapea *et al.*, 2016). The addition of durian seed flour to mayonnaise produced a protein content of 0.16% (Cornelia *et al.*, 2015). A combination of egg yolk and stabiliser resulted in protein levels of between 0.67 and 0.89% (Hutapea *et al.*, 2016). According to Goankar *et al.* (2010), the standard protein content of mayonnaise is 1.43%; the minimum protein content is 0.9%. The protein content of apple peel flour is 4.3 g/100g dry matter (DM).

Table 2 showed that the average fat content of mayonnaise decreased with the addition of apple peel flour. This is because vitamin C in apple peel flour affects fat oxidation, while fibre in apple peel flour binds the fat in mayonnaise. The fat content in egg yolk comprises of 65.5% triglycerides, 5.2% cholesterol, and 28.3% phospholipids. Triglycerides and phospholipids function to provide energy for daily activities, while cholesterol regulates body functions (Magistri, Yaswi & Alioes, 2016). Vegan mayonnaise containing durian seeds also has a fat content of 60.36% (Cornelia *et al.*, 2015). The fat content of RFM that combines a concentration of egg yolk and a stabiliser ranges 37.24–37.39% (Hutapea *et al.*, 2016).

Based on preliminary research, apple peel flour has a water content of around 12.71%. Its hygroscopic nature means it can also absorb more water from the ingredients contained in the product. In terms of texture, the resulting mayonnaise was very dense and thick in texture; with more apple peel flour added, the thicker the texture of the mayonnaise. Despite the distinctive aroma of apple peel flour (Falah, Kurniaty & Aprilia, 2020), when combined with mayonnaise, the latter's own distinctive aroma was slightly disguised as it was absorbed by the apple peel flour. The more apple peel flour added, the more fragrant the aroma. Apple peel flour is yellowish brown in colour (Falah *et al.*, 2020); as such, mayonnaise containing a higher level of apple peel flour was darker in colour and appeared less attractive to the panellists. In terms of flavour, apple peel flour was slightly sour and slightly lowered the pH of mayonnaise. This is due to the presence of pectin from the apple peel flour, which has a D-galacturonic acid polymer (Amelia *et al.*, 2016). Hutapea *et al.*, (2016) reported that RFM containing different concentrations of egg yolk and stabiliser yielded organoleptic texture values of between 2.01 and 4.03, organoleptic aroma values of between 4.19 and 4.01, and organoleptic taste values of between 3.01 and 3.66.

Gas chromatography was used to determine the fatty acid profile of RFM at 3% addition of apple peel flour. Fatty acids can be distinguished from the number of carbon atoms (C) by the presence of bonds and the number and location of double bonds. Fatty acids are divided based on their chemical structure to give saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The properties of SFA include a lack of double bonds in the hydrocarbon chain, stability, and the fact that it is neither easily oxidised nor easily turned into trans fatty acid compounds or other harmful compounds; while PUFA has characteristics such as double bonds, a liquid form at room temperature or lower temperatures, and lower melting points than MUFA and SFA. PUFA are also more susceptible to oxidation (Sartika, 2008). McClements & Decker (2000) stated that the speed of lipid oxidation is affected by the chemical structure of lipids, the presence of antioxidant and pro-oxidant compounds, the amount and type of oxygen, and the nature of the packaging material and storage temperature. The fatty acids of mayonnaise have been studied and yielded fatty acids with a composition of 27.2–39.5% PUFA, 33.2–41.7% MUFA, and 18.1–24.9% SFA (Nazari, Asgary & Sarrafzadegan, 2010). Rashed *et al.* (2017) found that palmitic acid was the dominant fatty acid in mayonnaise, by as much as 10.02 g/100 g. Oleic acid is a MUFA that can cause a bitter taste due to its binding to the hydrophobic component of the peptide.

CONCLUSION

Our research showed that apple peels-enriched mayonnaise had higher viscosity and protein content than control. Apple peel is also rich in polyphenols, mineral, and fibre, making it a potential and valuable stabiliser for reduced-fat mayonnaise production. The use of apple peel will contribute to solutions for environmental pollution. This research work concluded that the addition of apple peel flour improved the physicochemical, sensory attributes, and stability of mayonnaise.

Acknowledgement

We acknowledge the 2021 LK Doctoral Grant "Number: 1001/UN.10.F05/PN/2021" scheme as the source of funding for this research programme.

Authors' contributions

Herly E, created research concepts and designs, collected research data, reviewed and completed the manuscript; Agus S, conducted research, analysed data, created discussion narratives, wrote, translated and reviewed the manuscript.

Conflict of interest

We declare that there is no conflict of interest.

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Mal J Nutr 30(2): 203-215, 2024

Factors related to complementary feeding practices during the COVID-19 pandemic in Indonesia

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ABSTRACT

Introduction: Complementary feeding practice is critical for adequate growth and development in children. Appropriate practices should be maintained, especially during the COVID-19 pandemic. **Methods:** This cross-sectional study aimed to analyse complementary feeding practices and their related factors, including the impact of the COVID-19 pandemic on household economic aspects. Data were collected via online questionnaires involving 574 mothers of children aged 6–23 months in Indonesia from April to May 2022. **Results:** In total, 63.6%, 64.6%, 86.6%, and 57.7% of children met the criteria for timely introduction of complementary feeding (INTRO), minimum dietary diversity (MDD), minimum meal frequency (MMF), and minimum acceptable diet (MAD), respectively. Households that had no impact on their ability to provide food during the pandemic had higher odds of meeting MDD and MAD. Having a high household income level, high maternal education, and being a housewife increased the odds of INTRO. The odds of MDD increased in children who lived in Java, had older age, and whose mother had high education level. Older children had higher odds of having appropriate MMF. MAD was associated with mother's high education level and being a housewife. **Conclusion:** Several factors during the pandemic, including economic aspects, influenced complementary feeding practices. To prevent child malnutrition, besides ensuring household food security, other strategies to increase complementary feeding quality are also needed.

Keywords: complementary feeding, COVID-19, minimum acceptable diet, minimum dietary diversity, minimum meal frequency

INTRODUCTION

In 2020, a global outbreak of the coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), impacted countries including Indonesia (Liu, Kuo & Shih, 2020; Nugraha *et al.*, 2020). To prevent transmission of the disease, the government imposed physical restrictions on essential public places, including schools, workplaces, businesses and services, markets, and healthcare providers (WHO, 2020). Physical restrictions impacted the economic sector, which was depicted by the reduced number of working hours, the emergence of a work-from-home system, and layoffs (Nicola *et*

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doi: https://doi.org/10.31246/mjn-2023-0135

al., 2020). These conditions may lower household economic status and food security. Additionally, several health-related services were halted, which could limit the public's access to health information (Pires *et al.*, 2021). Due to such circumstances, people may be more susceptible to nutrition and health problems.

Children were one of the vulnerable groups affected during the COVID-19 pandemic, especially those in their first 1000 days of life. Currently, many malnutrition-related problems are still occurring in Indonesia, which include stunting, wasting, and underweight. Results of a survey prior to the pandemic in 2019 showed that the prevalence of stunting was 27.7%, which decreased to 24.4% in 2021; wasting decreased from 7.4% to 7.1%. However, the prevalence of underweight increased from 16.3% to 17.0% during the pandemic (National Institute of Health Research & Development, 2019; National Institute of Health Research & Development, 2021).

To ensure healthy growth and development of children aged 6–23 months, complementary feeding is given through a variety of nutritious foods introduced besides breast milk. To guarantee that the nutritional needs of infants and young children are met, complementary foods must be provided timely, adequately, safely, and appropriately (Binns *et al.*, 2020; UNICEF, 2020). To mirror the dietary quantity and quality of infants and young children, the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) have proposed several infant and young child feeding (IYCF) indicators, including the timely introduction of complementary feeding (INTRO), minimum dietary diversity (MDD), minimum meal frequency (MMF), and minimum acceptable diet (MAD) (WHO & UNICEF, 2021). Nevertheless, in Indonesia, complementary feeding requires optimisation. In the latest Indonesia Demographic and Health Survey, the proportion of MDD was reported to be 60%, MMF 72%, and MAD 40% (National Institute of Health Research & Development, 2018).

However, studies on the factors that influence complementary feeding practices in Indonesia during the COVID-19 pandemic are lacking. Therefore, this study aimed to analyse the indicators of complementary feeding practices and their related factors in Indonesia, which will serve as preparation for another possible outbreak in the future.

MATERIALS AND METHODS

This study was an online cross-sectional survey that involved a convenience sample of participants. A validated, self-administered online questionnaire was utilised to collect the data to be used for this study from April to May 2022, during which the COVID-19 pandemic took place. Data collection was carried out during the implementation of the physical restrictions policy by the government to prevent disease transmission, which included limiting access to essential public places, such as healthcare centres, workplaces, and markets. The following inclusion criteria were applied: living in Indonesia, able to complete the study questionnaire in the Indonesian language, have children aged 6–23 months, and could provide a consent form. In this study, 574 mothers of children aged 6–23 months in Indonesia were enrolled. An online questionnaire was distributed to several target populations in Indonesia via social media platforms, including Instagram, WhatsApp, Line, Facebook, and Twitter, and the Indonesian Breastfeeding Mothers' Association. This research was ethically approved by the Institutional Review Board (KE/AA/VI/10832/EC/2022), which complied with the Helsinki Declaration. The participants, before giving their informed consent, received written explanations about the study. Before they participated in the study, all participants electronically signed an online informed consent form. The identities of the participants were anonymised.

Outcome measures

Complementary feeding practices were measured using four indicators that reflected the children's nutrition adequacy based on the child's age, including INTRO, MDD, MMF, and MAD (WHO & UNICEF, 2021). A validated, online structured questionnaire was developed to assess complementary feeding consumption in the past 24 hours. To measure dietary diversity, a list-based approach was employed. The definitions of each indicator based on the WHO recommendation of IYCF are as follows:

– INTRO: Children who start receiving solid, semi-solid, or soft foods at six months of age besides breast milk.
– MDD: Children who had at least five out of eight defined food group consumptions during the previous day. The eight food groups include breast milk; grains, roots, and tubers; pulses, nuts, and seeds; dairy products; eggs; flesh foods (meat, fish, poultry, and organ meats); and vitamin A-rich fruits and vegetables.
– MMF: Children who had solid, semi-solid, or soft foods with a minimum number of times during the previous day. The minimum number of times such as two times feeding of breastfed infants aged 6–8 months, three times feeding of breastfed children aged 9–23 months, and four times feeding of non-breastfed children aged 6–23 months.
– MAD: Children who received at least the MDD and MMF during the previous day.

Explanatory variables

The questionnaire was utilised to collect information related to socio-demographic data and the impact of the COVID-19 pandemic on household economic aspects. Socio-demographic variables considered in this study were child, maternal, paternal, and household characteristics. Child characteristics included age (6–11, 12–17, and 18–23 months) and gender (male and female). Maternal characteristics included age (18–25, 26–35, and 36–40 years), level of education (low if junior high school and below, middle if senior high school, and high if college or above), mother's occupation (housewife/unemployed, government employees, and private employees), type of occupation (housewife/unemployed, work from home, and work from office), and source of complementary feeding information (healthcare providers such as paediatrician, nurse, nutritionist, or midwife; mass media including television, print, radio, or social media; and friends or family members such as neighbours or parents). Paternal characteristics included the father's occupation (unemployed, government employees, and private employees). Household characteristics included place of residence (Java Island and outside Java Island), household income level (household income quintiles: poorest, poorer, middle, richer, and richest), and family size (large, 7–10 people; middle, 4–6 people; and small, 2–3 people). Variables that were related to the impact of the COVID-19 pandemic on household economic aspects included impacts on household income, household ability to provide food, mother's employment status, and father's employment status. All variables of household economic

Table 1. The distribution of complementary feeding practices and characteristics of study participants (n=574)

Variable	*n*	%
Child characteristic		
Age (months)		
6-11	235	40.9
12-17	236	41.1
18-23	103	18.0
Gender		
Female	287	50.0
Male	287	50.0
Maternal characteristic		
Age (years)		
18-25	179	31.2
26-35	351	61.1
36-40	44	7.7
Level of education		
High	12	2.1
Middle	381	62.9
Low	201	35.0
Mother's occupation		
No occupation/ housewife	350	61.0
Government employee	82	14.3
Private employee	142	24.7
Type of occupation		
Housewife/ Unemployed	350	61.0
Work from home	74	12.9
Work from office	150	26.1
Source of complementary feeding information		
Healthcare providers	168	29.3
Mass media	391	68.1
Friends or family members	15	2.6
Paternal characteristic		
Father's occupation		
Unemployed	3	0.6
Government employee	165	28.7
Private employee	406	70.7
Household characteristic		
Place of residence		
Java	403	70.2
Outside Java	171	29.8

Table 1. The distribution of complementary feeding practices and characteristics of study participants (*n*=574) [cont'd]

Variable	*n*	%
Household income level†		
Richest (IDR >5000.000)	99	17.3
Richer (IDR >3500.000-5000.000)	130	22.7
Middle (IDR >3000.000-3500.000)	34	5.9
Poorer (IDR >2000.000-3000.000)	169	29.4
Poorest (IDR ≤2000.000)	142	24.7
Family size		
Small (2-3 people)	45	7.8
Medium (4-6 people)	381	66.4
Large (7-10 people)	148	25.8
Complementary feeding indicators		
INTRO	365	63.6
MDD	371	64.6
MMF	497	86.6
MAD	331	57.7

INTRO: Timely introduction of complementary feeding; MDD: Minimum dietary diversity; MMF: Minimum meal frequency; MAD: Minimum acceptable diet
†1 US dollar = IDR 14,340 IDR (as of April 20th, 2022)

aspects were data obtained from the participants in terms of their economic condition during the pandemic.

Statistical analysis

Using Pearson's chi-square test ($p<0.05$), a bivariate analysis of each complementary feeding indicator and the impact of the COVID-19 pandemic on household economic aspects was conducted. Variables with a 95% confidence interval and a p-value of less than or equal to 0.2 during the bivariate analysis were entered into the multivariate logistic regression analysis in order to determine the relative effect of confounding variables and the interactions of variables. All analyses were carried out using IBM SPSS Statistics for Mac version 26.0 (IBM Corp, Armonk, New York, USA).

RESULTS

The proportions of complementary feeding practices and characteristics of study participants are shown in Table 1. The proportion of infants and young children who received timely introduction of solid, semi-solid, or soft foods was 63.6%. The percentages of infants and young children meeting the MDD, MMF, and MAD were 64.6%, 86.6%, and 57.7%, respectively. Most of the infants and young children were aged below 18 months (82%). More than half of the mothers were aged 26–35 years (61.1%), completed middle education (62.9%), and were housewives (61.0%). Most fathers worked as private employees (70.7%). Complementary feeding information were mostly obtained from mass media (68.1%). Our study participants were mostly from outside Java Island (70.2%) and of medium-sized families (66.4%).

The results of multiple logistic regression of factors related to complementary feeding indicators are presented in Table 2. Timely INTRO was not associated with any child factor. At the maternal level, compared with those with low education, mothers with high education levels were more likely to have children meeting timely introduction of complementary foods (*AOR*=4.0; 95%*CI*:1.0–15.4). Unemployed mothers had a 2.6 greater chance of feeding their children timely than those who worked outside the house (95%*CI*:1.1–6.0). Children from the richest households were more likely to meet timely INTRO than those from other household income groups (*AOR*=1.6; 95%*CI*:1.2–8.9). There was no association between living residency and economic aspects related to the COVID-19 pandemic in terms of timely INTRO.

Children aged 18–23 months had a 2.8 greater chance of meeting MDD than younger children (95%*CI*:1.0–7.2). There was a dose–response relationship between maternal education level and MDD. Mothers with high and middle education levels had a greater chance of feeding their children with diverse foods, with odds of 6.0 (95%*CI*:1.8–8.2) and 5.1 (95%*CI*:1.2–7.9), respectively. Children who lived on Java Island were more likely to eat a more diversified diet than those who lived outside Java Island (*AOR*=2.2; 95%*CI*:1.2–4.1). Households with increased income during the pandemic tended to meet the MDD (*AOR*=1.3; 95%*CI*:1.1–3.9) compared to households with decreased income. Meanwhile, if the households were not impacted in their ability to provide food, the odds of meeting MDD increased 1.3 times (95%*CI*:1.1–3.8).

There were no associations between household, maternal, and paternal characteristics with MMF. MMF was only associated with a child's age. Children aged 12–17 months old tended to have appropriate meal frequency (*AOR*=2.0; 95%*CI*:1.3–2.9). The odds was 2.9 times higher in children aged 18–23 months (95%*CI*:1.7-4.9). Economic aspects related to the COVID-19 pandemic had no impact on MMF.

Mothers with high and middle education levels had a greater chance of meeting the MAD; the odds were 5.9 (95%*CI*:1.5–8.7) and 6.1 (95%*CI*:1.5–7.9), respectively. Mothers who were housewives were more likely to have appropriate MAD than mothers with informal work outside the home (*AOR*=2.1; 95%*CI*:1.0–4.1). Among all aspects related to the economic impacts of the COVID-19 pandemic, only impact on household ability to provide food had a significant association with MAD (*AOR*=1.6, 95%*CI*:1.4–3.9).

Family size, source of complementary feeding information, child's gender, and father's occupation had no significant associations with complementary feeding indicators. Furthermore, the impact of COVID-19 on mother's and father's employment statuses was also not affected by complementary feeding practices.

DISCUSSION

Based on the results, household income during the COVID-19 pandemic and maternal education level impacted INTRO, MDD, and MAD. Previous studies have also shown that household economic level predicts complementary feeding quality (Mitchodigni *et al.*, 2017; Berbari *et al.*, 2021). There was a decrease in support for proper IYCF practices, especially during the lockdown period, such as the ability to access healthcare service facilities to increase knowledge regarding nutrition and child health. In Indonesia, several health services related to growth monitoring and nutrition

Table 2. Multilevel logistic regression: Factors related to complementary feeding practice indicators

Variables	*INTRO*		*MDD*		*MMF*		*MAD*	
	COR (95% CI)	*AOR (95% CI)*	*COR (95% CI)*	*AOR (95% CI)*	*COR (95% CI)*	*AOR (95% CI)*	*COR (95% CI)*	*AOR (95% CI)*
Household characteristic								
Place of residence								
Java	1.6 (1.0-2.4)*	1.3 (0.80-2.3)	3.2 (2.0-5.3)***	2.2 (1.2-4.1)*	1.1 (0.7-1.5)		1.5 (1.0-2.1)*	1.3 (0.9-2.0)
Outside Java	1	1	1	1	1		1	1
Household income level								
Richest	2.0 (1.0-3.9)*	1.6 (1.2-8.9)*	2.6 (1.3-5.2)**	1.9 (0.9-4.0)	0.6 (0.4-1.1)	0.9 (0.6-1.6)	0.9 (0.5-1.4)	
Richer	1.5 (0.6-4.0)	1.1(0.8-3.0)	1.5 (0.8-2.8)	1.2 (0.4-3.5)	1.0 (0.6-1.7)	0.9 (0.6-1.2)	1.0 (0.6-1.6)	
Middle	1.8 (0.9-3.4)	0.3 (1.2-4.6)	1.1 (0.4-2.9)	1.4 (0.7-3.0)	0.8 (0.4-1.7)	0.7 (0.3-1.1)	0.9 (0.4-1.8)	
Poorer	0.9 (0.5-1.7)	0.9 (0.4-2.0)	1.7 (0.8-3.5)	1.5 (0.6-3.9)	0.9 (0.6-1.6)	0.9 (0.7-2.0)	1.3 (0.8-2.1)	
Poorest	1	1	1	1	1	1	1	
Family size								
Small	0.7 (0.6-1.6)		1.1 (0.4-2.7)		1.2 (0.8-1.8)		1.3 (0.9-3.2)	0.7 (0.3-1.3)
Medium	0.9 (0.3-1.4)		0.8 (0.3-2.2)		1.3 (0.7-2.5)		1.5 (0.8-2.8)	0.9 (0.4-1.8)
Large	1		1		1		1	1
Source of complementary feeding information								
Healthcare providers	2.4 (0.8-7.5)	1.8 (0.5-6.2)	3.0 (0.9-10.5)	3.1 (0.7-12.9)	0.6 (0.2-1.8)		1.1 (0.7-2.8)	
Mass media	2.1 (0.7-6.3)	1.8 (0.5-5.9)	2.2 (0.7-7.2)	3.0 (0.7-12.1)	1.1 (0.4-3.4)		1.8 (0.4-3.2)	
Friends or family members	1	1	1	1	1		1	
Child characteristic								
Age (months)								
18-23	0.4 (0.4-1.4)		3.0 (1.2-7.4)*	2.8 (1.0-7.2)*	3.0 (1.7-5.0)***	2.9 (1.7-5.0)***	3.0 (1.8-4.9)***	1.1 (0.4-3.5)
12-17	1.0 (0.6-1.9)		1.1 (0.7-1.8)	2.2 (0.8-5.8)	1.9 (1.3-2.8)**	2.0 (1.3-2.9)**	1.8 (1.2-2.6)**	1.9 (0.6-6.0)
6-11	1		1	1	1	1	1	1
Gender								
Male	1.2 (0.8-1.8)		0.9 (0.6-1.6)		1.0 (0.7-1.4)		1.0 (0.7-1.4)	
Female	1		1		1		1	

Table 2. Multilevel logistic regression: Factors related to complementary feeding practice indicators (cont'd)

Variables	*INTRO*		*MDD*		*MMF*		*MAD*	
	COR (95% CI)	*AOR (95% CI)*	*COR (95% CI)*	*AOR (95% CI)*	*COR (95% CI)*	*AOR (95% CI)*	*COR (95% CI)*	*AOR (95% CI)*
Maternal characteristic								
Age (years)								
36-40	1.5 (0.8-1.8)		0.7 (0.3-1.6)	0.5 (0.2-1.4)	1.3 (0.7-2.7)		1.2 (0.6-2.2)	
26-35	1.4 (0.81-1.9)		1.8 (1.1-3.1)*	2.6 (0.9-6.7)	1.2 (0.8-1.7)		1.1 (0.4-1.6)	
18-25	1		1	1	1		1	
Level of education								
High	0.3 (0.1-1.1)	4.0 (1.0-15.4)*	6.4 (2.2-23.3)**	6.0 (1.8-8.2)**	1.2 (0.9-1.7)		1.2 (0.9-1.8)	5.9 (1.5-8.7)*
Middle	0.9 (0.6-1.5)	1.5 (0.5-5.4)	7.2 (2.2-23.3)***	5.1 (1.2-7.9)*	1.2 (0.4-4.3)		0.3 (0.1-1.1)	6.1 (1.5-7.9)*
Low	1	1	1	1	1		1	1
Occupation								
Unemployed	1.9 (1.2-3.0)**		1.8 (0.8-4.1)	2.2 (0.8-6.4)	1.2 (0.8-1.8)		1.5 (1.0-2.2)*	1.9 (1.8-3.2)*
Private employee	0.9 (0.5-1.9)	0.9 (0.9-3.6)	1.7 (0.9-2.9)	1.8 (0.9-3.7)	0.8 (0.5-1.4)		0.9 (0.5-1.7)	2.0 (1.2-3.4)
Government employee	1	1	1	1	1		1	1
Type of occupation								
Housewife	2.4 (1.5-3.8)***	2.6 (1.1-6.0)*	1.7 (0.9-2.8)	1.9 (0.7-5.0)	1.5 (0.9-2.2)	1.4 (0.8-2.6)	1.9 (1.3-2.8)**	2.1 (1.0-4.1)*
Work from Home	2.5 (1.2-5.1)*	1.0 (0.3-2.9)	1.6 (0.7-3.6)	2.3 (0.1-3.1)	1.5 (0.8-2.7)	1.3 (0.8-1.9)	1.9 (1.1-3.4)*	1.2 (0.8-4.2)
Work from Office	1	1	1	1	1	1	1	1
Paternal characteristic								
Occupation								
Unemployed	3.6 (0.6-22.5)	1.3 (0.1-16.9)	0.7 (0.1-6.2)	1.6 (0.8-3.2)	2.6 (0.4-15.8)		1.9 (0.3-12.2)	
Private employee	2.6 (0.4-15.9)	2.1 (0.1-11.8)	1.4 (0.8-2.5)	3.4 (0.2-4.4)	2.9 (0.5-12.3)		2.1 (0.3-12.6)	
Government employee	1	1	1	1	1		1	

Table 2. Multilevel logistic regression: Factors related to complementary feeding practice indicators (cont'd)

Variables	*INTRO*		*MDD*		*MMF*		*MAD*	
	COR (95% CI)	*AOR (95% CI)*	*COR (95% CI)*	*AOR (95% CI)*	*COR (95% CI)*	*AOR (95% CI)*	*COR (95% CI)*	*AOR (95% CI)*
Impact of COVID-19 pandemic								
Impact on household income								
Increased income	4.0 (1.5-11.1)**	0.6 (0.3-1.2)	2.7 (1.9-7.9)*	1.3 (1.1-3.9)*	1.0 (0.4-2.8)		0.6 (0.2-1.6)	
No change in income	5.9 (2.2-16.1)***	2.1 (0.1-4.4)	4.6 (1.6-13.0)**	2.2 (0.9-5.5)	1.0 (0.7-1.5)		0.8 (0.6-1.2)	
Decreased income	1	1	1	1			1	
Impact on household ability to provide food								
Not impacted	1.3 (0.8-2.1)	1.1 (0.5-2.3)	2.9 (1.8-4.7)***	1.3 (1.1-3.8)**	1.3 (0.9-1.9)	0.9 (0.6-1.4)	2.0 (1.4-2.9)***	1.6 (1.4-3.9)**
Impacted	1	1	1	1	1	1	1	1
Impact on mother's employment status								
Not impacted	0.8 (0.6-1.3)		2.1 (1.2-3.5)**	1.1 (0.4-1.5)	1.1 (0.8-1.5)		1.2 (0.9-1.7)	
Impacted	1		1	1	1		1	
Impact on father's employment status								
Not impacted	1.4 (0.9-2.2)	1.1 (0.6-2.2)	2.5 (1.5-4.2)***	1.6 (0.3-1.7)	1.2 (0.8-1.8)		1.2 (0.8-1.8)	
Impacted	1	1	1	1	1		1	

INTRO: Timely introduction of complementary feeding; MDD: Minimum dietary diversity; MMF: Minimum meal frequency; MAD: Minimum acceptable diet; COR=Crude Odds Ratio; AOR=Adjusted Odds Ratio; CI=Confidence Interval
*statistically significant at $p<0.05$, **significant at $p<0.01$, *** significant at $p<0.001$

counselling for children were also limited; many respondents from low-income households experienced difficulties in accessing healthcare service facilities. Results further show that household economic levels affect healthcare access (Filha *et al.*, 2022). High-income households can use professional expert guides related to the first INTRO; while low-income households tend to receive knowledge only from their parents, friends, or family (Abate, Hassen, & Temesgen, 2023). Our results indicated that the richest households had 1.6 times greater odds of meeting the INTRO indicator.

Maternal education level was associated with most complementary indicators, including INTRO, MDD, and MAD. High maternal education level has a positive impact on health-seeking behaviours (Khasanah *et al.*, 2023; Yugistyowati & Marza, 2018). Those with high levels of education and household income tend to use webinars/expert recommendations, internet/applications, professional experts, and other telehealth services to increase their knowledge (Nurhayati *et al.*, 2023; Thomson *et al.*, 2021). High awareness and understanding of the benefits and quality of complementary feeding practices will protect mothers and children against external interference and pressure from the environment or family concerning food taboos (Andualem *et al.*, 2020). Most respondents in this study obtained information regarding complementary feeding during the pandemic via social media.

The Indonesian government has since optimised its social media platforms for health promotion during the pandemic.

Furthermore, the lockdown period during the COVID-19 pandemic has had a negative impact on the food supply chain (Bustos-Arriagada *et al.*, 2022). Due to the high cost of food caused by this condition, dietary preferences changed, especially in households with middle to lower economic status. People tended to choose foods with low prices per calorie and generally non-perishable foods, including starchy food groups (Laborde *et al.*, 2021). Household income level determines food availability and food insecurity. Food insecurity has led people to consume low diversity diets (Nofitasari *et al.*, 2023; Sidebottom *et al.*, 2022; Zhao *et al.*, 2020).

A child's dietary diversity is also significantly increased by a mother's education. High maternal education levels tend to open up more employment opportunities and higher economic status than low maternal education levels. A higher economic level will increase food purchasing power and the ability to provide a variety of foods for children (Tegegne *et al.*, 2017). Meanwhile, of all complementary feeding indicators, MMF had no significant relationship with household income levels during the COVID-19 pandemic. Previous studies also had the same result (Mitchodigni *et al.*, 2017). Household income was an insignificant predictor of MMF, possibly because meeting MMF is less resource-dependent.

The place of residence differed in relation to MDD compliance. The COVID-19 pandemic reduced the amount of food that was distributed in markets, which had a major impact on rural areas. Poor food access in rural areas reduced food diversity. Food groups that were most limited included dairy products and several types of animal-source foods (Sidebottom *et al.,* 2022). Furthermore, numerous foods were imported from the urban area. During the pandemic, numerous areas in Java (urban areas in Indonesia) had a high incidence of COVID-19 and social restrictions or lockdowns had to be implemented. This condition caused interruptions in the food chain. However, the emergence of food ordering and delivery services or online grocery shopping became a viable solution to maintain a stable food supply and access to adequate food during the COVID-19 pandemic because it can maintain dietary diversity and has the potential to reduce the spread of the disease through physical distancing (Zhao *et al.*, 2020). In Java, many online food delivery services were available, which might have increased dietary diversity of complementary feeding compared to outside Java Island, where not all regions had these services.

Mother's occupation has a relationship with INTRO and MAD. To protect their children from COVID-19, more housewives breastfed their infants more frequently during the pandemic, which allowed for timely INTRO (Holand *et al.*, 2022). Housewives have enough time to practise exclusive breastfeeding for 6 months compared to working mothers, whose time is spent mostly outside (Tušl *et al.*, 2021). Women are a vulnerable group to likely experience high levels of depressive symptoms and anxiety related to breastfeeding during the COVID-19 pandemic, especially working mothers (Lauzon-Guillain *et al.*, 2019). Due to work pressure, working mothers may not have enough time to pay attention to their children. As demonstrated in this study, compared with housewives, lower MAD was found among children with working mothers.

In this study, the age of the child was associated with MMF and MDD. Appropriate complementary feeding practices increase as the child gets older. As children get older, the frequency of eating increases and the types of foods that are introduced to them become more diverse (Tegegne *et al.*, 2017).

Due to physical restrictions during the pandemic, we employed self-administered online questionnaires, which is a limitation of this study. Given the diversity of the Indonesian region, our sample may not have been as representative. The only mothers included in this study were those who had access to the internet and completed the online survey.

CONCLUSION

In conclusion, complementary feeding practice indicators were altered during the pandemic. To prevent child malnutrition, strategies to increase complementary feeding quality are needed. The economic aspect is one of the factors in fulfilling indicators for appropriate complementary feeding practices. During the pandemic, the government must ensure household food security issues, such as a safety net. Furthermore, mothers' knowledge on the importance of appropriate complementary feeding practices must be enhanced.

Acknowledgement

We would like to express our gratitude to the enumerators and AIMI (Asosiasi Ibu Menyusui Indonesia) for their assistance to distribute survey link all around Indonesia.

Authors' contributions

Rahayu HK, principal investigator, conceptualised and designed the study, data analysis and interpretation, prepared the draft of the manuscript, and reviewed the manuscript; Paratmanitya Y, conceptualised and designed the study, led the data collection, and reviewed the manuscript; Herawati HD, led the data collection and reviewed the manuscript; Nurhayati E, advised on data analysis and interpretation, and reviewed the manuscript; Nuryani R, led the data collection.

Conflict of interest

The authors declare no conflicts of interest.

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Mal J Nutr 30(2): 217-229, 2024

Relationship between macro- and micronutrient intakes with undernutrition among toddlers aged 12-23 months in Aceh, Indonesia

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ABSTRACT

Introduction: Foods consumed contain macro- and micronutrients necessary for the growth and development of children. This study aimed to analyse the relationship between macronutrient and micronutrient intakes with undernutrition in toddlers aged 12-23 months. **Methods:** This cross-sectional study was conducted in 18 Integrated Healthcare Centres *(Posyandu)* in Simpang Tiga, Aceh Besar Regency. A total of 138 toddlers were selected using simple random sampling technique. Data collected included family demographics, characteristics, nutritional status of children, and food consumption for macro- and micronutrient intake analyses. **Results**: Prevalence of malnutrition in toddlers was relatively high – severe underweight and underweight totaled 23.2%, severe wasting and wasting 18.1%, and severe stunting and stunting 27.5%. Significant associations (p<0.05) were found between underweight and intakes of energy, protein, carbohydrates, fat, calcium, phosphorus, zinc, thiamin, and niacin. Wasting was significantly associated (p<0.05) with intakes of energy, protein, and vitamin A. Stunting was significantly associated (p<0.05) with intakes of energy, protein, carbohydrates, fat, calcium, phosphorus, iron, calcium, zinc, vitamin A, thiamine, riboflavin, niacin, and vitamin C. **Conclusion**: Certain macro- and micronutrient intakes were correlated with undernutrition in toddlers. The results of this study can be used as a reference for planning the development of supplementary feeding programmes for children aged 12-23 months, considering that the critical period of child growth and development is in the first 1000 days of life.

Keywords: macronutrient intake, micronutrients, toddlers, undernutrition

INTRODUCTION

The World Health Organisation (WHO) defines malnutrition as a condition of cellular imbalance between the supply of energy and nutrients that the body needs to ensure growth, nutrition, and certain functions (deOnis, 2015). Currently, nutritional problems in Indonesia is still one of the serious issues in the field of public health. In 2018, the prevalence of stunting in Indonesia was 30.8%, with underweight and wasting rates at 17.7% and 6.7%, respectively. Stunting is a serious public health problem, while underweight and wasting are considered moderate problems (Balitbangkes, 2018).

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doi: https://doi.org/10.31246/mjn-2023-0118

Aceh is one of the provinces in Indonesia with a high undernutrition rate and has the tenth highest underweight rate at 23.5% (Balitbangkes, 2018). The results of the Indonesian Toddler Nutrition Status Survey in 2021 showed that the prevalence of undernutrition in Aceh Besar Regency was relatively high, reaching 29.0% (MOH RI, 2021). The WHO defines the prevalence of undernutrition (with a *z*-score below -2 SD) at 20-29% as a critical public health problem that must be addressed immediately (WHO, 1995). Indonesia has 8.05% of its total population of 276.4 million aged <5 years. This shows that this generation determines Indonesia's future development.

During the toddler years, brain growth accelerates dramatically, making it a critical phase. The reason is because neural cells in the brain are growing fast, with branching speeds of up to a thousand per second. As a result, children beyond the age of two who experience developmental delays are often challenging to treat or cure (Prado & Dewey, 2014). Undernutrition is a particular concern for toddlers since it can cause short-term physical health problems, affecting growth and nutritional status, as well as long-term psychological health problems, impacting cognitive, mental, and psychomotor developments (Kristjansson, 2016). According to Grantham-McGregor *et al.* (2007), undernourished toddlers are most likely associated with permanent physiological and metabolic changes that result in impairment of the child's physical and intellectual developments in the future.

Macro- and micronutrients required for children's growth and development are found in the foods they consume. Each age has its own nutritional needs (Savarino *et al.,* 2021). Macronutrients, including carbohydrates, proteins, and fats, are the main energy supply for humans. They can be considered as the main components of different tissues and represent the total amount of caloric intake (Carreiro *et al.,* 2016). In addition, micronutrients are components of intake that do not contribute significantly to caloric intake, but can still be considered essential for health and vital functions, even if only required in smaller amounts. Adequate nutrition ensures physiological growth and prevents disease. Both macronutrients and micronutrients are essential for proper and balanced child nutrition. In addition, micronutrients are also essential because the lack of one of these nutrients can have significant consequences, such as disrupting growth, delaying maturity, or causing deficiency diseases such as rickets, scorbutus, and cretinism (Savarino *et al.,* 2021).

Many studies have been conducted regarding malnutrition and nutritional intake in children aged 6-23 months (Lewa *et al.,* 2019; Limardi *et al.,* 2022; Sewenet *et al.,* 2022); however, there are still limited studies that focus specifically on children aged 12-23 months. When children reach the age of 12-23 months, there is a transition in providing complementary feeding (CF) from porridge to family food. As a result, feeding problems and malnutrition are common while children's nutritional needs are increasing (WHO, 2009). Providing optimal and age-appropriate complementary foods will affect children's nutritional intake and determine whether or not they accept healthy meals (WHO, 2009). Therefore, this study aimed to analyse the relationship between macronutrient and micronutrient intakes with undernutrition in children aged 12-23 months.

MATERIALS AND METHODS

This cross-sectional study was conducted in Aceh Besar Regency, Indonesia, from

June to July 2023. Using simple random sampling, a total of 138 mothers and toddlers from 18 *Posyandu* in the working area of Public Health Centre *Simpang Tiga,* Aceh Besar Regency, Indonesia, were chosen to participate in this study. The number of samples was calculated with a 95% confidence interval and 10% absolute precision, with the proportion of malnourished children under five in Aceh at 29% (MOH RI, 2019). Public Health Centres *(Puskesmas)* with high frequency of underweight toddlers were purposively selected. Furthermore, all *Posyandu* that were available in the working area of *Puskesmas Simpang Tiga* were chosen. Toddlers between the ages of 12 and 23 months were the target subjects for this study. To be eligible to participate, they had to be willing to participate and not be ill at the time of the study.

Data were collected through interviews using a structured questionnaire for family characteristics (parents' age, occupation, education, income, and total number of children in the family) and child characteristics (gender, weight, birth weight, immunisation status). Food consumption data were collected using a 2x24-hour food recall questionnaire to find out data on macro- and micronutrient intakes. In order to assess the nutritional status of toddlers, anthropometric measurements were conducted to measure their body weight and body length. Body weight measurements were carried out using digital scales that have been calibrated first. Mother's weight was subtracted from the combined weight of the child and mother to determine the child's weight. Meanwhile, body length measurements were carried out using a multifunctional measuring instrument, with an accuracy of 0.1 cm. The final body weight and body length measurements were identified from the average value of the child's measurements done twice.

The obtained data were initially verified by a number of processes including entry, coding, cleaning, and analysis. The assignment of numbers or codes made data entry into computers quicker. This process is known as coding. Data entry involved inputting survey answers, as well as additional information that have been identified as primary data for each variable. To ensure that the information gathered were accurate, cleaning was done. Each variable was analysed once the data were processed and examined. Data processing and analysis were done using Microsoft Office Excel 2010 software (Microsoft Corporation, Redmond, Washington, USA) and IBM SPSS Statistics for Windows version 21.0 (IBM Corporation, Armonk, New York, USA). The relationship between toddlers' nutritional status with their macro- and micronutrient intakes was examined using the Spearman's Rank Correlation Test at a 95% confidence level.

Before the collection of data, respondents were given information about the study and a consent form indicating their willingness to participate. An informed consent form was given to respondents to complete and sign. This research was approved by the Ethics Commission of the Research and Community Service Institute at Health Polytechnic of Aceh, Ministry of Health, Aceh (No reference: Dp.04.03/12.7/078/2023).

RESULTS

Data from a total of 138 respondents were successfully collected. Data on family characteristics were obtained from mothers or respondents who had toddlers aged 12-23 months. Socioeconomic characteristics data collected included father's and mother's age, education, job, income, family size, and number of children under five.

Table 1 showed that the average age of fathers was 35 years old and mothers was 30 years old. More than half of the fathers and mothers had a high school education. Most of the mothers did not work or were only a housewife (81.9%), while the fathers worked as construction workers, tailors, drivers, and other

Table 1. Sociodemographic and socioeconomic characteristics of families

Family demographic characteristics	*n*	%
Father's age (years)		
≤24	3	2.2
25-28	27	19.7
29-32	28	20.4
≥33	80	57.9
Mother's age (years)		
≤24	19	13.8
25-28	39	28.3
29-32	18	13.0
≥33	61	44.2
Number of children under five (person)		
≤1	122	88.4
>1	16	11.6
Mother's education		
≤Elementary School	10	7.2
Junior High School	15	10.9
Senior High School	78	56.5
University	35	25.4
Father's education		
≤Elementary School	10	7.2
Junior High School	15	10.0
Senior High School	78	56.5
University	35	25.4
Father's occupation		
Entrepreneur	25	19.5
Civil servant	9	6.5
Farmer/Fisherman	27	19.6
Construction labourer	15	10.9
Service/Driver	60	43.5
Mother's occupation		
Not working/Housewife	113	81.9
Working	25	18.1
Family size (person)		
Small (≤4 people)	91	65.9
Medium (5-6 people)	43	31.2
Large (≥7 people)	4	2.9
Family income (IDR/month)†		
≤Regional minimum wage 3,413,000	112	81.1
>Regional minimum wage 3,413,000	26	35.4

†1 US dollar =16,095 IDR (as of May 14, 2023)

Table 2. Characteristics of toddlers

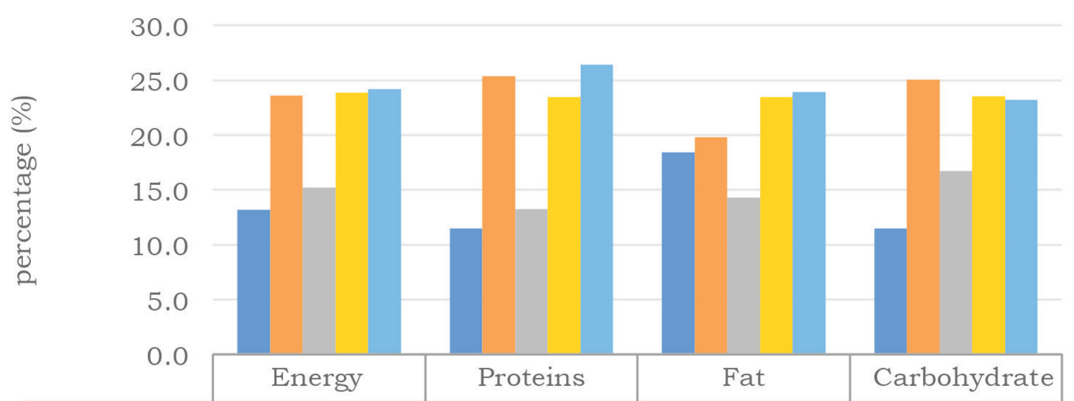
Variables	*n*	%
Age (months)		
12-18	76	55.0
19-23	62	44.9
Gender		
Boy	83	60.1
Girl	55	39.9
Birth weight (g)		
Low birth weight (≤2500)	1	0.7
Normal (>2500)	137	99.3
Immunisation status		
Complete	101	73.2
Not complete	37	26.8
WAZ status		
Severe underweight	2	1.5
Underweight	30	21.7
Normal	106	76.8
HAZ status		
Severe stunting	10	7.2
Stunting	28	20.3
Normal	100	72.5
WHZ status		
Severe wasting	5	3.6
Wasting	20	14.5
Normal	105	76.1
Overweight /obesity	8	5.8

WAZ: Weight-for-age; HAZ: Height-for-age; WHZ: Weight-for-height

related jobs. The average family income was Rp. 2,595,564 or below the standard Regional Minimum Wage. The average total number of family members was four, with an average of one toddler.

The characteristics of toddlers that were collected included age, gender, birth weight, immunisation status, and nutrition status. The subjects in this study were 12-23 months old and more than half of them were 12-18 months old (55.0%). Table 2 showed that a significant proportion of toddlers in the study areas were males (60.1%), had no history of low birth weight (99.3%), and were fully immunised (73.2%). Data on malnutrition in toddlers were measured using the indices of weight-for-age (WAZ), weight-for-height (WHZ), and height-for-age (HAZ). The average values for WAZ, WHZ, and HAZ indicators obtained from the analysis of z-scores were -1.15±1.20, -0.90±1.87, -0.95±1.18. Table 2 showed that the prevalence of malnourished children was relatively high, with severe underweight and underweight amounting to 23.2%, severe wasting and wasting 18.1%, and severe stunting and stunting 27.5%. Overweight or obesity, also a form of malnutrition based on weight-for-length indicator, was 5.8%.

Table 2 showed that children aged 12-23 months had energy intakes below the nutritional adequacy level, with an average energy intake of 836 kcal/day, while the recommended nutritional adequacy level was 1350 kcal/day. The average macronutrients intake, which included protein, reached the recommended level (25 g/day), while fat



	Energy	Proteins	Fat	Carbohydrate
breast milk	13.2	11.5	18.4	11.5
breakfast	23.6	25.4	19.8	25.1
snack	15.2	13.2	14.3	16.7
lunch	23.8	23.5	23.5	23.5
dinner	24.2	26.4	23.9	23.2

Figure 1. Contribution of mealtime to total energy and macronutrient intakes (%)

and carbohydrates (CH) were below the recommended levels (31.7 g/day and 106.4 g/day, respectively). The average micronutrients intake of toddlers did not meet the adequate daily intakes of zinc (1.6 g/day), calcium (387 mg), sodium (298 mg), potassium (733.9 mg), iron (1.6 g), thiamin (0.4 mg), vitamin A (205 RE), and vitamin C (16.1 g).

The nutritional intake of toddlers in this study was determined from the contributions of breast milk and complementary feeding intake, as shown in Figure 1. The intake of complementary feeding was calculated from the contribution of mealtime to energy and nutrients. This study only presented the contribution of mealtime to total energy and macronutrients. The contributions of macronutrient adequacy level from breast milk were 13.2% energy, 11.5% protein, 18.4% fat, and 11.5% carbohydrates, while the contributions of complementary feeding were 86.8% energy, 88.5% protein, 81.6% fat, and 88.5% CH. It can be seen from Figure 1 that the contribution of mealtime to total energy was mostly at dinner time (24.2%), followed by lunch (23.8%), breakfast 23.6%, and snack 15.2%. Meanwhile, the contribution of mealtime to total protein was mainly at dinner time (26.4%), followed by breakfast (25.4%) and lunch (23.5%), and finally, snack (13.2%). The contribution of mealtime to total fat was mainly at dinner (23.9%), followed by lunch (23.5%), breakfast (19.8%) and snack (14.3%). The contribution of mealtime to total carbohydrates was mainly at breakfast (25.1%), followed by lunch 23.5%, dinner 23.2%, and snack 16.7%.

The results of the association analysis (Table 4) showed that macronutrient intakes, such as energy, protein, fat, and carbohydrates, were significantly related to underweight, stunting, and wasting; only fat intake was not significantly related to wasting (p=0.113). Macronutrient intakes

Table 3. Proportion of macro- and micronutrient intakes according to type of undernutrition

Nutrition adequacy level (%RDA)	*Underweight*		*Wasting*		*Stunting*	
	Yes (n=32) n (%)	*No (n=106) n (%)*	*Yes (n=25) n (%)*	*No (n=113) n (%)*	*Yes (n=38) n (%)*	*No (n=100) n (%)*
Energy						
Severe deficit (<80)	32 (100)	93 (87.7)	24 (18.7)	101 (89.4)	37 (97.4)	88 (88.0)
Mild deficit (80-89)	0 (0.0)	8 (7.5)	1 (14.3)	7 (6.2)	1 (2.6)	7 (7.0)
Normal (>89)	0 (0.0)	5 (4.8)	0 (0.0)	5 (4.4)	0 (0.0)	5 (5.0)
Protein						
Severe deficit (<80)	25 (78.1)	22 (20.8)	13 (52.0)	34 (30.1)	20 (52.6)	27 (27.0)
Mild deficit (80-89)	3 (9.4))	17 (16.0)	1 (4.0)	19 (16.8)	5 (13.2)	15 (15.0)
Normal (>89)	4 (12.5)	67 (63.2)	11 (44.0)	60 (53.1)	13 (34.2)	58 (58.0)
Fat						
Severe deficit (<80)	30 (93.8)	63 (59.4)	18 (72.0)	75 (66.4)	32 (84.2)	61 (61.0)
Mild deficit (80-89)	1 (3.1)	8 (7.5)	1 (4.0)	8 (9.0)	1 (2.6)	8 (8.0)
Normal (>89)	1(3.1)	35 (33.0)	6 (24.0)	30 (26.5)	5 (13.2)	31 (31.0)
Carbohydrate						
Severe deficit (<80)	32 (100)	103 (97.2)	25 (100)	110 (97.3)	38 (100)	97 (97.0)
Mild deficit (80-89)	0 (0.0)	1 (0.9)	0 (0.0)	1 (0.9)	0 (0.0)	1 (1.0)
Normal (>89)	0 (0.0)	2 (1.9)	0 (0.0)	2 (1.80	0 (0.0)	2 (2.0)
Calcium						
Deficit (≤77)	30 (93.8)	79 (74.5)	19 (76.0)	90 (79.6)	34 (89.5)	75 (75.0)
Normal (>77)	2 (6.3)	27 (25.5)	6 (24.0)	23 (20.4)	4 (10.5))	25 (25.0)
Sodium						
Deficit (≤77)	32 (100)	100 (94.3)	23 (92.0)	109 (96.5)	37 (97.4)	95 (95.0)
Normal (>77)	0 (0.0)	6 (5.7)	2 (8.0)	4 (3.5)	1 (2.6)	5 (5.0)
Phosphorus						
Deficit (≤77)	16 (50.0)	29 (27.4)	8 (32.0)	37 (32.7)	15 (39.5)	30 (30.0)
Normal (>77)	16 (50.0)	77 (72.6)	17 (68.0)	76 (67.3)	23 (60.5)	70 (70.0)
Zinc						
Deficit (≤77)	11 (34.4)	6 (5.7)	2 (8.0)	15 (13.3)	12 (31.6)	5 (5.0)
Normal (>77)	21 (65.6)	100 (94.3)	23 (92.0)	98 (86.7)	26 (68.4)	95 (95.0)
Iron						
Deficit (≤77)	29 (90.6)	79 (74.5)	20 (80.0)	88 (77.9)	34 (89.5)	74 (74.0)
Normal (>77)	3 (9.4)	27 (25.5)	5 (20.0)	25 (22.1)	4 (10.5)	26 (26.0)
Vitamin A						
Deficit (≤77)	30 (93.8)	88 (83.0)	21 (84.0)	97 (85.8)	34 (89.5)	84 (84.0)
Normal (>77)	2 (6.3)	18 (17.0)	4 (16.0)	16 (14.2)	4 (10.5)	16 (16.0)
Thiamin						
Deficit (≤77)	31 (96.9)	50 (47.2)	17 (68.0)	64 (56.6)	32 (84.2)	49 (49.0)
Normal (>77)	1 (3.1)	56 (52.8)	8 (32.0)	49 (43.4)	6 (15.8)	51 (51.0)
Riboflavin						
Deficit (≤77)	12 (37.5)	19 (17.9)	6 (24.0)	25 (22.1)	13 (34.2)	18 (18.0)
Normal (>77)	20 (62.5)	87 (82.1)	19 (76.0)	80 (77.9)	25 (65.8)	82 (82.0)
Niacin						
Deficit (≤77)	8 (25.0)	8 (7.5)	4 (16.0)	12 (10.6)	7 (18.4)	9 (9.0)
Normal (>77)	24 (84.0)	98 (92.5)	21 (84.0)	101 (89.4)	31 (81.6))	91 (91.0)
Vitamin C						
Deficit (≤77)	27 (84.4)	89 (84.0)	21 (84.0)	95 (84.1)	36 (94.7)	80 (80.0)
Normal (>77)	5 (15.6)	17 (16.0)	4 (16.0)	18 (15.9)	2 (5.3)	20 (20.0)

Table 4. Nutrient intake and its relationship with undernutrition of toddlers

Nutrient intake	*Mean*	*SD*	*%RDA*	*Underweight (p-value)*	*Stunting (p-value)*	*Wasting (p-value)*
Energy (kcal)	836	171	61.9	<0.001**	<0.001**	0.004**
Carbohydrate (g)	106.4	23.0	49.5	<0.001**	<0.001**	0.049*
Protein (g)	25.0	7.7	96.2	<0.001**	<0.001**	0.006**
Fat (g)	31.7	12.7	70.5	<0.001**	0.010**	0.113
Calcium (mg)	387.0	241.2	59.5	0.061	0.001*	0.826
Phosphorus (mg)	474.1	158.8	103.0	0.017*	0.035*	0.114
Iron (mg)	4.3	1.3	62.1	0.011*	0.040*	0.072
Sodium (mg)	298.4	158.8	37.3	0.042*	0.489	0.066
Potassium (mg)	733.9	232.5	28.2	0.002**	<0.001**	0.632
Zinc (mg)	1.6	1.2	54.9	<0.001**	<0.001**	0.158
Vitamin A (RE)	205.6	119.7	51.4	0.517	0.042*	0.047*
Vitamin B1 (mg)	0.4	0.1	74.3	0.006**	0.005**	0.066
Vitamin B2 (mg)	0.6	0.4	134.6	0.104	0.024*	0.847
Vitamin B3 (mg)	7.2	2.8	120.9	0.002**	0.031*	0.086
Vitamin C (mg)	16.6	16.1	40.4	0.109	0.006**	0.796

Spearman's Rank Correlation Test; *p<0.05&**p<0.01

associated with underweight included energy, carbohydrates, protein, and fat, while micronutrient intakes included phosphorus, sodium, calcium, zinc, vitamin B1 or thiamine, and B3 or niacin. In stunting, macronutrients that were significantly related were energy, carbohydrates, protein and fat, while micronutrients included calcium, phosphorus, iron calcium, zinc, vitamin A, vitamin B1 or thiamine, vitamin B2 or riboflavin, vitamin B3 or niacin, and vitamin C. For wasting, macronutrients that were significantly related were energy, carbohydrates, and protein, while fat was not significantly related. The only micronutrient that was significantly associated with wasting was vitamin A.

DISCUSSION

A child who has a body weight that is low for his/her age is called underweight, a height that is too short for age is called stunting, and a body weight that is low for height is called wasting (Sguassero *et al.,* 2012). Height and weight are the most commonly used indicators of nutritional status among toddlers. According to WHO, appropriate HAZ reflects a child's linear growth and can measure long-term growth faltering or stunting, while appropriate WHZ reflects proper body proportion or the harmony of growth. WHZ is particularly sensitive to acute growth disturbances and is useful to detect the presence of wasting. WAZ represents a convenient synthesis of both linear growth and body proportion, thus can be used for the diagnosis of underweight children. Undernutrition is a condition in which a child has a body weight below normal standards; based on WHO standards, a toddler is said to be undernourished if the z-score value of WAZ or WHZ is between -2SD to >-3SD. WHO classifies the problem of moderate malnutrition as underweight (WAZ, Z-3.0 SD to <-2.0 SD), wasting (WHZ, Z-3.0 SD to <-2.0 SD), less upper arm circumference or LUAC (<125 mm), and stunting (HAZ, Z-3.0 SD to <-2.0 SD) (WHO, 2012).

The growth rate in a child's first two years of life is lower than in the first year of life (WHO, 2009). In the first year, a child's weight increases threefold, reaching an average of 9.5 kg and length increases by 25%. By the second year, the child weighs about 12.0 kg in males and 11.5 kg in females, with an average increase of 25% in the second year. Length increases by an average of 15% in both boys and girls. From ages 12 to 24 months, children are called toddlers because there are changes in body composition, reduction of subcutaneous tissue, increases in weight and length, maturation of all organs, and development of new neurocognitive and motor functions.

It is advised that children consume a balanced diet that contains a variety of foods from each of the food groups, including flour and cereals, vegetables and fruits, dairy products, meat and alternative meats, as well as fats and sugars (Schönfeldt & Hall, 2012). The diet of toddlers is outlined in the guidelines for child feeding practices (WHO, 2009), with a recommended frequency of three main meals, 1-2 snacks, and breastfeeding as desired by the child. According to the Ministry of Health Republic of Indonesia (MOH RI, 2023), the percentage of toddlers' needs from breast milk is 30%, while from complementary feeding is above 70%. Therefore, providing a balanced intake of breast milk, meals (breakfast, lunch and dinner), and snacks will determine the adequacy of macro- and micronutrient intakes in toddlers.

The energy intake of toddlers in the study was close to the results of the South East Asian Nutrition Survey (SEANUTS) study in Indonesia, showing that more than half of the children had nutritional intake below the recommended dietary allowance (RDA), with an average energy intake of only 840 kcal/day in children aged 6-23 months (Sandjaja *et al.,* 2013). A study conducted in Aceh on toddlers aged 12-23 months had an average energy intake of 584 kcal per day (Suryana *et al.,* 2016). Toddlers need high energy and nutrient intakes because intakes of these nutrients from breast milk begins to decrease as they enter the age of 12-23 months; breast milk is only able to fulfil energy needs ≤50%, so more intake is needed from complementary feeding (WHO, 2009). Infants and toddlers need energy from food for activities, growth, and normal development (EFSA, 2017).

Inadequate energy intake leads to various physiological adaptations, including growth restriction, loss of fat, muscle, and visceral mass, decreased basal metabolic rate, and reduced total energy expenditure (Dipasquale *et al.,* 2020). Pathophysiological mechanisms in acute malnutrition involve metabolic, hormonal, and glucoregulatory mechanisms. In the initial phase, there is rapid gluconeogenesis, with consequent loss of skeletal muscle due to amino acid, pyruvate, and lactate utilisation. There is then a phase of protein conservation, with fat mobilisation causing lipolysis and ketogenesis, including major changes in electrolyte content, such as sodium retention and intracellular potassium depletion (Grover & Ee, 2009).

The results of this study implied that the intake of macronutrients and micronutrients is important to overcome the problem of undernutrition in children. A child with macronutrient deficiency is more likely to become stunted (Fikawati *et al.* 2021). Inadequate consumption of protein and energy can cause children to experience micronutrient deficiencies (Pem, 2015). Intakes of energy, protein, fat, carbohydrates, calcium, phosphorus, iron, potassium, zinc, vitamin A, thiamine, riboflavin, niacin, and vitamin C are factors associated with stunting. Low energy and protein intake is a risk factor for stunting in children, with a 4-6 times risk of being stunted compared to children with normal or adequate intake (Nguyen *et al.,* 2020).

The carbohydrate adequacy for children aged 1-3 years, as determined in the Indonesian RDA, is 215 g/day (MOH, 2019). Another recommendation for carbohydrate intake in children aged >1 year is suggested to be around 130 g/day, which is estimated to be sufficient to reach the glucose needs of a child's brain, according to the European Food Safety Authority (EFSA, 2017). An intake of 50 to 100 gram per day will prevent ketosis (EFSA, 2017). Study results from Nguyen *et al.* (2020) showed that carbohydrate intake or its subtypes in early childhood was not associated with body composition, but higher carbohydrate intake in infancy, especially monosaccharides and disaccharides, were associated with higher triglyceride levels and lower high-density lipoprotein (HDL). However, a previous study by Sawe & Keino (2022) found that underweight, wasted, and stuntied children had lower levels of carbohydrate consumption.

Protein-energy malnutrition can damage several human tissues, such as the brain, immune system, and intestinal mucosa (Grover & Ee, 2009). Protein deficiency in infants and toddlers not only causes growth impairment and increases the risk of infectious diseases, but also contributes to other nutrient deficiencies (including vitamin A and iron) and deteriorates their metabolic profiles (Wu, 2016). On the contrary, excessive protein intake (>15%E) appears to be associated with the risk of overweight or obesity in adulthood, which may also increase the risk of developing chronic diseases (Lagstro *et al.,* 2013). A protein intake of 15% is recommended as an upper limit in children aged 12 months, as there is no risk of protein intake being too low at this level, but there may be an increased risk of being overweight in the future with higher intakes.

Fats are an essential source of solid energy and facilitate the absorption of fat-soluble food components such as vitamins (EFSA, 2017). Fats and oils are also important sources of essential fatty acids. It is vital to consider the quality of fat rather than the amount of fat in the consumption of fat among toddlers. In early childhood, the quality of fat consumed is more important than the quantity (Savarino *et al.*, 2021). Fat intake is very important in the first two years of age to support brain growth and development (Savarino *et al.*, 2021). The high fat intake of infants during the breastfeeding period can be reduced progressively from the beginning of complementary feeding until the age of three years. Total fat intake for infants aged 6-12 months is 40%, while at 2-3 years of age is 35-40%. Fat intake below 25% is associated with low vitamin levels in some toddlers (EFSA, 2017). The consumption of relatively excessive fat in toddlers does not promote overweight and obesity, as measured by the adiposity index at a later age.

Micronutrients are an important part of the intake of infants and toddlers. The necessity of most micronutrients increases with increasing age of the child. A stable supply of vitamins and minerals from the diet is necessary for optimal growth and function. Micronutrients are dietary components that do not contribute significantly to caloric intake, but are essential for health and vital functions. Micronutrients are required in smaller amounts, but deficiencies can have serious consequences (Carreiro *et al.,* 2016).

Nutrition has a crucial impact on a child's growth, especially in infants and children. Optimal growth requires a proper diet so that macronutrient and micronutrient intakes are well fulfilled. Macronutrients are compounds that humans consume in large amounts, including carbohydrates, proteins and fats; while micronutrients are needed in small amounts and are essential for children's growth, especially zinc, iron, and vitamin B (Savarino *et al.,* 2021). The intake of microminerals, such as vitamins and minerals, in underweight, wasted, and stunted children generally comes from vegetables and fruits. However, a previous study has shown that underweight, wasted, and stunted children have lower intake of vegetables and fruits (Khan *et al.,* 2022). A study conducted in Zambia found that micronutrient deficiencies in undernourished children were associated with intake of B vitamins, which are biotin, vitamin B12, folate, niacin, pantothenic acid, vitamin B6, riboflavin, and thiamin (Titcomb *et al.,* 2018). In addition, low iron consumption was found in underweight children (Sawe & Keino, 2022). Adequate iron intake with zinc can affect nutritional status and significantly changes a child's height (Alfonso Mayén *et al.,* 2022). Zinc and vitamin A intakes are correlated with children's nutritional status, such as underweight and stunting.

This research has limitations in collecting data on nutritional intake of toddlers. The collection of food consumption data in this study relied on the food recall method. It is known that this method is limited by the mother's memory to provide information related to her toddler's food consumption. Thus, data collection for this research used tools from food photo books (Ministry of Health) and trained enumerators to minimise data bias.

CONCLUSION

This study showed the relationships between undernutrition among toddlers and intakes of energy, macronutrients, and micronutrients. The high prevalence of malnutrition and low intake of macronutrients (except protein) and micronutrients in children aged 12-23 months indicated that there is a need for attention from parents and the government to improve the food consumption of toddlers to increase balance and diversity in terms of both quality and quantity. The results of this study can be used as a reference for planning the development of supplementary food programmes for children aged 12-23 months as the critical period of child growth and development is in the first 1000 days of life.

Acknowledgement

We are grateful to the Ministry of Education, Culture, Research and Technology research grant funders. We thank the 138 respondents who participated in the study, namely mothers of children aged 12-23 months, the Head of the Aceh Besar District Health Office, the enumerators and supervisors (*Puskesmas* nutrition staff), cadres in 18 *Posyandu* in *Puskesmas Simpang Tiga* working area, Aceh Besar District for all the support and assistance in collecting the research data presented in this paper.

Authors' contributions

Marliyati SA, writing and original draft preparation, conceptualised and reviewed the manuscript; Suryana, writing and original draft preparation, data analysis and interpretation, reviewed the manuscript; Khomsan A, data analysis and interpretation, review and editing, supervision; Dwiriani CM, methodology, review and editing, supervision. All authors discussed the results and contributed to the final manuscript.

Conflict of interest

The authors state that there are no potential conflicts of interest associated with the research, authorship, or publication of this article.

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Mal J Nutr 30(2): 231-244, 2024

Implementation of nutrition care process in Indonesian athletes and its effect on nutritional status and aerobic capacity performance

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ABSTRACT

Introduction: Despite the critical function of a nutritionist, only a few sports training centres for students in Indonesia have one. This study aimed to determine the effect of the nutrition care process (NCP) on athletes' nutritional status and aerobic capacity performance. **Methods:** This cohort study was conducted in 2022 (May–August) in four training centres (TC) in Indonesia. Subjects were athletes who have been dwelling in TC for at least three months, excluding those absent for >14 days due to a competition or other commitments during the data collection period. NCP included the assistance of trained sports nutritionists. In total, 114 athletes participated in this study: 90 strength athletes and 24 endurance athletes. The participants were aged 14-19 years old with approximately *(mean±SD)* 6±2.5 years of experience in specific sports. **Results:** After three months of NCP, knowledge of nutrition (p=0.013), body fat composition (p<0.001), skinfold thickness scores (p<0.001), and performance (p<0.001) of athletes significantly improved. In spite of good intakes of protein and fat, none of the intakes showed significant changes (p>0.05). Furthermore, improved knowledge of sports nutrition and exercise science had a positive impact on dormitory meal choices. **Conclusion:** Overall, three months of NCP had a significant effect on athletes' knowledge of nutrition, body fat composition, skinfold thickness, and also VO_2Max.

Keywords: athlete, nutrition care process, sports

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doi: https://doi.org/10.31246/mjn-2023-0096

INTRODUCTION

The nutritional needs of young athletes exceed those of non-athletes. Adequate nutrition is important to meet their daily training demands and for the continuation of other daily activities such as attending school. Generally, the nutrition problems of young athletes are diverse. These include the female athlete triad, iron deficiency anaemia, underweight, obesity, and gastrointestinal disorders (Cabral *et al.*, 2022). Nutritional diagnosis is established according to individual problems, which can be identified based on the results of nutritional assessment. Therefore, sports nutritionists aim to design ideal individual nutrition plans based on the nutrition care process (NCP), of which determining the nutrition problems among young athletes is key to the process.

Nutrition plans should be specific, especially in terms of the type and timing of meals and snacks based on training and competition. Thus, maintaining regular meal patterns preserves lean body mass and replenishes glycogen. Nascimento *et al.* (2016) showed that providing four individual nutrition counselling sessions over a period of eight months improved daily water intake and meal frequency (three hours apart), which could prevent gastrointestinal distress (Nascimento *et al.*, 2016). It also significantly improved the athletes' mid-arm muscle circumference and their body mass before and after the intervention. Another study showed that nutrition intervention could be an effective strategy to improve athletes' behaviours, eating habits, nutrition knowledge, and body composition (Fahrenholtz *et al.*, 2023). A case control study reported that six weeks of nutrition intervention in athletes positively altered their overall body composition, including body fat by 1% and the ratio of mass to fat by 0.3 (Rosimus, 2018).

Daily nutrient intake has a strong influence on athletes' performance and many studies have explored the effects of nutrition interventions. A recent study by Rossi *et al.* (2017) showed an improvement in athletes' performance on the 5-10-5 shuttle run, accompanied by a reduction in fat mass during the 12-week nutrition intervention (Rossi *et al.*, 2017). Evidence shows that good knowledge of nutrition is associated with better diet quality (Fahrenholtz *et al.*, 2023). In this case, the role of a nutritionist as an educator is important, ensuring that athletes receive trusted information. A systematic review on nutrition education in athletes found that 17 out of 22 papers included a nutritionist or dietitian in their research (Boidin *et al.*, 2021). This means that nutritionists and dietitians have an important role as educators for athletes.

Although the importance of nutrition interventions for young athletes has been well established, the role of sports nutritionists in sports schools and training centres in Indonesia is limited. Some studies in Indonesia found that the absence of nutritionists affected athletes' nutrition knowledge, resulting in unhealthy dietary habits (Dewinta *et al.*, 2022). Additionally, even if the athletes already have good nutrition knowledge, they might not implement it into their dietary habits. Athletes' adherence to nutrition advice was found to be seasonal due to the emotional obstacles of a high-performance environment, limiting their opportunity to develop their meal plans (Bentley *et al.*, 2021). We suggest that the implementation of NCP in athletes will improve education about nutrition, body composition, and performance. Thus, this study aimed to evolve an evidence-based approach to the impact of a three-month nutrition care process (NCP) on the body composition, nutrition knowledge, and performance of young athletes in Indonesia.

MATERIALS AND METHODS

Ethics and design

This was a prospective cohort study among Indonesian youth athletes in four regions of Indonesia (Capital City Jakarta, West Java, West Nusa Tenggara, and Special Region Yogya). Data collection took place in May–August 2022. Ethical clearance was obtained from the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Indonesia (KE-FK-0512-EC-2022).

Participants

The total number of athletes is described in Figure 1. The inclusion criteria were athletes between 14 and 19 years old who had been living in the training centres in those four regions for at least three months prior to the initial data collection. The exclusion criteria were injury or sickness that disallowed active training, or athletes who had a competition at another location, requiring them to leave the training centre for >14 days. At the beginning of the study, 274 athletes were enrolled. Some subjects failed to complete the study because they were required to take time off to compete at another location. Additionally, some of the participants graduated from school and left the dorm, while other athletes were unable to follow the full programme due to ill health. At the end, the total number of subjects involved in this research was 114 athletes.

Intervention

The intervention employed in the present study consisted of placement of nutritionists or dietitians at each training centre for three months. During this period, nutritionists and dietitians were responsible for conducting NCP for athletes, which was established in 2021 (Penggalih *et al.*, 2021). The athletes were given intervention in the form of a nutritional guideline programme. The assessments carried out in this study included body fat, body muscle, skinfold, and daily nutrient intake.

The interventions provided included meal assistance, training assistance, and nutrition-related education for athletes. Nutrition education was carried out in large and small groups. Large-group education provided information related to nutrition for different types of sports and hydration. Small-group education provided information regarding portion sizes. The tools used for large-group education were modules that contained nutrition information, while a nutrition profile containing dietary recommendations for each athlete was used for small-group education. NCP was carried out by a sports nutritionist. The number of sports nutritionists in each region was dependent on the number of athletes, with a ratio of 1 sports nutritionist per 30 athletes.

Measurements

In the present study, changes in athletes' performance were based on data on maximal oxygen uptakes (VO_2 max-ml/kg-min). VO_2 max was measured using the Balke and Bleep test. To define, the Balke test was running for 15 minutes, then the test results were adjusted to existing norms (Herdiles, Cholil & Komarudin, 2017). Meanwhile, the Bleep test, which is also known as a shuttle trial or Yo-Yo, was running back and forth from one point to another, which were placed 20 meters apart on a flat surface. This running test used the software sound "beep" as a sign that athletes start running and touch another point, at least one foot. This test

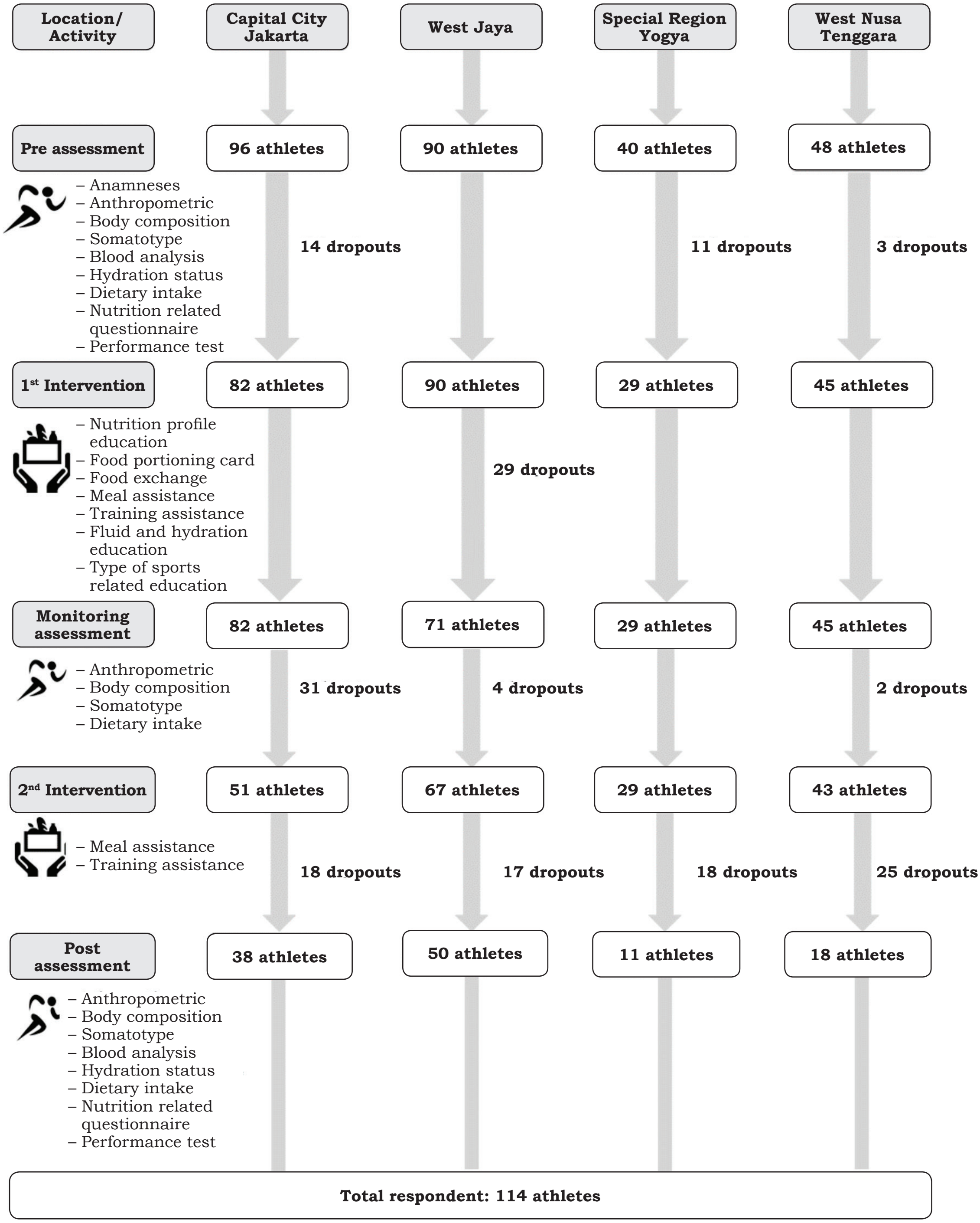

Figure 1. Data collection flow

consisted of 21 levels in which the "beep" tone became sooner. The score was decided by the accomplished level and the number of shuttles (total running) that were reached before they failed to adjust to the "beep" sound (Buttar, Saboo & Kacker, 2019). Aerobic capacity measurement used two methods for verification; this was essential because several athletes lived scattered across Western, Central, and Eastern Indonesia. Hence, to get valid data, the Bleep and Balke test methods were used. The tests were conducted at the beginning and at the end of the study by coaches.

The nutritional status of athletes included body fat (%), body muscle (%), skinfold (mm), fulfilment of nutrient intake compared with nutrient adequacy (%), and score of nutrition education. Body fat and body muscle were measured via bioelectrical impedance analysis (BIA) using OMRON Karada Scan HBF-375 (Omron, China) with 0.01 kg precision. Skinfold was measured using a Harpenden skinfold calliper (0.1 mm precision). BIA and skinfold measurements were taken in the morning after the athletes woke up (around 6.30 am). In this study, fat-free mass was measured with BIA and skinfold. Using both measurements provided a more accurate and comprehensive look at fat distribution. Measurement with BIA was used to see the distribution of total fat, while skinfold was used to describe the distribution of fat on specific parts of the body, such as the triceps, subscapular, suprailiac, and calf (Penggalih *et al.*, 2021).

Nutrient intake was measured by two methods - visual food Comstock and SQ-FFQ (semi-quantitative food frequency questionnaire). The former was used to quantify the percentage of dorm food intake obtained by comparing the ingested food to the individual portion recommendation. Individual nutritional needs were calculated based on the Recommended Dietary Allowance (RDA) from the Ministry of Health Indonesia 2019. The latter was to measure the intake of non-dorm foods (Penggalih *et al.*, 2021). All activities were collected directly by the sports nutritionists in each dormitory. Nutrition education was evaluated with questionnaires both prior to and after the intervention programme. Meal assistance was provided to ensure that athletes were eating the correct portions and small group discussions were used to convey the information. Meal assistance was carried out once per month for three main meals and two snacks. Nutritionists matched the meal recommendations with the portions that athletes took at the eatery. If their portion sizes were inappropriate, athletes were encouraged to adjust the portion of food according to their needs.

Training assistance was carried out to evaluate patterns of fluid consumption during exercise. Before and after training, athletes measured their body weight to monitor changes. The athletes were also questioned about the amount of fluid consumed during exercise.

Protocol

The study began with recruiting sports nutritionists, which consisted of several processes: administrative, cognitive, interview, training, and skills assessment. Eligible nutritionists had academic qualifications equivalent to Diploma-IV or Strata-I from tertiary institutions with a minimum accreditation of B. Cognitive selection was made via questions related to sports nutrition. Interviews were conducted to determine the communication style and commitment of prospective nutritionists. The trainings aimed to provide the same standards for all prospective nutritionists regarding the management of NCP that will be carried out on athletes, which

Table 1. Characteristics of respondents

Athlete's characteristic	*Number of athletes (%)*		
	Strength category	*Endurance category*	*Total*
Age (years)			
14	7 (7.7)	-	7 (6.1)
15	8 (8.8)	4 (16.7)	12 (10.5)
16	29 (32.2)	5 (20.8)	34 (29.8)
17	26 (28.8)	11 (45.8)	37 (32.5)
18	18 (20.0)	3 (12.5)	21 (18.4)
19	2 (2.5)	1 (4.2)	3 (2.7)
Total	90 (100)	24 (100)	114 (100)
Gender			
Boy	42 (46.7)	13 (54.1)	55 (48.2)
Girl	48 (53.3)	11 (45.9)	59 (51.8)
Total	90 (100)	24 (100)	114 (100)
Duration of playing sport (years) (*Mean*±*SD* = 6.0±2.5 years)			
<3	5 (5.6)	1 (4.2)	6 (5.3)
<5	23 (25.6)	2 (8.4)	25 (21.9)
<10	53 (58.9)	17 (70.7)	69 (60.5)
>10	10 (9.9)	4 (16.7)	14 (12.3)
Total	90 (100)	24 (100)	114 (100)
Smoker			
Yes	3 (3.3)	-	3 (2.7)
No	87 (96.7)	24 (100)	111 (97.3)
Total	90 (100)	24 (100)	114 (100)
Injury history			
Yes	45 (50)	22 (91.7)	67 (58.7)
No	45 (50)	2 (8.3)	47 (41.3)
Total	90 (100)	24 (100)	114 (100)

ended with a skills assessment to ascertain the skill levels of nutritionists.

Shortly after the placement of sport nutritionists, baseline measurements were taken at each site for performance and nutritional status. Following baseline measurements, the intervention began with the sport nutritionists designing a NCP for each athlete that lasted for three months. During the intervention, anthropometry, somatotype, body composition, and dietary intake were measured once. Finally, post-intervention measurements were taken for all variables that were measured at the beginning of the study.

Statistics

Statistical analysis was performed using IBM SPSS Statistics for Windows version 23.0 (IBM Corp, Armonk, New York, USA). Prior to data analysis, a normality test was conducted for each dataset using the Shapiro–Wilk test. A mixed model analysis of variance (ANOVA) was used to compare the mean differences between groups (within and between subjects). Analysis of "Time" was measured to compare before and after intervention (pre – post). Analysis of "Group" was measured to compare between strength and endurance sports categories. "Time X Group" analysis was used to measure the interaction for each variable.

RESULTS

Characteristics of respondents

This was a cohort study of 114 Indonesian youth athletes aged 14 to 19 years old, of whom 90 were strength athletes and 24 were endurance athletes. Subjects were followed for two months of intervention based on a NCP for athletes. Informed consent was obtained from all respondents prior to the study. Majority of the subjects were 16-17 years old, with an even split between girls and boys. The sports type was divided into strength and endurance. The strength categories were archery, taekwondo, martial arts, weightlifting, gymnastics, athletic sprinting, and karate. The endurance categories were swimming, football, badminton, climbing, and athletics on medium distance. The strength category contained more girls at 53.3%, while the endurance category involved more boys at 54.1%. The average time for which athletes had been involved in their chosen sport was 6+2.5 years. More than 97% of the athletes do not smoke. Both strength and endurance athletes suffered from injuries (58.7%). All these characteristics can be seen in Table 1.

Nutrition care process for athletes

All of the athletes in each location were included in the study. Figure 1 shows the data collection flowchart for respondents. Initially, the pre-assessment stage was carried out one month prior to the intervention to conduct initial measurements, prepare nutrition profiles, and practise food portioning among athletes. Monitoring was conducted monthly; the earliest session took place one month after the intervention and comprised education on general nutrition science, nutritional status, food recommendations or food portioning, meal assistance, and training assistance.

Descriptive data on nutrient intake, body composition, and performance

Following the NCP for two months, it was expected that athletes could adequately fulfil their energy and nutritional needs. As shown in Table 2, the trend of overeating reduced considerably from 11.5% to merely 2.6%. Meanwhile, adequate intake of energy recorded a slight increase of 2%.

Regarding protein intake, more athletes (33.3%) consumed adequate protein after the NCP, followed by a reduction in the number of athletes with excessive or deficient consumption. Even slightly, there was a 29.8% increase in adequate consumption of fats and a 5% decline in subjects with excessive intake. The majority of athletes had been lacking in carbohydrate intake, which reached more than 70% after the NCP.

Difference on nutrition education during programme

Table 3 shows the results from the mixed model ANOVA analysis, which was matched by the researchers. The indicators used for matching were body mass index (BMI)-for-age and total physical activity of athletes. The results of nutrition education in Table 3 showed significant differences before and after intervention (p=0.023), but no difference between strength and endurance groups (p=0.734). Nutrition education consisted of counselling for each athlete and group class. The nutrition knowledge consisted of queries about general nutrition and sports nutrition science.

Differences on fulfilment of nutrient intake during programme

As shown in Table 3, we compared the nutrient fulfilment data to RDA according to athlete's age and gender. Statistically, none of the intakes showed significant changes (calorie intake p=0.509; protein intake p=0.261; fat intake p=0.301; carbohydrates intake p=0.776) before and after intervention. There were also no significant changes for calorie, protein, fat, and carbohydrates (p=0.796; p=0.983; p=0.742; p=0.983, respectively) between groups.

Table 2. Pre- and post-test of nutrient intake, body composition, and performance

Variable	*Strength, n (%)*		*Endurance, n (%)*		*All respondents, n (%)*	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*
Energy						
Deficient	54 (60)	61 (67.8)	14 (58.3)	15 (62.5)	68 (59.6)	76 (66.7)
Adequate	26 (28.8)	27 (30.0)	7 (29.2)	8 (33.3)	33 (28.9)	35 (30.7)
Over	10 (11.2)	2 (2.2)	3 (12.5)	1 (4.2)	13 (11.5)	3 (2.6)
Protein						
Deficient	62 (68.9)	56 (62.2)	14 (58.3)	12 (50.0)	76 (66.7)	68 (59.6)
Adequate	20 (22.2)	27 (30.0)	8 (33.3)	11 (45.8)	28 (24.6)	38 (33.3)
Over	8 (8.9)	7 (7.8)	2 (8.4)	1 (4.2)	10 (8.7)	8 (7.1)
Fat						
Deficient	33 (36.7)	34 (37.7)	8 (33.3)	8 (33.3)	41 (35.9)	42 (36.8)
Adequate	23 (25.6)	32 (35.6)	6 (30.0)	2 (8.4)	29 (25.4)	34 (29.8)
Over	34 (37.7)	24 (26.7)	10 (36.7)	14 (58.3)	44 (38.7)	38 (33.4)
Carbohydrates						
Deficient	64 (71.1)	65 (72.2)	16 (61.6)	20 (83.2)	80 (70.3)	85 (74.6)
Adequate	18 (20.0)	23 (25.6)	6 (30.0)	4 (16.8)	24 (21.0)	27 (23.7)
Over	8 (8.9)	2 (2.2)	2 (8.4)	-	10 (8.7)	2 (1.7)
% Body fat†						
Low	22 (24.4)	19 (21.1)	2 (8.3)	3 (12.5)	24 (21.1)	22 (9.3)
Normal	63 (70.0)	67 (74.4)	18 (75.0)	20 (83.3)	81 (71.1)	87 (76.3)
Over	5 (5.6)	4 (4.4)	4 (16.7)	1 (4.2)	9 (7.9)	5 (4.4)
% Muscle‡						
Low	5 (5.6)	3 (3.3)	3 (12.5)	-	8 (7.0)	3 (2.6)
Normal	54 (60.0)	60 (66.7)	18 (75)	22 (91.7)	72 (63.2)	82 (71.9)
High	31 (34.4)	27 (30.0)	3 (12.5)	2 (8.3)	34 (29.8)	29 (25.4)
Performance (VO_2max)§						
Poor	-	-	-	-	-	-
Below average	5 (5.6)	1 (1.1)	-	-	5(4.4)	1 (0.9)
Average	26 (28.9)	19 (21.1)	2 (8.3)	3 (12.5)	28(24.6)	22 (19.3)
Good	55 (61.1)	65 (72.2)	16 (66.7)	9 (37.5)	71(62.3)	74 (64.9)
Excellent	4 (4.4)	5 (5.6)	6 (25.0)	12 (50.0)	10(8.8)	17 (14.9)

†Percentage of body fat was categorised in consideration of sex [boys 18-38 years old: low (<10), normal (10-20), high (20-25), very high (≥25); girls 18-38 years old: low (<20), normal (20-30), high (30-35), very high (≥35)] (Penggalih *et al.*, 2021)

‡Percentage of muscle was categorised in consideration of sex [boys 18-38 years old: low (33.3), normal (33.3-39.3), high (39.4-44.0), very high (≥44.1); girls 18-38 years old: low (<24.3), normal (24.3-30.3), high (30.4-35.3), very high (≥35.4)] (Penggalih *et al.*, 2021)

§Maximal aerobic capacity was categorised in consideration of sex [boys <29 years old: poor (<24), below average (25-33), average (34-42), good (43-52), excellent (≥53); girls <29 years old: poor (<23), below average (24-30), average (31-37), good 38-48(), excellent (≥49)] (Penggalih *et al.*, 2021)

Differences in skinfolds and body composition during programme

From the information supplied in Table 3, there was a significant decrease in all parameters of skinfold and body fat percentage in athletes (body fat p=0.004; triceps skinfold p=0.003; subscapular skinfold p=0.016; suprailiac skinfold p<0.001; calf skinfold p<0.001) before and after intervention. There was a significant difference between the endurance and strength groups for triceps and calf skinfolds (p=0.032; p=0.049, respectively).

Table 3. Associations between type of sports with performance and nutritional status

Variable	*Group*	*Pre*	*Post*	*p-value*	
		Mean±SD	*Mean±SD*		
Performance (VO_2max)	Strength	41.5±6.5	42.7±6.3	Time	0.012*
	Endurance	41.2±7.7	43.7±5.7	Group	0.867
	Time	41.5±6.8	42.9±6.2	Time x group	0.344
Score of nutrition knowledge	Strength	9.3±4.9	10.1±4.0	Time	0.023*
	Endurance	8.4±4.7	11.7±6.4	Group	0.734
	Time	9.1±4.9	10.5±4.7	Time x group	0.146
Body fat composition (%)	Strength	19.3±6.5	19.3±6.5	Time	0.004*
	Endurance	20.5±5.4	18.7±5.2	Group	0.860
	Time	19.6±6.2	19.2±6.1	Time x group	0.002*
Triceps skinfold (mm)	Strength	12.5±4.4	10.5±4.2	Time	0.003*
	Endurance	9.8±3.3	8.5±3.5	Group	0.032*
	Time	11.9±4.2	10.0±4.1	Time x group	0.503
Subscapular skinfold (mm)	Strength	11.9±3.6	10.9±3.7	Time	0.016*
	Endurance	10.9±2.5	9.8±2.5	Group	0.264
	Time	11.7±3.3	10.6±3.5	Time x group	0.999
Suprailiac skinfold (mm)	Strength	14.2±6.3	11.9±5.3	Time	0.000*
	Endurance	12.6±6.2	9.4±4.3	Group	0.206
	Time	13.8±6.3	11.3±5.1	Time x group	0.463
Calf skinfold (mm)	Strength	9.9±3.7	8.6±4.3	Time	0.001*
	Endurance	7.8±3.5	6.4±3.7	Group	0.049*
	Time	9.5±3.8	8.1±4.2	Time x group	0.911
Calorie intake to RDA (%)	Strength	76.2±26.2	73.1±24.3	Time	0.509
	Endurance	77.8±30.8	74.3±22.1	Group	0.796
	Time	76.5±27.1	73.4±23.6	Time x group	0.955
Protein intake to RDA (%)	Strength	69.1±27.9	74.5±29.3	Time	0.261
	Endurance	68.4±23.0	75.5±28.2	Group	0.983
	Time	68.9±26.6	74.8±28.8	Time x group	0.880
Fat intake to RDA (%)	Strength	99.1±48.1	112.9±26.2	Time	0.301
	Endurance	105.6±62.6	115.5±57.9	Group	0.742
	Time	100.7±51.5	113.5±66.9	Time x group	0.863
Carbohydrate intake to RDA (%)	Strength	68.4±26.2	64.5±22.7	Time	0.776
	Endurance	65.6±25.3	66.9±20.5	Group	0.983
	Time	67.7±25.8	65.05±22.1	Time x group	0.562

Values are expressed as mean±*SD*

Greenhouse-Geisser p-levels are reported with univariate analyses for time, group, and time x group interactions for each variable; *p<0.05 was significant.

Differences on performance during programme

According to Table 3, the athletes' performances revealed a significant increase in oxygen uptake or VO_2 max (p=0.012) during the intervention period. There was no significant difference between groups or interactions for each variable (p=0.867 and p=0.344).

DISCUSSION

The present study was conducted on young athletes from four training centres across Indonesia to assess how an intensive nutrition care process provided by nutritionists and dietitians affected athletes' diet, body composition, and performance. The nutrition care process was established in 2021 in collaboration with the Ministry of Sports in Indonesia (Penggalih *et al.*, 2021). This study reported that a 3-month nutrition care process reduced athletes' body fat composition. Besides that, it also improved health-related fitness component (body composition, cardiorespiratory fitness, muscle strength, and muscular endurance). In this case, we measured VO_2max as it is known as one of the many indicators to predict cardiorespiratory fitness or aerobic performance in athletes (Hoeger *et al.*, 2015; *Buttar et al.*, 2019).

One of the components of the NCP is providing nutrition education via nutritionists and dietitians to all athletes, both in class and in small groups. In the present study, the assessment of athletes' nutrition knowledge resulted in a significantly increased score (13%) among athletes. The results in Table 3 showed a significant difference before and after intervention (p=0.023), but there was no difference between strength and endurance groups (p=0.734). Similar to our results, four sessions of 30-minute nutrition education weekly were shown to increase sports nutrition knowledge in young, male, Chinese soccer athletes, while no change was observed in dietary intake (Zeng *et al.*, 2020). Research conducted by Zaman *et al.* (2021) showed something similar, whereby nutrition education increased knowledge, attitude, and practice (KAP), but not actual dietary intake. A systematic review showed that although majority of studies reported a significant change in the level of nutrition knowledge, but changes in dietary intake were inconsistent (Boidin *et al.*, 2020).

A study has shown the correlation between nutrition knowledge and dietary intake in adolescent athletes (Noronha *et al.*, 2020). Previously, the implementation of a slightly longer nutrition education programme (six weeks) led to improvements in dietary intake (Borjloo *et al.*, 2021). This means that changing habits requires more effort. A study of Malaysian athletes reported improved dietary intake only after eight weeks. The programme, although carried out over a shorter duration than the present study, provided education sessions more frequently: 1-2 hours per week (Mohd Elias *et al.*, 2018). Thus, the duration and frequency of our programme might have been sufficient to elicit changes in athletes' knowledge level, but not at the behaviour level.

The implementation of NCP did not statistically improve any parameters of the athlete's diet, which was measured as a percentage of RDAs fulfilled for calories, carbohydrates, fat, and protein. However, it is worth noting that despite this, most of the athletes's average dietary fulfilment for all macronutrients, both before and after the intervention, was between 80 and 110% of recommendation, which can be classified as adequate. Furthermore, categorisation of athletes' dietary fulfillments after intervention indicated some improvements in terms of the proportion of athletes with adequate intakes of total calories, protein, fat, and carbohydrates (Table 2).

Through this intervention, the researchers did not make changes to the food budget because this has been regulated by the government. The feeding programme in the athletes' dormitories also could not be adjusted to individual needs based on the respective sports and training period when providing food service for a large group. The modification of eating intervention was carried out by calculating the athlete's meal portion according to individual needs. Each athlete received information regarding an individual meal plan through counselling by a nutritionist. This is also in line with research conducted on athletes in Italy, where athletes received nutrition education related to food and had a positive effect on changes in eating patterns (Terenzio *et al.*, 2021).

While dietary intake remained unchanged, the present study indicated that after three months of intervention, an improvement in subcutaneous fat composition was observed. Although total body composition remained unchanged after the intervention, measurements of skinfold at four sites—triceps, subscapular, suprailiac, and calf were all significantly lower than baseline measurements. Remarkably, after the intervention, the present study also reported improved performance, measured as VO_2max, by nearly 2 ml/kg/minute. Several studies previously conducted reported that low body fat was correlated with higher physical performance, particularly cardiovascular and muscle endurance (Lubis *et al.,* 2022; Aikawa *et al.*, 2020; Ferreira *et al.*, 2016). Body fat captures non-metabolically active tissue that does not contribute to the physiological capacity to produce force (Ruiz-Castellano *et al.*, 2021), thus increasing adiposity may interfere with muscular performance and impact slow movement in athletes. On the contrary, a lower composition of body fat may be associated with the delayed onset of fatigue and durable endurance capacity (Ruiz-Castellano *et al.*, 2021).

Body composition of young athletes is also influenced by their growth and maturity status. Understanding the expected changes in body composition that accompany normal growth and maturation is essential. Several studies showed that growth spurts in body composition, especially in athletes, occur at the age of 12 years in girls and 14 - 15 years in boys (Desbrow, 2021; Campa *et al.*, 2019). At this age, the growth of fat-free mass (FFM) reaches its highest peak and fat mass (FM) decreases. However, when this study was conducted, the majority of athletes were 16 – 17 years old, so they had already gone through growth spurts for FM and FFM.

This study also aimed to compare the changes before and after intervention between endurance and strength athletes, as well as changes within the same group. It is interesting to note that strength athletes showed a lesser reduction in their skinfold thickness for upper extremity sites (triceps) compared to endurance athletes (Table 3). Indeed, a previous meta-analysis evaluating the effects of strength and endurance training revealed that endurance training led to a higher decrement in body fat, whereas strength training augmented muscle hypertrophy more effectively (Gorner & Reineke, 2020). Additionally, when comparing the baseline data between groups, strength athletes had relatively lower subcutaneous fat compared to endurance athletes. This may be why athletes in this group had lesser reduction in skinfold thickness. It is also worth mentioning that in spite of the minimal reduction in skinfold thickness, strength athletes still significantly improved their performance at post-intervention measurement. This might be explained by the improvement in nutrition knowledge, which led to improved skinfold thickness. VO_2max at the end of the study did not differ between groups.

Boullosa *et al.* (2020) stated that genetic, nutrition, training, psychological, and physiological factors can affect the performance of VO_2 max. In this study, the respondents were athletes in the same age range and experienced the same physical activity of more than 1000 METS (unpublished data), meaning that their trainings were quite similar. Researchers screened athletes with a cut-off of physical activity at 1000 METS. This is to equate the characteristics of athletes so that confounding variables during statistical analysis can be suppressed. According to the research, physical activity above 1000 METS is in the moderate to high category (Strasser & Burtscher, 2018). Apart from physical activity, the researchers also conducted matching with BMI-for-age. This indicator was used because athletes were still in their growth phase. Athletes who fell into the abnormal category (underweight and overweight) were not included in the analysis. The aerobic capacity of athletes was measured using two methods (Balke test and Bleep test) for verification. A study by Herdiles *et al.* (2017) stated that these two methods gave the same effect on endurance football players. As such, improvement in VO_2 max performance was affected by the nutritional status of athletes in this study.

NCP for athletes is a comprehensive process to assist athletes from a nutritional perspective. A study stated that there are three approaches in which NCP in athletes can affect athletes' performance, namely (1) addressing the determinants of eating behaviours, (2) addressing eating behaviours and dietary intake, and (3) addressing the consequences of dietary intake in relation to health and sports performance (Iwasa-Madge & Sesbreno, 2022). Based on the NCP chart in athletes (Figure 2), this study examined the eating behaviours of athletes during dietary assessment. The results of the assessment were then poured into nutrition interventions, where athletes were given education about nutrition profiles, nutrition and sports, as well as portion size of each meal according to their needs. Not only that, the researchers also conducted meal assistance, where nutritionists accompanied athletes at mealtimes. This was a way to improve eating behaviours in athletes who were still lacking.

The design of the NCP in the present study did not solely focus on providing nutrition education and meal assistance; training assistance was also provided monthly to athletes, which consisted of monitoring their hydration status. A study reported that athletes improved their training consistency, suffered from fewer injuries and illnesses, and demonstrated improved resilience after the athletes and coaches received education from nutritionists (Logue *et al.*, 2021). This could be one of the reasons for improved performance in this study, since athletes were given meals and training assistance by nutritionists, who also coordinated with their coaches.

To our knowledge, the present study is the first to implement a comprehensive NCP, which integrated nutritional assistance during mealtimes and training sessions by employing nutritionists or dietitians in government training centres for three full months. While this study also tried to differentiate its impact between endurance and strength athletes, we were aware of the unmatched number of participants between the two groups that might be unfavourable to statistical analysis. Additionally, it must be noted that our intervention did not include tailoring the same training programme for all types of sports. We let the training programmes run as naturally as possible, as designed by the coaches, so that any changes present in the results would be due to the implementation of NCP. Although the nutritionists or dietitians provided regular nutrition reports and recommendations to the coaches and managers of the training centres, they did not have the authority to modify the training programmes. Therefore, training programmes might serve as an uncontrolled confounder, since athletes from different sports might have different training volumes, affecting their body composition and performance outcomes. Though the type of training programmes varied between training centres, the total physical activity of the athletes was similar, at more than 1000 METS (unpublished data).

CONCLUSION

In conclusion, three months of NCP significantly improved the knowledge of nutrition, body fat composition, skinfold thickness, and performance of athletes. Despite the good intakes of protein and fat, none of the intakes showed significant changes. Furthermore, knowledge of sports nutrition and exercise science had a positive impact on dormitory meal choices. It is imperative that the NCP programme be carried out continuously with the assistance of nutritionists so that all existing parameters reach their expected optimal results based on the targets of each sport.

Acknowledgement

The authors convey their appreciation to all partners and enumerators who supported this research. The authors would also like to thank Lembaga Pengelola Dana Pendidikan (LPDP/Indonesia Endowment Fund for Education), Ministry of Finance, Republic of Indonesia for supporting this research through the Riset Inovatif Produktif (RISPRO/Productive Innovative Research) 2020-2022 (PRJ-106/LPDP/2019) grant.

Authors' contributions

Mirza HSTP, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; Zaenal MS and Laksono T, prepared the draft of the manuscript and reviewed the manuscript; Edi NS and Ernawaty, led the data collection in the Javanese region; Bayu R, Margono, Dadi S, and Raden I, led the data collection in the Lombok and Sulawesi regions; Kurnia MS, Rahadyana M, Naila AS, Veronika DPP, conducted the study, data analysis and interpretation, assisted in drafting of the manuscript, and reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Mal J Nutr 30(2): 245-256, 2024

Therapeutic diet plate waste and satisfaction among adult patients

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ABSTRACT

Introduction: There are limited studies on plate waste and satisfaction levels among adult patients receiving therapeutic diets in hospitals, particularly in the local context. Therefore, this study aimed to determine the percentage of plate waste and the level of satisfaction among adult patients receiving therapeutic diets, as well as the contributing factors. **Methods**: This cross-sectional study was conducted at a university hospital during lunch and dinner hours on eleven types of therapeutic diets. Food weighing and photography-assisted visual estimation by the Comstock scale were used to measure plate waste. Interviews were conducted with patients using the Acute Care Hospital Foodservice Patient Satisfaction Questionnaire (ACHFPSQ) to collect information about their satisfaction towards food quality and service. **Results:** Mean overall plate waste rate for 96 patients receiving therapeutic diets was 43.6% (40.0% for lunch, 47.3% for dinner), with the highest rate of wastage in a combined diet low in fat and salt. Overall score for patient satisfaction was 3.7 out of 5. Patients had higher satisfaction levels with staff and food service (4.0) compared to food quality (3.4). Food quality negatively contributed to the rate of plate waste in terms of taste (r=-0.107, p=0.035), presentation (r=-0.078, p=0.043), and texture (r=-0.052, p=0.020). **Conclusion:** Therapeutic diet plate waste among adult patients in this study was high and primarily attributable to food quality. Efforts to minimise therapeutic diet plate waste should be made by improving food quality for the best possible outcomes for patients.

Keywords: food service, hospital, plate waste, satisfaction level, therapeutic diet

INTRODUCTION

Food waste can be defined as the amount of food that is served, cannot be consumed by the consumer, and thus thrown away. According to the Food and Agriculture Organization of the United Nations, as much as 17.0% of the world's food, which is equivalent to approximately 1.3 billion tons, is wasted every year (FAO, 2019). As much as 22.0% of the 1.3 billion tons of food is wasted at the end of the food supply chain, which includes settings such as households, the food service industry, and the retail sector (Cook *et al.*, 2023). Although waste still occurs everywhere,

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doi: https://doi.org/10.31246/mjn-2023-0128

food waste in hospitals can reach up to 50.0% of the total waste generated from the wards (Alam *et al.*, 2008).

According to Williams & Walton (2011), food waste generated by hospitals is the most challenging for the implementation of food waste reduction strategies. There are three types of food waste produced in the hospital setting: food preparation waste, tray waste (food left unserved), and plate waste (Williams & Walton, 2011). Among these, plate waste contributes the most to the rate of food waste in hospital institutions (Goonan, Mirosa & Spence, 2014). Leftover plate in hospitals is defined as the amount of food that has been served and can be eaten, but not eaten by patients. Plate waste of more than 30.0% in a hospital setting is generally agreed upon as high (Edwards & Nash, 1997). Out of 17 observational studies that have been conducted in Indonesia, the median plate waste in hospital institutions was reportedly 27.6% (ranging from 14.8 % - 88.7%), which was caused by several factors such as patients' clinical conditions, food taste, and hospital food service environment (Diana *et al.*, 2022). In addition, a study showed that the rate of hospital food waste in Malaysia ranged from 17.0% to 67.0%, with the highest rate of food waste at dinner time (Zakiah, Saimy & Ah, 2005). A high percentage of plate waste can potentially be a major factor related to nutritional malpractice in hospitals and incurring hospital management with higher financial and environmental costs (Williams & Walton, 2011).

There are various factors that contribute to high plate waste in hospitals. Most of the factors that contribute to the generation of plate waste in hospitals are related to elements such as the appearance and characteristics of the foods served to patients, patient's medical condition, medication, hospital environment, and also food service management. A study showed that the quality of food service greatly affects the level of patient satisfaction in hospitals (Amany *et al.*, 2012). Another study by Aminuddin, Vijayakumaran & Abdul Razak (2018) in East Malaysia's government hospitals discovered that the average plate waste was 36.0% in 2017. Recently, Razalli *et al.* (2021 found that hospitalised patients who were on a textured modified diet had a significantly higher plate waste of 47.5%. The study identified blended diet as the most wasted (65.0%), followed by minced diet (56.0%), and mixed porridge (35.0%). Additionally, a local study conducted by Jamhuri *et al.* (2017) revealed that food wastage among cancer patients was considerable, with rates of 59.3% using the observational method and 41.9% using the weighing method, respectively.

In addition, the level of patient satisfaction is also one of the factors that greatly influences food intake among patients. Research findings suggested factors such as meal taste, menu variety, and staff responsiveness as contributors to patients' satisfaction with food service in hospital settings. A study done by Sahin *et al.* (2006) found that 48.7% of hospital patients were mainly dissatisfied with the taste and appearance of foods served by hospital food service. A more recent study by Nakamura (2021) suggested that the characteristics and appearance of food play a very important role in the level of acceptance and satisfaction of patients towards the food that has been served to them.

Despite extensive studies on hospital plate waste globally, research on plate waste in therapeutic diets and its link to patient satisfaction is limited. Therapeutic diets, prescribed by doctors and planned by dietitians, control specific nutrients and foods based on patients' medical conditions.

These diets modify macronutrients (carbohydrates, protein, fats) and micronutrients (vitamins, minerals) to suit patients' needs, including allergens and texture. In Malaysia, the common types of therapeutic diets include calorie modification diet/diabetic diet, protein modification diet, low-fat diet, low/high-fibre diet, micronutrient modification diet, and other diets (Ministry of Health Malaysia, 2016). A study by Norshariza *et al.* (2019) among patients in Malaysian public hospitals revealed that 47.4% of therapeutic diets were wasted. However, the study did not investigate patient satisfaction levels. Furthermore, Rattray, Desbrow & Roberts (2017) found that the energy and protein intakes of acute care patients on therapeutic diets were poor, with only 18.0% meeting their estimated needs. This issue is linked to factors such as low nutrient availability, high patient nutritional needs, and reduced intake. They suggest several strategies to improve nutritional intake and prevent or treat malnutrition in these patients.

MATERIALS AND METHODS

Study design

A cross-sectional study was conducted among 96 patients aged 18 years old and above who received therapeutic diets in the wards at Universiti Kebangsaan Malaysia (UKM) Medical Centre. Ethical approval was obtained from the Research Ethics Committee, Universiti Kebangsaan Malaysia (UKMPPI/111/8/JEPUKM_JEP-2023-286). Information sheets of the study were distributed and written consent was obtained from all subjects prior to data collection.

Sampling and subjects

Subjects were selected from the Department of Dietetics and Food Services using the Caring Hospital Enterprise System (C-HETS) via convenience sampling. The inclusion criteria were adult patients aged 18 years old and above who received at least one full day of nutrient modification therapeutic diet meals (breakfast, lunch, and dinner), able to give feedback on the level of satisfaction of food service, and fully capable of undergoing height and weight measurement procedures. Exclusion criteria included patients receiving a therapeutic diet of texture modification, i.e. mixed porridge diet or a minced diet, patients from psychiatric wards, intensive, semi-intensive, and those infected with COVID-19. A total of 11 types of therapeutic diets were assessed in this study, which consisted of three single nutrient modification diets (diabetic diet; low-sodium diet; low-purine diet) and eight multiple nutrient modification combination diets (diabetic, low-sodium diet; diabetic, low-fat diet; diabetic, low-sodium, low-fat diet; diabetic, low-sodium, low-potassium diet; diabetic, low-sodium, low-potassium, low-phosphate diet; low-sodium, low-fat diet; low-potassium, low-phosphate diet; low-sodium, low-phosphate diet).

Data collection

Kish's (1965) formula determined a minimum sample size of 96 patients for this study with 95% confidence. Data were collected through a questionnaire, which subjects completed after explanation from researchers. The structured questionnaire had five sections: sociodemographic information, anthropometry, food intake, clinical information, and food service quality.

Data collection on sociodemographic information was done by collecting name, age, race, education level, type of ward admission, and others. Some were collected through interviews or medical records. Nutritional status was assessed using anthropometric measurements, including height and weight. The subjects were classified according to body mass index (BMI) categories based on the World Health Organization (2007) classifications. For the clinical part, information on signs and symptoms of disease, appetite, consumption of oral nutrition supplements (ONS), and whether subjects were taking outside food were collected from patients.

Two methods were employed to assess the amount of plate waste – weighing and photography-assisted visual estimation, which were conducted by two trained undergraduate researchers. However, each patient's tray was only evaluated by a single researcher. For the weighing method, a Tanita KD 160WH digital food weighing scale (Itabashi-ku, Tokyo, Japan) with a weight capacity of 2 kg and weight graduation of 1 gram was used. A standard weight of the therapeutic diet on the tray was first measured and recorded. The remaining weight of the therapeutic diet was measured again and compared with the standard weight to obtain percentage plate waste. This method involved collecting all food waste from each patient and separating it into four different food components: starch, protein, vegetables, and fruits/dessert before weighing them accordingly. The following formula was used to determine the rate of plate waste:

Rate of plate waste (%) = Amount of plate waste(g)/Standard food weight(g) x 100%

Visual estimation of waste expressed on a 6-point scale developed by Comstock *et al.* (1981) was used to measure approximately what proportion of food was left. Food waste was separated into four different food components, then observed and recorded in different values from 0%, 25%, 50%, 75%, 90%, 100% (all consumed to none consumed). Digital photography was also used to assist in the recording of plate waste, which can minimise errors and allow unhurried estimates of portion sizes at a later time. Both of these methods were carried out within one day with two main dishes, which were lunch and dinner, with a clear classification of food according to food groups.

Information on patient satisfaction level was measured using the Acute Care Hospital Foodservice Patient Satisfaction Questionnaire (ACHFPSQ) (Capra *et al.*, 2005). The Malay version of the questionnaire (Aminuddin *et al.*, 2018) was approved for use in this study and modified according to the needs of this study. The questionnaire consisted of 5 parts: (A) respondent information; (B) evaluation of food quality; (C) evaluation of food service quality; (D) evaluation of staff and service issues; and (E) evaluation of the physical environment. Scoring was done on a 5-point scale: 1-strongly disagree, 2-disagree, 3-neutral, 4-agree, 5-strongly agree. Satisfaction scores were then analysed in two main domains: (i) food quality and (ii) staff and food service. A higher mean score correlated with better results and indicated greater satisfaction.

Statistical analysis

Data were analysed using IBM SPSS Statistics for Windows Version 27.0 (IBM, Armonk, New York, USA). The statistical significance level used was 0.05. Descriptive analyses were employed to assess sociodemographic and anthropometric profiles, plate waste percentages for therapeutic diets at lunch and dinner using weighing and visual methods, and satisfaction scores across various domains. Means and standard deviations were calculated for therapeutic diets and satisfaction scores. Additionally, the non-parametric Spearman's correlation test was conducted to examine the relationship between percentage of total plate waste and its influencing factors due to non-normal distribution. This test also assessed the link between plate waste and patient satisfaction levels. Multiple linear regression analysed the association between percentage plate waste and significant satisfaction factors.

RESULTS

A total of 96 patients receiving therapeutic diets were included in this study and their sociodemographic profile is presented in Table 1. The average age of the patients was 65.3 years old, with a higher number of males (*n*=52, 54.2%) compared to females (*n*=44, 45.8%). In terms of length of stay, more than half of the patients (*n*=60, 62.5%) had stayed in the hospital for more than two days. For anthropometric data, the average BMI of the patients was 23.9 kg/m^2, which fell within the normal category.

Table 1. Socio-demographic and anthropometric profiles of subjects [represented as mean±*SD* or *n* (%)]

Parameters	*Total (n=96)*
Age	65.3±13.7
Gender	
Male	52 (54.2)
Female	44 (45.8)
Race	
Malay	61 (63.5)
Chinese	19 (19.8)
Indian	16 (16.7)
Education level	
Lower secondary	20 (20.8)
Upper secondary	47 (49.0)
Diploma	11 (11.5)
Degree	18 (18.8)
Length of stay	
>2 days	60 (62.5)
>7 days	23 (24.0)
>2 weeks	8 (8.3)
>1 month	5 (5.2)
Anthropometry	
Height (cm)	163.0±7.6
Weight (kg)	63.4±10.7
Body mass index (kgm^{-2})	23.9±3.7

Table 2 shows the percentage of plate waste for therapeutic diets using the weighing method and the Comstock visual estimation method according to each meal time. During lunch time, the weighing method revealed high plate waste percentages for diabetic diet (33.7%), low-sodium diet (43.0%), and low-purine diet (46.3%). Meanwhile, plate waste for combinations of diets were also high at more than 30% for diabetic, low-sodium diet (40.9%); diabetic, low-fat diet (51.5%); diabetic, low-sodium, low-fat diet (41.0%); diabetic, low-sodium, low-potassium diet (42.6%); low-sodium, low-fat diet (51.8%); low-potassium, low-phosphate diet (72.7%); and low-sodium, low-phosphate diet (39.1%). Diabetic, low-sodium, low-potassium, low-phosphate diet had a slightly lower percentage of plate waste at 24.4% compared to other therapeutic diet combinations.

As for dinner time, high plate waste percentages were also recorded for diabetic diet (42.4%), low-sodium diet (32.0%), and low-purine diet (70.0%). Diet combinations also had a high percentage of plate waste: diabetic, low-sodium diet (58.1%); diabetic, low-fat diet (42.1%); diabetic, low-sodium, low-fat diet (44.1%); diabetic, low-sodium, low-potassium diet (31.9%); diabetic, low-sodium, low-potassium, low-phosphate diet (42.2%); low-sodium, low-fat diet (72.5%); and low-potassium, low-phosphate diet (28.3%); except for low-sodium, low-phosphate diet (4.7%).

In detail, using the Comstock visual estimation method, the percentages of plate waste during lunch time were recorded high for diabetic diet (34.7%), low-sodium diet (43.3%), and low-purine diet (43.8%). Plate waste percentages were also high, exceeding 30% for most combinations of diet such as diabetic, low-sodium diet (40.4%); diabetic, low-fat diet (54.0%); diabetic, low-sodium, low-fat diet (41.1%); diabetic, low-sodium,

Table 2. Percentage of plate waste for therapeutic diet during lunch and dinner by using weighing method and visual estimation method (mean±*SD*)

Types of therapeutic diet	*Weighing method*		*Visual estimation method*	
	Lunch (n=96)	*Dinner (n=96)*	*Lunch (n=96)*	*Dinner (n=96)*
Diabetic diet (*n*=39, 40.6%)	33.7±25.3	42.4±31.5	34.7±26.3	42.7±32.0
Low sodium diet (*n*=5, 5.2%)	43.0±22.0	32.0±22.1	43.3±21.8	31.3±20.7
Low purine diet (*n*=2, 2.1%)	46.3±10.0	70.0±3.3	43.8±8.8	72.1±3.0
Diabetic, low sodium diet (*n*=20, 20.8%)	40.9±34.0	58.1±33.2	40.4±33.9	58.4±32.9
Diabetic, low fat diet (*n*=5, 5.2%)	51.5±34.7	42.1±33.2	54.0±34.0	43.8±34.0
Diabetic, low sodium, low fat diet (*n*=8, 8.3%)	41.0±27.7	44.1±27.9	41.1±30.0	45.4±25.3
Diabetic, low sodium, low potassium diet (*n*=2, 2.1%)	42.6±8.4	31.9±12.2	39.4±2.7	30.0±15.9
Diabetic, low sodium, low potassium, low phosphate diet (*n*=4, 4.2%)	24.4±41.7	44.2±40.5	22.8±37.8	44.1±37.9
Low sodium, low fat diet (*n*=7, 7.3%)	51.8±24.6	72.5±35.8	50.5±27.2	73.1±34.9
Low potassium, low phosphate diet (*n*=3, 3.1%)	72.7±20.3	28.3±35.9	70.4±21.3	29.6±36.2
Low sodium, low phosphate diet (*n*=1, 1.1%)	39.1±0.0	4.7±0.0	37.5±0.0	6.3±0.0
Total (*n*=96, 100%)	39.9±28.3	47.1±32.3	40.0±28.5	47.5±32.2
Overall	43.6±15.7		43.8±13.0	

low potassium diet (39.4%); diabetic, low-sodium, low-fat diet (50.5%); low-potassium, low-phosphate diet (70.4%); and low-sodium, low-phosphate diet (37.5%); except for low-potassium, low-phosphate diet (22.8%).

Meanwhile, for dinner time, the percentages of plate waste using the Comstock visual estimation method were recorded as similarly high for diabetic diet (42.7%), low-sodium diet (31.3%), and low-purine diet (74.1%). Most combination diets also had percentages of plate waste at almost 30.0% or more during dinner time: diabetic, low-sodium diet (58.4%); diabetic, low-fat diet (43.8%); diabetic, low-sodium, low-fat diet (45.4%); diabetic, low-sodium, low-potassium diet (30.0%); diabetic, low-sodium, low-potassium, low-phosphate diet (44.1%); low-sodium, low-fat diet (73.1%); and low-potassium, low-phosphate diet (29.6%); except for low-sodium, low-phosphate diet (6.3%).

Overall, the mean plate waste for therapeutic diets in this study using the weighing method was 43.6%, where the wastage of food occurred mostly during dinner time compared to lunch time (47.0% for dinner time vs. 39.9% for lunch time). On the other hand, mean plate waste of therapeutic diets measured using the Comstock visual estimation method was 43.8%, mostly contributed by dinner time (47.5%) and the remaining produced during lunch time at 40.0%.

An additional statistical analysis was conducted to determine if there was any significant difference between the results obtained from the weighing method and the Comstock visual estimation method for both lunch and dinner times. Data from the Comstock visual estimation was compared with data from the weighing method since weighing food waste from individual plates using a scale is considered the gold standard method (Allison, 1995). From the paired sample *t*-test that was conducted, the difference between the average plate waste obtained from the weighing method and the Comstock visual method for lunch was not significantly different (*p*=0.820). Similarly, there was no significant difference between both methods for dinner time (*p*=0.312). As a result, the overall mean percentages of plate waste for therapeutic diets using both the weighing method (43.6%) and the Comstock visual estimation method (43.8%) were almost the same, showing only a slight difference of 0.2%, which was not statistically significant.

Table 3. Satisfaction scores according to different criteria of satisfaction domains (mean±*SD*)

Criteria	*Mean score*
Satisfaction level to food quality	3.4±0.6
Taste	2.3±0.7
Vegetables	3.3±1.0
Variety of choices	2.8±0.8
Texture	3.6±1.0
Appearance	3.7±1.1
Portion	3.5±1.0
Temperature	4.3±0.7
Satisfaction level to staff and food service	4.0±1.0
Cleanliness	3.5±1.8
Attire	4.2±0.6
Manners	4.0±0.7
Ward surrounding smell	4.4±1.1
Food service quality	3.8±1.0
Overall satisfaction level	3.7±0.8

Patients who were receiving therapeutic diets in the hospital were assessed for their satisfaction levels in two main domains: food quality and staff and food service (Table 3). Under the domain of food quality, there were seven items, including taste, vegetables, variety of choices, texture, appearance, portion, and temperature. In comparison, the domain of staff and food service consisted of five main items: cleanliness, attire, manner, smell, and overall satisfaction level. The findings revealed that patients had higher satisfaction levels with staff and food service (mean=4.0, standard deviation, *SD*=1.0) compared to food quality (mean=3.4, *SD*=0.6). Among the specific aspects of food quality, temperature received the highest mean satisfaction score (mean=4.3, *SD*=0.7). On the other hand, surrounding smell received the highest mean satisfaction score under the domain of staff and food service (mean=4.4, *SD*=1.1). Overall, the mean satisfaction score from both domains (food quality and staff and food service) was 3.7±0.8 out of 5.

The study employed the Spearman's correlation test to assess the relationship between factors influencing plate waste and percentage of total plate waste (Table 4). The results revealed that there was no significant correlation for the overall factor. However, within the domain of food quality, three specific aspects – taste (*r*=-0.107, *p*=0.035), appearance (*r*=-0.078, *p*=0.043), and texture (*r*=-0.052, *p*=0.020) – exhibited significant and negative correlations with percentage of total plate waste. This suggested that as the quality of taste, appearance, and texture improved, there was a decrease in the amount of plate waste. On the other hand, the overall satisfaction level had a weak and negative association with percentage of total plate waste, but this association was not statistically significant (*r*=-0.152, *p*=0.139). This implied that overall satisfaction did not have a significant impact on the amount of plate waste.

Next, the study utilised multiple linear regression to examine the relationship between percentage of total plate waste and seven different factors related to food quality. The results, as presented in Table 5, indicated that only the appearance of food was significantly associated with the total amount of plate waste.

DISCUSSION

The overall mean plate waste among patients who took therapeutic diets at the UKM Medical Centre was 43.6%, which was considerably high given that plate waste of more than 30.0% in a hospital setting is generally considered high (Edwards & Nash, 1997). The result obtained was almost identical to a study carried out by Zakiah *et al.* (2005), where plate waste was 43.3%. The mean percentage of plate waste produced during lunch time was approximately 40.0%, whereas the average percentage of plate waste produced during dinner time was 47.3%. This showed that plate waste contributed during dinner time was relatively higher compared to plate waste produced during lunch time. The above study findings are almost identical to those of another study done by McCray *et al.* (2018), in which hospital plate waste fell within the range of 21.0% to 65.0%, with a median of 34.0%. However, the overall mean plate waste obtained in this study was slightly low compared to the findings of Norshariza

Table 4. Correlations between percentage of total plate waste with contributing factors and satisfaction level (*n*=96)

Factors	*Percentage of total plate waste (%)*	
	r	*p-value*
Gender	0.152	0.139
Race	0.094	0.364
Education level	0.092	0.372
Length of stay	-0.088	0.391
Type of therapeutic diet	0.102	0.323
Appetite	0.081	0.549
Ward discipline	-0.062	0.597
Signs/Symptoms	-0.055	0.342
Age group	0.102	0.453
Body mass index (BMI)	-0.098	0.546
Oral nutrition supplement consumption	-0.051	0.654
Outside food consumption	-0.011	0.780
Satisfaction level to food quality		
Taste	-0.107*	0.035
Appearance	-0.078*	0.043
Texture	-0.052*	0.020
Vegetables	-0.116	0.259
Temperature	-0.080	0.440
Variety of choices	-0.003	0.979
Portion size	-0.069	0.503
Satisfaction level to staff and food service		
Cleanliness	0.005	0.962
Attire	-0.067	0.517
Manners	-0.170	0.098
Surrounding smell	-0.145	0.158
Overall satisfaction level	-0.152	0.139

*Statistically significant at $p<0.05$, analysed using Spearman's correlation test

Table 5. Multiple linear regression analysis of total plate waste as dependent variable and satisfaction factors as independent variables

Variables	*Percentage of total plate waste (%)*		
	Regression coefficients	*t-value*	*p-value*
Taste	-0.115	0.169	0.447
Appearance	0.230	-0.078*	0.032
Texture	0.032	0.219	0.828
Vegetables	-0.165	-0.130	0.262
Temperature	-0.050	-0.434	0.658
Variety of choices	0.053	0.434	0.658
Portion size	-0.001	-0.100	0.992

*Statistically significant at $p<0.05$
Statistical model $R^2=0.260$

et al. (2019), which was 47.4%. In that study, various factors were found to have contributed to plate waste of therapeutic diets among patients in Malaysian public hospitals, including patients sleeping or were not present in the ward, lacked appetite during the times when meals were served to them, and their physical condition, which contradicted their ability to consume their meals in comfort.

The percentage of plate waste did not show a significant correlation with the contributing factors identified in the study. This finding contradicts the results of Razalli *et al.* (2022), who found a negative association between contributing factors related to appetite and mean percentage of plate waste. Our questionnaire results revealed that approximately 52.1% of patients on a therapeutic diet had a moderate level of appetite, which could explain the difference in findings. Additionally, satisfaction level with food quality and food service was found to be an important factor contributing to plate waste. Taste, appearance, and texture were negatively associated with plate waste, aligning with the findings of Chik *et al.* (2018), who identified taste as a significant contributor to plate waste.

This study observed that the combination of a low-sodium and low-fat diet yielded the highest percentage of plate waste. Several factors may contribute to elevated plate waste levels among patients receiving low-fat, low-salt meals in hospital settings. These factors encompass dissatisfaction with taste and texture, limited menu variety, and the challenge patients encounter in adapting their palate to alterations in fat content, which affects mouthfeel, and sodium level, which influences taste perception. Acknowledging the temporal nature of patients' adjustment to these changes underscores the significance of incorporating food preferences into meal planning to mitigate plate waste and bolster patient satisfaction. While published evidence is limited regarding patients receiving low-fat diets, a study in Indonesia reported a significant, negative relationship between plate waste and food taste, as well as plate waste and food appearance among patients receiving low-sodium diets (Saragih, 2020). Specifically, some respondents in this study commented that the food was tasteless, which can be attributed to the fact that most respondents were on a diabetic and low-salt diet, resulting in higher amounts of plate waste due to unmet taste expectations.

Furthermore, the low-sodium, low-fat diet combination (during dinner time) together with the combinations of low-potassium, low-phosphate diet (during lunch time) and low-purine diet (during dinner time) were found to be highly wasted at 70% or more. Low-potassium, low-phosphate diet is normally prescribed for patients with renal disease (Ministry of Health Malaysia, 2016). In a study conducted at an American hospital, significantly lower intake and higher plate waste were reported for patients receiving renal diet compared to regular non-therapeutic diet (Sargent, 2010). Potassium restriction typically involves abstaining from high-potassium vegetables, particularly green leafy varieties (Ministry of Health Malaysia, 2016) and replacing them with harder textured vegetables. This substitution may potentially lead to increased plate waste, particularly among elderly patients or those experiencing dental issues. Low-purine diet is characterised by a diet low in purine prescribed for patients with gout and hyperuricemia (Ministry of Health Malaysia, 2016). No previous studies have reported a high percentage of plate waste among patients receiving this kind of diet. However, it might be possible that the limitation of protein sources, such as fish, chicken, meat, and seafood, might affect patients' acceptance.

Among all, low-sodium, low-phosphate diet (during dinner time) had the lowest percentage of plate waste (4.7% by weighing, 6.3% by visual estimation). This is in contrast with the data of this study, where any diet combination with sodium restriction was normally highly wasted. However, generalisations cannot be made since this low-sodium, low-phosphate diet was only consumed by one patient.

Results of the multiple linear regression analysis revealed a significant relationship between the appearance of food and plate waste. This finding is consistent with the findings of Zaid *et al.* (2019), who also found that the appearance of the food served was associated with higher plate waste. Previous studies have emphasised the importance of meal appearance in generating and maintaining appetite (Navarro *et al.*, 2014. The presentation of food plays a crucial role in stimulating patients' eagerness to try food, which in turn affects their food intake and nutritional benefits. When less appealing foods are presented, patients are more likely to ignore them or look up for more appealing options such as fruits, leading to increased plate wastage in hospitals. In order to determine the optimal food service system for hospitals, it is important to comprehensively measure all key outcomes related to the food service system, as highlighted by the study conducted by McCray *et al.* (2018). Additionally, Sonnino & McWilliam (2011) stated that improving communication between patients and hospital staffs is essential for better understanding the reasons behind food wastage and its consequences.

To address the issue of excessive plate waste, it is crucial to implement a systematic and effective intervention strategy through a sequence of trials. This approach will provide opportunities for quality improvement in food services and reduction of plate waste. However, it is important to recognise that tackling this complex problem requires collaboration from all individuals involved. Everyone, including hospital staffs, patients, and food service providers, must work together to find sustainable solutions. It is important to acknowledge that this issue cannot be fully resolved overnight and will require ongoing efforts and continuous improvement.

There were several limitations present in this study. One of those limitations was that this study only recorded and interpreted plate waste produced during lunch and dinner times due to logistic challenges for the undergraduate researchers. Plate wastage contributed by patients during breakfast and teatime was not recorded, thus making it less accurate to represent the actual plate waste among patients who consumed therapeutic diets. Another limitation that affected the findings of this study was the fact that certain patients' food might have also been consumed by their family or caretaker; therefore, the percentage of plate waste could actually be higher than recorded. Furthermore, plate waste assessment for each patient's tray was performed by a single rater, potentially introducing rater's bias during visual observation. Nonetheless, both undergraduate researchers who conducted the assessments had received prior training and the Comstock visual estimation data did not exhibit any statistical variance when compared to data from the weighing method, which served as the gold standard for plate waste measurement (Allison, 1995). Other than that, this study was a single-centre study, thus making it less suitable to generalise the total plate waste to those produced by patients in Malaysia. On that account, for more precise findings regarding plate waste, it is suggested that a study be carried out to identify the differences in plate waste produced during every menu rotation that is set up by the hospital food service.

CONCLUSION

The overall mean plate waste among adult patients who received a therapeutic diet at UKM Medical Centre was high. Furthermore, there was a significant, negative relationship between food quality and percentage of therapeutic diet plate waste. Urgent measures must be taken to address the high plate waste rates among patients on therapeutic diets. Prioritising patient satisfaction with food quality and service is crucial for reducing waste in hospital settings. This involves regular plate waste audits to pinpoint menu underperformance, menu revisions, and providing kitchen staffs with training to enhance meal quality in terms of taste, texture, and presentation.

Acknowledgement

We would like to express our heartfelt gratitude and thanks to all subjects and their caregivers who willingly participated in this study. A special mention to all officers and staffs from the Department of Dietetics and Food Services, UKM Medical Centre for extending us support and encouragement throughout the study.

Authors' contributions

Moorthy JM & Ruzalee RS, conducted the study, data analysis and interpretation, prepared the draft of the manuscript; Hafizon DMP, assisted in data collection, advised on data analysis and interpretation, and reviewed the manuscript; Razalli NH, principal investigator, conceptualised and designed the study, edited the draft of the manuscript, and reviewed the manuscript.

Conflict of interest

This study has no conflict of interest.

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Effects of LED treatments on the growth and nutritional content of lettuce (*Lactuca sativa*) in a hydroponic vertical farming system

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ABSTRACT

Introduction: LED-integrated hydroponic vertical farming system (HVFS) may address food security challenges arising from urbanisation, while antioxidants in vegetables may prevent cardiovascular disease. This study aimed to explore the LED effects on yield, antioxidant properties, and vitamin C content in Butterhead lettuce (BL) and Italian lettuce (IL). **Methods:** Three LED combinations were used: 77% red, 14% green, and 9% blue lights (77R:14G:9B); 7% red, 41% green, and 52% blue lights (7R:41G:52B); and 25% red, 67% green, and 8% blue lights (25R:67G:8B). The concentration and pH of nutrient solution in HVFS ranged from 0.878-2.061ppm and 6.0-6.6, respectively, with a 12:12 light-dark cycle. Chlorophyll and anthocyanin contents were measured using chlorophyll and anthocyanin meters, respectively. Total phenolic and vitamin C content were measured using Folin-Ciocalteu colorimetric assay. Total flavonoid and antioxidant activity were determined through aluminium chloride colorimetric assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) colorimetric assay, respectively. Completely randomised design was adapted with three replications. **Results:** BL exhibited greater yield, anthocyanin and chlorophyll contents, while IL had higher vitamin C content. Significantly ($p<0.001$) higher yields were produced under 77R:14G:9B and 25R:67G:8B. Significantly higher phenolic ($p<0.001$) and flavonoid ($p=0.003$) contents were produced under 25R:67G:8B. Vitamin C was significantly ($p=0.037$) higher under 25R:67G:8B than 7R:41G:52B. **Conclusion:** LED treatment with higher proportion of red light generated greater yield, whereas higher proportion of green light gave higher phenolic, flavonoids, and vitamin C accumulation. This study provided preliminary data on boosting crop yield and phytochemical contents, which may improve food self-production and population health.

Keywords: antioxidant, butterhead, food security, phytochemicals, yield

INTRODUCTION

Urbanisation is a complicated socio-economic process that modifies the built environment, changing previously rural towns into urban settlements while simultaneously shifting the spatial distribution of a population from rural to urban areas (United Nations, Department of Economic and Social Affairs and Population Division, 2019). The global urban population has increased from 0.75 billion in 1950 to 4.22 billion in 2018, and it is predicted to continue growing until 6.68 billion in 2050 (United Nations, Department of Economic and Social Affairs and Population Division,

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doi: https://doi.org/10.31246/mjn-2023-0107

2019). With the increasing rate of urbanisation, more lands are diverted from agricultural production and used for urban development, resulting in less food production. At the same time, increasing population leads to higher demands for food and subsequently resulting in food insecurity. Therefore, light-emitting diode (LED)-integrated hydroponic vertical farming system (HVFS) can be considered as a way to solve this problem because it can be used to grow foods in nutrient solution on vertically stacked layers, producing higher yields than traditional farming. It has also been shown that greater yield could be produced under LED treatment than under natural sunlight (Chua *et al.*, 2020).

Besides, it was reported that about 32% of deaths worldwide in 2019 were attributable to cardiovascular disease (CVD) (World Health Organization, 2021). Plant-based foods are excellent sustainable food sources and they are rich and safe sources of natural antioxidants. Bioactive substances, such as polyphenols, flavonoids, anthocyanins, and chlorophylls, in plant-based foods are crucial as they may help to reduce the prevalence of CVD. Antioxidants could help control the oxidation of low-density lipoprotein, hence improving lipid profile and significantly control the emergence and progression of CVD (Ciumărnean *et al.*, 2020).

Whether growing plants indoors or outdoors, light is a crucial environmental component influencing plant growth and development. Therefore, LED technology can be used to improve the effectiveness of indoor plant growth where sunlight may be deficient. LED lights are more environmentally friendly than other artificial light sources because of their great light efficiency, low heat emission, and low power consumption. Also, combinations of various LED lights can deliver high flux and specialised wavelengths for plant culture, which trigger plant development (Monostori *et al.*, 2018). While light intensity affects photosynthesis the most, it was found that spectral composition plays a role as well in modifying carbon dioxide assimilation and electron transport processes for photosynthesis (Monostori *et al.*, 2018). However, the precise lighting condition, light intensity, and spectrum makeup necessary for the optimal growth and nutritional value of various plant species are still not well understood.

Most research done so far has focused on the importance of red (600-700nm) and blue (400-500nm) lights when used separately or in combination due to the high absorption by photosynthetic pigments at these wavelengths. For instance, a study conducted by Spalholz, Perkins-Veazie & Hernández (2020) has discovered higher total phenolic, anthocyanin, and chlorophyll contents in lettuce under combinations of red and blue lights than under sunlight. Green light (500-600nm) has recently been given a more important function to play. It is essential for fostering biomass formation in deeper leaf and lower canopy regions where other lights are deficient (Smith, McAusland & Murchie, 2017). Photosynthesis rate and fresh weight were found to be significantly greater when supplementing red-blue light with appropriate quantities of green light (Smith, McAusland & Murchie, 2017).

Lettuce is a popular greenhouse vegetable widely consumed and it serves as a model crop for studies on how plants react to light quality (Li & Kubota, 2009). According to Brazaitytė *et al.* (2022), it is one of the highly valuable crops grown in controlled environment agriculture, which is rich in fibre, minerals, various vitamins (vitamin C, vitamin E), bioactive compounds such as folate, and phenolic compounds like flavonoids and anthocyanins. In addition to its rich content and wide consumption, cultivating lettuce in a closed production system, such as HVFS, may also contribute to the efforts to meet the world's rising food demand (Rehman *et al.*, 2017). Thus, implementing LED-integrated HVFS can help in achieving easier and faster food self-production, as well as promoting antioxidant intake, which is beneficial for a wide variety of non-communicable diseases (e.g., CVD) at the same time. The only barrier that limits the widespread implementation of LED-integrated agriculture is its high initial installation cost (Rehman *et al.*, 2017). Nonetheless, this cost may be justified if we take into account its long lifetime (Rehman *et al.*, 2017). A study has also estimated that the power consumption for eight hours a day of LED lights is just about 32.12 kWh/year (Khorasanizadeh *et al.*, 2015).

In this study, we hypothesised that growing crops on the LED-integrated HVFS would positively affect the fresh yields and antioxidant content of lettuce cultivars. Thus, the objectives of this experiment were to investigate the effects of different LED treatments on the yield, phytochemicals, and vitamin C contents of lettuce cultivars, namely Butterhead lettuce (BL) and Italian lettuce (IL).

MATERIALS AND METHODS

Experimental site

This research project was carried out at the LED-integrated HVFS located in Block C at University of Nottingham Malaysia. The average room temperature was about 27°C. From sowing until data collection, this experiment took place from 27 October 2022 until 21 February 2023.

Experimental materials

Seeds of BL (*Lactuca sativa var. capitata*) and IL microgreens (*Lactuca sativa L. var. ramose*) were obtained from Baba™ Smart Grow and New Trio Products, respectively. Fertilisers used were manufactured by SQM Holland BV and distributed by Agroniche Sdn Bhd. EC meter (TDS&EC, China) and pH meter (Danoplus Ltd., Hong Kong) used for monitoring the electrical conductivity (EC) and pH of nutrient solution in HVFS were obtained from the Biosciences Lab at University of Nottingham Malaysia.

Germination test

Ten-day germination test was done to determine the quality of seeds. Seeds of each type were placed onto moistened filter papers separately in different agar plates and observed for germination in darkness for two days, followed by eight days in the presence of light.

Nursery management

Nursery management was done to ensure that the seedlings could survive and be able to grow well when transplanted onto the HVFS. First, seeds of the two cultivars were soaked separately in two agar plates with water and kept in the chiller at 5°C for 24 hours. After 24 hours, the seeds were removed from the chiller and prepared for sowing. Each type of seed was sown in 70 germination sponge cubes with 2-4 seeds per cube. The seeds in sponge cubes were then allowed to germinate in a plastic tray indoors away from sunlight for the first two days, followed by outdoor germination under the exposure of sunlight. Watering and monitoring were carried out daily to prevent seedlings from drying out. At nine days after sowing (DAS), thinning was done to ensure only one seedling germinated in each cube. At 12DAS, 27 seedlings of each type were selected and transplantation onto the HVFS was carried out. Nine seedlings of each lettuce cultivar were put into individual net pots and transplanted onto each row of the three LED treatments at 12DAS.

Treatments

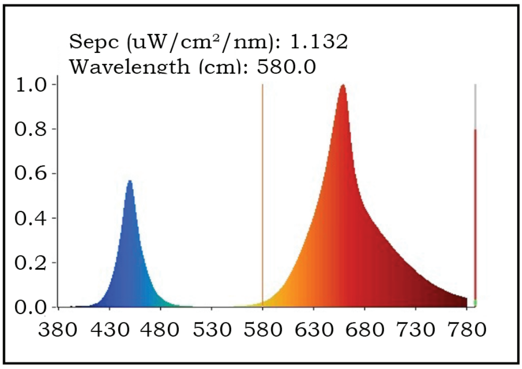
The LED light combinations and light intensity were measured using a spectral irradiance colorimeter (OHSP-350C, Hangzhou HopooLight and Color Technology Co. Ltd., China). Three different LED treatments were set up, with combinations of red, blue, and green lights: 77% red, 14% green, and 9% blue lights (77R:14G:9B) with a light intensity of 250fc; 7% red, 41% green, and 52% blue lights (7R:41G:52B) with a light intensity of 145fc; 25% red, 67% green, and 8% blue lights (25R:67G:8B) with a light intensity of 635fc. 25R:67G:8B served as the control treatment. The emission spectra of each light treatment are shown in Figure 1.

Setup of HVFS

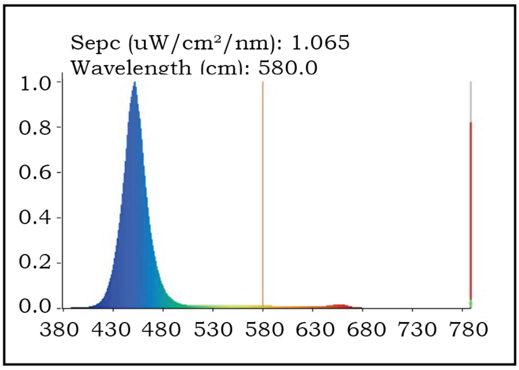
The HVFS was mainly made up of polyvinyl chloride (PVC) pipes, stainless steel rack, digital timers, LED light tubes, and water pump, with a reservoir tank at the bottom. All tubes were interlinked and eventually linked to the water reservoir tank to ensure that the water flowing down would be pumped up by the water pump again for nutrient solution recirculation. Fertilisers were added to supply nutrients for plant growth. About 6250 grams of solid Fertiliser A (Ultrasol® Calcium, SQM Holland BV, Westdorpe, Netherlands) and 6250 grams of solid Fertiliser B (Ultrasol® Lettuce Hydroponica, SQM Holland BV, Westdorpe, Netherlands) were dissolved in 25 litres of water, respectively, in two individual HDPE jerry cans as stock solutions. The compositions of fertilisers are shown in Table 1. 150mL of Fertiliser A (EC=5.434ppm) and 150mL of Fertiliser B (EC=9.747ppm) were added and mixed with about 50 litres of water in the reservoir tank, producing an electrical conductivity (EC) of 0.920ppm.

Maintenance of conditions in HVFS

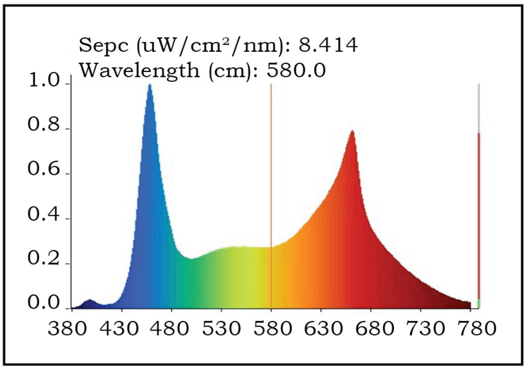
The HVFS was run indoors with an average temperature of 27±1°C. Digital timers were used to switch on the LED lights from 6 a.m. to 6 p.m., providing a 12-hour-light/12-hour-dark photoperiod cycle as study has shown that such photoperiod cycle promotes better lettuce growth (Hang *et al.*, 2019). The water pump was switched on for 24



a. 77R:14G:9B



b. 7R:41G:52B



c. 25R:67G:8B

Figure 1. Spectra distribution of three LED treatments on HVFS. **a.** 77R:14G:9B, **b.** 7R:41G:52B, and **c.** 25R:67G:8B. 77R:14G:9b=77% red, 14% green, and 9% blue lights; 7R:41G:52B=7% red, 41% green, and 52% blue light; 25R:67G:8B= 25% red, 67% green, and 8% blue light.

Table 1. Content of fertiliser A (Ultrasol® Calcium) and fertiliser B (Ultrasol® Lettuce Hydroponica)

Content	*Fertiliser A*	*Fertiliser B*
% Total nitrogen (N)	15.5	7.8
% Nitric nitrogen	14.3	7.8
% Ammoniacal nitrogen	1.2	
% Calcium oxide (CaO) water-soluble	26.3	
% Water-soluble phosphorus pentoxide (P_2O_5)		9.6
% Phosphorus pentoxide (P_2O_5) soluble in neutral ammonium citrate and in water		9.6
% Water-soluble potassium oxide (K_2O)		40.9
% Water-soluble sulphur trioxide (SO_3)		11.3
% Water-soluble boron (B)		0.04
% Water-soluble copper (Cu)		0.006
% Copper (Cu) 100% chelated by EDTA		0.006
% Water-soluble iron (Fe)		0.207
% Iron (Fe) 100% chelated by EDTA		0.207
% Water-soluble manganese (Mn)		0.035
% Manganese (Mn) 100% chelated by EDTA		0.035
% Water-soluble molybdenum (Mo)		0.009
% Water-soluble zinc (Zn)		0.042
% Zinc (Zn) 100% chelated by EDTA		0.042

EDTA: Ethylenediamine tetraacetic acid

hours for continuous recirculation of the nutrient solution. The pH of nutrient solution was maintained between 6.0 to 6.6. The EC of nutrient solution was measured daily and increased according to plant growth from about 1ppm to 2ppm. Modification of EC was done by adding dissolved fertilisers or tap water to the nutrient solution. Increment of pH from acidic condition was done by adding 1M NaOH.

Chlorophyll and anthocyanin contents

Chlorophyll and anthocyanin contents were measured using a handheld chlorophyll meter (SPAD-502, Konica Minolta Inc., Tokyo, Japan) and a portable anthocyanin meter (ACM-200, Opti-Science Inc., NH, USA), respectively, at 2 weeks after transplanting (WAT), 3WAT, and 4WAT by clamping the sensor compartment of the meter onto three different leaves on each plant. Then, the average measurement was calculated.

Fresh weight

At 4WAT, plants were harvested. The roots of lettuce were removed using scissors, leaving only the shoot part. Fresh weight was determined immediately using an electronic weighing balance (GF-61000, A&D Ltd, Japan).

Sample extraction

BL and IL under each treatment were separated into individual paper bags and dried in an oven (Memmert GmbH + Co. KG, Schwabach, Germany) set at 60°C for five days and two days, respectively, until a constant weight was achieved. The dried BL samples were ground into powders using a blender (HR2096/01, Koninklijke Phillips N.V., Amsterdam, Netherlands), while dried IL samples were ground into powders using a set of mortar and pestle. The powders were stored in individual zip bags, with three bags of powders for each type of lettuce under each treatment. The zip bags were stored in a freezer at -20°C until needed for the preparation of plant extracts. Preparation of plant extracts was done by dissolving the sample powder in 30% v/v of ethanol in 1:10 ratio (powder: solvent) (Hefnawy & Ramadan, 2013). In this case, 0.2 grams of powder was mixed with 2ml solvent and stirred using a spatula for even dissolution. 6 ml of distilled water was then added to dilute the homogenates. The mixture was poured into individual 50ml falcon tubes and incubated in hot water bath at 80°C for an hour, followed by centrifugation at 27°C and 10 000rpm for 10 minutes using a centrifuge (Eppendorf AG 22331, Eppendorf SE, Hamburg, Germany). After incubation, filtration was done using Filtres Fioroni filter papers grade 601 (150mm) to obtain the liquid extracts.

Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu colorimetric assay modified from Javanmardi *et al.* (2003). 75μl of extracts was mixed with 75μl of 0.2M Folic-Ciocalteu reagent and left still for three minutes. Then, 100μl of 7.5% w/v aqueous sodium bicarbonate was added and incubated at room temperature for an hour. 250μl of each mixture was pipetted into a 96-well plate (SPL Life Sciences Co., Ltd, Korea) with flat bottom. Absorbance was measured using a microplate reader (Epoch, BioTek Instruments Inc, Vermont, USA) at 760nm, with distilled water as the blank and ethanol as the negative control. The standard calibration curve was generated using 50-300mg/L gallic acid solutions. Total phenolic content was expressed as microgram of Gallic Acid Equivalents (mg GAE)/g of extract.

Total flavonoid content

Total flavonoid content of sample extracts was determined using a modified colorimetric method by Heimler *et al.* (2005). 0.25ml of sample extract was added into a mixture of 0.75ml of 5% sodium nitrate, 0.15ml of 10% aluminium chloride, and 0.5ml of 1M sodium hydroxide solutions in a 5ml Eppendorf tube, followed by the addition of distilled water until the volume reached 2.5ml. Incubation of the mixture was done at room temperature for 5 minutes. The absorbance was measured at 520nm using UV spectrophotometer (Libra S12, Biochrom Ltd, Cambridge, England) against distilled water as the blank. Total flavonoid content (mg QE/0.25ml extract) was determined by comparing the absorbance rate of the sample with the standard curve of quercetin hydrate at different concentrations (0 to 10000μM).

Total vitamin C content

Modified from Jagota & Dani (1982), 0.5ml of extract was mixed with 0.8ml of 10% trichloroacetic acid, shaken vigorously and kept on ice for 5 minutes. Centrifugation was done at 4°C and 10,000rpm for five minutes. Next, 0.2ml of the mixture was diluted using 2ml of distilled water. 0.2ml of 0.2M Folin-Ciocalteu reagent was added and shaken vigorously. The mixture was left at room temperature for ten minutes and the absorbance was read at 760nm using a UV spectrophotometer (Libra S12, Biochrom Ltd, Cambridge, England) against distilled water as the blank. Vitamin C content was estimated using an ascorbic acid standard curve (0-0.03g/ml).

Antioxidant activity

Modified DPPH radical scavenging method from Zhang & Hamauzu (2004) was used to measure antioxidant activity. 0.2ml of sample extract was added into 1ml of 0.1mM DPPH. Absorbance was measured immediately at 521nm using a UV spectrophotometer (Ultrospec 8000, Biochrom Ltd, Cambridge, England) against distilled water as the blank, and after three minutes at room temperature.

The sample extract was replaced by distilled water to act as negative control.

Antioxidant activity was estimated using the following equation:

$$\text{Percentage Inhibition DPPH} = \frac{\text{Initial absorbance} - \text{Absorbance after 3 min}}{\text{Initial absorbance}} \times 100$$

Experimental design and statistical analysis

This experiment adapted a completely randomised design and triplicate measurements. Data were analysed using IBM SPSS Statistics for Windows version 28.0 (IBM Corp, Armonk, New York), with normally distributed data subjected to two-way ANOVA and Tukey's test at 5% significance level. Data of vitamin C content was subjected to $\log^{10}$ transformation before two-way ANOVA analysis. Chlorophyll and anthocyanin contents were not normally distributed and were subjected to Independent-Samples Kruskal-Wallis Test. Table 2 provides an overview of all the data collected.

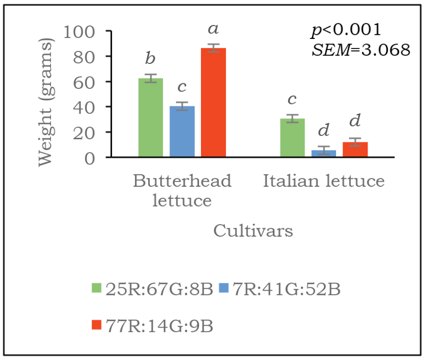
RESULTS

Fresh yield

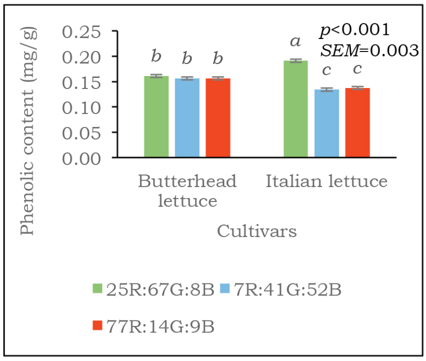
BL produced significantly ($p<0.001$) higher fresh yields than IL. Overall, 77R:14G:9B and 25R:67G:8B treatments generated significantly ($p<0.001$) greater fresh yields than 7R:41G:52B treatment. The interactions between cultivars and LED treatments were significant ($p<0.001$). As shown in Figure 2a, BL and IL responded differently to LED treatments, in which the highest fresh weight of BL was produced under the 77R:14G:9B treatment, while the highest fresh weight of IL was produced under the 25R:67G:8B treatment.

Chlorophyll content

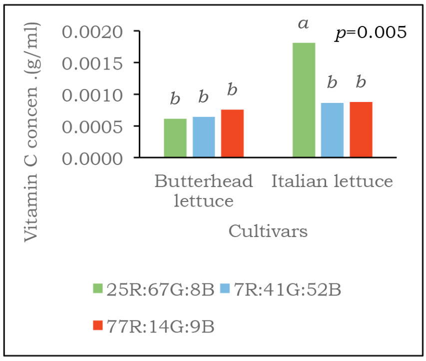
The chlorophyll content of BL was significantly ($p<0.001$) higher than IL. No significant effect of LED treatments on chlorophyll content was observed.



a. Fresh yield



b. Phenolic content



c. Vitamin C concentration

Figure 2. Effect of interaction between cultivar and LED treatment on **a.** fresh yield, **b.** phenolic content, and **c.** vitamin C concentration (n=3). Bars with the same letter are not significantly different using Tukey's test at 5% significance level. Error bars are SEM values. 25R:67G:8B= 25% red, 67% green and 8% blue light; 7R:41G:52B=7% red, 41% green, and 52% blue light; 77R:14G:9B= 77% red, 14% green, and 9% blue light. SEM=standard error of mean.

Table 2. Effect of cultivars, LED treatments, and their interactions on the fresh weight, chlorophyll content, anthocyanin content, phenolic content, antioxidant activity, vitamin C concentration and flavonoid content of lettuce after harvest (n=3)

Treatment	*Fresh weight (grams)*	*Chlorophyll content (SPAD)*			*Anthocyanin content (ACI)*			*Phenolic content (mg GAE)/g*	*Flavonoid content (mg QE/0.25ml extract)*	*Vitamin C concentration (g/ml)*	*Antioxidant activity (% inhibition DPPH)*
		2WAT	*3WAT*	*4WAT*	*2WAT*	*3WAT*	*4WAT*				
Cultivars (C)											
Butterhead lettuce (BL)	63.0^{a}	37.4^{a}	39.8^{a}	39.2^{a}	5.00^{a}	5.20^{a}	6.20^{a}	0.158	4.26	0.000667^{b}	15.2
Italian lettuce (IL)	16.0^{b}	15.7^{b}	16.7^{b}	20.7^{b}	1.90^{b}	1.90^{b}	2.50^{b}	0.154	4.14	0.001109^{a}	17.6
SEM	1.77	-	-	-	-	-	-	0.002	0.140	-	1.77
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.200	0.558	<0.001	0.369
LEDs											
77R:14G:9B	49.2^{a}	24.4	25.4	31.7	3.35	3.35	4.25	0.147^{b}	3.87^{b}	0.000813^{ab}	12.7
7R:41G:52B	22.8^{b}	27.7	28.0	29.5	3.45	3.70	3.90	0.145^{b}	3.92^{b}	0.000745^{b}	18.2
25R:67G:8B (Control)	46.4^{a}	30.6	29.5	29.0	3.70	3.95	4.45	0.176^{a}	4.81^{a}	0.00105^{a}	18.3
SEM	2.17	-	-	-	-	-	-	0.002	0.171	-	2.18
p-value	<0.001	0.431	0.717	0.522	0.696	0.764	0.699	<0.001	0.003	0.037	0.162
Interaction (*p*-value)											
C x LEDs	<0.001	-	-	-	-	-	-	<0.001	0.060	0.005	0.975

WAT: Week after transplanting; GAE: Gallic acid equivalent; QE: Quercetin equivalent; *SEM*: Standard error of mean.

Means in a column indicated by the same letter are not significantly different using Tukey's test at 5% significance level. Vitamin C content was not normally distributed and the data shown were back-transformed from log^{10} transformation method. Medians for chlorophyll and anthocyanin contents indicated by the same letter are not significantly different using the Independent-Samples Kruskal-Wallis test at 5% significance level. 77R:14G:9B= 77% red, 14% green, and 9% blue light; 7R:41G:52B=7% red, 41% green, and 52% blue light; 25R:67G:8B= 25% red, 67% green, and 8% blue light.

Anthocyanin content

Overall, BL contained significantly ($p<0.001$) higher concentration of anthocyanin than IL. However, the distribution of anthocyanin content was the same across all three LED treatments.

Phenolic content

The phenolic contents of BL and IL were not significantly different. The effects of different LED treatments on phenolic contents were significantly ($p<0.001$) different, with 25R:67G:8B treatment resulting in significantly higher concentration than both 77R:14G:9B and 7R:41G:52B treatments, which were not significantly different from each other. The interaction between types of cultivars and LED treatments was significant too. Referring to Figure 2b, BL was found to respond similarly under all three LED treatments, while IL under the 25R:67G:8B treatment was found to produce significantly higher phenolic concentration than 77R:14G:9B and 7R:41G:52B treatments.

Flavonoid content

The flavonoid contents of BL and IL were not significantly different. LED treatments had significant ($p=0.003$) effects on the average flavonoid content, with 25R:67G:8B treatment resulting in the highest content, followed by 7R:41G:52B and 77R:14G:9B treatments, which had no significant difference between them. Also, BL and IL responded similarly to different LED treatments.

Vitamin C concentration

IL had significantly ($p<0.001$) higher vitamin C than BL. LED treatments also had significant ($p=0.037$) effects on vitamin C accumulation in lettuce, with 25R:67G:8B treatment resulting in the highest content and 7R:41G:52B treatment resulting in the lowest content, while the effect of 77R:14G:9B treatment was not significantly different from them. The two cultivars were also found to respond significantly ($p=0.005$) differently to the treatments. As shown in Figure 2c, BL responded similarly to all three treatments, whereas IL under the 25R:67G:8B treatment produced significantly higher vitamin C than 77R:14G:9B and 7R:41G:52B treatments.

Antioxidant activity

Neither types of cultivars nor LED treatments showed significant effects on antioxidant activity and their interactions. Therefore, antioxidant activity was not influenced by all these factors.

DISCUSSION

Fresh yield

The high proportion of red light (R) (600-700nm) in the 77R:14G:9B treatment could promote the conversion of inactive phytochrome (red light-absorbing form) to active phytochrome (far-red light-absorbing form), which regulates the downstream components of the light signalling pathway and plays a role in initiating germination, de-etiolation, chlorophyll biosynthesis, and leaf expansion (Tripathi *et al.*, 2019). For 25R:67G:8B treatment, the high proportion of green light (G) could penetrate deep into the mesophyll layer of the leaf and increase carbon dioxide fixation rate, where R and blue lights (B) are deficient to drive photosynthesis (Smith *et al.*, 2017). The lower result of 7R:41G:52B treatment might be related to overstimulation of cryptochrome (CRY1) by B, hence inhibiting gibberellin biosynthesis, which is essential for plant growth (Matsuo *et al.*, 2019).

Chlorophyll and anthocyanin contents

Chlorophyll and anthocyanin contents were not affected by LED treatments. These results might be due to some limitations in this experiment such as light intensity and genetic variation. A

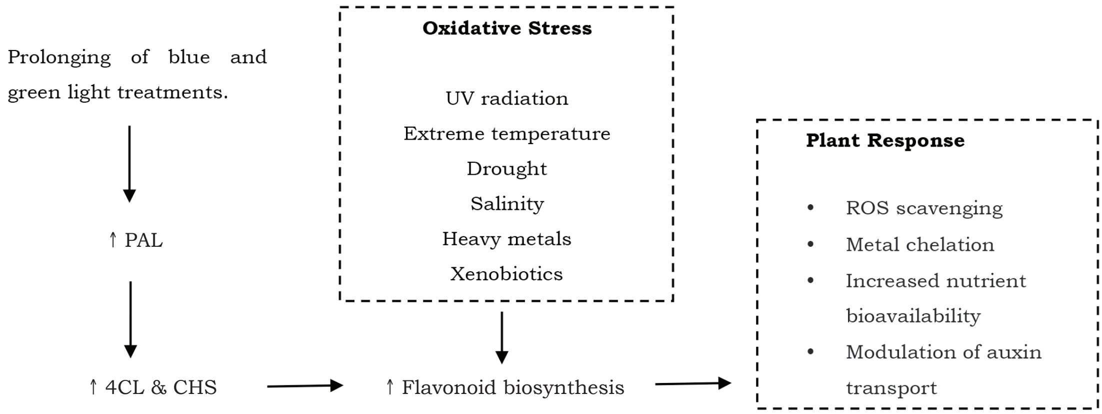


Figure 3. The impacts of light and oxidative stress on the production of flavonoids and plant response. Adapted from Liu *et al.* (2018) and Ghitti *et al.* (2022).
PAL=phenylalanine ammonia lyase; 4CL=4-coumaroyl CoA-ligase; CHS=chalcone synthase; ROS=Reactive Oxygen Species

study has shown that the accumulation of chlorophyll in lettuce was promoted by a light intensity of 350µmol/m^2/s to 600µmol/m^2/s at 23°C to 30°C, so the accumulation in our study might have been influenced by the low light intensity provided for the growth of lettuce (Zhou, Li & Wang, 2022). Meanwhile, the low, insignificant difference of anthocyanin present in both lettuce cultivars might be due to the lack of anthocyanin pigments, which are responsible for the purple or blue colour in green leaves.

Phenolic content

Higher phenolic content was observed under the 25R:67G:8B treatment. Our result was consistent with a study done by Fazal *et al.* (2016), which showed enhanced total phenolic production by G in a medicinal plant known as self-heal. According to Son *et al.* (2012), phenolic concentration and antioxidant capacity are effectively promoted by monochromatic B due to the activation of the phenylalanine ammonium lyase (PAL) gateway enzyme during the phenolic compounds' biosynthesis. Meanwhile, a study has shown that supplementation of G to B could help phenolic compound accumulation, as shorter G wavelengths of less than 530nm are also recognised as a part of the canonical cryptochrome and phototropin B response, while longer G wavelengths of about 570nm could counteract B cryptochrome activation and hence phenolic compound accumulation (Brazaitytė *et al.*, 2022). Therefore, higher phenolic content might have been induced as a G wavelength of less than 530nm was provided to the lettuce in this experiment. Shading by other plants on the HVFS might be a reason for the insignificant difference in BL.

Flavonoid concentration

Higher flavonoid concentration was found under the 25R:67G:8B treatment. B and G were shown to increase flavonoid biosynthesis through the mechanisms shown in Figure 3 (Liu *et al.*, 2018; Ghitti *et al.*, 2022). In this experiment, higher production of flavonoids was found under the 25R:67G:8B treatment than the 7R:41G:52B treatment, which might be due to the higher light intensity of the former treatment. Also, studies have shown that the production of flavonoids is induced by certain factors to protect against harsh environments (Figure 3) like drought, cold, heat, UV radiation, and pathogens, acting as detoxifying agents, allelopathic compounds, and signalling molecules (Liu *et al.*, 2018; Ghitti *et al.*, 2022). The potential stress factor in our study might be the temporarily insufficient nutrients provided during 2WAT. Therefore, the additive effects of LED treatment with different oxidative stress factors might have resulted in different levels of flavonoid production, as obtained by other studies. A similar result was obtained by Klimek-Szczykutowicz *et al.* (2022) that a combination of 50% G, 35% R, and 15% B, which was similar to the 25R:67G:8B treatment in this experiment, had resulted in a significantly higher flavonoid concentration in watercress.

Vitamin C concentration

Higher vitamin C concentrations were found under the 25R:67G:8B and 77R:14G:9B treatments. Many studies have shown different results under different LED treatments, which might be due to different stress factors that have occurred. The stress factors that might have occurred in our study were suboptimal temperature (27±1°C) and light intensity. A study has shown higher vitamin C accumulation in sweet corn seedlings at a cultivation temperature of 40°C (Xiang *et al.*, 2020). Another study has also found higher vitamin C accumulation in tomatoes at higher light levels (Ntagkas *et al.*, 2019). In our study, 25R:67G:8B and 77R:14G:9B treatments provided stronger light intensities than the 7R:41G:52B treatment, which resulted in higher vitamin C accumulation.

Antioxidant activity

Antioxidant activity was not affected by LED treatments. Several research studies have also revealed the negligible impact of various lighting conditions on antioxidant properties. For example, the antioxidant potential of purple basil callus under different lighting conditions was not much different as compared to other similar studies conducted using DPPH assay (Nazir *et al.*, 2020). This might be due to certain limitations of the DPPH assay, in which the yellowish-green pigments or colours of the extract might have affected the light absorption in the spectrophotometer, hence interfering with the radical scavenging potential determination.

It might also be due to the development of both non-enzymatic and enzymatic antioxidant systems in plants to combat excessive reactive oxygen species. It has been established that LED light affects not only antioxidant contents, but also antioxidative enzymes, of which mechanisms are yet to be discovered (Adil, Haider Abbasi & Ul Haq, 2019). Since no test was conducted to measure the antioxidative enzymes in this study, the phenolic compounds, flavonoids, and vitamin C concentrations measured might not reflect the actual total antioxidant activity. When processed with heat and consumed, both non-enzymatic and enzymatic antioxidant compounds might be affected or reduced.

Strengths and limitations

This research has implemented an innovative approach to grow crops indoors in a vertical way, which has proven the practicality of the HVFS, which is both energy-efficient and space-saving. It has been carried out under a well-controlled, chemical-free, and pest-free environment, which significantly reduced the effects of various external factors that may be encountered using the normal farming method.

Despite the significant findings, several limitations should still be addressed. Firstly, the small sample size used might not represent the general growth or phytochemical contents of lettuce cultivars. We have adapted the sample size of n=3 due to practical constraints, such as resource availability and time constraint, though the experiment was conducted under controlled, homogeneous conditions.

Secondly, shading by self-leaves due to overgrowth might have affected photosynthesis and hence nutrient accumulation. Thirdly, tip burn on lettuce was observed due to insufficient calcium supplied, but calcium fertiliser was added once this was realised. Next, the temperature for plant growth might not be optimal as the experiment was conducted indoors with an average temperature of 27±1°C. A study conducted by Choi, Paek & Lee (2000) found that the optimum day growing temperatures for butterhead and leaf lettuces were 22 to 26°C and 20 to 24°C during the early and middle growth stages, respectively, while the optimum night temperature was 15 to 20°C. Also, the long storage time of samples after harvest might have caused some degree of phytochemical degradation.

CONCLUSION

In conclusion, this study observed that BL outperformed IL in terms of yield, chlorophyll and anthocyanin contents, while for vitamin C content, it was the opposite. Also, a higher R proportion (77R:14G:9B) positively affected the lettuce's fresh yield, while a higher G proportion (25R:67G:8B) positively affected phytochemical (phenolic, vitamin C, and flavonoid contents) accumulation. No significant difference was observed for chlorophyll content, anthocyanin content, and total antioxidant activity under different LED treatments.

To address the issue of food insecurity related to rising urbanisation rate, cultivating lettuce cultivars under the 77R:14G:9B or 25R:67G:8B treatment might aid in producing the greatest yield of crops. To address the issue of rising CVD, cultivating lettuce under the 25R:67G:8B treatment could aid in producing the greatest phenolic, vitamin C, and flavonoid contents. Thus, this study provided preliminary data for optimising LED treatment combinations for optimal plant growth and phytochemical content, which are essential for addressing food security and health issues. This study also added to the understanding of LED-integrated HVFS, and the inclusion of variable indicators has provided a more comprehensive knowledge of how LED treatments affect the quality of crops. Aside from LED-integrated HVFS, future research can also be done to look into the potential use of solar photovoltaic hydroponic system in order to achieve a more sustainable form of agricultural production.

Acknowledgement

This research was fully funded by School of Biosciences, University of Nottingham Malaysia. I would like to express my sincere gratitude to Dr. Ajit Singh for the great support and contributions throughout the study, which has been pivotal in the completion of this work.

Authors' contributions

Koh MX, the main investigator, conceptualised, designed, and conducted the study. Koh MX also led the data collection, data analysis and interpretation, drafted and reviewed the manuscript. Singh A contributed to the design of the study, data analysis and interpretation, drafting and review of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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Effect of Bambara groundnut (*Vigna subterranea*) on Firmicutes and Bacteroidetes, *FGF21* gene expression, and liver histopathology in mice with low-protein diet

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ABSTRACT

Introduction: The number of Firmicutes and Bacteroidetes in the intestine is influenced by diet. Gut microbiota and fibroblast growth factor 21 (*FGF21*) form a liver-gut axis that mediates the body's response to protein restriction. Bambara groundnut (*Vigna subterranea*), which contains high levels of amino acids, has the potential to be a source of protein. This study aimed to determine the effect of Bambara groundnut on the number of Firmicutes and Bacteroidetes, *FGF21* gene expression, and liver histopathology in mice fed with a low-protein diet. **Methods:** A total of 25 mice were divided into five groups: normal protein diet (N), low-protein diet (LP), and low-protein diet with supplementation of 100 g (LPLB), 200 g (LPMB), and 300 g (LPHB) of Bambara groundnut, respectively. After 2 months of intervention, mice were sacrificed, the number of Firmicutes and Bacteroidetes in the intestines and faeces, as well as *FGF21* gene expression were analysed, while liver histopathology was visualised. **Results:** Results showed that Bambara groundnut supplementation increased the growth of Firmicutes and Bacteroidetes, decreased *FGF21* gene expression, and reduced liver inflammation caused by a low-protein diet. **Conclusion:** Bambara groundnut supplementation has the potential to increase the amount of Firmicutes and Bacteroidetes, decrease the expression of adaptive stress gene *FGF21*, and improve the degree of liver inflammation in a low-protein diet.

Keywords: Bacteroidetes, bambara groundnut, *FGF21*, Firmicutes, low-protein diet

INTRODUCTION

Dietary protein has been known to influence the composition of microbiota in the digestive tract, including Firmicutes and Bacteroidetes. Studies on mice have provided evidence that dietary protein can affect not only the diversity, but also the overall microbial biomass (Bartlett & Kleiner, 2022). A low-protein diet decreases the number of

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doi: https://doi.org/10.31246/mjn-2024-0012

Firmicutes and Bacteroidetes, which is related to homeostasis and pathological pathways. Therefore, many therapeutic strategies are suggested to achieve a proper Firmicutes and Bacteroidetes ratio to maintain homeostasis (Abenavoli *et al.*, 2019). Zhao *et al.* (2020) found that 2% dietary protein withdrawal markedly reduced the ratio of Firmicutes to Bacteroidetes. The response of the microbiota to host food produces metabolites that have significant physiological effects on the host. Thus, gastrointestinal microbiota can be used as a parameter of host nutrition.

In addition, a low-protein diet also affects the expression of fibroblast growth factor 21 (*FGF21*) gene (Zhao *et al.*, 2020), which plays a role in controlling nutritional status by regulating glucose, lipid, and energy metabolisms (Tezze *et al.*, 2019). *FGF21* is expressed in several metabolically active organs, including liver, and is only released during a low-protein diet (Solon-Biet *et al.*, 2016), so consumption of a high-protein diet reduces *FGF21* expression (Alemán *et al.*, 2019). Protein restriction activates the GCN2/pEIF2α/ATF4 pathway involved in upregulating *FGF21* gene expression; high availability of amino acids can suppress the signalling pathway (Laeger *et al.*, 2014).

Bambara groundnuts (*Vigna subterranea*) are a source of vegetable protein, which contain 15–25% protein (Oluwole *et al.*, 2022). Bambara groundnuts have a high content of essential amino acids, including isoleucine, leucine, methionine, phenylalanine, threonine, and valine (Mune Mune *et al.*, 2011). Therefore, this study examined the benefits of Bambara groundnuts on the number of intestinal microbiota, especially Firmicutes and Bacteroidetes, and the expression of the *FGF21* gene, as well as the liver histopathological appearance of mice given a low-protein diet.

MATERIALS AND METHODS

Materials

This research used Bambara groundnuts with black testa. Bambara groundnuts in the form of fine powder were sent for proximate analysis at the Centre of Food and Nutrition Studies. The fine powder was also dissolved in ethanol solvent for phenolic compound analysis using high resolution mass spectrometry (HRMS) at the Integrated Research and Testing Laboratory (Q Exactive™ High Resolution Mass Spectrometer, Thermo Fisher Scientific, Waltham, MA, USA).

Animals

In this study, the number of animals needed was calculated by the Federer formula, which is $(n - 1)(t - 1) > 15$. The n value determines the number of animals per group, while the t value determines the number of treatment groups. Based on the calculation, a total of 25 mice (*Mus musculus*) of the DDy strain, four weeks old, with a body weight of ±20 grammes (g), were divided into five groups: normal protein diet (N); low-protein diet (LP); low-protein diet with low 100 g (LPLB); moderate 200 g (LPMB); and high 300 g (LPHB) Bambara groundnut supplementation. The mice were obtained from the integrated research and testing laboratory and the study was done after receiving approval from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (Ref. No. KE/FK/0879/EC/2021). The diet compositions are presented in Table 1.

All study groups were acclimatised for one week with standard care (dark cycle: light 12:12 hours and room temperature 22-25°C) and fed with the standard diet of modified AIN 93 M formulation. After the acclimatisation period, the mice were given dietary treatment according to their groups for

Table 1. Diet composition per 1 kg

Ingredients	*Treatment groups*				
	N (g)	*LP (g)*	*LPLB (g)*	*LPMB (g)*	*LPHB (g)*
Maizena	621	661	561	461	361
Casein	140	100	100	100	100
Sucrose	100	100	100	100	100
Bambara groundnut	0	0	100	200	300
Corn/Soybean oil	40	40	40	40	40
Alpha cell	50	50	50	50	50
Mineral mix	35	35	35	35	35
Vitamin mix	10	10	10	10	10
Methionine	2	1.8	1.8	1.8	1.8
Choline chloride	3	2.5	2.5	2.5	2.5
Tert-Butylhydroquinone	0.00	0.01	0.01	0.01	0.01
Total	1,000	1,000.3	1,000.3	1,000.3	1,000.31

N: Normal protein (14%), LP: Low-protein (10%), LPLB: Low-protein (10%) with 100g of Bambara groundnut supplement, LPMB: Low-protein (10%) with 200g of Bambara groundnut supplement, LPHB: Low-protein (10%) with 300g of Bambara groundnut supplement

two months. Feed intake was calculated based on the amount of feed given and then deducted by the amount of feed remaining the next day. The amount of protein intake was calculated based on the percentage of protein composition in the feed for each group as follows: N 14% (140 g/kg), LP 10% (100 g/kg), LPLB 11.2% (112.89 g/kg), LPMB 12.5% (125.78 g/kg), and LPHB 13.8% (138.67 g/kg). Around 0.5 g of faeces was collected at the end of the experiment by removing the mice for 5-10 minutes, then waiting for the mice to excrete their faeces. Faeces was collected in a sterile tube containing preservation buffer (PB) and stored at -20^{o}C. The PB contained 20 mM ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate, 25 mM sodium citrate trisodium salt dihydrate, and 5.3 M ammonium sulphate. For each treatment, around 2 ml of PB buffer was used. The duration between collection and analysis was about 2-3 hours. After that, all mice were terminated and anaesthetised with a combination injection of 0.5 ml of Ketamine and 0.25 ml of Xylazine. Mice were euthanised by neck dislocation. Liver tissue and intestines were then collected for further analysis.

Examination of gastrointestinal microbiota

Deoxyribonucleic acid (DNA) was extracted from faeces using the Quick DNA Faecal/Soil Microbe Miniprep Kit (D6010; Zymo Research, Irvine, CA, USA). The relative abundance levels of Firmicutes and Bacteroidetes were determined using the quantitative real-time polymerase chain reaction (qRT-PCR) SensiFAST™ SYBR® No-ROX Kit (BIO-98005; Bioline, London, UK).

Primer sequences for Firmicutes were:

Sense : 5'-GTCAGCTCGTGTCGTGA-3'

Antisense : 5'-CCATTGTAACGTGTGT-3'

Primer sequences for Bacteroidetes were:

Sense : 5'-AGCAGCCGCGGTAAT-3'

Antisense : 5'-CTAGCATTTCACCGCTA-3'

Examination of *FGF21* gene expression

Total RNA was extracted from liver cells using the Direct-zol RNA Miniprep Plus (R2071; Zymo Research, Irvine, CA, USA) according to manufacturer's protocol. It was then reverse transcribed to complementary DNA using the SensiFAST cDNA Synthesis Kit (Bio-65054; Meridian Bioscience, Cincinnati, OH, USA) according to manufacturer's protocol. The expression levels of *FGF21* were determined using the quantitative real-time polymerase chain reaction (qRT-PCR) SensiFAST™ SYBR® No-ROX Kit (BIO-98005; Bioline, London, UK). Relative expression was calculated by the 2–ΔΔCt method.

Primer sequences for *Mus musculus FGF21* were:

Sense :
5'-GAGGATGGAACAGTGGTAGG-3'

Antisense :
5'-CAAAGTGAGGCGATCCATAGAG-3'

Primer sequences for Mus musculus Beta Actin were:

Sense :
5'-AAGATCAAGATCATTGCTCCTCC-3'

Antisense :
5'-TAACAGTTCCGCCTAGAAGCA-3'

Statistical analysis

The data collected were analysed using IBM SPSS Statistics for Windows version 20.0 (IBM Corp., Armonk, New York, NY, USA). Data with normal distribution were analysed using the one-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) post-hoc test. Data with skewed distribution were analysed using the Kruskal-Wallis test. The *p*-value was considered significant if it was lower than or equal to 0.05 ($p \leq 0.05$).

Results of the gene expression of intestinal and faecal Bacteroidetes and Firmicutes showed normally distributed data (indicated by $p>0.05$). Because the data were normally distributed, a homogeneity test was carried out ($p>0.05$ indicated that the data were homogeneous). Next, a follow-up test was carried out with Tukey's HSD test ($p<0.05$). Meanwhile, the results of *FGF21* gene expression showed abnormally distributed data, therefore analysis was carried out using a non-parametric test (Kruskal-Wallis test) ($p<0.05$). To determine significant notation, the Mann-Whitney test was carried out ($p<0.05$).

Histopathology

Hepatic tissues were fixed in a neutral buffered formalin solution (10%) and embedded in paraffin. Then, 4-μm thick sections were placed on adhesive slides and stained with haematoxylin-eosin. Samples were visualised using an Olympus microscope (Olympus CX21) at 100x-400x magnification using standard procedure. All imaging was performed with the group identity blinded. Images were then quantified using imaging software (Optilab; Miconos, Yogyakarta, Indonesia). Histologic examination of the hepatic cells was reviewed by a single expert pathologist who was blinded to all other features of the sample's characteristics.

All samples were evaluated for the presence or absence of the following liver damage features: sinusoidal dilatation, cloudy swelling, and inflammation. Centrilobular sinusoidal dilatation and cloudy swelling were graded according to the altered hepatic lobule zone. Liver inflammation was graded as mild if it only extended to the periportal area, mild if it extended to the periportal and intraparenchymal areas, and severe if it had reached the periportal and intraparenchymal areas with bridging necrosis (Trefts, Gannon & Wasserman, 2017).

RESULTS

Analysis of Bambara groundnut

Proximate analysis showed that Bambara groundnuts contained 10.61% water, 3.15% ash, 12.89% protein, 7.24% fat, and 66.11% carbohydrate (by difference). In proximate analysis, the Soxhlet method with Weibull modification was used for fat content analysis, the Kjedahl method for protein content analysis, the gravimetric method for water and ash analyses, and for analysis of carbohydrate content, it was a deduction of all proximate components. Amino acid analysis showed that L-lysine was the highest amount of essential amino acid in Bambara groundnuts (8,021.00 mg/kg), followed by L-histidine (4,109.70 mg/kg), L-phenylalanine (3,255.80 mg/kg), L-leucine (2,716 mg/kg), L-isoleucine (2,452.20 mg/kg), L-valine (549.10 mg/kg), L-methionine (222.60 mg/kg), L-tryptophan (93.30 mg/kg), and L-threonine (78.10 mg/kg). High Resolution Mass Spectrometry (HRMS) analysis also detected some phenolic

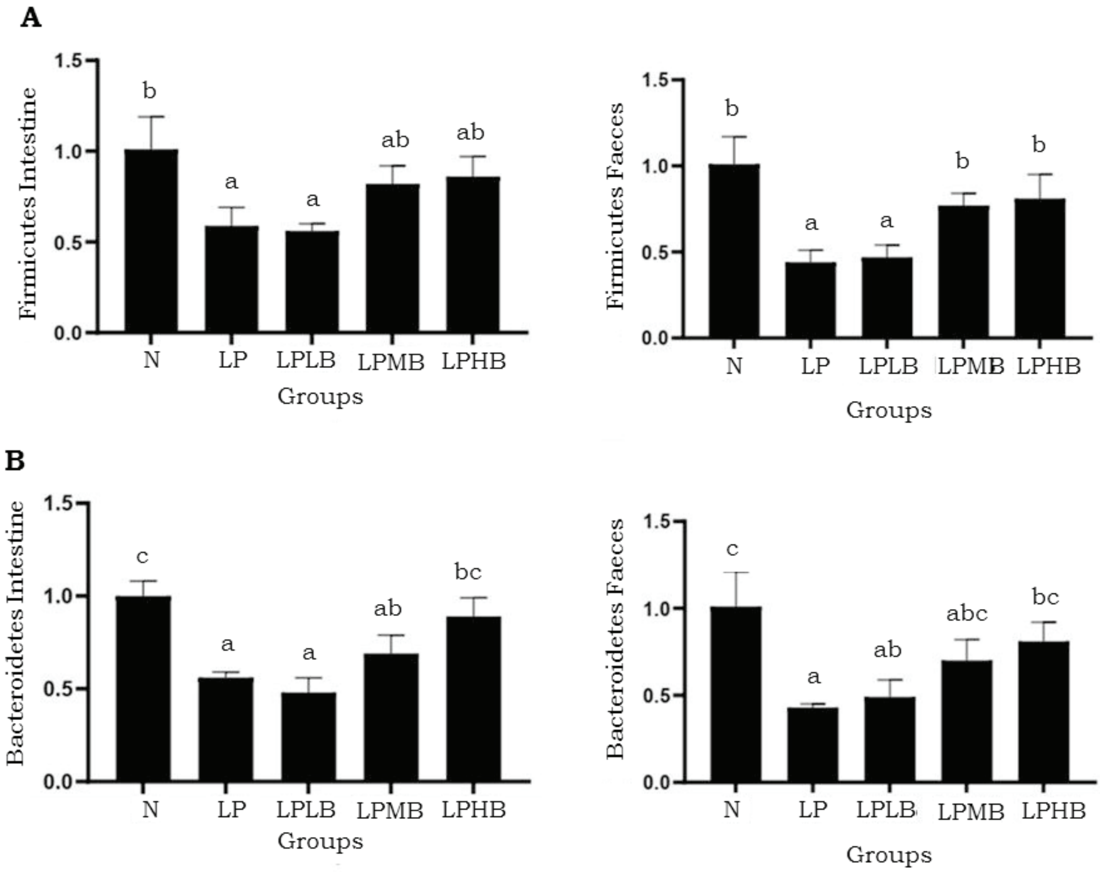

Figure 1. The effect of Bambara groundnut supplementation on the gene expression of Firmicutes (A) and Bacteroidetes (B) in low-protein diet mice

Note: (N) Normal protein diet; (LP) Low-protein diet; (LPLB) Low-protein diet + 100g of Bambara groundnut; (LPMB) Low-protein diet + 200g of Bambara groundnut; (LPHB) Low-protein diet + 300g of Bambara groundnut. Data are shown as means±standard deviation. Different letters of a, b, and c indicate significant differences between groups ($^{*}p<0.05$).

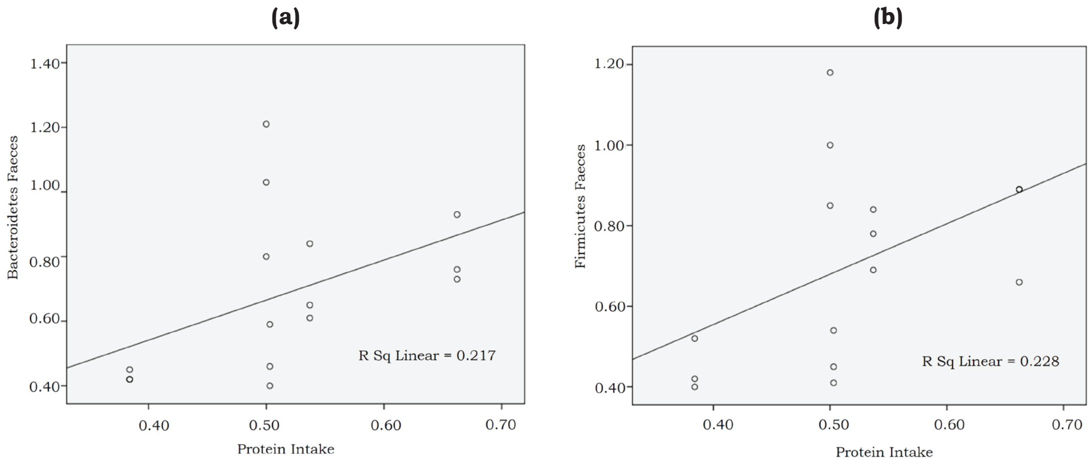

Figure 2. Correlation between protein intake and the number of (a) Bacteroidetes and (b) Firmicutes in faeces.
Correlation coefficient: 0.400*, Sig. (2-tailed): 0.048

compounds in Bambara groundnuts, such as genistin (432.10 g/mol), daidzin (416.11 g/mol), myricetin (318.03 g/mol), genistein (270.05 g/mol), and daidzein (254.06 g/mol).

Effect of Bambara groundnuts on the number of Firmicutes and Bacteroidetes in low-protein diet mice

This research revealed that a low-protein diet caused the number of Firmicutes and Bacteroidetes to greatly decrease. However, the supplementation of Bambara groundnuts did not cause significant changes to the Firmicutes and Bacteroidetes ratio in the intestine and faeces of mice (Bartlett & Kleiner, 2022). Figure 1 shows a significant increase in the number of Firmicutes and Bacteroidetes in the intestine and faeces after Bambara groundnut supplementation. The supplementation of Bambara groundnuts as much as 200 g (LPMB) and 300 g (LPHB), respectively, was proven to give better results compared to supplementing Bambara groundnuts at only 100 g. As shown in Figure 2, it appears that the higher the protein intake, the higher the number of Firmicutes and Bacteroidetes.

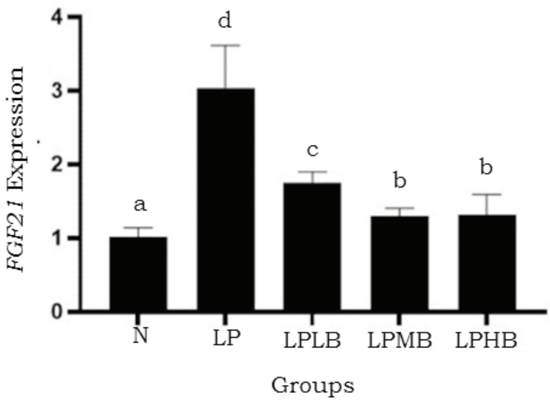

Figure 3. The effect of Bambara groundnut supplementation on the gene expression of *FGF21* in low-protein diet mice
Note: (N) Normal protein diet; (LP) Low-protein diet; (LPLB) Low-protein diet + 100g of Bambara groundnut; (LPMB) Low-protein diet + 200g of Bambara groundnut; (LPHB) Low-protein diet + 300g of Bambara groundnut. Data are shown as means±standard deviation. Different letters of a, b, c and d indicate significant differences between groups (*$p<0.05$).

Effect of Bambara groundnuts on *FGF21* gene expression in low-protein diet mice

A low-protein diet could significantly increase *FGF21* gene expression compared to control group (Figure 3). All low-protein diet groups supplemented with Bambara groundnuts showed a decrease in *FGF21* gene expression. Both Bambara groundnut supplements at 200 g and 300 g, respectively, were found to give better results than Bambara groundnuts supplement at 100 g.

Effect of Bambara groundnuts on liver histopathology

Figure 4 shows that Bambara groundnut supplementation reduced inflammation features in liver histopathologic findings. Mice on a low-protein diet with Bambara groundnut supplementation had lower scores of sinusoidal congestion, cloudy swelling, and inflammation compared to low-protein diet mice.

DISCUSSION

Bacteria in the intestine are represented by more than 1000 species that belong to six dominant phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Bacteria from the phyla Firmicutes and Bacteroidetes are the most common, representing 90% of intestinal microbiota (Rinninella *et al.*, 2019). The ratio between these two phyla has been associated with maintaining homeostasis and changes in this ratio can lead to various pathologies and disorders (Lopetuso *et al.*, 2013; Statovci *et al.*, 2017; Park & Kim, 2020). Balancing the intestinal ecosystem is an important aspect of maintaining normal human body function (Abenavoli *et al.*, 2019). In addition to being a regulator of digestive processes, the intestinal microbiota also plays a role in metabolic processes and systemic immune responses (Statovci *et al.*, 2017). Several studies suggest that changes in the composition of intestinal microbiota (increasing F/B ratio) can be influenced by dietary factors such as nutrients and bioactive compounds from foods, contributing to direct and indirect effects on the intestinal microbiota (Zou *et al.*, 2020). This is in accordance with this study, where a low-protein diet significantly decreased the number of Firmicutes and Bacteroidetes compared to the control mice group (adequate protein diet) and supplementation of Bambara groundnuts in low-protein diet mice increased the number of Firmicutes and Bacteroidetes. Several studies regarding low-protein diets stated that protein deficiency will affect the intestinal microbiota (Hsu *et al.,* 2021; Masuoka *et al.*, 2020) and research on mice showed that protein levels in the diet not only change the diversity, but also the overall biomass of microbes in the intestine (Bartlett & Kleiner, 2022).

To understand the interactions between dietary protein and the microbiota, one must first consider the process of protein digestion. Protein digestion and absorption influence how much protein reaches the colon, where most of the microbiota resides. This undigested dietary protein serves as a substrate for microbial metabolism. Undigested dietary protein that reaches the large intestine is hydrolysed into peptides and amino acids, which may be used by the microbiota as a source of carbon, nitrogen, and energy through some diverse metabolic pathways, thus influencing the abundance of the microbiota (Moreno-Pérez *et al.*, 2018; Oliphant & Allen-Vercoe, 2019). Furthermore, a study from Masuoka *et al.* (2020) showed that dietary protein quantity was influential on microbiota, particularly through providing nitrogen, a limiting nutrient for the intestinal microbiota. Similar evidence was found

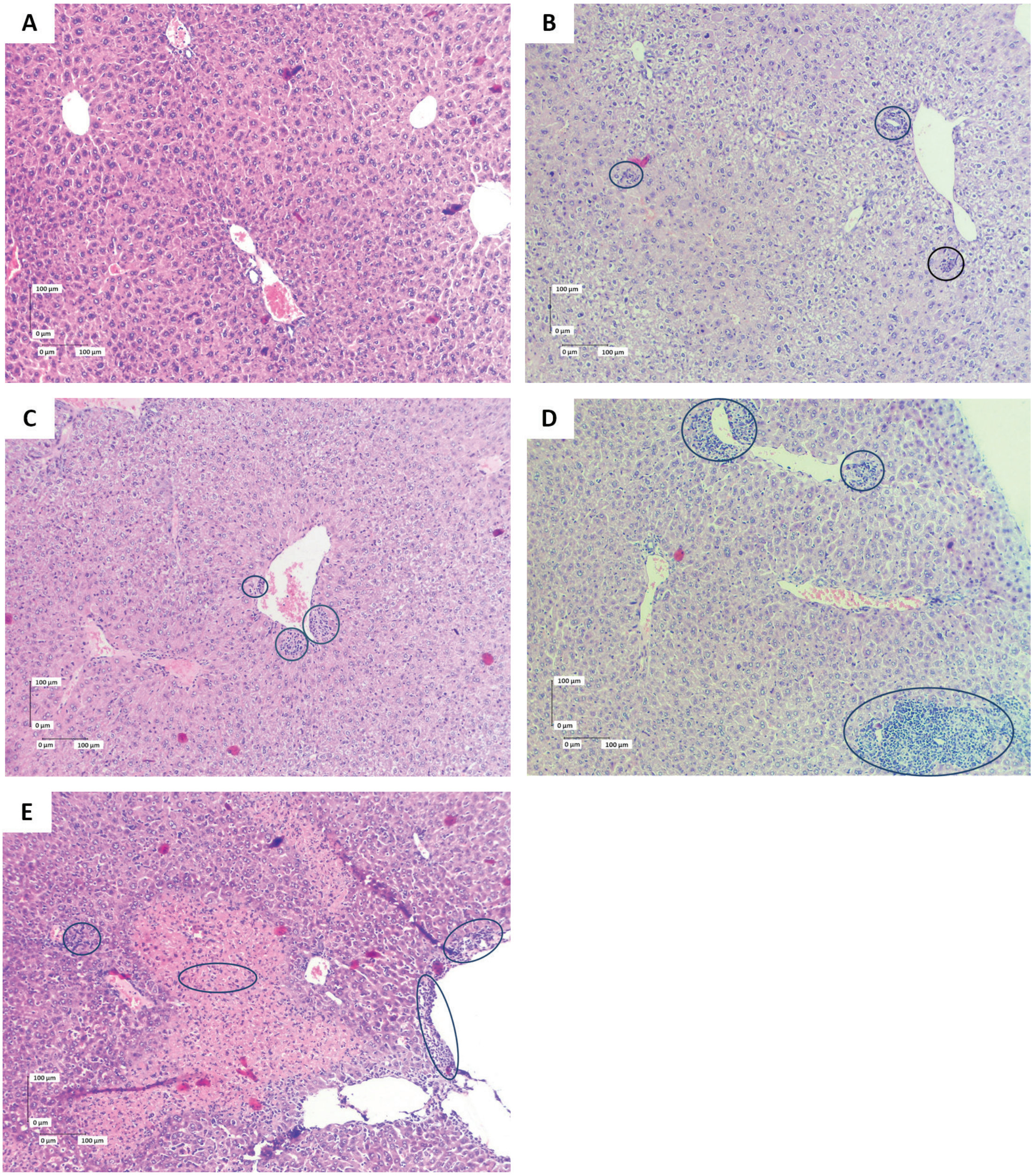

Figure 4. Liver histopathology under light microscope with 100x magnification (scale bar 100 µm): normal (A), mild inflammation in LPMB (B), moderate inflammation in LPHB (C), severe inflammation in LPLB (D), and severe inflammation with necrosis in LP (E). The inflammatory area is marked with a black circle. Swollen liver cells are surrounded by lymphocyte cells. In the area of necrosis, many liver cells appear damaged and shrunken, and lymphocyte cells are clustered in this area. Note: (N) Normal protein diet; (LP) Low-protein diet; (LPLB) Low-protein diet + 100g of Bambara groundnut; (LPMB) Low-protein diet + 200g of Bambara groundnut; (LPHB) Low-protein diet + 300g of Bambara groundnut.

in this study, where a very low number of Firmicutes and Bacteroidetes were detected in the low-protein mice group compared to the control group with a normal protein diet. Meanwhile, the low-protein diet mice groups supplemented with Bambara groundnuts at 200 g per kg feed (LPMB) and 300 g per kg feed (LPHB), respectively, showed a higher amount of Firmicutes and Bacteroidetes compared to the control group.

Low-protein diet leads to reduced delivery of amino acids to the liver, activating the kinase general control nonderepressible 2 (GCN2), leading to increased eukaryotic initiation factor 2α (eIF2α) phosphorylation and activation of activating transcription factor 4 or 5 (ATF4/ATF5). ATF4/5 binds amino acid response elements (AAREs) within the *FGF21* promoter, leading to increased liver *FGF21* production and increased circulating *FGF21* (Laeger *et al.*, 2014). *FGF21* is a liver-derived circulating hormone (hepatokine) belonging to the FGF family and it has been shown to respond to different nutritional signals. *FGF21* is expressed in several metabolically active organs and interacts with different tissues. It regulates nutritional status through the control of glucose, lipids, and energy metabolisms (Tezze *et al.*, 2019). Increased *FGF21* is related to liver inflammation and the lower the protein content in the diet, the more severe the degree of inflammation in the histopathological appearance of the liver. Previous studies reported that *FGF21* is elevated when dietary protein is low (Solon-Biet *et al.*, 2016; Li *et al.*, 2019). This is consistent with our findings, where a low-protein diet significantly increased *FGF21* gene expression compared to the control group.

During long-term dietary protein restriction, the intestinal microbiota undergoes metabolic adaptations that stimulate hepatic *FGF21* adaptive metabolic pathways (Wu *et al.*, 2022). In this study, the LPMB mice group that consumed feed supplemented with 200 g of Bambara groundnuts per kg of feed had much lower *FGF21* expression than mice fed a low-protein (LP) diet. Bambara groundnuts, as a source of protein, have a high content of essential amino acids, including isoleucine, leucine, methionine, phenylalanine, threonine, and valine (Tan *et al.*, 2020). This research showed that consumption of foods with sufficient or high protein levels reduces *FGF21* expression. This result is in line with the findings of Alemán *et al.* (2019). Chalvon-Demersay *et al.* (2016) also reported that a high-protein diet decreased the expression and circulating levels of *FGF21*.

Based on the HRMS examination, Bambara groundnuts contained isoflavones. The current study affirms the finding from Okafor *et al.* (2022), who reported that Bambara groundnuts are rich in polyphenolic compounds, including flavonoid subgroups such as isoflavones. Isoflavones, the most well-known subgroup of phytoestrogens, play protective roles against chemically induced liver injuries through several molecular mechanisms. The hepatoprotective effects of isoflavones are partly associated with their antioxidant, anti-inflammatory, immunomodulatory, and anti-fibrotic properties. Moreover, isoflavones can reduce gut-derived endotoxins, accelerate alcohol metabolism, stimulate detoxification of hepatotoxic chemicals, suppress the bioactivation of these chemicals, inhibit hepatocyte apoptosis, and restore autophagy activity during chemically induced liver diseases (Sarhan *et al.,* 2012; Yao *et al.*, 2021). This is a possible mechanism by which in this study, supplementation of Bambara groundnuts in mice with a low-protein diet had the effect of reducing the severity of sinusoidal congestion, swelling, and cloudy inflammation. This finding is in line with Folayan *et al.* (2022), who showed that liver damage can be improved by supplementing feed with antioxidant effects. Sarhan *et al.* (2012) also reported that soy supplementation rich in isoflavones showed some protective effects against liver damage in rats due to its antioxidant activity.

CONCLUSION

Overall, this study revealed that Bambara groundnut supplementation in low-protein diet mice has the potential to increase the number of Firmicutes and Bacteroidetes, decrease the expression of adaptive stress gene *FGF21*, and improve the inflammation degree of liver histopathologic features.

Acknowledgement

We thank Universitas Gadjah Mada for the grant support (5075/UN1.P.II/Dit-Lit/PT.01.01/2023), Aretha Medika Utama for the assistance with laboratory resources, Roro Wahyudianingsih and Demes Chornelia Maratantiningtya for the assistance in research discussion, Khoiruddin Anshori and Anisah Hanif Abiyu for the assistance in managing the experiment.

Authors' contribution

Gunanegara RF, conceptualisation, formal analysis, original draft preparation; Dewanto A, project administration; Sunarti, writing, reviewing, and editing of manuscript.

Conflict of interest

Authors declare that there is no conflict of interest in this study.

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