Volume 16, Number 2, July 2021

ISSN 1978-1059
EISSN 2407-0920

JURNAL GIZI DAN PANGAN

INDONESIAN JOURNAL OF NUTRITION AND FOOD

Articles

Published by:

Food and Nutrition Society of Indonesia

Department of Community Nutrition, Faculty of Human Ecology, IPB

Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, July 2021, 16(2):63-70***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2021.16.2.63-70*
ISSN 1978-1059 EISSN 2407-0920

Validation of Iron-Food Frequency Questionnaire for Assessing Iron Intake in Women of Reproductive Age: A Cross-Sectional Study on Female Undergraduate Students in Indonesia

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ABSTRACT

The aim of this study is to develop and assess the validity of the Iron-Food Frequency Questionnaire (Iron-FFQ) in assessing the iron intake of reproductive age women. This cross-sectional study involved randomly selected female under graduate students at Jantinangor, Sumedang district, West Java (n=94) as respondents. The validity test compared the iron intake using Iron-FFQ with the 3-Days Food Diary (FD). The iron contents of each food from both methods were obtained from Indonesian Food Composition Table (2017). Iron contents were analyzed using the Wilcoxon signed rank test and Spearman's rank correlation. The results showed significant correlation in iron content between Iron FFQ and the 3 Days FD with r=0.25 for the tubers, nuts, and vegetables group, and r=0.21 for milk and fish ($p<0.05$). While, statistically non-significant correlations were found for the rest of the food groups (cereal, meat, poultry, eggs, fruits, fats, sugar, syrup, confectionary, and spices) with r ranged from r=0.19 to r=0.01. The average assessment value using Iron-FFQ was lower than the 3-Days Food Diary, especially in the food and beverages group (-100%), sugar, syrup, and confectionary (-93.8%), and vegetables (-88.5%). In conclusion, Iron-FFQ can be declared valid for measuring iron intake from some food group such as starchy tubers, fish, shellfish and shrimp, nuts, vegetables, and milk as they have an acceptable correlation value.

Keywords: food frequency questionnaire, iron intake, reproductive age woman, validation study

INTRODUCTION

Women of reproductive age are those belong to 15–49 year old female group, either married or not (BKKBN 2015). Based on the 2017 Indonesia Health Profile, their number in Indonesia was 69,739,202 or approximately 53.5% of all women (MoH RI 2018b). In addition to their reproductive roles, they have an important role in economic productivity (Pasricha *et al.* 2014). There is a massive increase in number of working women in Indonesia. There were 2.12 million working women in 2007 (Fitriananto *et al.* 2018), and based on the 2018 *Profil Perempuan Indonesia*, 48.12% of Indonesian women were working (Hakiki *et al.* 2018) or 52.33 million based on BPS Indonesia (BPS 2020).

Reproductive age women are prone to develop anemia and globally, 496 million (29%) of non-pregnant women suffering from anemia (Pasricha *et al.* 2014), while in Indonesia the prevalence of anemia in the same group was 22.7% (MoH RI 2013). Further, the risk increased during pregnancy, in Indonesia 48.9% of pregnant women were found anemic (MoH RI 2018a). Anemia increases the risk of low birth weight, postpartum bleeding and maternal mortality (Nechitilo *et al.* 2016). In addition, anemia in women of reproductive age can lead to restricted fetal growth and development, impeded immune, cognitive, motor and social-emotional development in children (Nechtilio *et al.* 2016). Thus meeting the nutritional needs of this demography will prevent nutrition related

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(Received 22-03-2021; Accepted 11-06-2021; Published 29-07-2021)

diseases for themself and future generation (Perdana *et al.* 2014).

Iron plays an important role in human health and deficiency of this element in daily intake might result in iron deficiency anemia (Pasricha *et al.* 2014). According to the World Health Organization (WHO) publication in 2021, the most common causes of anemia include nutritional deficiency particularly iron (WHO 2021). Nutritional approach is can be an effective way to prevent iron deficiency anemia, hence assessment of iron intake is pivotal (Lopez *et al.* 2018). Therefore, establishing simple individual iron intake assessment tool is needed to support this stage (Głąbska *et al.* 2017). The Food Frequency Questionnaire (FFQ) is a tool that fits these assessments. This tool is easy, valid and can be used in large populations (Fahmida and Dillon 2011). However, few tools are specifically designed to assess iron intake (Głąbska *et al.* 2017). Therefore, the aim of this study is to assess the validity of Iron-FFQ to measure the iron intake in reproductive age women in West Java, Indonesia.

METHODS

Design, location, and time

This cross-sectional study was conducted between April and November 2018 at the Faculty of Medicine, Universitas Padjadjaran, Jatinangor campus. The research was conducted after obtaining permission from the Ethics Commission of the Faculty of Medicine, Universitas Padjadjaran with an approval number 959/UN6.KEP/EC/2018.

Sampling

Respondents who participated in this study were 100 students of the Faculty of Medicine, Universitas Padjadjaran batch 2016, 2017, and 2018. The research participants were recruited using systematic random sampling. A total of 505 female students were gathered as study population; 100 students who met the inclusion criteria and eliminated by the exclusion criteria were selected as study subject. The age range of respondents who participated in this study was 17–22 years. All of the participants had been provided with information and consented to participate in this study. The participants of this study were female undergraduate students of Universitas Padjadjaran, Jatinangor Campus, in the reproductive age range of 15–49 years (MoH RI 2018b). Students who were pregnant, had impaired red blood cell production or chronic diseases, lived with their parents, and were fasting when the data were taken were excluded. During the data quality control stage, it was found that 6 students were fasting or on a diet thus they were excluded and the final analysis only involved 94 participants.

Data collection

The data obtained in this study were general characteristics of respondents, respondents' anthropometry and iron intake obtained from the FFQ and Food Diary / Weighed Food Records. Standardization training for the data collection method was carried out by Nutrition Working Group Faculty of Medicine Universitas Padjadjaran.

We trained and briefed the respondents aiming for standardized result. On the first day, respondents was trained about, how to fill iron Food Frequency Questionnaire (FFQ), how to fill 3 days Food Diary (FD), including how to use the kitchen scale and assessed their anthropometry. At the end of the session respondents was given the FD and kitchen scale to be used at home. On the 8th day, respondents collected the FD back to the research team, re-briefed about how to fill the iron FFQ, then filled the FFQ.

Anthropometric measurement in this research included body height, body weight, and BMI. Body height measuring instrument used was SECA stadiometer and body weight was measured using digital scale Tanita SC240 following the protocol in Gibson's Nutritional Assessment 2005 (Gibson 2005).

Iron-FFQ was a food questionnaire which includes food and beverage list. The list was consisting of foods containing iron including both of raw and cooked food. The questionnaire was self-administered where respondents received a short briefing by the researcher beforehand which includes a mini trial. The questionnaire was immediately returned to the researcher after completion.

Food and beverages list in the questionnaire was developed from Food Diary (FD) which was collected in prior research conducted in 2017 (Anugerah 2018). Thirty-four FDs were collected. Raw and cooked food and beverage names were extracted and listed (132 items) in the FFQ. Foods and beverages included in the

FFQ was those containing iron more than 0.1 mg per 100 grams of food (Głąbska *et al.* 2017) and base on Anugerah (2018) being consumed by more than 10% respondent (n=34).

Food and beverages list obtained was then divided into 13 groups based on the Indonesian Food Composition Table 2017 (TKPI) (MoH RI 2018c). The food groups were cereals and cereal products, starchy tubers and their processed products, meat, poultry and their processed products, fish, shellfish, shrimp and their processed products, nuts and their processed products, eggs and their processed products, vegetables and their processed products, fruits and their processed products, milk and their processed products, fats and their processed products, sugar, syrup and confectionery, spices, and drinks.

The portions served are based on most of the portions listed in the food diary which were previously used to list the names of food and drinks. Portions are served in the form of portions, pieces, packs, or other household sizes such as rice scoops, tablespoons. The frequencies used in this iron questionnaire are "never", "1 per month", "2–3 per month", "once a week", "2–4 times a week", "5–7 times a week" , "Once a day", "2–3 times a day", "4–6 times a day", ">6 times a day" (Willet 1990).

Food Diary (FD) is chosen as a comparison tool. In the diary, there are empty columns which respondents can use to record every food and drink consumed. The columns consist of the schedule column for writing the day, date, and time; the food type column to write the food/ beverage and food ingredients, the amount that is served and wasted; and the last is the cooking method. To measure the amount of the weight served and wasted in grams, respondents were given a digital food scale, SF-400 kitchen scale, which has a capacity of up to 10 kg with a precision of 0.001 kg.

Food Diary was distributed a week before FFQ. At the beginning, the researcher provided respondents with FD and digital food scales to help respondents in filling the diary. Respondents were asked to fill in this diary for 3 days, consisting of 2 working days or weekdays and 1 holiday or weekend. They were given 1 week to fill in this FD. Then, after a week the researcher returned to take the FD and the scales, and then gave Iron-FFQ to fill in. The mini trial was done in the time of FD and weighing scale distribution, and after briefed by Nutrition Working Group team from Faculty of Medicine Universitas Padjadjaran.

Data analysis

Iron content from food or drinks listed in these two methods was then measured using the Indonesian Food Composition Table 2017 (TKPI 2017). Mixed foods or drinks that were not listed in the TKPI 2017 or without iron content data were analyzed based on the ingredients and referring to TKPI 2017. Iron content was generated from total iron from each ingredient.

Iron-FFQ data were processed by converting the respondent-chosen frequency into daily frequency. Frequency was multiplied to foods or beverages portion in gram and the multiplied again to iron content per 100 g, then divided to 100. The result was stated with [daily frequency x food/beverages portion x portion weight (g) x iron content per 100 g (mg) / 100)]. After iron content was obtained from each food, the iron content average was calculated.

Furthermore, for FD, iron content from every food or drink listed in the form was collected. Just like the FFQ, the iron that had been obtained was then averaged according to the food group. After that, each food group from each day was calculated.

The data normality was tested using Saphiro-wilk. The mean difference between two iron intakes from both methods (each food group in FFQ and FD) was tested using Wilcoxon – signed rank test with significant value cut off between two methods of $p<0.05$. (Głąbska *et al.* 2017). Correlation analysis between the means of iron intake per food group obtained from both methods using the Spearman's rank correlation, with <0.2 a bad outcome, 0.2 to 0.49 an acceptable outcome, and 0.5 and above is a good outcome (Lombard *et al.* 2015).

RESULTS AND DISCUSSION

This study involved 100 respondents who were reproductive age women in the age range of 15–49 years. Six respondents were excluded because there were changes in diet (diet and fasting) within 3 days of taking the Food Diary data. Therefore, only 94 respondents were included in the analysis.

Age characteristics and nutritional status. Table 1 shows the general characteristics of the

study respondents by age as well as nutritional status determined by BMI. The study respondents who were 19 years old were dominant, 32 respondents (34.04%) and only one subject were aged 22 years old.

In this study, it is found that 52 of 94 respondents (55.31%) had a good nutritional status, based on their body mass index, while 42 other respondents had a low body mass index that (10.63%) and excess (34.04%) based on WHO - Asia Pacific BMI grouping WHO (2000). The subject with body mass index (BMI) ≥25 kg/m^2 was 17.02%. This result is similar to the obesity proportion in study conducted by Lopez-Arana *et al.* (2013) involving reproductive age women in 33 low income countries, that was lies between 3.4% to 73.7%. The data also close to the proportion in Indonesia which is 21,8% that published in Riskesdas 2018.

Iron intake and RDA percentage. Based on this research, it was found that the daily iron intake of respondents was significantly lower than the standard proposed by the government (MoH RI 2019). Based on Table 2, the mean daily iron intake of respondents using food diary were 10.09 mg per day which is much lower than the RDA; only 19 respondents consumed adequate iron each day. Research by Angraini *et al.* (2018) reported that 95.6% subject had low iron intake while only eight out of 183 women of reproductive age had sufficient iron intake. This research data supports the importance of the currently running national program of iron and folic acid supplement for pregnant women (MoH RI 2013).

Iron content–food frequency questionnaire. In this validation study, Food Diary or Weighed Food Record was used for 3 days, consisting of 2 days of weekdays and 1 day of weekends or working days. This method used by Steinemann and Grize (2017) and Głąbska *et al.* (2017). Filling in the questionnaire for 3 days was chosen because filling in 4 days or more can produce unfavorable results since respondents will feel tired in filling out the food diary (Thompson & Subar 2008). Further, according to Shim *et al.* (2014), filling the food diary is indeed burdensome to respondents.

Table 3 shows data analysis results using the Wilcoxon signed rank test for each food group. It was found that there were no significant differences of iron intake using both methods for the meat, fish, shellfish, shrimp, and processed food groups. However, there were significant differences in several food groups for cereals, tubers, poultry, nuts, eggs, vegetables, and several other food groups. The difference between these two methods occurred due to the difficulty experienced by respondents in estimating the number of portions, because portions can differ depending on from where the food is obtained. Sauvageot *et al.* (2014) in their study stated that

Table 1. Age and body mass index of respondent

Characteristics	Validation study (n=94)	%
Age		
17	4	4.25
18	23	24.47
19	32	34.04
20	28	29.78
21	6	6.38
22	1	1.06
BMI		
Underweight (<18.5 kg/m^2)	10	10.63
Normal (18.5–22.9 kg/m^2)	52	55.31
Overweight (23.0–24.9 kg/m^2)	16	17.02
Obese I (25.0–29.9 kg/m^2)	9	9.57
Obese II (>30.0 kg/m^2)	7	7.44

BMI: Body Mass Index

Table 2. Iron intake and %RDA

Age group (years)	Mean iron intake (mg/day)	RDA (AKG 2019) (mg/day)	% RDA
16–18	11.13±4.87	15	63.21
19–29	9.88±3.95	18	51.86
All	10.19 ±4.18		

%RDA: Average of %RDA in age group
RDA: Recomended Dietary Allowance

Table 3. Food group validity measured with FFQ and FD, and difference test using wilcoxon signed rank test (p^a), correlation between both methods measured with Spearman's rank correlation (r^b), and it's significancy (p^c)

Food group	FFQ iron intake			Food diary iron intake			Mean difference (FFQ-3 D FR)		Validation statistics operation		
	Mean	Median	IQR	Mean	Median	IQR	Mean	%	p^a	r^b	P^c
Cereals and processed products	0.36	0.30	0.22– 0.46	2.58	2.38	1.67– 3.14	-2.22	-86	0.000	0.01	0.889
Starchy tubers and processed products	0.15	0.07	0.37–0.17	0.11	0.00	0.00– 0.08	0.04	36.4	0.001	0.25	0.017
Meats and processed products	0.18	0.12	0.07–0.20	0.52	0.00	0.00– 0.63	-0.34	-65.4	0.674	0.10	0.334
Poultry and processed roducts	0.26	0.19	0.12–0.28	2.13	1.26	0.64–3.15	-1.87	-87.8	0.000	0.07	0.516
Fish, shellfish, shrimp and processed products	0.11	0.08	0.04–0.13	0.40	0.00	0.00–0.41	-0.29	-72.5	0.314	0.21	0.047
Nuts and processed products	0.17	0.10	0.05–0.25	0.59	0.21	0.00–1.04	-0.42	-71.2	0.000	0.25	0.015
Eggs and processed products	0.14	0.12	0.05–0.20	0.31	0.00	0.00–0.53	-0.17	-54.8	0.028	0.09	0.354
Vegetables and processed products	0.10	0.07	0.04–0.12	0.87	0.34	0.00–1.11	-0.77	-88.5	0.000	0.25	0.013
Fruits and processed products	0.09	0.06	0.03–0.12	0.56	0.21	0.00–0.87	-0.47	-83.9	0.000	0.18	0.073
Milk and processed products	0.30	0.31	0.06–0.62	1.11	0.93	0.00–1.83	-0.8	-72.9	0.000	0.21	0.041
Fat and processed products	0.02	0.01	0.00–0.02	0.02	0.00	0.00–0.00	0.0	-34.2	0.000	0.09	0.368
Sugar, syrup, and confectionary	0.06	0.03	0.20 –0.80	0.90	0.24	0.00–0.80	-0.9	-93.8	0.000	0.19	0.068
Spices	0.03	0.02	0.10–0.30	0.04	0.00	0.00	0.0	-22.1	0.000	0.06	0.571
Beverages	0.00	0.00	0.00	0.04	0.00	0.00	0.0	-100.0	0.028	-	-

FD: Food Diary; FFQ: Food Frequency Questionnaire; IQR: Inter-Quartile Range: RDA: Recomended Dietary Allowance

using photos for portion estimation can overcome this problem while in this study, no photos were used as an additional method.

Table 3 also presents the correlation coefficients obtained from the statistical operations of Spearman's rank correlation. In this validation study, the food groups of starchy tubers, fish, shellfish, and shrimp, nuts, vegetables, and milk can be accepted because they have correlation coefficients of 0.25, 0.21, 0.25, 0.25, and 0.21 respectively ($p<0.05$). These food groups were declared valid and can be used for measurement of iron intake, despite the low degree of correlation.

Aligns with Steinemann and Grize (2017), the finding could be because the food in the list was not consumed very often by respondents. In this study, the food list was obtained from the FD taken in 2017. The food has not been adapted to Jatinangor food list which has been developed since 2016. For further studies, it is necessary to update the food list so that the food types that

are included in the FFQ are those frequently consumed and contain significant amounts of iron. The recommended list of food types that need to be included in the FFQ include common mixed food available in Jatinangor area. Some spices such as bay leaves and lemongrass, as well as beverage ingredients such as jasmine tea and cocoa powder also contain high iron per 100 grams. However, the amount of iron consumed cannot be assessed exactly because what is consumed is the water extract, not the ingredients that are listed.

Buscemi *et al.* (2015) in their study stated that newly designed FFQ will produce a higher correlation coefficient value compared to the questionnaire adapted from previous studies. In the same study, it was also stated that the FFQ filling method also had an effect on the correlation coefficient. The validation of the study was critically determined by the administration method of FFQ filling. Interviewer-administered method increased the correlation coefficient value, whereas in this study respondents filled out their own FFQ and it relied on their memory to complete the form.

In Table 3, almost all of the mean differences are negative. It shows that the iron intake as measured by the Iron Food Frequency Questionnaire is lower when compared to the Food Diary. This is also stated by Streppel *et al.* (2013) in their study which aimed to observe relative validity of FFQ, that the Food Frequency Questionnaire measures food intake is lower than the comparison method. Sauvageot *et al.* (2014) in their study stated that these differences could be caused by respondents' difficulty in quantifying the consumption of food and drink. Most of the food and drinks in the FFQ are served in the form of single food, but there are some types of foods that are usually consumed together with other foods or mixed dishes so that it will be difficult for respondents to estimate the frequency of their meals. In addition, the study conducted by Sauvageot also stated that filling out the FFQ depends on the memory of respondents, so that it can affect the filling of this food questionnaire. The statement was in line with what Fahmida and Dillon (2011) propose in nutritional handbook.

The limitation of this study is that this study does not perform test-retest reliability. The food list in the FFQ is also incomplete; there are some common mixed food types potential to be completed in further research. The use of photos for portion estimation is not carried out in this study, thus hindering respondents from estimating the portions they ate, and this can cause difference in measurement of FD and FFQ. The administration method that can increase the correlation coefficient results, interviewer - administered is recommended to perform in further studies.

CONCLUSION

This food questionnaire can be declared valid for measuring iron intake in the starchy tubers, fish, shellfish and shrimp, nuts, vegetables, and milk food groups because they have an acceptable correlation value. The results of measurements using the Iron Food Frequency Questionnaire are lower than the Food Diary or Weighed Food Records. Further research is needed to make adjustments to the resulting figures. It is recommended to develop FFQ data collection method with pictures guidance to help enumerators and respondents to estimate the portion size of foods and beverages to increase validity.

ACKNOWLEDGEMENT

The author would like to thank the Department of Public Health, Faculty of Medicine Universitas Padjadjaran and Department of Child Health, Faculty of Medicine Universitas Padjadjaran-Hasan Sadikin General Hospital and students of the Faculty of Medicine, Universitas Padjadjaran, batch 2016–2018 who have contributed to this research. Also, we would like to thank Putri Halleyana AR, MD for the support in proofreading this article.

AUTHOR DISCLOSURES

The authors have no conflict of interest.

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ISSN 1978-1059 EISSN 2407-0920

J. Gizi Pangan, July 2021, 16(2):71-80
DOI: https://10.25182/jgp.2021.16.2.71-80

Field Trial of Local Nutrition Plans and Programs Monitoring and Evaluation Protocol in the Philippines

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ABSTRACT

The field trial was conducted to establish the reliability in producing similar results between evaluators of the proposed new tools for Monitoring and Evaluation (M&E) of the nutrition plans and programs in the Local Government Units (LGUs). To do this, orientation activities were conducted to familiarize the 46 M&E team (MET) members evaluating the provincial, municipal, city, and barangay levels in two regions with the proposed tools during the field trial. After the event, the perceptions of the MET members of the tools were gathered by asking them to rate the tools through a self-administered questionnaire, and by noting their written and verbal commentaries about the proposed system. During the field trial, each MET member, as well as the member of the Project Team (PT), individually evaluated the LGUs using the tools. Secondary data on the LGUs performance scores using the old system were also gathered. The MET members' perception was examined based on the median rank of their ratings and content analysis of their insights about the tools, whereas the reliability of the tools was assessed based on the interrater reliability of the MET members' scores for the LGUs analyzed through paired samples t-Test, Pearson correlation coefficient, intraclass correlation coefficient, and technical error of measurement. The weighted scores of the MET and PT members were also compared. Moreover, the difference in the generated scores between the old and the new system was determined. The findings revealed that the MET members generally have a positive perception of the new system but raised some issues and concerns. Although the reliability of the tools was generally observed, actions are warranted for improvement. The tools generated statistically different scores when used by MET and PT members, and when compared to the existing system. Steps should be taken to improve the reliability of the proposed tools.

Keywords: interrater reliability, LGNMES, MELLPI Pro, monitoring and evaluation system, nutrition plans and programs

INTRODUCTION

Mandated to monitor and evaluate the implementation of the Philippine local food and nutrition plans and programs, the National Nutrition Council (NNC) has been implementing the Monitoring and Evaluation of Local Level Plan Implementation (MELLPI) system since 1978. This was employed to assess the efficiency and effectiveness of the LGUs in planning and implementing local nutrition programs. However, in 2015, the NNC initiated the enhancement of the system to cover the assessment of the local nutrition policy and legislation initiatives, service delivery, and capacity-building, and include nutrition outcomes among pregnant mothers, in addition to infants and young children to make mobilization for nutrition more facilitative for LGUs. Consequently, an updated M&E protocol with the appropriate tools was developed

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(Received 08-12-2020; Accepted 17-06-2021; Published 29-07-2021)

adapting a more results-based, integrated, and comprehensible approach (Gawe 2015) and is anchored on the various hierarchies of nutrition action plans. The updated M&E protocol was based on the Local Government Nutrition Monitoring and Evaluation System (LGNMES) of the Nutrition Results Framework (NRF) that is developed by the University of the Philippines Los Baños (UPLB). One of its components is the Local Nutrition Organizational Capacity Assessment Component (LNOCAC) which assesses the nutrition plans and programs of the LGUs.

The updated M&E system was pre-tested for feasibility, relevance, and comprehensiveness but a field trial was needed to assess the reliability and replicability of the tools. A field trial can provide relevant information before making any public health decisions and optimizing national health programs (Piedra-Fernández & Ganoza-Guerrero 2016). It establishes whether a change in the system can lead to a more desirable outcome ensuring that the new protocol will not fail or even worsen the existing one before its wide-range implementation (Smith *et al.* 2015; Wandner 2017). Furthermore, since the M&E information generated would be the basis for nutrition-specific planning, intervention, and policymaking (Vidyarini *et al.* 2021; Jefferds & Flores-Ayala 2016), it is vital to ensure that data produced are reliable and reproducible over time (George *et al.* 2013). To do this, the interrater reliability of the personnel in using the M&E tools must be established to assure consistency in the evaluation of a particular object or event (Drummond & Murphy-Reyes 2017).

Hence, this study aimed to conduct a field trial of the LNOCAC of LGNMES in the M&E of nutrition plans and programs at the different levels of LGUs. Specifically, it aimed to report the perceptions of the evaluators about the proposed protocol. It also assessed the reliability of the new system in evaluating the local nutrition plans and programs. Lastly, it determined the implication of the adoption of the proposed system in the performance evaluation of the LGUs.

METHODS

Design, location, and time

Two regions were selected jointly by NNC and UPLB as the study areas for the field trial. The criteria used for the selection of provinces, cities, municipalities, and barangays were as follows: has records of 2016 MELLPI scores; with active and organized local Nutrition Plans and Programs (NPP) evaluators or monitoring and evaluation team (MET); Local Chief Executive's (LCE) approval of participation; and willingness of the MET members to be part of the study. Using these criteria, the NNC Regional Offices were consulted for the identification of the study areas as well as participants. The field trial was conducted from April to May 2018 in four provinces, two cities, and eight municipalities in the two regions.

Sampling

Upon coordinating with NNC Regional Offices, 48 MET members qualified and were thereby invited to participate in the study. The qualified MET members were those active and organized evaluators of local NPP in the selected areas identified by the NNC Regional Offices. Among the invited MET members, 46 agreed to participate. The selected MET members evaluated the LGUs corresponding to their levels of M&E protocol using the LNOCAC tools during the field trial: the Regional M&E Team (RMET) was assigned to evaluate a province or a city; the Provincial M&E Team (PMET) was assigned to a municipality; and the City/Municipality M&E Team (C/MMET) was assigned to a barangay. A total of six RMET members, 11 PMET members, six CMET members, and 23 MMET members participated in the study. All the MET members were asked to sign an informed consent stating their willingness to participate in the study.

Data collection

Before the field trial, the Project Team (PT) conducted activities to orient the MET members about the new LGNMES tools and protocol. These activities included the evaluation of LGUs corresponding to their levels of M&E protocol using the LNOCAC of the LGNMES, the focus of this paper.

After the orientation activities, the MET members were asked to answer a self-administered questionnaire about their perception of the LNOCAC protocol. Participants accomplished the questionnaire by expressing their agreement or disagreement with each statement based on a 5-point Likert scale. Each item was rated on a 1 to 5 response scale where; 1=strongly

disagree; 2=disagree; 3=agree; 4=strongly agree; and 5=neither agree nor disagree. The said questionnaire captured the whole aspects of understanding and using the evaluation tools. It inquired about the understandability of the words used in the tools, the scoring system, easiness in interpreting the results, easiness in explaining to Local Nutrition Committees (LNCs)/LCEs, its relevance, comprehensiveness, clarity, applicability to the full range of intended uses, concreteness, parsimony, ease of use, and if fairness were integrated. The MET members' written remarks and verbal commentaries during the orientation activities were also noted.

Following the LGNMES protocol, the METs were asked to do a certain sequence of activities during the field trial: travel to the site or venue of evaluation; conduct of courtesy call to the LNCs and orientation; desk review and scoring the LGUs' performances using the LNOCAC tools/forms; the processing of the team; and feedbacking of results to the LNCs. The LGUs were requested to make the relevant documents available during the scheduled MET visit to facilitate the evaluation process.

During the scoring process, each MET member individually evaluated the LGUs corresponding to their level of M&E protocols. This was done to determine the Interrater Reliability (IRR) between the MET members in using LNOCAC forms of the LGNMES for evaluating LGUs. A PT member assigned to the area also evaluated the LGUs using the tool to serve as a source of comparison. Both teams scored the performance of the LGUs based on the following dimensions in the LNOCAC forms/tools: Vision and Mission, Nutrition Laws and Policies, Governance and Organizational Structure, Local Nutrition Communication Management Functions, and Nutrition Intervention under its Organizational Component, and Prevalence of Underweight 0-<5 Children, Prevalence of Stunted 0-<5 Children, Prevalence of Wasted 0-<5 Children, Prevalence of Overweight and Obesity among Children, Prevalence of Wasted School-Age Children, and Prevalence of Nutritionally At-risk Pregnant Women under its Nutrition Situation Component.

Lastly, the data on the 2016 MELLPI scores of the LGUs were also gathered during the visit. These were collected to determine the implication of the adaption of the LGNMES in the performance scores of LGUs. This is vital to evaluate the two systems based on the generated performance scores (Figure 1).

Data analysis

Perception of the MET members. The data on the participants' perceptions were gathered to determine their view of the proposed system and the possible implication of its implementation in different levels of LGUs. For the analysis of the perception of the MET members on the LNOCAC tools and protocol, the median rank of their ratings per item was examined by region. The median rank of ratings in the two regions was also statistically compared using the Mann-Whitney U-test at a 5% level of significance. The common themes in the written remarks in the questionnaire and verbal commentaries during the orientation activities of the participants were also determined through content analysis.

Reliability of the LNOCAC tool. Reliability is an important indicator to assess the usefulness of a tool. In this study, the reliability of the proposed tool in monitoring and evaluating the performance of the LGUs was estimated to describe the capacity of the said tool to produce almost similar results (precision) across several evaluators. To assess the reliability of the LNOCAC forms in evaluating the LGUs' performance, the IRR of the MET members based on their scores in using the tools were analyzed through t-test for paired samples, Pearson correlation coefficient, Intraclass Correlation Coefficient (ICC), and Technical Error of Measurement (TEM). The weighted mean performance scores of the MET and PT members for the different levels of LGUs were also compared to describe the precision or capacity of the tools to produce precise results when used by different groups of evaluators.

The paired t-test was used to describe the agreement between two MET members' performance scores for an LGU. A $p>0.05$ indicates that the scores given by the two MET members are in agreement. The Pearson correlation coefficient, on the other hand, measured the degree of association between two MET members' performance scores for an LGU. A positive Pearson correlation coefficient indicates a direct association in the scores of the two MET members, while a negative coefficient indicates an opposite direction implying poor association. The closer its value is to one, the

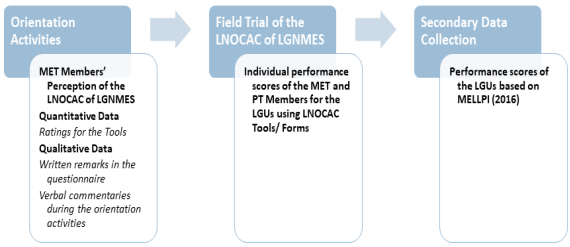

Figure 1. Flowchart summarizing the information gathered in each phase of data collection

stronger the association of the scores. Moreover, the ICC was computed to evaluate the degree of reliability of the two MET members' performance scores for an LGU: a negative coefficient indicates no reliability; coefficients less than 0.5 are indicative of poor reliability; 0.5 to <0.75 is moderate reliability; 0.75 to 0.90 is good reliability; and coefficient more than 0.90 indicates excellent reliability. It reflected both degrees of correlation and agreement between measurements, and thus, was used as an index. Lastly, the TEM was computed to indicate the IRR of the MET members in the absence of paired t-test, Pearson correlation coefficient, and ICC results due to constant scores between two evaluators. The TEM was used to indicate the variability between the two MET members' performance scores for an LGU. The lower the computed value, the more the two evaluators are in agreement. The percentage distributions of the acceptable IRR of the MET members by dimensions of the LNOCAC were then computed to describe the reliability of the tools.

In comparing the weighted mean performance scores of the MET and PT members for the different levels of LGUs, a significant differentiation of their scores indicates poor reliability of the tools. This comparison of the scores was also based on the paired t-test, Pearson correlation coefficient, ICC, and TEM.

Comparison of M&E systems. The LGUs' weighted mean performance scores based on the LNOCAC tools as evaluated by the MET and PT members were compared with the corresponding 2016 MELLPI scores. The weights were assigned to generate comparable performance scores. The comparison was done to determine the possible implication in the performance scores of LGUs upon the adaption of the LNOCAC as the new M&E system of the implementation of NPP at the local levels in the country. The paired t-test was used to determine the differences between the weighted mean performance scores for the LGUs based on the two M&E systems. The data on this study were analyzed using the Statistical Package for Social Science (SPSS) version 23.

RESULTS AND DISCUSSION

MET members' perceptions of the new system

Table 1 shows the perception of the MET members on the LNOCAC as an M&E system for the NPP of the LGUs. The MET members in Region B generally had higher median ranks of ratings on positive statements about the new system than in Region A. This indicates that MET members in Region B agree to these statements more than those in Region A, albeit statistically insignificant.

On the other hand, the results of the median rank of ratings on the negative statements that compare the LNOCAC to MELLPI indicate that the proposed protocol takes less effort to use and that it is preferred in the M&E of the nutrition situation the LGUs in both regions. Moreover, the MET members in both regions had median ranks of ratings of three in the "bored on the system of evaluation" statements indicating the general agreement to the statement. Significant differences in the median ranks of ratings given by MET members in the two regions were only observed in the statements "The evaluation is costly." ($p=0.013$) and "It is too long to finish the evaluation." ($p=0.001$). The result in the statements about the costliness indicates that MET members in Region A agree that the LNOCAC is costly while MET members in Region B believe otherwise. On the other hand, the evaluators in both regions disagreed on the statement regarding the duration of the process, although MET members in Region B had a significantly stronger disagreement with the said statement compared to Region A.

Furthermore, based on the content analysis of the MET members' written remarks and verbal commentaries during the orientation activities, the LNOCAC is more comprehensive compared to MELLPI because of its broad range of organizational dimensions while also considering the changes in nutritional status as an indicator of nutrition programs' effectiveness like MELLPI. However, they also highlighted that the proposed tools were designed to be used only to monitor

Table 1. The median rank of the ratings given by MET members for the LGNMES in evaluating LGUs

Perceptions	Region A	Region B	p-value
Positive statements			
The evaluation is relevant to improve the nutrition situation in the LGU	3	4	0.352
The evaluation is comprehensive	3	3	0.154
The evaluation will be useful for the LGU	3	4	0.206
The evaluation will be useful for the LCE	3	4	0.298
The evaluation will be useful for the LNC	3	4	0.212
The evaluation will be useful for the nutrition workers in the LGU	3	4	0.358
The words used in the form are easy to understand.	3	3.25	0.181
The criteria are easy to interpret	3	3	0.904
The form is easy to fill out	3	3.5	0.824
The instructions on how to use the form are clear	3	3	0.993
The criteria used is fair from LGU to LGU	3	3	0.201
The results are easy to interpret	4	4	0.703
The results are easy to explain to the LNC	3	3.5	0.675
I enjoy using and filling out the forms	3	3	0.939
Training is not needed to use in this evaluation system, orientation is enough	3	3	0.802
Negative statements			
The evaluation is costly	3	2	0.013*
It is too long to finish the evaluation	2.5	1	0.001*
It takes more effort to use this evaluation system than MELLPI	2	2	0.061
I prefer MELLPI over this new tool	2	2.25	0.941
I am bored with this system of evaluation	3	3	0.029

*Significant at 5% level; MET: Monitoring and Evaluation Team; LGNMES: Local Government Nutrition Monitoring and Evaluation System; LGU: Local Government Units; LCE: Local Chief Executive's; LNC: Local Nutrition Committees; MELLPI: Monitoring and Evaluation of Local Level Plan Implementation

and evaluate local NPP implementation while the results of the MELLPI system are used as a basis for giving awards or recognition.

The MET members suggested that perhaps the MELLPI is still needed to be used in tandem with the proposed tool. The two tools may be harmonized by either merging their important elements or by simply making the proposed tool a continuation of MELLPI. They added that it may be necessary to retain the data validation processes and the presence of the Barangay Nutrition Council (BNC) members protocol during tnhe evaluation. Moreover, they mentioned that there is a need to synchronize the LNOCAC forms with other health-related forms being used by LGUs. They also claimed that the Department of the Interior and Local Government (DILG), the executive department of the national government for strengthening LGUs, should be part of the MET so that it will have a strong impact.

The participants identified some factors that needed to be considered before the nationwide implementation of the LGNMES. They say that the proposed system may require added manpower and better logistics implementation from the LGUs. The tools must also be further improved as it includes parameters that did not apply to all LGUs such as protracted disaster areas, but does not consider ordinances related to health, solid waste, and sanitation which are relevant to the overall nutrition situation of the communities. Additionally, the MET members deemed that the answer for minimum change in nutritional status could be manipulated and that the new tools are costly due to their numerous pages.

Moreover, they mentioned that the tools would be much better if it was shorter and more specific to the area being evaluated. The criteria in each dimension should also be improved by identifying a basis for comparison or which data to use. Lastly, contrary to the overall result of the self-administered assessment of the LNOCAC system, some MET members still think that intensive orientation training should be provided.

Reliability of the LNOCAC tools

Interrater reliability of the MET members. The results in the t-test for paired evaluators revealed that all MET members had a hundred percentages of acceptable IRR, except for the Nutrition Intervention dimension among MMET which had 80%, indicating their high degree of agreement with each other in their scores for the LGUs in all the dimensions of the LNOCAC tools. The Nutrition Situation Component of the tools had generally better reliability than its Organizational Component as lower percentages of acceptable IRR among MET members were observed in the latter. The reliability of the tools was also more evident in Region A based on the higher percentages of acceptable IRR observed among MET members than in Region B.

Furthermore, the results showed that the RMET members had better IRR with one another in using the LNOCAC tools than in other groups. This indicates that the tools used for the M&E of NPP of the provinces and cities are more reliable than the LNOCAC tools used in other levels of LGUs. The Nutrition Situation Component of the tools for the barangays in cities also showed notable reliability based on the high percentages of acceptable IRR of the CMET members (Table 2).

Moreover, the Nutrition Intervention dimension under the Organizational Component had 100% percentages of acceptable IRR consistently among the MET members based on ICC while high percentages of acceptable IRR among the MET members were commonly observed in Prevalence of Overweight and Obesity among Children and Prevalence of Wasted School-Age Children dimensions under the Nutrition Situation Component. The lowest percentages of acceptable IRR among the MET members based on ICC were generally recorded in the Vision and Mission, Nutrition Laws and Policies, and Local Nutrition Communication Management Functions dimensions under the Organizational Component. On the other hand, the MET members also had the lowest percentages of acceptable IRR in the dimension under the Nutrition Situation Component, particularly in using the tools for the M&E of provinces, cities, and municipalities NPP. These results indicate that the LNOCAC tools for evaluating local NPP are least reliable in these dimensions.

The reliability of the LNOCAC tools when used by different groups. The results of the comparison of the MET and PT members' weighted mean performance scores for the LGUs using the LNOCAC tools are summarized in Table 3. Based on the paired t-test analysis, an agreement was observed between the MET and PT members based on their weighted mean performance scores for the LGUs, except for the barangay levels in Region A ($p=0.003$).

Excellent IRR was observed between the two groups based on their weighted mean performance scores for the provinces in Region B as evidenced by the computed value of ICC. This indicates the reliability of the LNOCAC tool for evaluating NPP at the provincial levels when used by different groups of evaluators. The two groups also had the smallest differences in the scores when the tool was used in these areas based on the TEM. However, contradicting results were found based on the tool when used by the two groups in Region A; the mean performance scores of the two groups for the provinces had a negative correlation and ICC. The highest variability in the scores was also observed when the tool was in these areas based on the TEM.

Moreover, the two groups had moderate reliability in using the LNOCAC tools for evaluating the barangays in both regions and municipalities in Region B, while poor reliability for evaluating the municipalities in Region A and cities in Region B based on ICC. To sum, the results showed that the LNOCAC tools were reliable in varying degrees when used by different groups for evaluating the NPP of different levels of LGUs, except for provinces in Region A when the tools were found to have no reliability based on the ICC.

Comparison of LGNMES and MELLPI

The adjusted mean performance scores of both the MET and PT members using the LNOCAC tool were compared to the corresponding 2016 MELLPI scores of the LGUs

Table 2. Percentage distribution of acceptable interrater reliability of MET members

Dimension	Region A			Region B			Region A			Region B		
	Corr coeff	ICC	TEM	Corr coeff	ICC	TEM	Corr coeff	ICC	TEM	Corr coeff	ICC	TEM
	MMET						PMET					
Organizational component												
VM	80	80	70	83.3	83.3	66.7	83.3	50	66.7	100	100	75
NLP	70	60	100	83.3	66.7	50	100	66.7	66.7	50	50	50
GOS	80	70	80	66.7	58.3	50	100	83.3	50	25	75	25
LNCMF	90	70	70	58.3	41.7	75	83.3	83.3	33.3	50	50	66.7
NI	100	100	60	75	66.7	91.7	100	100	16.7	100	100	100
Nutrition situation component												
PUW	90	90	70	83.3	83.3	66.7	66.7	66.7	66.7	100	100	75
PS	100	100	50	66.7	83.3	50	100	100	66.7	75	75	75
PW	90	90	70	91.7	91.7	50	66.7	66.7	33.3	100	100	75
POO	80	80	60	75	66.7	50	100	100	16.7	100	100	75
PWS	80	80	60	75	75	50	100	100	33.3	100	100	75
PNAP	80	80	70	83.3	83.8	75	100	100	50	75	75	75
	CMET						RMET					
Organizational component												
VM	100	100	33.3	33.3	33.3	0	100	100	100	100	100	33.3
NLP	100	100	100	33.3	33.3	33.3	100	100	100	100	100	100
GOS	100	100	66.7	66.7	66.7	0	66.7	66.7	0	100	100	100
LNCMF	100	66.7	33.3	33.3	0	0	33.3	33.4	33.3	100	100	100
NI	100	100	100	100	66.7	0	100	100	100	100	100	100
Nutrition situation component												
PUW	100	100	100	100	100	100	100	66.7	100	100	100	100
PS	100	100	100	100	100	100	100	100	100	100	75	100
PW	100	100	100	100	100	0	100	66.7	100	100	100	100
POO	100	100	100	100	100	33.3	100	100	33.3	100	100	100
PWS	100	100	100	100	100	33.3	100	100	33.3	100	100	100
PNAP	100	100	100	100	100	33.3	100	100	100	100	75	100

GOS: Governance and Organizational Structure; ICC: Intraclass Correlation Coefficient; LNCMF: Local Nutrition Communication Management Functions; NI: Nutrition Intervention; NLP: Nutrition Laws and Policies; PNAP : Prevalence of Nutritionally At-risk Pregnant Women; POO: Prevalence of Overweight and Obesity among Children; PS: Prevalence of Stunted 0-<5 Children; PUW: Prevalence of Underweight 0-<5 Children; PW: Prevalence of Wasted 0-<5 Children; PWS: Prevalence of Wasted School-Age Children; TEM: Technical Error of Measurement; VM: Vision and Mission; MMET: Municipality Monitoring and Evaluation Team CMET: City Monitoring and Evaluation Team; PMET: Provincial Monitoring and Evaluation Team; RMET: Regional Monitoring and Evaluation Team

to implications of the adaption of the LGNMES as the new M&E system for evaluating the local NPP (Table 4). Results showed that the MET and PT members' adjusted scores were consistently lower than the 2016 MELLPI scores across all the LGUs evaluated. However, these differences were only found significant for the performance scores of barangays in both regions, and the city in Region B.

Furthermore, significant differences were also observed between the 2016 MELLPI weighted score and PMET members' mean performance score for the municipalities, and between the 2016 MELLPI weighted score and PT members' mean performance score for the provinces in Region A. No significant differences were observed in the performance scores for municipalities and provinces in Region B. The overall results indicate that LGUs' performance scores would significantly become lower when the LGNMES tool is adapted as the new M&E system, particularly at the barangay levels.

The MET members had a generally positive perspective on the LNOCAC of LGNMES,

Table 3. Comparison of the MET vs PT members' consensus scores using the LGNMES tool

	Mean Scores (Standard deviation)		p-value (t-test)[a]	Corr coeff	ICC	TEM
	MET Members	PT Members				
Region A						
Barangay	51.78 (17.54)	42.04 (16.51)	0.003*	0.805	0.699	98.35
Municipality	60. 61 (22.79)	65.80 (20.85)	0.664	0.357	0.396	259.3
City[b]	44.37	48.82	-	-	-	9.9
Province	55.35 (12.32)	38.19 (16.85)	0.412	-0.966	-0.890	426.2
Region B						
Barangay	61.35 (17.08)	51.03 (21.42)	0.059	0.796	0.697	112.6
Municipality	72.02 (15.92)	60.95 (17.15)	0.070	0.749	0.632	119.0
City[b]	58.40 (14.53)	44.78 (17.12)	0.151	0.608	0.473	168.3
Province	55.54 (3.83)	54.72 (3.95)	0.065	1.000	0.978	1.4

*Significant at 5% level; a) using t-test for paired samples; b) with one observation only; MET: Monitoring and Evaluation Team; PT: Project Team; ICC: Intraclass Correlation Coefficient; TEM: Technical Error of Measurement

the proposed M&E system for evaluating local NPP. However, they raised some concerns such as implied logistics challenges, additional manpower requirements, cost implications, and technical issues about the tools that need to be resolved and considered prior to its nationwide implementation. The reliability of the tools was generally observed but is more evident in the Nutrition Situation Component. The tools also had better reliability when used in Region A. The reliability of the LNOCAC tool for assessing provinces and cities as well as the Nutrition Situation Component of the tool for assessing barangays in cities were more prominent than in other tools. Nevertheless, results indicate that the reliability of the tools needs improvement particularly for evaluating lower levels of LGUs and in dimensions where the MET members had low percentages of IRR. Moreover, the reliability of the LNOCAC tools was observed when used by different groups, although inconsistent results were found when used to evaluate NPP in the provinces. The tools also generated lower performance scores for the LGUs compared to the existing M&E system, particularly at the barangay levels.

The general reliability of the LNOCAC tools observed may be attributed to the training workshop conducted among the MET members. According to Sattler *et al.* (2015), training can improve the IRR of raters as it leads to a common understanding of the definitions and meaning of the rating scale. However, there is still a need to improve the reliability of the tools in producing consistent results for evaluating local NPP among MET members as low IRR was observed in their performance scores for the LGUs in some dimensions, which may be due to inconsistent implementation of a rating system (Lange 2011).

To improve the reliability of the tools, the IRR of the MET members must be improved. Based on the literature, this can be achieved through tool revisions and repeated instructions (Blick *et al.* 2018). Hence, further refinement and emphasis during the training activities of the tool dimensions in which the MET members had low percentages of acceptable IRR are warranted to reduce frequent rater errors and achieve a desirable level of reliability of the tools. Moreover, the reliability of the tools can also be secured by providing a more elaborate description of the dimensions used. Further, the evaluators may be given or shown an actual LGU scenario for each rating category in each dimension during the training for them to have a better knowledge base of the tools.

Additionally, the construction of the manual of operations and procedures, random testing for the percentage of agreement among the end-users, written and verbal communication options for end-users to address questions and problems, and considering fatigue relative to the time of use can enhance the IRR of the tool

Table 4 . Comparison of the evaluation scores using the LGNMES tool and the MELLPI

	Mean (Standard deviation)			p-value	
	MET	PT	MELLPI	MET vs MELLPI	PT vs MELLPI
Region A					
Barangay	51.78 (17.54)	42.04 (16.51)	88.28 (10.01)	<0.0001*	<0.0001*
Municipality	60.61 (22.79)	65.80 (20.85)	91.87 (7.18)	0.020*	0.051
City	44.37	48.82	87.87	-	-
Province	55.35 (12.32)	38.19 (16.85)	87.25 (8.55)	0.081	0.028*
Region B					
Barangay	61.35 (17.08)	51.03 (21.42)	91.38 (21.61)	0.002*	0.006*
Municipality	72.02 (15.92)	60.95 (17.15)	86.36 (19.21)	0.108	0.052
City	58.40 (14.53)	44.78 (17.12)	87.99 (3.54)	0.034*	0.011*
Province	55.54 (3.83)	54.72 (3.95)	90. 98 (2.29)	0.077	0.077

*Significant at 5% level; MET: Monitoring and Evaluation Team; PT: Project Team; LGNMES: Local Government Nutrition Monitoring and Evaluation System; MELLPI: Monitoring and Evaluation of Local Level Plan Implementation

end-users (Burns 2014). Perhaps, developing a guideline for the MET members in using the LNOCAC tools to clarify the definition of its dimensions would improve the reliability of the tools. Periodic assessment of the IRR of the evaluators in using the tools and setting a limit for M&E sessions to avoid fatigue may also help reduce disagreement among evaluators and increase the reliability of the tools.

Using reliable M&E tools is essential to assess the effectiveness of nutrition programs, identify and address problems in program implementation, and disseminate data for public health actions to improve overall health (Jefferds & Flores-Ayala 2016). Other countries like Bangladesh were able to increase the stakeholders' commitment to community-based nutrition project and their understanding of its progress and evaluation activities by adopting a collaborative M&E system (Kang *et al.* 2021) that is supposed to result in a stronger evaluation design, enhanced data collection and analysis, and M&E data that stakeholders understand and use (O'Sullivan 2012). Moreover, a study showed that strengthening the M&E system could improve the performance of health-related projects (Micah & Luketero 2017).

However, it is important to note that the new system can generate significantly lower performance scores, especially at the barangay levels. Hence, caution should be taken when comparing the annual performance scores of the LGUs that were based on different M&E systems. Nevertheless, its adoption would allow the assessment of the LGUs' performance in relation to the quality standards and evidence-based measures. It will also promote joint discussion among MET members and the LGUs assessed for learning and action plans for nutrition. Moreover, the system is consistent with the Philippine Plan of Action for Nutrition's strategic thrusts and can generate information that would be useful for planning programs for nutritionally at-risk pregnant women and young children.

The study provides evidence on the reliability of the proposed M&E tools in efforts to support the NNC's plan for updating the current M&E system. It is also supplemented with qualitative data regarding the insights from the target end-users of the tools which are deemed useful for future enhancement, updating, and nationwide implementation of the new system. However, the field trial of the tools was only limited to two regions in this study. Other concerns and issues regarding the system may still emerge particularly in LGUs with different organizational settings and M&E practices to the areas included in the study.

CONCLUSION

The MET members generally had a positive perception of the proposed M&E system for the evaluation of local NPP, although they

also raised critical issues and concerns that must be considered to minimize possible problems in the future. The reliability of the LNOCAC tools was also observed in general, but various steps are still warranted for improvement. Moreover, the adaption of the proposed M&E system may have implications for the performance scores of the LGUs.

ACKNOWLEDGEMENT

This research was funded by the NNC Central Office. The authors would like to thank the NNC Regional Offices for helping in the facilitation of the study as well as the MET members and the LGUs who participated in this study.

AUTHOR DISCLOSURES

The authors declare that they have no competing interests.

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J. Gizi Pangan, July 2021, 16(2):81-90
DOI: https://10.25182/jgp.2021.16.2.81-90

Factors Associated with Stunting among 24–35-Month-Old Kalinga Indigenous Children in Pinukpuk, Kalinga, Philippines: A Case-Control Study

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ABSTRACT

The study identified the risk factors associated with stunting among 24–35 months indigenous children in Pinukpuk, Kalinga using case control design; 174 children (87 cases and 87 controls) were randomly selected and information were collected through interview and anthropometric measurements. Odds ratio and 95% confidence interval were used to measure association. Low birth weight, child drinking brewed or commercial coffee, no nutrient supplement intake since birth, and incomplete immunization were child risk factors of stunting. Exclusive breastfeeding from 0–6 months and weaning at >12 months have protective effect. Antenatal visits <4 times, father's height <5 feet, parent's education below secondary level were parental risk factors. Nuclear household and size of less than five members have protective effect against stunting. Having food restrictions among lactating mothers was cultural risk factors. Mothers' insufficient knowledge on exclusive breastfeeding, frequency of and proper way of breastfeeding, continuance of breastfeeding beyond 6 months, benefit of exclusive breastfeeding for six months to mothers and low self-confidence in preparing complementary food were all associated with stunting. Mother's positive attitude on benefits of frequent feeding was found to have protective effect against stunting. Thus, these family factors could be used when designing an action plan to address the problem of stunting among the indigenous Kalinga children.

Keywords: case-control, family factors, stunting

INTRODUCTION

Stunting remains a global issue, recent 2018 statistics from the World Health Organization showed that 22% or 149 million children under 5 years old are stunted. While the number has decreased from 2013 with 165 million or 25%, the number is still quite alarming particularly for the low- and middle-income countries (Black *et al.* 2013). Stunting is defined as having Height-for-Age Z-score (HAZ) that is less than two negative standard deviations below the age-sex median for a well-nourished reference population. Children affected with stunting have an increased risk of dying and suffering from other adverse consequences throughout life (UNICEF 2015) and is one of the leading causes of the global burden of diseases in childhood and 80% of this burden is in developing countries (Save The Children 2012). Several studies showed similar results that stunting before the age of two predicted poorer cognitive development and poor educational outcome in later childhood and has significant educational and economic consequences at the individual, household, and community level (Adair *et al.* 2013; Black *et al.* 2013; Martorell *et al.* 2010). Poor socioeconomic conditions and an increased risk of frequent and adverse conditions such as illness and inappropriate feeding practices may give rise to this high level of stunting. Stunting is noticeably high among indigenous people and, in rural communities. The probability of stunting and underweight among indigenous children is almost three times higher than non-indigenous children; the prevalence is highest among those in the Northern Region and South America (Horta *et al.* 2013; Tanner *et al.* 2014).

In the Philippines, stunting is a public health problem. Results of the National Nutrition Survey showed that the prevalence of stunting among 0–59 months children is 30%. The country ranked number 9 in terms of number of stunted

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(Received 08-12-2020; Accepted 28-06-2021; Published 29-07-2021)

children over 200 countries all over the world (UNICEF 2015). Stunting prevalence in the country among less than 5 years old was highest among the poorest wealth quintile and in rural communities (FNRI-DOST 2015). The Cordillera Administrative Region (CAR), where most of its populations are indigenous people has a 32% and 37% prevalence of stunting among under five children in the year 2013 and 2015 NNS, respectively. Children over the age of 24 months have a significantly higher risk of stunting and the likelihood of being stunted was highest for age groups 24–35 months, followed by 36–47 months and 48–60 months (Habimana & Biracyaza 2019). In Kalinga province, a landlocked and culture-rich province in the Philippines situated within the Cordillera Administrative Region in Luzon, the prevalence of stunting among children under five is 34% and 35% based on the NNS survey in year 2013 and 2015, respectively. If this increasing prevalence of stunting would not be addressed in the early years of life, stunted children would be at risk of the previously mentioned consequences.

Knowledge and information on the relative risk factors associated with stunting would assist policymakers and stakeholders to come up with appropriate interventions to hopefully eradicate stunting problems among the Kalinga Indigenous People (IP) community. Therefore, this study aimed to determine risk factors associated with stunting among 24–35 months old Kalinga in Pinukpuk, Kalinga.

METHODS

Design, location, and time

A retrospective case-control study was conducted in Pinukpuk, Kalinga, Philippines from August to October 2018. The municipality is bounded by the municipality of Balbalan on the west; Conner, Apayao on the north, Tabuk on the south, and the municipality of Rizal and partly by the municipality of Tuao, Cagayan on the east. Agriculture is the main livelihood of the inhabitants in the area. Poultry raising and fishing were supplemental livelihoods of the people in the municipality. The ethical clearance was obtained from Cordillera Regional Health and Research Development Consortium – Ethical Review Committee (CRHDRC-ERC) with CRHDRC No. 2018-01. Furthermore, approval from the National Commission on Indigenous People was sought.

Sampling

A complete list of 594 children 24–35 months old in Pinukpuk, Kalinga was obtained from the Municipal Nutrition Office. The total number of children eligible for the study was 447; 327 were non-stunted and 120 were stunted. The study covered native Kalinga IP in Pinukpuk, Kalinga. Children and mothers with special health conditions and disability were excluded in the study. In this study, stunting was defined based on the WHO Child Growth Standard. Children with a cut-off score of <-2 SD for height-for-age were considered stunted and included in the study.

Sample size was estimated using the following value, confidence level= 95%; relative precision=50%; case-control ratio=1:1; OR=2. From the sample size that was computed (case=76, control=76, Total=152), a 15% non-response rate was used in the study. The total sample size for both case and control children was 87. Simple random sampling (draw lots) was used to select participants. The case and control were matched based on sex and age. The control was composed of non-stunted children. However, no other inclusion criteria were observed in choosing the control group.

Data collection

Prior to collection of data, written consent was obtained from mothers or main caregivers of under five children. The data collection techniques employed were interview and anthropometric measurement of height of children. Height of the child was measured using microtoise. All measuring devices were properly verified. Anthropometric measurement standard of Food and Nutrition Research Institute (FNRI) was adopted to obtain the desired precision and accuracy of height data. Independent variables such as child, parental, household, socioeconomic, community, and cultural characteristics, maternal knowledge, practices, and attitude, and Infant and Young Child Feeding (IYCF) was collected using structured questionnaire via face-to-face interviews with mothers or main caregiver of children between 24–35 months. Child characteristic consisted of sex, type and place of delivery, birth weight, health care, supplementation and immunization. The parental, household, and socioeconomic factors included mother's age of conception, prenatal and antenatal care, parental height,

educational attainment, employment status, type and size of household, annual income, dwelling status, and appliance and vehicle ownership. The community and cultural factors composed of accessibility to water, water treatment, health access, and food beliefs. Mother's knowledge, attitude, and IYCF practices were also collected. Questions on IYCF questionnaire were adopted from the 2013 Philippines National Demographic and Health Survey (NDHS) and 8th National Nutrition Survey questionnaire which includes exclusivity and duration of breastfeeding, age of weaning and complementary feeding. This questionnaire was pretested to ensure reliability (α=0.78). The data were collected by four Barangay (village) Nutrition Scholars who can speak "Kalinga and Ilokano", the dialects in the locale. They had a one-day training to learn the study objectives, apply proper interview techniques, and do anthropometric measurement. Filled questionnaires were checked for its completeness every after-interview session.

Data analysis

The data obtained from the questionnaires were encoded, processed, and categorized using Microsoft Excel. Descriptive statistics, frequency distribution in particular were used to compare the characteristics of the households, children, parents, practices, among others in the case and control groups. Odds ratio and 95% confidence interval was used to measure association of stunting risk factors and measure the strength of the association. Binary Logistic Regression was used to determine odds ratio in this study. Matching sex and age of participant was used to control confounding variables. All analysis was done in SPSS version 16.

RESULTS AND DISCUSSION

Characteristics of the case and control households

Child characteristics. Higher proportion of the children was females in both cases (55%) and controls (55%). Also, majority of the children in both cases and control were born via normal delivery (92%, 90%) and were taken to hospitals or clinics when ill (95, 95%). Also, children delivered at home and with low birth weight were higher among cases (20%, 31%) than in controls (15%, 11%). More children in the control group had complete immunization (97%) and had taken nutrient supplements since birth (92%) compared to the case group that had more children who undergone medication since birth (72%). Finally, more than 80 percent of main caregivers in both groups were mothers.

Parental characteristics. In terms of parental factors, ages of mothers upon conception ranged from 12 to 45 years old. Specifically, mothers younger than 18 years old and older than 35 years old upon conception were higher among cases (33%) than in controls (22%). It is worth noting that almost all the mothers had prenatal check-up in both groups. Mothers who had their prenatal check-up at less than 4 months of pregnancy were higher among controls (90%), and mothers who had equal or less than four times antenatal check-up were higher among cases (51%). On parental height, mothers and father with height below five feet were higher among cases (63%, 18%) than in controls (49%, 5%). In terms of education, mothers and fathers who attained below secondary level were higher among cases (18%, 31%) than in controls (11%, 28%). Meanwhile, in terms of working conditions, there were more working mothers among cases than in controls.

Household characteristics. More than half of the households in the study were nuclear family; it is higher among the cases (68%) than controls (53%). Households with members greater than five were higher in controls (64%) than in cases (62%). Majority of the households has annual per capita income above the annual poverty threshold in CAR. Almost all households owned the dwelling they were occupying; however, 11% of the case and 8% of the control households had no electricity.

Socioeconomic characteristics. Majority of the households in the study owned livestock. Households owning land were higher among controls (54%); whereas, households renting land (34%) and landless (23%) were higher in cases. Majority (89%) of the households in the study owned functioning appliances, higher (91%) among controls compared to cases (87%). More than one third (38%) of the households, in both groups, owned transport vehicles; however, four-wheel transport vehicles owned by controls were two times higher (18%) than cases (6%).

Community characteristics. The sources of drinking water of the households in the study

were mostly dug well (56%), water from spring (45%), and refill (6%). Majority (80%) of the case households applied water treatment to make their water safer to drink. The number of households using water from refilling stations was higher (8%) in control than case group (4%). There were instances that prevented pregnant mothers to access health services. More respondents in cases (7%) mentioned that it was a big problem for them to get permission to go to the doctor for prenatal care compared to control respondents (1%); one third (33%) of the cases said getting money for medication was a big problem; more than one fifth (24%) said that distance to health facility was a big problem, and 8% said that not wanting to go alone was a problem respectively higher than controls.

Regarding food access, majority of both case and control groups claimed that their food comes from their own produce (84%, 82%) and nearby store (94%, 92%). More of the case households (57%) were located greater than five kilometers away from the wet market compared to controls (47%). Households with backyard gardens were higher (83%) among controls than cases (79%); most of the produce for both groups were for human consumption (87%, 80%); and most of them started before the year 2015 (70%, 72%).

Cultural characteristics. In terms of cultural belief, more than half of respondents do not believe on food that could cure sickness, and majority (98%) do not believe that there is food in their community that could cause adverse health effects. For newborns, 12% of the case respondents believed that vegetable or fruit extract should be given to newborns for cleansing. Regarding food taboo among pregnant and young children, more respondents (21%) in control have food taboos compared to cases (12%). Respondents in case household believed that stunting is caused by genetics (51%), malnutrition (32%), and sickness (3%). The same causes were identified by respondents in control but more mentioned genetics. There were more respondents in case (30%) than in control (22%) who had no idea about stunting.

Maternal knowledge, attitudes and practices on IYCF

In terms of IYCF practices in both case and control households, majority of the children were ever breastfed (98%); breastfed immediately or within 30 minutes after delivery (70%); and given colostrum (>75%). Ever exclusive breastfeeding was lower in cases (82%) than in control (95%). Less than half of the children in both groups were exclusive breastfed less than 6 months old and 55% were exclusively breastfed greater than or equal to six months old. For the pre-lacteal feeds, 24% were given pre-lacteal food among cases while only 18% in controls. For complementary feeding practices, 11% of mothers started giving complementary food at six months. Weaning started earlier in control groups while more children were already coffee drinkers in case group (70%).

Generally, knowledge of mothers regarding infant and young child feeding were remarkably more sufficient among controls compared to cases. The highest score among mothers in the control group came from the statement that as the child's age increases the number of feeding times should also be increased; while the lowest came from the statement that an infant should be breastfed at least 8 times per day. Mothers' attitudes towards ideal nutrition-related practices on IYCF were more positive among controls compared to cases. More mothers in cases claimed that it is difficult to continue breastfeeding beyond 6 months and to breastfeed on demand. There were more mothers in the case group who were not confident to breastfeed while there were more mothers who were not confident to give complementary food in the control group (Table 1).

Factors associated with stunting among the 24–35 months old indigenous children in Pinukpuk, Kalinga

Odds ratio estimation revealed a strong association of stunting with child risk factors including low birth weight, incomplete immunization, and no food supplement intake since birth (Table 2). The odds of getting stunted was 3.5 times (OR=3.5; 95% CI:1.57–7.71) higher among low-birth-weight children than those children with normal weight at birth. In related studies, children perceived to be very small or small at birth were more likely to be stunted than those perceived to be large at birth (Chirande *et al.* 2015; Semali *et al.* 2015). Also, low birth weight infants with IUGR have poor growth and neurodevelopmental outcomes (Sharma *et al.* 2015). This might be due to maternal nutrition and antenatal care during pregnancy.

Table 1. Maternal knowledge and attitude on infant and young child feeding in both case and control household

Variables	Case (n=87)		Control (n=87)	
	n	%	n	%
Maternal knowledge on IYCF				
Knew that a neonate should start breastfeeding with in 1 hour after birth				
Yes	59	68	65	75
No	28	32	22	25
Knew that an infant should be breastfeed exclusively for the first 6 months				
Yes	52	60	65	75
No	35	40	22	25
Knew that an infant should breastfeed at least 8 times/day				
Yes	48	55	64	74
No	39	45	23	26
Knew that the start of complementary feeding for infants should be at the age of 6 months				
Yes	57	66	73	84
No	30	34	14	16
Knew that an infant should continue breastfeeding until two years or more				
Yes	56	64	73	84
No	31	36	14	16
Knew that a breastfeed infants should take complementary food 2-3times/day at 6-8 months				
Yes	47	47	73	84
No	40	40	14	16
Knew that as the child age increases the number of time of feeding should be increased				
Yes	55	63	77	89
No	32	37	10	11
Maternal attitude towards ideal IYCF				
Exclusive breastfeeding for 6 months				
Perceive Benefits				
Good	84	97	85	98
Not good	3	3	2	2
Perceive Barriers				
Difficult	11	13	9	10
Not difficult	76	87	78	90
Breastfeeding in Demand				
Perceive benefits				
Good	86	99	86	99
Not good	1	1	1	1
Perceive Barriers				
Difficult	17	80	11	13
Not difficult	70	20	76	87
Self-Confidence				
Breastfeeding				
Confident	81	93	79	91
Not confident	6	7	8	9
Complementary feeding				
Confident	76	87	85	98
Not confident	11	13	2	2
Continue breastfeeding beyond 6 months				
Perceive benefits				
Good	84	97	85	98
Not good	3	3	2	2
Perceive barrier				
Difficult	25	29	16	18
Not difficult	62	71	76	87

IYCF: Infant and Young Child Feeding

Table 2. Factors associated with stunting among Kalinga children

Variables	OR	95% CI
Child factors		
Low birth weight	3.5	1.57–7.71**
Ever exclusively breastfed within the 1st 6 months	0.2	0.07–0.67**
Child has not taken supplements since birth	3.2	1.27–8.05**
Incomplete immunization	4.9	1.35–17.94**
Parental factors		
Full term number of prenatal check-up less than 4 times	2.6	1.39–4.94**
Height of father below 5ft	4.7	1.49–14.63**
Mother's educational attainment below secondary level	2.4	1.28–4.60**
Father's educational attainment below secondary level	5.3	2.16–12.90**
Households factors		
Nuclear/single family	0.5	0.29-0.99*
Household members greater than five	0.6	0.24–0.87**
Cultural factors		
Nutritional beliefs and restrictions		
Have food restriction for pregnant woman	0.6	0.28–1.37
Have food restriction for lactating woman	2.0	1.11–3.78**
Have food restriction for young children	0.5	0.09–2.74

*p<0.05; **p<0.001

Children who did not receive the required complete immunization were 4.9 times (OR=4.9; 95% CI:1.35–17.94) more likely to develop stunting than their counterparts. Similar findings were observed in Southern Ethiopia in which children who did not receive any vaccine were 6.4 times more likely to be under-nourished as compared to those who were completely vaccinated (Batiro *et al.* 2017). This was probably because the unvaccinated or incompletely vaccinated children were more prone to infectious diseases like pneumonia, diarrhea, and measles, which might have exposed them to undernutrition and growth retardation. Children who never had food supplement intake since birth were 3.2 times (OR=3.2; 95% CI:1.27–8.05) more likely to be stunted than children who had taken food supplements since birth. According to the European Food Safety Authority (nd), food supplement is defined as concentrated sources of nutrients or other substances with a nutritional effect that are marketed in dose form. Sufficient food intake and adequate supply of calories, vitamins and minerals are essential for growth and development and the majority of malnourished children fail to achieve their full genetic body growth both linear and ponderal due to the lack of such intake. In this study, micronutrient supplements are from government programs in the form of vitamin A capsules, ferrous sulfate syrup or capsules, and iodine in liquid form, and commercially bought supplements often prescribed by physician to treat or prevent a micronutrient deficiency in young children.

The present study also showed that parental risk factors were significantly associated with stunting like full-term total number of maternal prenatal check-ups less than four times, height of father below 5ft, and mother and father's educational attainment below secondary level. Children of mothers who had less than four times prenatal check-up until delivery were 2.6 times (OR=2.6; 95% CI:1.39–4.94) more likely to be stunted than their counterparts. A similar study showed that a child of mother who had fewer than four government antenatal care visits during pregnancy was more likely to be malnourished (Hamel *et al.* 2015). Other authors have also found a positive association between antenatal care services and improved birth outcomes (Kunt & Volmer 2017). Antenatal visits are indicators of contact with health services and health-seeking behavior which may be associated with better care and feeding practices for young children. Children of father whose height is below 5 ft were 4.7 times (OR=4.7; 95% CI:1.49–14.63) more likely to develop stunting than children of father whose height is greater than 5 ft. Parental height

influences the offspring's linear growth. Child's linear growth is not only influenced by nutritional status during growth phase but is also affected by genetics (Martorell *et al.* 2010; Prentice *et al.* 2013). Thus, a shorter parent is more likely to have shorter children. Children of mothers (OR= 2.4; 95% CI:1.28–4.60) and fathers (OR=5.3; 95% CI:2.16–12.90) with educational attainment below secondary school were more likely to develop stunting than children of parents who attained above secondary education. This finding is similar to other studies conducted in different countries (Biswas & Bose 2010; Jesmin *et al.* 2011; Mayhar *et al.* 2010; Maulundyani *et al.* 2012). Parental education is associated with improved child-care practices related to health and nutrition and better ability to access and benefit from intervention (Black *et al.* 2013). Educated mothers have better resource allocation and understanding of nutrition information than the less educated. Household risk factors were also found significantly associated with stunting like type of household and household size (Table 2). In this study, it was found that children living in a nuclear household (OR=0.5; 95% CI:0.29–0.99) were significantly less likely to develop stunting than children living in an extended household. This finding is consistent with the study conducted in South Africa where stunted children are common with large household members (Mayhar *et al.* 2010). Association of large number of household members might be due to resource depletion and decrements of food availability and more competition for available food.

The study also demonstrated that cultural risk factors like nutritional beliefs and restrictions for lactating mothers were shown to have statistically significant association with stunting. Children of mothers who believed and practiced cultural nutritional beliefs and restrictions during lactating period were 2.0 times (OR=2.0; 95% CI:1.11–3.78) more likely to be stunted compared to children of mothers with no nutritional restrictions while lactating (Table 2). This might be because food restriction might limit nutritional intake which could contribute to lactation difficulty or failure.

Maternal infant and young child feeding practices found to be statistically and significantly associated with stunting were giving of at least 1 cup or more of coffee to young children, ever exclusively breastfed in the first six months of life and weaning at age greater than 12 months. In this study, mothers who gave their child at least 1 cup or more of coffee a day made their children 2.6 times (OR=2.6; 95% CI:1.41–4.91) more likely to develop stunting than children of mothers who did not give coffee to their young children. (Table 3) In related studies, caffeine-containing beverage such as coffee can inhibit calcium absorption and contributes to calcium excretion leading to poor bone growth due to inadequate calcium supply (NIH 2021).

In this study, children who were ever exclusively breastfed in the first six months of their life were significantly less likely to develop stunting as compared to non-ever exclusively breastfed (OR=0.2; 95% CI:0.07–0.67). Breast milk is the best food for infants. It provides a unique nutrient composition needed for cell function and growth. Exclusive breastfeeding up to six months of age helps in the attainment of child growth and development and visual acuity (Pem 2015). On the contrary, a study conducted in Libya and Nepal showed that infants exclusively breastfed for either less than six months or more than eight months were more likely to be stunted (Taguri *et al.* 2008). Ideally, breastfeeding should be exclusively practiced during the first six months of life. In this study, children who were weaned at the age greater than 12 months were less likely to be stunted as compared to children weaned at the age below 12 months (OR=0.5; 95% CI:0.26–0.95). Yet, other studies showed that children breastfed for more than 12 months were significantly more likely to be stunted than those breastfed up to 12 months (Akombi *et al.* 2017; Tiwari *et al.* 2014). This might be a result of cultural influences, quality of complementary feed, availability of food in the household, and maternal knowledge.

Maternal knowledge of IYCF is an important factor for successful child feeding. In this study, maternal knowledge on IYCF was found statistically significant risk factors associated with stunting. Table 3 shows that children of mothers who had insufficient knowledge on the duration of exclusive breastfeeding, proper frequency of breastfeeding, and proper way of administering breastfeeding, right age of child to receive complementary food, proper amount of complementary food per day depending on the age of the child, continue breastfeeding until two years or more and benefit

Table 3. Maternal infant and young child feeding KAP associated with stunting

Variables	OR	95% CI
Maternal practices on IYCF		
Ever exclusively breastfeed within the 1st 6 months	0.2	0.07–0.67**
Age of weaning ≥12 months	0.5	0.26–0.95*
Child drinks one or more than a cup of coffee per day	2.6	1.41–4.91**
Maternal knowledge on IYCF		
Did not know that an infant should be breastfed exclusively for the first 6 months	2.0	1.04–3.79*
Did not know that an infant should breastfeed at least 8 times/day	2.3	1.20–4.27**
Did not know that an infant should finish one breastmilk before switching to another breast	2.5	1.27–4.99**
Did not know that the start of complementary feeding for infants should be at the age of 6 months	2.7	1.33–5.65**
Did not know that an infant should continue breastfeeding until two years or older	2.9	1.40–5.93**
Did not know that a breastfed infant should take complementary food 2–3 times/day at 6–8 months	4.4	2.18–9.03**
Did not know that as the child's age increases the number of feeding times should also be increased	4.5	2.03–9.87**
Did not know that exclusive breastfeeding during the first 6 months is beneficial to lactating mothers	2.8	1.27–6.10**
Maternal attitudes towards ideal		
Not confident preparing complementary food	6.0	1.32–28.64*
Positive attitudes towards perceived benefits of frequent feeding	0.1	0.01–0.94*
Difficult to feed child frequently	2.0	0.76–5.31

*p<0.05; **p<0.001; IYCF: Infant and Young Child Feeding; KAP: Knowledge, Attitude, and Practices

of exclusive breastfeeding for six months to lactating mothers were significantly more likely to be stunted as compared to their counterpart. Similar study was observed in Bangladesh that showed significant association between nutritional status and maternal child care. Unadjusted mean of height-for-age Z-scores of children born to mothers having high level of maternal child care knowledge were significantly higher than those children of mothers who had lower knowledge levels in child care practices (Saaka 2014). Knowledge of mothers on IYCF recommendations has statistically significant association with their IYCF practices. Mothers who had insufficient knowledge of IYCF recommendation were more likely to have inappropriate feeding practices (Demilew 2017).

Table 3 also shows that maternal attitudes towards ideal child feeding and nutrition-related practices were found to be significant risk factors for stunting. Children of mothers who were not confident that they prepared complementary food for their child correctly were 6.1 times more likely to be stunted as compared to their counterparts. Mothers who had positive attitude towards the benefits of frequent child feeding made their children less likely to develop stunting . Positive attitude towards infant feeding is an important component in child nutritional health.

CONCLUSION

Stunting was found to be a result of multiple factors such as low birth weight, incomplete immunization, no food supplement intake, household type and size, and maternal and infant and young child practices particularly exclusive breastfeeding, and maternal knowledge. The study implied that preventing stunting requires multi-disciplinary approach, the study suggests that public health intervention programmer should focus on these risk factors to reduce or

alleviate stunting. Maternal knowledge on IYCF plays an important role on the prevention of stunting. Mothers/caregivers should be educated on the importance of exclusive breastfeeding with its proper mechanics, methods of complementary feeding, and maternal care and nutrition during pregnancy and lactation. Programs or advocacies promoting proper nutrition, breastfeeding, multiple micronutrient supplementation, immunization, periodic growth monitoring, as well as knowledge of mothers on their child's nutritional status should be strengthened to address the risk factors of stunting.

ACKNOWLEDGEMENT

The researcher would like to extend its gratitude and appreciation to National Commission on Indigenous People which permitted to conduct the study and the Accelerated Science and Technology Human Resource Development Program-National Science Consortium (ASTHRDP-NSC) under the Department of Science and Technology (DOST) which has provided funding for this research.

AUTHOR DISCLOSURES

The authors have no conflict of interest.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, July 2021, 16(2):91-100***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://10.25182/jgp.2021.16.2.91-100*
ISSN 1978-1059 EISSN 2407-0920

Child Development and Nutritional Status of Children Under Five: A Cross-Sectional Study of a Fishermen Community in Terengganu, Malaysia

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ABSTRACT

This study aims to determine child development, nutritional status and the association between child development and nutritional status. This cross-sectional study was conducted among 60 fishermen's children under five years old in selected districts in Terengganu. All respondents were assisted by their mothers during the assessment. Child development was evaluated through the Denver II Development Screening Test. Nutritional status was determined via the anthropometry assessment (BMI-for-age and height-for-age). Preponderantly, the prevalence of suspected development delay of children under-five in Terengganu was 31.7%. The prevalence of suspected delay for language, fine motor-adaptive, and personal-social skills were 15.0%, 1.7%, and 16.7%, respectively. BMI-for-age z-score and height-for-age z-score of these children were -1.62±1.23 and -0.27±1.41, respectively, indicating a normal range; nonetheless, there were still children that were wasted (23.3%), severely wasted (13.3%), stunted (5.0%), severely stunted (1.7%), and at risk of being overweight (1.7%). However, the chi square test showed there was no association found between child development and nutritional status, BMI-for-age and height-for-age ($p>0.001$), among fishermen's children under-five in this study. The nutritional status had no effect on the development of these fishermen's children, but may have been influenced by other factors such as stimulating surroundings, parenting abilities, and culture.

Keywords: child development, children under five years, fishermen, nutritional status

INTRODUCTION

Extant research recognizes early child development's critical role in a person's imminent trajectory and life course (Huiracocha-Tutiven *et al.*2019). An increasing body of evidence indicates that children from low-income families have slightly slowed development growth (Grantham *et al*. 2014; Rao *et al.* 2019; Rubio-Codina *et al.* 2016). Child development has received much too little attention in these low-income households, especially in fishing communities, until now. Floods and monsoon seasons affect this community's income, particularly in Terengganu, resulting in inadequate food supplies and damaged infrastructure, leading to food shortages, poor nutrition, and health issues (Capanzana *et al.* 2018; FAO 2000; Sanusi *et al.* 2018; Atiah & Asma' 2021). Although most Terengganu fishermen's household income was over the poverty level, they still lacked health, education, and insurance (Chua *et al.* 2018; Wei & Ali 2018; Sanusi *et al.* 2018). The majority of the parents and children in this neighbourhood only completed secondary school (Wong *et al.* 2014). In terms of occupation, the majority were occupied in fishing, while some were involved in agricultural or labour activities. As for their wives, more than half of them were unemployed and worked as housewives in Terengganu (Wei & Ali 2018; Wong *et al.* 2014).

Child developmental delay is the inability of children under the age of five to reach developmental milestones associated with cognitive and motor disabilities, resulting in limitations in life activities (NHMS 2016b). In 2016, the incidence of developmental delay among these children ranged from 5% to 16% worldwide, depending on environmental and genetic factors. In 2015, the prevalence of overall developmental delay in children in Malaysia was 3.3%. The prevalence of developmental delay in speech, social skills, fine motor, and gross motor was 1.7%, 1.2%, 0.7%, and 0.6%, respectively,

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(Received 29-04-2021; Accepted 07-07-2021; Published 29-07-2021)

at the national level. There was also a higher incidence of developmental delay in children whose mothers had low educational levels and whose parents were unemployed (NHMS 2016b). This demonstrates the significance of a family's socioeconomic status in a child's growth.

The national prevalence of underweight, stunting, and wasting in Malaysia in 2015 was 13%, 13.4%, and 7.9% for these children, respectively (NHMS 2015). Meanwhile children under the age of five in rural areas had a higher prevalence of stunting and a lower prevalence of wasting than children in urban areas (NHMS 2015). A comparable study conducted in 2016 discovered that the national prevalence of underweight climbed to 13.7% among children under the age of five, the national prevalence of stunting increased swiftly to 20.7%, and the national prevalence of wasting increased marginally to 11.5% (NHMS 2016b). According to UNICEF Malaysia (2018), stunting among children under five increased alarmingly from 2006 to 2016, rising from 17.7% to 20.7%. According to these three reports, the prevalence of underweight, stunting, and wasting among Malaysian children under five has grown, signaling the need for intervention initiatives to reduce its prevalence. More recently, research has been published that indicates a clear correlation between child development and nutritional status. According to studies, when a child's nutritional condition is excellent, its growth is more substantial (Huiracocha-Tutiven *et al*. 2019; Hurley *et al.* 2016; Jimoh *et al.* 2018; Warsito 2012). Malnutrition affects a child's growth because the body is unable to develop due to a lack of nutrition (Capanzana *et al.* 2018). Terengganu had among the highest rate of stunting in 2016, at 26%, which was significantly higher than the national rate of 13.4% (UNICEF 2018). The growing incidence of stunting in Malaysia, especially in Terengganu, is concerning because it will have a negative impact on children's future health because they are still in the critical growth stage. Additionally, there is a dearth of knowledge on Terengganu children's growth and a scarcity of data on the nutritional status of children under the age of five living in fishing communities in Terengganu. Thus, in light of the aforementioned issues, it is critical to conduct this study on child development and its relationship to nutritional status among fishermen's children under the age of five in Terengganu. In keeping with the aims of the study, the following research question was addressed: Is there any significant relationship between child development and nutritional status among fishermen's children under the age of five in this study? The hypothesis for this study is that children under the age of five who live in low-income households have poor child development and nutritional status.

METHODS

Design, location and time

This cross-sectional study was conducted in Kuala Nerus and Kuala Terengganu (these two districts in Terengganu reside a large number of fishermen). The respondents involved were voluntarily mothers, and had healthy children aged 2 years to 4 years 11 months old from a fishermen household. Ethical approval was obtained by the Human Ethics Board of Committees of Universiti Malaysia Terengganu with reference number: JKEPM/2019/37.

Sampling

According to the Malaysian Open Data Portal (2019), the total population of Kuala Terengganu and Kuala Nerus in 2016 was 367,600, while the number of children aged 0 to under five years old was 38,000 in both districts, accounting for 10.33% of the total population. Meanwhile, the total number of fishermen in Kuala Terengganu and Kuala Nerus in 2016 was 2,567. This study covers a selected population to match the purpose of the study, with responses ranging in age from 2 years to under five years. The children in this age group were chosen for consistent and accessible anthropometric measurement since they can stand without the assistance of others for the determination of nutritional status.

Respondents are excluded from this study if they are under two years old considering them as infants. As the age range is further lowered to 2 to under five, the possibilities of obtaining respondents are further lessened. Due to the scarcity of respondents who met the criteria, healthy 2 to 5-year-old children from the fisherman community, snowball sampling was used. Researchers went to each fisherman's ports in these districts and asked the fishermen if they knew of any eligible candidates to find a suitable candidate. The chosen respondents were also asked if they knew any other qualified people for

future data gathering. Given the 95% confidence interval, and the prevalence of stunting of children under five in Terengganu was 20.7% (UNICEF 2018), the minimum sample size derived from Cochran's formula was 60 respondents. Data was collected between July to October 2019.

Data collection

The data was collected via home visits to fishermen homes. The subjective information sheet and informed consent were given to the respondents' moms before data collection. Once mothers agreed to participate voluntarily, a questionnaire was to mothers. In Section 1, the moms were given questionnaires to collect information about their socio-economic situation. The researchers then measured the respondent's weight and height, which were reported in Section 2. Following that, in Section 3, researchers interviewed mothers about their child's development. A token of appreciation was handed at the end of the session. The questionnaire consists of three sections: 1- Socio-economic status; 2- Child development (Denver II Development Screening Test); and 3- Nutritional Status (BMI-for-age z-score and height-for-age z-score). It was administered during a face-to-face interview with parents and their children.

Meanwhile, for child development, the parameters used were gross motor, language, fine motor-adaptive, and personal-social skills. The children were assessed on their ability to perform certain items or activities by listening to the researcher's instructions or parents. The questions were adapted from the Ministry of Health's Baby and Children Health Records book and the National Health and Morbidity Survey questionnaire (NHMS 2016a). The scoring system and interpretation were based on the Denver II Development Screening Test. Denver II Development Screening Test is solely for screening purposes to detect whether the child is suspected of delay in development. Therefore, it does not utterly confirm that a child is indeed delayed. Normal indicates that the child is undergoing normal development with no delays for their age. Suspect indicates that there is a probability of the child undergoing delay in development for their age and are recommended to see an expert for re-screening.

The children's weight and height were measured using an electronic weighing scale (Tanita Bioelectrical Impedance Analysis BC-541, Japan) and SECA portable stadiometer 225 (SECA, Hamburg, Germany), respectively. Without shoes, the respondent stands upright with arms at his sides, feet together, and heels and back against the wall. The measurement should be taken from a line drawn on Plexiglas and should be accurate to within 0.5 cm. Ascertain that the weighing scale is on a flat, hard surface and that it reads zero when there is no weight. The respondent is measured when dressed in light clothing and without shoes. Weight measurements should be taken to the nearest 0.1 kg. The measurement taken was analyzed using a WHO Anthro version 3.2.2 software to assess the children's nutritional status in z-score (BMI-for-age and height-for-age). The child's BMI-for-age was calculated using z-scores to decide if he or she is overweight, obese, or wasting. The height-for-age z-scores are used to decide whether a child is stunted. The height-for-age and BMI-for-age indicators were determined using the WHO (2006) cut-off points. When the reading is >3 SD z-score, the child is classified as tall for his or her age or obese for BMI-for-age. When a child's reading score is greater than 1 or 2 SD in z-scores, he or she could be at risk of being overweight or obese, respectively (for BMI-for-age). Meanwhile, if the height-for-age z-score reading falls below -2 or -3 SD, the children are classified as stunted or severely stunted, respectively. In terms of BMI-for-age z-scores, children who fall below the -2 and -3 SD z-scores, respectively, are considered to be wasting.

Data analysis

The SPSS (IBM SPSS Statistics for Windows, Version 23) was used with the significance level of $p<0.05$. A normality test was carried out before data analysis. Descriptive data for socio-demographic characteristics, child development and nutritional status was calculated in terms of mean, median, frequency, percentage, standard deviation, and interquartile range. Chi-square test was used to determine the association between child development and nutritional status of fishermen's children under-five, significant at $p<0.05$.

RESULTS AND DISCUSSION

Sociodemographic data

Among the 60 respondents, 30 (50.0%) were boys, while the remaining 30 (50.0%)

were girls. All of the respondents were Malay 60 (100.0%) and Muslim 60 (100.0%), as shown in Table 1. Majority were aged 3 years - 4 years 11 months (n=35, 58.3%) followed by 2 years - 2 years 11 months (n=25, 41.7%). Most of the parents had a secondary school education (78.3%). Monthly household incomes are typically less than RM1,500 (93.3%), with households of 5 to 8 family members (46.7%), and more than half reported not receiving any financial aid (55.0%). Overall, the sociodemographic status of fishermen's households in Kuala Nerus and Kuala Terengganu is low. In the majority of these families, the parents' highest standard of schooling was just secondary school. It's upsetting because education is so important in everyone's life. It's also worrying because children have a proclivity to follow in their parents' footsteps. Higher education contributes to higher earnings, which can improve their socioeconomic status by raising their monthly household income by at least RM1,000. Furthermore, most mothers of the respondents were housewives who depended solely on their husbands' income.

Child development of the children

The parameters employed in this child development assessment were based on gross motor, language, fine motor-adaptive, and personal-social skills. The majority of respondents had a normal overall development (n=41, 68.3%). The suspected development delay was 31.7% (n=19) in this study, which is considered to be comparatively high in comparison to studies conducted in Malaysia (3.3%) (NHMS 2016b), in Norway (5.7% to 7.0%) (Valla *et al.* 2015), in the United Arab Emirates (8.4%) (Eapen *et al.* 2006), and in Ecuador (11.7%) (Huiracocha-Tutiven *et al.* 2019). It is likely that the findings of these studies were affected by the techniques used in the various studies. The Denver Development Screening Test was used in this study, while other studies have used the New Child Health Record Book (NHMS 2015), the Schedule of Growing Skills II (Jimoh *et al.* 2018), and the Ages and Stages Questionnaire (Valla *et al.* 2015; Zhang *et al*. 2018). Two critical factors may have contributed to this suspected developmental delay among fishermen's children in this study: socioeconomic status and parenting skills. These respondents live in households with a low socioeconomic status, which is considered to have a negative effect on children's growth (Grantham *et al.* 2014; Rao *et al*. 2019; Rubio-Codina *et al.* 2016; Treanor *et al.* 2012) due to the fact that poverty can result in cognitive and behavioural delays (UNICEF 2018; Shong *et al.* 2018). Numerous studies have found that socioeconomic status (i.e., income and education) has the greatest negative effect on child growth (Grantham *et al.* 2014; Rao *et al*. 2019; Rubio-Codina *et al.* 2016; Treanor *et al*. 2012).

The prevalence of suspected developmental delay in gross motor and fine motor skills was 0.0% and 1.7%, respectively, among fishermen children under the age of five in Kuala Nerus and Kuala Terengganu. This result is consistent with NHMS (2016), which found that the prevalence of developmental delay in gross motor skills was 0.6% and 0.7% respectively, among Malaysian children. Contradicts to a finding by Duarte *et al.* (2017), children from low-income households have a higher risk of motor developmental delay. Given that the fishermen's children came from low-income families (93.3% came from households earning less than RM1500 per month), it is impressive that their motor production was not delayed. Duarte *et al.* (2017) stated that the atmosphere in which children grow plays a role in their motor growth, with the more stimulation in their immediate environment, the more developed their motor skills (Duarte *et al.* 2017). This statement is confirmed by Venetsanou and Kambas (2010), who discovered that positive environmental benefits these children's motor growth, especially gross motor development. Additionally, they noted that a household's low socioeconomic status results in a lack of available space and a lack of variety of toys, which can have a negative effect on motor growth (Venetsanou & Kambas 2010). Toys, such as building blocks, may assist children in improving their fine motor-adaptive skills. In comparison, children in fishermen households may have a low income and live in a small home, but they have access to spacious outdoor areas such as the playground, the yard, and the beach. Additionally, they may lack toys, but they are exposed to other resources that can be used to play, such as tag and chase, hide and seek, or building structures with sand to improve motor skills (Venetsanou & Kambas 2010), which may explain why some of them advanced in gross motor (5.0%) and fine motor-adaptive (33.3%) growth. Additionally,

Table 1. Sociodemographic characteristics of under five and respondents in Terengganu (n=60)

Characteristics	Distribution n (%)	Mean±SD/ Median (IQR)
Gender		
Boy	30 (50.0)	
Girl	30 (50.0)	
Race		
Malay	100 (100.0)	
Religion		
Islam	100 (100.0)	
Age		37.93±10.49 months
2 years–2 years 11 months	25 (41.7)	
3 years–4 years 11 months	35 (58.3)	
Highest education level (Mother)		
Primary school	7 (11.7)	
Secondary school	47 (78.3)	
Certificate/STPM/Diploma	4 (6.7)	
Degree/Higher education	2 (3.3)	
Highest education level (Father)		
No formal education	2 (3.3)	
Primary school	11 (18.3)	
Secondary school	44 (73.3)	
Certificate/STPM/Diploma	3 (5.0)	
Number of family members		5 (3)
1–4	25 (41.7)	
5–8	28 (46.7)	
>9	7 (11.7)	
Monthly household income		
≤RM1,500	56 (93.3)	
RM1,500–RM2,999	4 (6.7)	
Mother's occupation		
Run small business	2 (3.3)	
Store assistant	3 (5.0)	
Caregiver	1 (1.7)	
Housewife	50 (83.3)	
Others	4 (6.7)	
House ownership		
Bought	3 (5.0)	
Rent	12 (20.0)	
Owned or passed down by parents	45 (75.0)	
Financial aid receiver		
Yes	27 (45.0)	
No	33 (55.0)	
Food aid receiver		
Yes	2 (3.3)	
No	58 (96.7)	

RM: Ringgit Malaysia

Duarte *et al.* (2017) discovered that children who have more siblings have greater gross motor development than children who have fewer siblings. The median number of family members in a fishermen's household was five, suggesting that most of them have siblings to play with, which aids in gross motor growth. This will account for the fishermen's children's superior motor growth.

As seen in Table 2, both language development (n=9, 15.0%) and personal social skills (n=10, 16.7%) faced substantial delays. This, however, contradicts the results of a previous national survey, which showed that only 1.7% of children had a delay in language development (NHMS 2016b). There are several possible reasons for this contradiction, including the sample size, respondents' sociodemographic characteristics, and respondents' age. The previous study was a large-scale study that included respondents from a number of sociodemographic backgrounds and a wide range of ages. Numerous previous studies established a strong correlation between language development and socioeconomic status, and home climate (Rubio-Codina *et al.* 2016; Bradley *et al.* 2002; Pem 2015). This is because parents of low socioeconomic status are less likely to purchase products that encourage brain growth, such as reading and learning materials. Additionally, they frequently have no say about how much time their children spend watching television. Parenting abilities are a factor in a child's language growth (Bradley *et al.* 2002). Communicating with and engaging in simple activities with their children, such as reading, will aid in brain development, including language development (Bradley *et al*. 2002).

As shown, the prevalence of suspected personal social skills development delay in fishermen's children was 16.7%. This research contradicts the results in NHMS (2016b), which suggested a substantially lower prevalence of social skill developmental delay among children in Malaysia (1.2%). Personal social skills refer to children's ability to care for themselves (e.g., putting on or removing clothes independently) and social skills (such as making friends and playing with anyone). However, few studies exist that examine the factors influencing children's development of personal social skills, primarily since most studies classified self-care as a fine motor skill. Though, there is an explanation for the high prevalence of suspected development of

personal social skills among fishermen's children. According to Venetsanou and Kambas (2010), cultures often play a role in child growth, as various cultures emphasize different development aspects while undermining others.

Nutritional status of the children

As shown in Table 3, these children's BMI-for-age and height-for-age z-scores were -1.62±1.23 and -0.27±1.41, respectively, implying normal BMI and height. In Table 3, the fact that approximately 36.6% of respondents were wasted or severely wasted stands out. This study showed that the prevalence of wasting was higher than the national prevalence of 11.5% to 20.7% (NHMS 2015; NHMS 2016b). Wasting occurs when a child's BMI-for-age falls below -2SD of the WHO z-score for growth development, according to the World Health Organization (1997). It is often linked to acute and/or severe disease, as well as chronic unfavourable conditions like inadequate hygiene, which may make children more susceptible to disease. Low daily energy intake, particularly during the monsoon season, is one possible explanation for our findings, which can lead to wasting. During the monsoon season, about 98.8% of the fishermen households in Terengganu were severely food insecure, and the remainder were moderately food insecure, according to a study conducted by Sanusi *et al.* (2018) in Terengganu. Food coping mechanisms were most widely used in the Sanusi study by reducing the amount of food cooked for meals and decreasing daily/monthly food expenditure. As a consequence, it's likely that wasting would occur among the children in this sample.

However, there are other possible explanations for the higher prevalence of wasting in this study. Prior studies have noted the importance of a mother's education and practices (Bandoh *et al.* 2018; Capanzana *et al.* 2018; Cheah e*t al.* 2012; Khan *et al*. 2019; Mzumara e*t al.* 2018). The prevalence of wasting was shown to be strikingly higher among mothers with lower education level (Adnan & Muniandy 2012). A study discovered that mothers with low BMI during pregnancy have a greater risk of having wasting and underweight children (Khan *et al.* 2019). These findings may be somewhat limited by the nutritional status of mothers during their pregnancy. Low income is one of the key factors in poor nutritional status since they have restricted access to good food resources, which leads to poor food composition in their diet, low food consumption, and even limited access to health services (Chua *et al.* 2018; Wong *et al.* 2014; Cheah *et al.* 2012). To add, as fishers were commonly poor, their household expenditure and unavailability of land for home agriculture activities, especially during monsoons, lead to minimal access to food. The same study by Cheah *et al.* (2012) discovered that other vital factors might affect these children's nutritional status, such as age, where younger children have a high prevalence of underweight, stunting and wasting.

In contrast, older children and adolescents have a high prevalence of overweight and obesity. Other than that, the children's breastfeeding duration has significantly affected the prevalence of underweight, stunting, and wasting. Meanwhile, a mother's nutrition and low birth weight also impact children's nutritional status. Imbalanced feeding and low-energy diet food due to mothers' lack of nutrition education also play

Table 2. Overall child development and it's parameter among under five children in Trengganu (n=60)

Items	Distribution n (%)
Overall development	
Normal[a]	41 (68.3)
Suspect[b]	19 (31.7)
Gross motor	
Advanced	3 (5.0%)
Normal	57 (95.0%)
Caution	0 (0.0%)
Delayed	0 (0.0%)
Language	
Advanced	0 (0.0%)
Normal	50 (83.3%)
Caution	1 (1.7%)
Delayed	9 (15.0%)
Fine motor-adaptive	
Advanced	20 (33.3%)
Normal	39 (65.0%)
Caution	0 (0.0%)
Delayed	1 (1.7%)
Personal-social skill	
Advanced	0 (0.0%)
Normal	47 (78.3%)
Caution	3 (5.0%)
Delayed	10 (16.7%)

[a] Normal indicates that the child is undergoing normal development with no delays for their age

[b] Suspect indicates that there is a probability of the child undergoing delay in development for their age and are recomended to see an expert for re-screening

Table 3. Nutritional status of under five children in Terengganu (n=60)

Characteristics	Distribution n (%)	Mean±SD
BMI-for-Age (z-score)*		-1.62 ±1.23
Above 3 SD (Obese)	0 (0.0)	
Above 2 SD (Overweight)	1 (1.7)	
Above 1 SD (Possible risk of overweight)	0 (0.0)	
0 (Median) (Normal)	19 (31.7)	
Below -1 SD (Normal)	18 (30.0)	
Below -2 SD (Wasted)	14 (23.3)	
Below -3 SD (Severely wasted)	8 (13.3)	
Height-for-Age (z-score)		-0.27 ±1.41
Above 3 SD (Tallness)	2 (3.3)	
Above 2 SD (Normal)	1 (1.7)	
Above 1 SD (Normal)	7 (11.7)	
0 (Median) (Normal)	34 (56.7)	
Below -1 SD (Normal)	12 (20.0)	
Below -2 SD (Stunted)	3 (5.0)	
Below -3 SD (Severely stunted)	1 (1.7)	

*Cut-off point is based on WHO (2006)

a significant role in children's nutritional status. The children's appetite too impacted them as they consume low calorie that prevents their growth and development (Cheah *et al.* 2012).

A total of 6.7% of respondents were stunted or seriously stunted. Stunting is when the height-for-age of children is below -2SD of WHO growth standard z-score. Stunting is caused by prolonging insufficient consumption of nutritious food and a poor-quality diet that leads to a lack of nutrient intake (Mzumara *et al.* 2018). This result is somewhat counterintuitive because stunting is lower than in another Terengganu study (Wong *et al*. 2014), the national prevalence of stunting (13.4%) (NHMS 2015), and Terengganu's prevalence of stunting (18.2%) (NHMS 2015). This variance may be explained by variations in the number of respondents, geographic location, socioeconomic status (NHMS 2015; NHMS 2016b; Wong *et al.* 2014; Cheah *et al*. 2012), and the age range of the samples involved (NHMS 2016b). Geographical areas and various socioeconomic status are also the cause of the differences in the prevalence of wasting and stunting as this study only include fishermen communities, where other studies involved both rural and urban areas (NHMS 2015; NHMS 2016b; Wong *et al.* 2014; Cheah *et al*. 2012). Another reason for the differences in the broad age range of samples involved where this study only involves children aged 24 to 59 months, while other studies involve children aged 0 to 59 months (NHMS 2016b). This study has a lower percentage of stunting concerning other studies, primarily may due to higher accessibility to fish consumption. Fishes are an excellent protein source, Polyunsaturated Fatty Acids (PUFA), vitamins, and iodine. Protein and amino acids assist in tissue growth and development, while polyunsaturated fatty acids assist in vitamin A and K absorption. Iodine assists with the mental development of children's cognitive abilities (Mlauzi *et al.* 2017). More work needed to be done exploring the relationship between particular food group intake and its effects on reducing stunting prevalence.

Association between nutritional status and child development

The chi-square test for independence shows no significant association between BMI-for-age and child development, χ^2 (1, n=60)=0.167; p=0.682; OR=0.793, meanwhile, there was also no association between height-for-age and child development, p>0.001, OR=0.919, using Fisher exact test as shown in Table 4. The study has indicated that although wasting was prevalent, statistically, it was not evident that their nutritional status was associated with their overall development. This finding is contrary to previous studies which have suggested that better nutritional status leads to lower chances of developmental delay (Huiracocha-Tutiven *et al.* 2019; Hurley *et al.* 2016; Jimoh *et al.* 2018).

Table 4. Association between nutritional status and overall development among children under five in Terengganu (n=60)

Nutritional status	Child overall development		χ^2	Suspect delay OR	p
	Normal n (%)	Suspect n (%)			
BMI-for-age[c] (ref: normal)					
Normal	26 (70.3)	11 (29.7)	0.167[a]	0.793	0.682
Not Normal	15 (65.2)	8 (34.8)			
Height-for-age[d] (ref: normal)					
Normal	37 (68.5)	17 (31.5)	0.919		1.000[b]
Not normal	4 (56.7)	2 (33.3)			

*Significant at p<0.05; OR=Odds Ratio

[a]0 cells (0.0%) have expected count less than 5; The minimum expected count is 7.28

[b]1 cells (50.0%) have expected count less than 5; The minimum expected count is 1.90 (thus, use Fisher exact test)

[c,d] BMI-for-age and height-for-age were recoded into two dummy variables; Normal and not normal (any indicator that is not normal) to reduce higher possibility of any expected cell counts <5 is more than 20%

This rather contradictory result may be due to the different instruments used, number of respondents, and age of respondents (Jimoh *et al.* 2018). Hence, it could conceivably be hypothesized that child development among fishermen's children between 24 to 59 months in Kuala Nerus and Kuala Terengganu were associated with other factors such as socio-economic status, parental skills, stimulating environment and culture rather than nutritional status. Many constraints must be acknowledged. This research does not measure dietary intake, as this may add bias and complications. The respondents are young, and parents may not disclose their children's actual intake. As a result, further research is necessary to determine the viability of child development and its relationship to nutritional status. Future research would require a longer time span to create a complete image of child development growth, allowing for an in-depth examination of children's growth and development. In-depth investigations of each parameter are essential, as multiple factors influence various parameters of child growth.

This study has shed a contemporary light on the contentious issue highlighted in Nutrition Research Priorities (NRP) in Malaysia for 2016–2020 involving maternal and young child nutrition. This study should prove to be particularly valuable to the National Plan of Action for Nutrition for Malaysia, NPANM (2016–2025), as it demonstrates the prevalence of stunting and wasting for children under five in a vulnerable community. The NPANM goal is to have that prevalence of not more than 11% by 2025.

CONCLUSION

Around 30% of these children have been identified as having developmental delays. The majority of these children have a normal BMI and height for their age. In contrast to expectations, this study discovered no connection between child development and nutritional status of children under the age of five. As a result, further research is necessary to fully grasp child development and its relationship to nutritional status.

ACKNOWLEDGEMENT

This study was funded under the Fundamental Research Grant Scheme for Research Acculturation of Early Career Researchers (FRGS – RACER): RACER/1/2019/SKK06/UMT//1. This study is part of a larger study "Risk assessments of low cognitive performance among fishermen's children in Terengganu, Malaysia".

AUTHOR DISCLOSURES

The authors confirm that there are no known conflicts of interest associated with this publication.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, July 2021, 16(2):101-108***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://10.25182/jgp.2021.16.2.101-108*
ISSN 1978-1059 EISSN 2407-0920

Sauropus androgynus, Papaya Leaves, and Mung Beans as Mixed Galactagogue Drink for Urban Postpartum Mothers

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ABSTRACT

This study explored the effect of *Sauropus androgynus*, papaya leaves, and mung beans as mixed galactagogue drinks on breastmilk volume, frequency, and duration among urban postpartum mothers in Jakarta. A quasi-experimental study with 60 postpartum mothers divided in intervention and control groups was conducted. The intervention group was administered with a 400-cc traditional galactagogue drink daily within 4 weeks of postpartum, while the control group received 3 times breastfeeding counselling. The breastmilk volume was measured using the evaporative water loss method on mothers' weight at the first, second, third-, and fourth-week consumption. The mean difference of breastmilk volume, breastfeeding frequency, and duration between the intervention and control groups was calculated by bivariate analysis using an independent sample t-test. The breastmilk volume was not different between both groups on the first and second week (1st:622.93±289.24 and 507.68±231.28, p=0.094; 2nd:683.00±252.42 and 582.58±225.42, p=0.110), however, the intervention group had higher volume than the control group in the third and fourth week (3rd:801.43±273.35 and 656.24±214.43, p=0.026; 4th=908.52±271.27 and 756.69±196.29, p=0.016). No significant difference was observed in the breastfeeding frequency and duration among the groups. In conclusion, the new galactagogue mixed drink consumption has the potential to increase breastmilk production and enhance a mother's confidence to continue breastfeeding.

Keywords: breastfeeding, counselling, mung beans, polyphenol, *Sauropus androgynous*

INTRODUCTION

One of the global nutrition targets in Sustainable Development Goals (SDGs) is to promote the rate of mothers performing exclusive breastfeeding within 6 months up to at least 50% in 2025 (Fanzo *et al.* 2018). However, the proportion of 0–5 months old infants fed exclusively with breast milk has only been 40.7% and formula milk sales in developing countries are increasing gradually (Fanzo *et al.* 2018). In Indonesia, the exclusive breastfeeding rate is 37.3% (MoH RI 2018), despite the country's effort to follow the World Health Organization (WHO) recommendation and issued a Decree from the President of Republic Indonesia No.33/2012 which stipulated child feeding practices which included exclusive breastfeeding for the first 6 months of life.

Exclusive breastfeeding is essential for optimal child growth and development (Kuchenbecker *et al.* 2015). Inadequate practices of this contribute to more than ten thousand mother's and children's deaths yearly (Walters *et al.* 2016). While adequate practice can prevent both communicable and non-communicable diseases such as diarrhoea, pneumonia, and cancer (Walters *et al.* 2016). Breast milk nutritional composition correlates with the baby's physiological states and immune system to prevent infection and reduce risk of obesity (Tao *et al.* 2017; Sakka 2014). Furthermore, breastfeeding also provides comfort which bridges the differences between pre and postnatal life for infants, acted as a natural contraception for mothers and reduces risk of maternal cancers of reproductive organs (Newton 2018; Sakka 2014).

Commonly identified barriers on providing exclusive breastfeeding are identified i.e anxiety of inadequate production, premature delivery, serious medical concern, separation

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(Received 17-05-2021; Accepted 30-06-2021; Published 29-07-2021)

from the baby after birth, stress, and discomfort (Sakka 2014). Previous study reported that 38% of mothers stopped breastfeeding due to a lack of breast milk (Ghasemi *et al.* 2015). The two weeks after delivery are critical to determining the success of breastfeeding; since the mother may feel fatigued and painful nipples, at the same time the baby shows dissatisfaction with breast milk. Evidence proved that in the 48 hours after delivery, the infants often experience reduced weight, showed satiety rather than sleep well, produced insufficient urine and stool (Galipeau *et al.* 2017). These conditions can lead to anxiety and lack of self-confidence among mothers on their capacity to exclusively breastfeed their infants. Therefore, many preferred to either consume galactagogue or attend the counselling to improve their milk production and to smoothen the exclusive breastfeeding process especially for those that have delivered the first child (Nguyen *et al.* 2016; Foong *et al.* 2020).

One of the local wisdoms were elders or family providing treatment such as massage, acupuncture, herbal therapy meditation, and yoga for postpartum mothers especially for those living in the rural area. Through those, the mother could face easy post-delivery period and an easy breastfeeding (Mediastari 2020). Supporting the treatment, several food ingredients are expected to give a lactogenic effect including *Sauropus androgynous* or known locally as "katuk leaves" and papaya leaves. Sauropus androgynous is a potential commodity easily found in Indonesia, so far many Indonesian people had experienced the benefits, but the scientific evidence to measure its effect on breastmilk volume are still scarce (Indrayani *et al.* 2020; Santoso 2016; Suwanti & Kuswanti 2015; Pinem *et al.* 2019). Supplements containing these ingredients have been consumed among Indonesian mothers to increase breast milk production (Suwanti & Kuswati 2015). Furthermore, papaya leaves juice and mung beans also affect breastfeeding (Wulandari & Jannah 2015).

Mothers who live in a rural area that adhering tradition and whose education was lower and were unemployed have a higher rate of exclusive breastfeeding compared to working and educated mothers. In addition, mothers living in Jakarta have the lowest rate of exclusive breastfeeding practice compared to other provinces (Laksono *et al.* 2021). Further, there has been misconception on food containing galactagogue among urban mothers, they perceive it did not bring benefit to breastmilk production, while formula milk consumption brought healthier effect to baby due to heavier weight gain (Nuzrina *et al.* 2016). A dilemma of lactating mothers when they should return to work and leave their babies at home is also a common reason among urban mothers to stop breastfeeding (Sulaiman *et al.* 2018). Previous study stated that counselling through interpersonal discussion brought a more positive outcome on breastfeeding behaviour than only receiving a campaign through mass media (Nguyen *et al.* 2016). Finding professionals and receiving a recommendation from experts help to determine successful breastfeeding (Nyqvist *et al.* 2012). However, for some parents counselling can be burdensome due to the cost and time required. Thus, effective galactagogue may help to ease the burden or supplement the breastfeeding counselling when needed. Therefore, this study aims to compare the breastmilk volume, breastfeeding frequency, and duration after the administration of mixed galactagogue drink and receiving counselling only among urban postpartum mothers.

METHODS

Design, location and time

This study used a quasi-experimental design where two groups were assigned with random selection. It was conducted at the Maternity Home Integrated Primary Health Centre (*Puskesmas*) Kebayoran Lama District from August to November 2017. This was approved by the Ethical Review Committee for Human Research Health Polytechnic of Jakarta II under NO. LB.02.01/I/KE/31/287/2017.

Sampling

The inclusion criteria were healthy pregnant women in the third trimester with age range 20–35 years, not smoking, with a single pregnancy, routinely attended antenatal care, and voluntarily involved in this study. Mothers were excluded when she had serious medical conditions, food allergy, and drank other galactagogue supplements besides what was administered. All participants received an explanation of the study procedures and signed an informed consent form beforehand. In total, 60

pregnant mothers were recruited and randomly assigned into the intervention and control groups.

Data collection

Data including the respondents' age, education, occupation, household income, mother's weight and height were collected to determine the nutritional status using Body Mass Index (BMI) with the formula weight (kg)/height (m^2). Their body weights were measured before and after breastfeeding, while the frequency and duration were also recorded using the recall method.

The breastmilk volume measurement was performed using the evaporative water loss method on the participants at the first, second, third, and fourth week of postpartum. The mothers' weight was measured before and immediately after breastfeeding using an electronic weighing scale with +5 g accuracy (Tanita HD-378-Digital Scale) as also mentioned in the previous study (Scanlon *et al.* 2002). In addition, the compliance level of mixed drink consumption was recorded on the observation sheet. Information on a 24-hour breastfeeding frequency and duration was also collected through interviews.

Galactagogue Mixed Drink (GMD). The galactagogue mixed drink ingredients were *Sauropus androgynous* extract, mung beans, papaya leaves, tamarind, sugar and water. The first step is shorting the desirable Sauropus androgynous and papaya leaves which were neither too old nor too young, with fresh green colour, then washed them with clean water. The composition of one serving are 50 g Sauropus androgynous, 25 g Papaya leaves, 15 g mungbeans, 15 g sugar, 25 g tomatoes and 12 g tamarind. Second, the leaves were blanched at a temperature of 83°C–92°C for 3 minutes. After that, each Sauropus androgynous and papaya leaves were formed into a solution using a blender with leaves and water proportion were 1:2.

The solution was then filtered and mixed with mung bean porridge, sugar, and tamarind. Then it was heated to 60°C for 15 minutes. The mixed galactagogue drink is cooled at room temperature and the products were packaged into a ready-to-drink bottle with the same shape, size and packaging material. The nutrient and polyphenol contents per 100 grams are calorie (57.0 Cal), protein (0.9 g), fat (0 g), carbohydrate (13.5 g), water (85.3 g), ash (0.3 g), fiber (0.5 g), and polyphenol (574 mg).

Intervention group. The intervention groups were administered 400 cc mixed Sauropus androgynous leaf extract, papaya leaves, mung bean and turmeric mixed drink products (2 bottles) daily for 4 weeks of postpartum. One bottle of mixed drink (±200 ml) was consumed twice daily within the interval of the main meal and at night before sleeping. The participants started consuming the galactagogue drink immediately after delivery until the fourth week of exclusive breastfeeding.

Control group. Breastfeeding counselling was delivered for 30–45 minutes thrice after delivery within 2 months of observation (at birth, 7–14, and 35 days old). The material used in this process referred to the module from the Ministry of Health's 40-hour by a health worker or enumerator that had attended such counselling before. Mother's weight measurement to determine the breastmilk volume, breastfeeding frequency and duration was performed for the intervention group during counselling at the first, second, third and fourth week of postpartum. Before the intervention started, a socio-economic and nutritional intake screening process through 2x24 hours food recalled was performed to ensure that both groups had the similar characteristics.

Data analysis

All data were coded and analysed using SPSS software version 21. The univariate statistical analysis was used to determine the mean, median, and Standard Deviation (SD) for continuous variable. Participant's characteristics were analysed using chi-square test to ensure both groups having similar characteristics to prevent bias. The mean difference of breastmilk volume, breastfeeding frequency and duration between the intervention and control groups was calculated by bivariate analysis using independent sample t-test. The statistical result with a $p<0.05$ was considered significant. The mean difference of breastmilk volume between 1st and 2nd week, 2nd week to 3rd week, and 3rd week and 4th week in both groups were analysed using independent sample t-test.

RESULTS AND DISCUSSION

Characteristics of respondents

The characteristics of participants can be seen in Table 1. The majority were aged 21–30 years old, finished senior high school, housewives

and from higher-income family with 2,000,000 IDR monthly income in both groups, indicating no significant differences in this aspect $p>0.05$ (Table 1). The mean value of BMI in both groups was normal but near to overweight. In addition, infants' characteristics distribution was almost equal across genders with mostly had a term delivery and normal birth weight.

Breastmilk volume, breastfeeding frequency and duration

Breastfeeding practice is essential to child immunity against mild and severe infections, therefore, when mixed or non-exclusive it leads to higher risk (Tao *et al.* 2017). Supporting and facilitating the process tend to make it successful. This study analysed how mixed galactagogue drink and counselling influenced the exclusive breastfeeding practice among urban postpartum mothers. The result showed that rather than counselling, consuming galactagogue drink was more effective at increasing the breastmilk volume. The mean breastmilk volume in the third and fourth week after GMD consumption among the intervention group was higher than the control which was 801.4±273.3 and 908.5±271.3 ml/day, respectively. The breastmilk volume in the first week after the intervention was not significantly different from the second week ($p>0.05$). Compared to the second week, the breastmilk volume is higher in the third week. Another increase was observed from the third to the fourth week among intervention groups ($p<0.05$). Meanwhile, in the control group, the significant difference was only significant between the third and fourth weeks. Additionally, there was no significant difference between two groups in terms of breastfeeding frequency and duration (Table 2).

Commercial galactagogues consumption was often in the form of supplement or tea (Foong *et al.* 2020; Ghasemi *et al.* 2015). This supposed to make it easy-to-drink besides avoiding the unpleasant taste and smells due to the herbal ingredients. However, it is often inaccessible due to the expensive price. Thus, a traditional formulation was used by simply boiling the mixed herbs and vegetables altogether, the GMD can be produced and consumed daily without spending more. Additionally, a mixture of mung beans, tamarind, and a bit of sugar removed the bitter taste and enhanced the product acceptability.

Galactagogue is commonly consumed in the lactogenesis II stage or right after birth, when physiologically the mammary glands start breastmilk secretory activation (Newton 2018; Ghasemi *et al.* 2015; Foong *et al.* 2020). In this stage, a normal or delayed production tends to occur, since after placenta removal, progesterone decreases sharply as the prolactin, cortisol, and insulin levels increase (Pillay & Davis 2020).

The breastmilk volume among the groups increased gradually indicating that due to infant growth, they required more nutrient to stimulate breastmilk ejection (Table 2). Skin to skin contact and nipple stimulation as the infant's tip of the tongue touches the nipple for suckling, the afferent impulses from sensory nerve terminals stimulation in the areolas travel to the central nervous system, hence, promoting oxytocin secretion for breastmilk release (Newton 2018). Earlier and more frequent breastfeeding increases breastmilk production, while other factors such as primiparous women, having a caesarean delivery, retained placental fragments, diabetes, and stressful vaginal deliveries retain its ejection (Pillay & Davis 2020).

A previous study showed consuming galactagogues within certain periods during postpartum elevated milk productions than a placebo (Nguyen *et al.* 2016). The breastmilk volume in the second and fourth week was comparable to the previous study that used various natural oral galactagogues such as banana flower, fenugreek, ginger and moringa (Foong *et al.* 2020). The result showed that the breastmilk volume in this study was higher. This might be because each vegetable mixture used contains nutrients needed to stimulate the ejection process, hence, their combination might bring more galactagogue effects. This result is in agreement with a recent literature review that highlights the robust increment of breastmilk volume after consuming mixed natural oral galactagogues (Foong *et al.* 2020). Another study used lactating rats as experimental subjects which presented mixed galactagogue responses to increase milk production by regulating Aquaporins (AQP) in the mammary gland especially AQP-3 and AQP-5 protein levels which mainly controlled water movement (Liu *et al.* 2015).

Sauropus androgynus consumption orally after 24 hours of postpartum gave a 50.7% increase in breastmilk volume and reduced the

Table 1. Characteristics of respondents

Variable	Intervention group (n=30)		Control group (n=30)	
	n	%	n	%
Mothers' characteristics[a]				
Age				
≤20	2	6.7	2	6.7
21–30	18	60.0	21	70.0
31–40	8	26.7	6	20.0
≥41	2	6.7	1	3.3
Education				
Elementary	3	10.0	2	6.7
Junior high school	8	26.7	8	26.7
Senior high school	18	60.0	14	46.7
Diploma	0	0	1	3.3
Bachelor degree	1	3.3	5	16.7
Occupation				
Housewives	24	80.0	22	73.3
Private company	5	16.7	5	16.7
Trade	1	3.3	1	3.3
Entrepreneurs	0	0	1	3.3
Others	0	0	1	3.3
Household monthly income				
500,000–1,000,000 IDR	3	10.0	1	3.3
1,000,000–2,000,000 IDR	8	26.7	10	33.3
>2,000,000 IDR	19	63.3	19	63.3
Anthropometry				
Baseline weight (Mean ±SD)	63.7 ± 12.4		63.1 ± 7.7	
End-line weight (Mean±SD)	60.3 ± 13.1		58.6 ± 7.6	
Height (Mean±SD)	156.4 ± 5.7		157.6 ± 4.5	
BMI (Mean±SD)	24.5 ± 4.8		23.5 ± 2.7	
Infants' characteristics				
Parity				
Child number–1	5	16.7	15	50.0
Child number–2	17	56.7	8	26.7
Child number–3	7	23.3	5	16.7
Child number–4	1	3.3	2	6.7
Term delivery	28	93.3	24	80.0
Birth weight				
<2,500 g	2	6.7	2	6.7
≥2,500 g	28	93.3	28	93.3

BMI: Body Mass Index; IDR: Indonesian Rupiah; [a] Participant's characteristics both groups are statistically not significant p>0.05; Chi-square test

mother's perspective on less breastmilk (Suwanti & Kuswanti 2016). This linear to our current study, however, the difference identified after three weeks of consumption (Table 2). The result also showed a similar trend with another study, the difference of breastmilk production in mice between the intervention and control group occurs at least after the 6th day of consumption (Iwansyah *et al.* 2017). One possible reason might be because the GMD did not contain *Sauropus androgynous* leaves only, but the combination of more ingredients such as papaya leaves and mung beans.

Papaya leaves juice stimulates prolactin hormone level, while mung beans, besides having galactagogue effect, also contains thiamine or

Table 2. Breastmilk volume, frequency and duration of breastfeeding

Variable	Intervention group (n=30)	Control group (n=30)	p-value
Breastmilk Volume (ml/day±SD)			
First week	622.9±289.2	507.7±231.3	0.094
Second week	683.0±252.4	582.6±225.4	0.110
Third week	801.4±273.3[b]	656.2±214.4	0.026[a]
Fourth week	908.5±271.3[b]	756.7±196.3[b]	0.016[a]
Breastfeeding frequency (times/day±SD)			
First week	14.2±2.7	15.1±2.3	0.145
Second week	14.2±3.1	16.2±2.8	0.130
Third week	13.8±2.8	14.2±2.5	0.534
Fourth week	13.0±2.6	13.8±2.8	0.273
Breastfeeding duration (minutes/day±SD)			
First week	18.5±13.0	14.7±12.0	0.241
Second week	18.1±12.8	14.7±6.8	0.200
Third week	25.5±14.8	19.8±9.7	0.084
Fourth week	23.2±12.1	22.3±9.4	0.767

[a]Statistical analysis:Independent sample t-test; Significancy is at $p<0.05$

[b]Independent sample t-test significancy is $p<0.05$; 2nd and 3rd week, 3rd and 4th week in intervention group and 3rd and 4th week in control group

vitamin B1 which converts carbohydrates into energy and reduces stress, as well as triggers oxytocin secretion (Wulandari & Jannah 2015; Ikhlasiah *et al.* 2020). Previous studies reported that these effects were due to a dilate blood vessels on the mammary glands and secretory cell proliferation that increase blood flow (Indrayani *et al.* 2020; Foong *et al.* 2020). These are related to phytochemical groups' effect on galactopoietic (Mohanty *et al.* 2014). The GMD polyphenol content was 574 mg after the combined formulation that comparable with a previous study (8.80±0.01 mg) (Iwansyah *et al.* 2017). Therefore, it modulated the breastmilk production hormones in the lactogenesis and lactation process.

Along with the breastmilk volume, an infant need to be breastfed frequently as necessary without a strict schedule and when this is spontaneous, it prevents breastfeeding problems. The result showed that in the first and second week, breastfeeding frequency was more than in the third (14–16 times/day) and fourth (13–14 times/day). The duration varies according to their suction pattern, where the average was longer in the intervention group (18–23 minutes per breastfeeding) than in the control (14–22 minutes per breastfeeding) (Table 2). Nevertheless, the effect of counselling on breastfeeding frequency and duration was less apparent compared to the GMD group.

The only significant result found was breastmilk volume, where it was measured using Evaporative Water Loss (EWL) on mothers. This method allows a more accurate and precise weighing than on infants due to the unpredictable movement that caused an unstable weighing value. However, it required a strict attention thus mothers did not alter their body weight from consumption, excretion, clothing or physical activity. Another possible reason might be because the mothers had understood how to perform exclusive breastfeeding since they were mostly having the second, third or fourth child (Table 1).

CONCLUSION

Breastfeeding mothers given the mixture of three galactagogue ingredients mix, namely *Sauropus androgynous* and papaya leaves, as well as mung beans showed significantly higher breastmilk volume after three weeks of consumption compared to breastfeeding counselling. Considering the fact that the raw materials for this GMD are commonly found and the procedures to prepare it was easy for a household level preparation, this can supplement the breastfeeding counselling program trough

the Community Health Centre and Community Based Integrated Health Post (*Posyandu*) to improve exclusive breastfeeding practice in the country, alongside adequate food consumption.

ACKNOWLEDGEMENT

The authors are grateful to Mr. Joko Sulistyo, Directors of Health Polytechnics of Jakarta II and Directors of the Maternity Hospital of *Kebayoran Lama* Community Health Centre for their permission, support and cooperation. The authors are also grateful to Mrs. Betty Yosephin from Health Polytechnic for the suggestions provided to improve this study.

AUTHOR DISCLOSURES

No potential conflict of interest relevant to this article was reported.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019
ISSN 1978-1059 EISSN 2407-0920

J. Gizi Pangan, July 2021, 16(2):109-118
DOI: https://10.25182/jgp.2021.16.2.109-118

Development of Functional Beverage with Antioxidant Properties using Germinated Red Rice and Tempeh Powder Mixture

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ABSTRACT

The objectives of this study were to develop a functional beverage containing Germinated Red Rice (GRR) and tempeh powders and analyze the total soluble phenolic content, phenolics profile, in vitro antioxidant activity, and sensory evaluation. A mixture of GRR and tempeh powder at 1:2, 1:1, and 2:1 w/w ratios were mixed with water at 6% w/v concentration. The total soluble phenolic content and the antioxidant activity of the samples increased significantly ($p<0.05$) with the increasing level of GRR powder and the decreasing level of tempeh powder, whereas 2:1 w/w ratio of GRR and tempeh powder showed the highest total soluble phenolic content (79.79±12.10 µg/ml GAE) and in vitro antioxidant activity (68.84±1.56%). However, a control beverage containing only GRR powder and only tempeh powder had the highest and lowest total soluble phenolic content and antioxidant activity, respectively. Ferulic acid was detected in all samples containing GRR, while daidzein was not detected and genistein was only detected in 1:2 and 1:1 sample ratios. All formulated samples in lemongrass sugar solution were accepted by the panelists (score 5 out of 7). In conclusion, GRR was responsible to increase the total soluble phenolic content and antioxidant activity of the beverage. A loss of isoflavone in the tempeh-containing beverage samples suggested that optimizing the dose and processing method were important to achieve the optimum health benefits of the ingredients.

Keywords: daidzein, ferulic acid, genistein, HPLC, phenolic compounds

INTRODUCTION

Germination and parboiling of whole grain red rice could modify the texture of whole grain rice resulting in softer rice texture, higher bound phenolics content, and better palatability (Hu *et al.* 2017). The main bound phenolics in red rice were ferulic acid, syringic acid, trans-p-coumaric acid, and quercetin, while the main free phenolics were catechin, protocatechuic, and caffeic acids (Sumczynski *et al.* 2016). During germination, various biochemical and enzymatic reactions occurred which could increase the nutritional value and digestibility, release the bound phytochemicals in the substrate, and induce the biosynthesis of phenolic compounds potentially contributed to many beneficial biological activities, such as scavenging free radicals and protecting against oxidative-stress related diseases (Maksup *et al.* 2018). Germinated Red Rice (GRR) was usually consumed as steamed rice to replace white rice or processed into powder, cereal drink, and baby food.

Tempeh is an Indonesian indigenous food made from soybean fermentation using *Rhizopus spp.* mold (Ahnan-Winarno *et al.* 2021). Tempeh provides higher nutrition and isoflavones aglycone content than soybean due to its biochemical degradation during fermentation process (Astawan *et al.* 2015). Tempeh flour that was processed using steam blanching and oven drying at 60°C for 8 h still exhibited a high content of genistein, daidzein, and a high antioxidant activity (Astawan *et al.* 2020). Tempeh is seldom found in a beverage form. It is proposed that the addition of tempeh, instead of other soy products, in the GRR beverage could enrich not only the protein and vitamin B12, but also the isoflavone content (Ahnan-Winarno *et al.* 2021; Sethi *et al.* 2016).

Dietary polyphenols can be served in a beverage form (Shahidi & Ambigaipalan 2015). A plant-based beverage can be a milk alternative for those who have lactose intolerance, milk allergy, or are vegan. The design of a plant-based beverage, which is based on the cereal and legume mixture, that can provide

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(Received 18-12-2020; Accepted 09-04-2021; Published 29-07-2021)

complementary nutrition and various phenolic bioactive compounds is hypothetically able to provide a better delivery of phenolic compounds and enhance. This can be attained through the modification of the phenolic compounds bound to the food matrices. The enzymatic hydrolysis occurred during germination could liberate the ferulic acid and other phenolic acids that were bound to the bran layer of the cereals (Xu *et al.* 2020). The aglycone form of isoflavones were the most bioavailable phenolic compounds in tempeh (Ribas-Agusti *et al.* 2018). Based on the hypothesis above, this study was aimed to develop a functional beverage containing a mixture of GRR and tempeh powder and further analyze the total soluble phenolic content, phenolics profile, in vitro antioxidant activity, and sensory evaluation of the mixed beverage.

METHODS

Design, location, and time

The experimental study used a completely randomized design. The study was conducted in the Laboratory of Food Processing, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, started from September 2018 until June 2019.

Materials and tools

The main ingredients for the beverage, such as organic red rice (*Cap Kenari*, Jakarta, Indonesia), fresh lemongrass, and fresh tempeh (*Karya Abadi*, Tangerang, Indonesia) were purchased from the market at *Bumi Serpong Damai* area, South Tangerang. The reagents used for the chemical analysis were purchased from Merck (Germany) distributor in Jakarta, Indonesia. The standard chemicals for High Performance Liquid Chromatography (HPLC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay were purchased from Sigma-Aldrich (Singapore) distributor in Jakarta, Indonesia. The tools used were drying oven (Memmert, Germany), centrifuge (Eppendorf, Germany), pH meter (Mettler Toledo, USA), UV-Vis spectrophotometer (Thermo Fischer Scientific, USA), and HPLC with a UV-Vis detector (Agilent 1100 Series, USA).

Procedure

Preparation of GRR powder. Red rice (100 g) was rinsed with running tap water, drained, and soaked in sterile distilled water at 1:3 w/v ratio at room temperature (25°C) for 24 h, then drained and rinsed again with sterile distilled water. The rice was placed on a tray layered with a damp tissue paper and covered with a damp clean cloth. The germination process was performed in the dark at room temperature for 48 h following Anawachkul and Jiamyangyuen (2009) method. The covering cloth was sprayed with sterile water every 4 h to maintain humidity. GRR was oven-dried at 60°C for 12 h, then ground into powder using a food processor and sieved at 60 mesh. The GRR powder was stored at room temperature in a plastic zipped bag added with a silica gel sachet and used in the beverage formulation within 2 weeks.

Preparation of tempeh powder. Tempeh powder preparation followed the Bastian *et al.* (2013) method with a modification in drying time. Fresh bought tempe was sliced to 0.5–1 cm thickness, then blanched in hot water at 90°C for 15 min to reduce the beany flavor and inactivate oxidizing enzymes. Tempeh was oven-dried at 70°C for 12 h. Dried tempeh was ground into powder using a food processor and sieved at 60 mesh. The tempeh powder was packed in a plastic zipped bag added with a silica gel sachet and stored at a room temperature for use within 2 weeks.

Beverage formulation. The mixture of GRR and tempeh powder were rehydrated in water to reach 6% w/v powder concentration as having the best solubility and stability in the preliminary study. The formulation used three different ratios of GRR and tempeh powder mix, i.e. 1:2, 1:1, and 2:1 w/w. A plain formulation was used for the chemical analysis and phenolics profiling. A sugar formulated beverage was used for the sensory evaluation by adding 10% v/v lemongrass syrup to help masking the beany flavor of the beverage. As many as 1 kg of table sugars were dissolved in 500 ml of boiling water, then 8 lemongrass stalks were brewed into a sugar solution for 15 min and filtered out to make the lemongrass-infused syrup. Both the plain and sugar added beverage were subjected to sensory evaluation.

Sample preparation and pH analysis. Each formulation was sterilized with an autoclave at 121°C, 1 atm for 15 min. Samples of each formulation were centrifuged at 4,000×g for 7 min. The supernatant was collected, measured for pH using a pH meter, and stored in a freezer at -20°C prior to analysis.

Analysis of total soluble phenolics content. The total soluble phenolics content was measured using a method described by Agustinah *et al.* (2016). The absorbance of each sample was measured using a UV-Vis spectrophotometer at 725 nm wavelength. The absorbance was converted into total phenolics concentration and expressed in µg gallic acid equivalent per mL sample (µg/ml GAE).

Analysis of in vitro antioxidant activity. The antioxidant activity was measured using DPPH radical scavenging assay (Agustinah *et al.* 2016) with a modification of DPPH concentration at 0.2 mM in 96% ethanol. The absorbance (A) was read at 517 nm wavelength using a UV-Vis spectrophotometer. The antioxidant activity was expressed as % inhibition of DPPH radical formation and calculated using a formula:

$$\%Inhibition = \frac{A\ reaction\ control - A\ sample}{A\ reaction\ control} \times 100\%$$

Phenolics profiling by HPLC. The phenolics profile of each sample was determined using HPLC protocols as described by Agustinah *et al.* (2016) for phenolic acids and Lee *et al.* (2008) for isoflavones. A volume of 2 ml sample was mixed with methanol for ferulic acid profiling or acetonitrile for genistein and daidzein profiling at 2:1 v/v ratio, then centrifuged at 10,000×g for 5 min. The supernatant was diluted with HPLC-grade water at 1:1 v/v ratio, then filtered through a 0.2 µm syringe filter. Twenty microliters of filtered sample were injected into HPLC with a UV-Vis detector. The analytical column used was Zorbax XDB-C18 4.6x150 mm with packing material of 5 µm particle size.

The profiling of ferulic acid was conducted at a flow rate of 1 ml/min for 25 min with a gradient elution consisting of (A) 100% methanol and (B) 10 mM phosphoric acid (pH 2.5). The solvent system (%A/ %B) was run at 8 min (60/40), 7 min (100/0), 3 min (0/100), and 7 min (0/100). The chromatogram was recorded at 225 nm during each run.

The profiling of isoflavones was performed at a flow rate of 0.5 ml/min for 25 min with a gradient elution consisting of (A) 0.1% v/v glacial acetic acid in water and (B) 0.1% v/v glacial acetic acid in acetonitrile. The solvent system (%A/ %B) was run at 0 min (85/15), 5 min (70/30), and 20 min (35/65). The chromatogram was recorded at 254 nm during each run. Pure standards of ferulic acid, genistein, and daidzein were used to calibrate the standard curves and retention times.

Sensory evaluation. An affective test using a 7-scale hedonic score, i.e.: 1 (disliked very much); 2 (disliked); 3 (quite disliked); 4 (neutral); 5 (quite liked); 6 (liked); 7 (liked very much), was conducted to evaluate the acceptance (liking) of the taste, color, aroma, texture, and aftertaste of each beverage sample. There were 30 semi-trained panelists who were selected from the students of Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia and trained once on the specific sensory evaluation method that was used in this study. The acceptance test was done in two rounds, the first round used plain samples and the second round used the formulated beverage with lemongrass-infused syrup.

Data analysis

The experiment was performed in duplicate, except for phenolics profiling and sensory evaluation that were performed once. All analysis was done in triplicate, except for HPLC (phenolics profiling) and sensory evaluation which were done in duplicate. The data were presented in mean±standard deviation, further analysis of mean difference was done using one-way ANOVA and Duncan post-hoc test in Statistical Package for the Social Science (SPSS) software version 24. The confidence level set was 95% and the p-value less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

Total soluble phenolics content and pH

The formulation of GRR and tempeh powder beverage was provided in Table 1. As was shown in Figure 1, the total phenolics content of all 6% w/v mix beverage sample was at the same level which ranged between 63.65±7.81 to 79.79±12.10 µg/ml GAE. Tempeh as a protein-loaded food was reported to contain high amount of isoflavones aglycone, such as genistein and daidzein, with high antioxidant activity (Astawan *et al.* 2020). However, in this study tempeh powder had the lowest total phenolics content. The interaction between protein and phenolic compounds, via non-covalent or covalent processes, in tempeh could affect the detectability and bioavailability of phenolic

Table 1. Formulation of GRR and tempeh mix beverage

Ingredients	1:2 Mix	1:1 Mix	2:1 Mix	GRR	Tempeh
GRR powder (g)	4	6	8	12	0
Tempeh powder (g)	8	6	4	0	12
Water (ml)	188	188	188	188	188
Total (g)	200	200	200	200	200

GRR: Germinated Red Rice

compounds in tempeh (Ahnan-Winarno *et al.* 2021). Such protein-phenolics interaction could reduce protein solubility and digestibility and mask phenolics bioavailability and antioxidant capacity (Zhang *et al.* 2020).

The total phenolics content in a single GRR beverage sample was almost 1.8 fold higher than that in a single tempeh beverage sample. However, the increasing ratio of GRR powder and the decreasing ratio of tempeh powder did not change the total phenolics content in the mix beverage. Moreover, the total phenolics content of the 1:2 mixed sample (with higher tempeh powder ratio) was similar to that of single tempeh beverage sample. It was also shown in 1:1 and 2:1 mix samples, respectively, that by replacing 50% (6 g) and 67% (8 g) of tempeh powder with GRR powder, which contained approximately 0.44–0.59 mg/ml GAE, it could increase the total phenolics content of the mix sample to 1.5 to 1.6-fold as compared to a single tempeh beverage sample or reach the same level of phenolics content as in the single GRR beverage sample. This finding was higher than the study conducted by Sęczyk *et al.* (2017) that exhibited

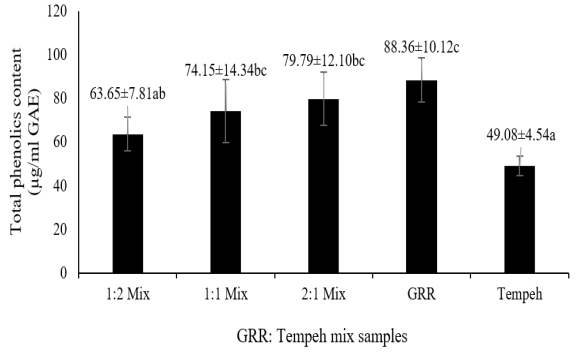

Figure 1. The total soluble phenolics content of beverage sample consisted of GRR and tempeh powder mix. Bars with different letters are significantly different ($p<0.05$)

only 1.2-fold increase of total phenolics content in the soymilk fortified with green coffee extract containing 0.5 mg/ml GAE of total phenolics content. The combination of various phenolics from two different substrates, such as in fruit juices, suggested a possible synergistic interaction which could increase the content and solubility of the phenolic compounds (Agustinah *et al.* 2016).

Based on the calculation from Figure 1, each 1:2, 1:1, and 2:1 mix beverage sample should contain the estimated 62, 69, and 75 µg/ml GAE, respectively. The experimental value was slightly higher than the estimated value of the total phenolics content. This result indicated a potential effect of mixing GRR and tempeh powder in the beverage in increasing the total phenolics content. However, further optimization study was required to increase the ingredients concentration to above 6% and solve the problem of powder solubility and stability in the beverage. The beverage which consisted of dissolved GRR and tempeh powder mix in water showed a mean pH of 6. The pH data was not shown as it was not significant among all samples.

Germination of cereal seeds was one of the non-thermal processing which resulted in the dynamic changes of various types of phenolic compounds (Xu *et al.* 2020). The total phenolics in this study was considered as soluble free phenolic compounds since ethanol was used in the method as a solvent (Agustinah *et al.* 2016). The increase of soluble free phenolic compounds could be resulted from the breakdown of soluble bound phenolic compound from the macromolecule complex in the endosperm through the activation of endogenous hydrolytic enzymes during germination (Xu *et al.* 2020). Additionally, heat treatment on the GRR, such as parboiling (Hu *et al.* 2017) or potentially oven-drying in this study, could also release the bound phenolics from the cell wall which resulted in the increasing of soluble free phenolics content. However, this study did not characterize the

bound phenolic compounds in the mixture; thus, indicating the need of further confirmation study.

Antioxidant activity (DPPH free radical scavenging activity)

Following the pattern of the total soluble phenolics content, the antioxidant activity of the mix beverage was also improved by the increasing ratio of GRR powder that reached 68.84±0.08% in 2:1 GRR: tempeh mix beverage (Figure 2). The GRR single beverage had the total phenolics content and antioxidant activity as high as in the 2:1 mix and higher than that of a single tempeh beverage. There was a potential positive relationship between the total phenolics content and the antioxidant activity of the plant samples as was also reported by Wahyuni *et al.* (2020) in the leaves extrats of Sundanese traditional salad.

Both the total phenolics content and the DPPH free radical scavenging activity in the mix beverage showed an increasing trend as the GRR powder content increased and the tempeh powder content decreased. The addition of tempeh powder did not seem to provide any additional benefits in the total phenolics content and antioxidant activity of the mix beverage, in contrast to our hypothesis. It was the GRR that contributed more to the total phenolics content and antioxidant activity of the mix beverage. There were some bioactive compounds in the ungerminated and germinated rice extracts, such as phenolic acids, flavonol, tannin, Gamma-amino Butyric Acid (GABA), and a-tocopherol (Kaur *et al.* 2017). The phenolic acids content such as ferulic acid, p-coumaric acid, 2,5-dihydroxybenzoic acid, sinapic acid, vanillic acid, and syringic acid was correlated with the antioxidant activity (Shao *et al.* 2018).

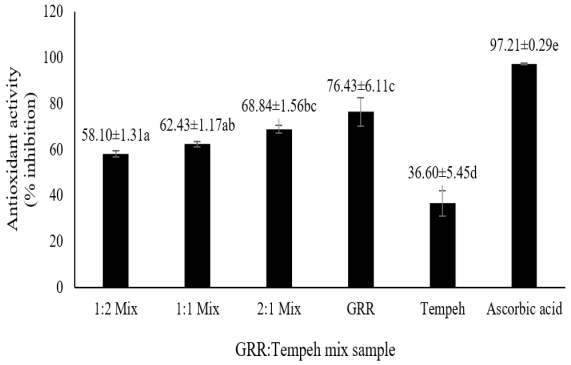

Figure 2. The antioxidant activity of beverage sample consisted of GRR and tempeh powder mix. Bars with different letters are significantly different ($p<0.05$)

The antioxidant activity of tempeh powder-only beverage was the lowest among all samples (Figure 2). Tempeh powder in this study was made on the same day when the commercial fresh tempeh was purchased from the supermarket, which was about 2 days after the production date. Tempeh that was fermented for 24–60 h (IC_{50} of 1 mg/ml) had a stronger antioxidant activity than that of 72 h (IC_{50} of 2 mg/ml) (Athaillah *et al.* 2019). The tempeh-only beverage in this study was made with 6% w/v (60 mg/ml) tempeh powder from approximately 48 h-fermented tempeh and only showed 36% antioxidant activity. It indicated that the powdering process could give negative impact to the antioxidant activity of tempeh powder by degrading or modifying the structure of the compounds that contributed to tempeh antioxidant activity, such as isoflavones, low molecular weight peptides (<3 kDa), several amino acids, particularly hydrophobic amino acids (Astawan *et al.* 2020), and 3-Hydroxyanthranilic Acid (HAA) as an intermediate metabolite of tryptophan (Ahnan-Winarno *et al.* 2021). The loss of isoflavone in tempeh-only beverage as was shown in this study (Table 2) and a low isoflavone content in another tempeh flour (0.62 mg genistein/g db) as was reported by Astawan *et al.* (2020) also supported this finding. The content of peptides, amino acids, and HAA were not analyzed in this study. Further, tempeh antioxidative properties might not derived from a high dose of single compounds, but rather a mixture of other bioactive compounds in a much lower dose (Ahnan-Winarno *et al.* 2021).

The addition of tempeh powder could still contribute to other nutritional content in the mix beverage. Tempeh powder provided 51% db protein, 5% db crude fiber, and high essential amino acids content with hypoglycemic and insulinotropic properties, such as arginine, alanine, phenylalanine, isoleucine, and leucine (Astawan *et al.* 2020). Further study on the nutritional content and other bioactive compounds, such as peptides and polysaccharides, could be conducted to elucidate the other health benefits of the GRR and tempeh mix beverage.

Phenolics profile

Ferulic acid, genistein, and daidzein were detected in the HPLC chromatograms at 2.1,

Table 2. Ferulic acid, genisten, and daidzein content in beverage samples

Sample (GRR:Tempeh)	Ferulic acid (µg/ml)	Genistein (µg/ml)	Daidzein (µg/ml)
1:2 Mix	239.03	2.99	ND
1:1 Mix	315.62	0.44	ND
2:1 Mix	233.73	ND	ND
GRR	163.85	ND	ND
Tempeh	ND	ND	ND

GRR: Germinated Red Rice; ND: Not Detected

14.0 and 15.8 min retention times, respectively (Figure 3 and Figure 4).

Ferulic acid could mainly be found in cereals, and to the lesser extent, legumes (Shahidi & Ambigaipalan 2015). Table 2 showed that ferulic acid was only detected in the GRR-containing beverage. The lowest and highest content of ferulic acid was found in the GRR only-containing beverage (163.85 µg/ml) and 1:1 GRR: tempeh mix beverage (315.62 µg/ml), respectively. Although the ferulic acid content in the 1:1 mix beverage sample increased by almost two-fold from the single GRR beverage, which could possibly suggest the transformation of the phenolics in the GRR and tempeh, the antioxidant activity of the 1:1 mix beverage sample was lower than the single GRR beverage sample (Figure 2).

Ferulic acid was reported to provide many physiological functions, such as antioxidant, antimicrobial, antiinflammatory, and anticancer properties, which could reduce the risk of several chronic oxidative-linked diseases (Shahidi & Ambigaipalan 2015). However, other bioactive compounds in GRR, instead of ferulic acid, could also contribute to the high antioxidant activity of the single GRR beverage sample, such as other phenolic compounds, γ-oryzanol, GABA, tocopherol, and tocotrienol (Kaur *et al.* 2017; Widyawati *et al.* 2014).

Tempeh fermentation could enhance the release of water-soluble phenolics, especially the isoflavone aglycones, which was due to the microbial hydrolysis action (Athaillah *et al.* 2019). However, genistein and daidzein were not detected in some tempeh powder-containing beverage samples. Genistein was only detected in the 1:2 and 1:1 mix beverage samples, while daidzein was not detected in all samples. Genistein was reported for its antioxidant, phytoestrogen, and cancer cell growth suppression activities at a low concentration (Athaillah *et al.* 2019), while daidzein was found to have antioxidant and antiviral activities by inhibiting the proteolytic activity of SARS-CoV-3C like protease (Lammi & Arnoldi 2021).

In contrast to this finding, Astawan *et al.* (2020) showed that tempeh flour which was made from ungerminated soybean contained 62 mg genistein and 54 mg daidzein per 100 g db. The beverage in this study was made with 12 g of tempeh, GRR, or mix powder in 200 ml of water. This would suggest that a very low concentration of genistein and daidzein might be present in the beverage which was below the detection capability of the profiling method. Therefore, increasing the tempeh powder concentration to above 6% w/v might increase the isoflavone aglycones content as well, but it would result in the decreased solubility that would require further formulation of the beverage. Moreover, the low concentration of genistein and daidzein in the soybean and commercial tempeh might also potentially contribute to the very low

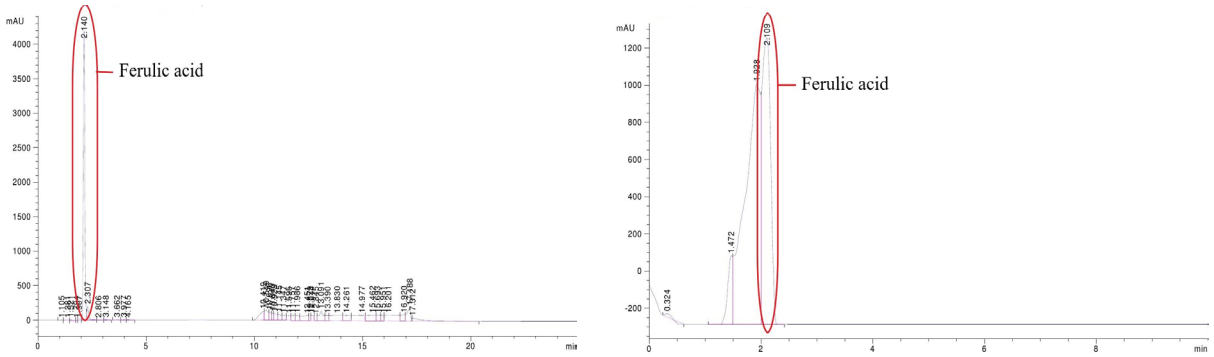

Figure 3. Chromatogram of (a) 400 µg/ml ferulic acid standard solution and (b) 1:2 GRR: Tempeh mix beverage sample

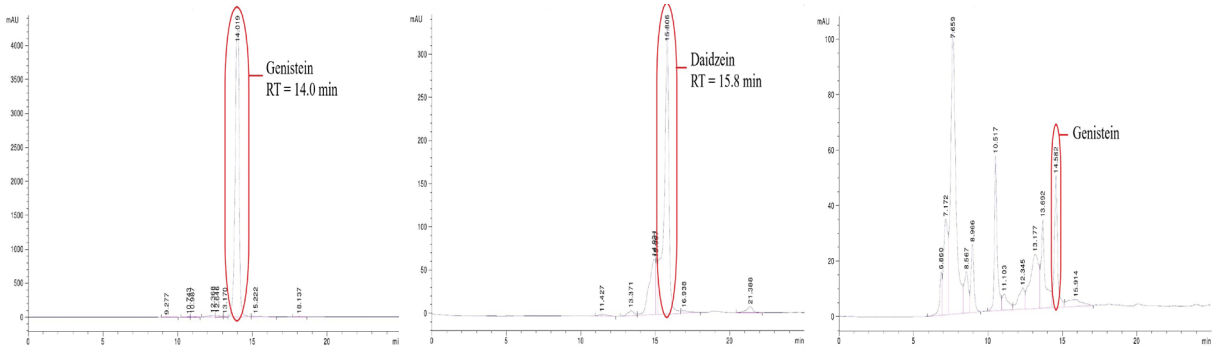

Figure 4. Chromatogram of (a) 100 µg/ml genistein standard solution, (b) 50 µg/ml daidzein standard solution, and (c) 1:1 GRR: Tempeh mix beverage sample

concentration of both compounds in the beverage samples. A selection of soybean variety with high isoflavones content might increase the isoflavone aglycones content in tempeh because soybean variety was the dominant contributor of the isoflavone aglycones content in tempeh (Athaillah *et al.* 2019).

A solid substrate fermentation, as was applied in tempeh production using *Rhizopus* spp., served as a good strategy to increase the total phenolics content and improve the antioxidant activity of the substrate, as was also observed in chickpea fermentation (Sánchez-Magana *et al.* 2014). Isoflavone aglycones, such as genistein and daidzein, were abundant in tempeh as compared to soybean which contributed to the higher bioavailability and antioxidant activity (Santos *et al.* 2018). Tempeh fermentation could initially transform the glycosides form of isoflavones to their aglycone form, then further cause the biotransformation of the isoflavones to polyhydroxylated isoflavones through microorganisms' action during tempeh fermentation (Chang 2014) which could result in the reduction of genistein and daidzein content.

Protein could protect the soy isoflavones from a thermal degradation (Malaypally & Ismail 2010). However, the heat treatment applied during tempeh powder production and beverage formulation in this study could degrade the protein, thus, exposing the isoflavones to the thermal degradation. Genistein and daidzein began to degrade at 95°C and drastically declined at a temperature above 200°C (Huang *et al.* 2006). The reduction and optimization of heat treatment in the production of functional beverage was, therefore, essential to maintain the stability of phenolic compounds with targeted health benefits.

Sensory evaluation

The acceptance test scores on the plain beverage samples were not different significantly among all samples (Table 3) which ranged from 2.90 to 4.27. Formulation of the beverage with lemongrass-infused syrup improved the acceptance particularly for the taste, texture, and aftertaste attributes which ranged from 4.93 to 5.63 (Table 4). However, such result was also not different significantly for each sensory attribute among all samples.

The taste, color, aroma, and aftertaste of GRR-containing beverage was more liked than tempeh single beverage in both plain and sugar added beverage. The beany flavor of

Table 3. The acceptance test result on plain beverage sample with a 7-scale hedonic score

Sample (GRR:Tempeh)	Taste	Color	Aroma	Texture	Aftertaste
1:2 Mix	2.90±1.42a	4.53±1.28a	4.27±1.48a	3.77±3.77a	3.07±3.07a
1:1 Mix	3.33±1.42a	4.50±1.22a	4.23±1.33a	3.97±1.47a	3.37±1.59a
2:1 Mix	3.40±1.59a	4.17±1.46a	4.08±1.46a	3.77±1.50a	3.73±1.46a
GRR	3.23±1.52a	4.83±1.39a	4.03±1.27a	4.27±1.51a	3.50±1.48a
Tempeh	3.07±1.64a	4.90±1.45a	4.23±1.45a	4.10±1.49a	3.23±1.43a

Values with similar letters within a column are not significantly different ($p>0.05$); GRR: Germinated Red Rice

Table 4. The acceptance test result on formulated beverage sample (after addition of lemongrass-infused syrup) with a 7-scale hedonic score

Sample (GRR:Tempeh)	Taste	Color	Aroma	Texture	Aftertaste
1:2 Mix	5.63±0.96a	5.07±1.17a	4.87±1.11a	5.13±1.28a	5.07±1.14a
1:1 Mix	5.07±1.46a	4.93±1.20a	4.60±1.25a	4.93±1.26a	5.00±1.49a
2:1 Mix	5.43±1.22a	4.97±1.38a	4.73±1.31a	5.37±1.19a	5.50±1.17a
GRR	5.60±1.25a	5.43±1.17a	5.13±1.07a	5.23±1.17a	5.37±1.19a
Tempeh	5.20±1.03a	5.30±1.18a	4.83±1.23a	5.40±1.04a	4.93±1.41a

Values with similar letters within a column are not significantly different ($p>0.05$); GRR: Germinated Red Rice

tempeh powder was detected and not liked by some panelists. The tempeh single beverage still exhibited beany flavor although it had been blanched and oven-dried during the tempeh powdering process. The beany flavor of soy-derived product was the result of volatile compounds that was released during the heating process and/or oxidation reaction, such as short chain fatty acids, sterols, and sulfur compounds (Sethi *et al*. 2016). Blanching could inactivate the lipoxygenase enzyme in tempeh; however, it was more effective to deodorize the beany flavor of soybean when it was applied in the soybean soaking step (Sethi *et al.* 2016). The increasing content of GRR and the decreasing content of tempeh in 2:1 mix beverage showed the highest score for the taste and aftertaste in the plain beverage. The lemongrass flavor and sugar addition was suitable to improve the sensory properties of the GRR and tempeh powder mix or single beverage by masking the beany flavor of tempeh powder. Lemongrass contained flavoring compounds, such as β-myrcene, α-pinene, and geraniol which could interact with the macromolecules in the beverage to entrap the beany flavor of tempeh (Natisri *et al.* 2014).

CONCLUSION

Mixing both GRR and tempeh powder at 6% w/v concentration as a beverage could be a good strategy to achieve the potential health benefits in terms of the phenolics and antioxidant activity. The 2:1 GRR : tempeh powder mix beverage could generate a total phenolic content and a DPPH radical scavenging activity as high as a single GRR powder beverage and 1.6-fold higher than that of a single tempeh powder beverage. GRR contributed to the increase of total phenolic content, ferulic acid content, and antioxidant activity of the beverage samples. A lemongrass syrup addition was suitable to improve the taste and aftertaste of the beverage formulation. A loss of isoflavone in the tempeh-containing beverage samples suggested that optimizing the dose and minimizing heat treatment during processing were important to achieve the optimal health benefits from both ingredients.

ACKNOWLEDGEMENT

The authors thank LPPM Atma Jaya Catholic University of Indonesia for providing research fund to this study under the 2018 decentralized grant which was managed by the Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia.

AUTHOR DISCLOSURES

The authors have no conflict of interest.

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Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://10.25182/jgp.2021.16.2.119-128*
ISSN 1978-1059 EISSN 2407-0920

Maternal and Cord Blood Saturated Fatty Acid Level and Infant Adiposity

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ABSTRACT

This study aimed to assess SFAs profiles in the maternal and cord blood, and the relationship of both SFAs levels with infant adiposity. As many as 99 mothers with singleton pregnancy and pre-pregnancy BMI ≥18.5 agreed to join the research and completed the data collection process. Maternal and cord blood erythrocyte SFAs profile was analyzed using Gas Chromatography-Flame Ionized Detector. Infant birth weight was measured at birth, while infant skinfolds were at 5–7 days postpartum. We used Aris *et al.* (2013) equation to assess the infant fat mass. The average maternal age was 29.62±5.84 years old, while the pre-pregnancy BMI was 22.87±3.90 kg/m^2. Infant birth weight was 3168.83±341.64 g, and fat mass was 9.39±3.52 %. Maternal total SFAs and palmitic acid (C16:0) concentration were higher than cord blood, while lignoceric acid (C24:0) was lower ($p<0.05$). Increased maternal caproic (C6:0), capric (C10:0), and lauric acids (C12:0) were associated with higher infant adiposity ($p<0.05$). Total SFAs, palmitic (C16:0), stearic (C18:0), and behenic acids (C22:0) in cord blood were negatively associated with infant adiposity ($p<0.05$). Elevated lauric (C12:0) and myristic (C14:0) acids in cord blood were associated with greater adiposity. In conclusion, we found a different SFAs profile between maternal blood during the third trimester of pregnancy and cord blood. Increased maternal caproic, capric, and lauric acids as well as cord blood's lauric and palmitic acids contribute to greater infant adiposity.

Keywords: cord blood, fat mass, infant adiposity, pregnant women, saturated fatty acids

INTRODUCTION

Obesity has been growing nutrition problem affecting both the adult and children population in Indonesia. In 2018, the national prevalence of childhood obesity reached 8% (MoH RI 2018). Determinants of Childhood obesity in developing countries are multifaceted, some are unhealthy diet (fast food consumption), physical inactivity, socioeconomic status, area of residency, age, gender (Gupta *et al.* 2012; Febriani & Sudiarti 2019). Recent evidence suggests that childhood obesity could also be predicted at birth by early Fat Mass (FM) deposition or neonatal adiposity (Moore *et al.* 2020). Excess neonatal adiposity is associated with an adverse health outcome in the future life. Hernandez-Trejo *et al.* (2020) showed that it might increase the pro-inflammatory cytokine levels at birth. Later during the childhood period, it contributes to the incidence of inflammatory diseases, such as atopic dermatitis (O'Donovan *et al.* 2016).

The adipose tissue serves as an energy storage and thermoregulator among mammals, including in human infant. Interestingly, it also acts as an endocrine organ which modulates a range of metabolic pathways and inflammation process. The human body will deposit energy surplus as triglyceride in adipose tissue through the lipogenic pathway. Conversely, when the body experiences energy scarcity, the lipolytic process will break the triglyceride deposit in adipose tissue down into glycerol and fatty acids. The released glycerol and fatty acids will be distributed to muscle and other organs and modulate the energy balance throughout the body (Luo & Liu 2016). It is a crucial mechanism to protect the infant from energy shortfall when breastfeeding has not been established yet, or during the transition to weaning food.

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(Received 01-05-2021; Accepted 15-07-2021; Published 29-07-2021)

On the other side, adipose tissue can maintain infant body temperature through non-shivering thermogenesis. This is an important mechanism since muscle function in new-born has not fully developed, making them unable to raise body temperature through shivering (Lichtenbelt & Schrauwen 2011).

SFAs is the most abundance component of lipid which play a major part on lipid metabolism in human body. However, evidence depicting the role of SFAs on neonatal adiposity are inconsistent and limited. Mennitti *et al.* (2015) reviewed several animal studies which concluded that excessive SFAs intake may lead to the development metabolic disease and obesity. Moore *et al.* (2018) studied the dietary pattern among pregnant women and concluded that intake of saturated fat contributed to higher dietary inflammatory index. This study further demonstrated that elevated dietary inflammatory index and pre-pregnancy Body Mass Index (BMI) increased the odds of large-for-gestational age infant. However, growing body of evidence also suggests that increased concentration of certain SFAs is not necessarily associated with increased adiposity. A recent study among Singaporean infants shows that Medium-Chain Fatty Acids (MCFA) are associated with lower infant skinfold thickness (Chia *et al.* 2020). MCFAs increases the intrinsic respiratory capacity of mitochondria which is beneficial to prevent lipid accumulation in adipose tissue (Montgomery *et al.* 2013).

The peak of the lipid accumulation process in infants happens during the third trimester of pregnancy. In this period, maternal fat deposit going through enhanced lipolysis. Fatty acids from maternal circulation will enter the fetal body through the placenta, and eventually the umbilical cord. The placenta has its regulatory mechanism to select materials that will pass through the fetal body, including fatty acids. Placenta can also perform de novo synthesis of SFAs (Chavan-Gautam *et al.* 2018). It suggests that SFAs profile in the placenta and umbilical cord blood is not necessarily similar to that of maternal blood.

Therefore, in this study, we assessed SFAs profiles in the mother's blood during the third trimester of pregnancy and cord blood. The SFAs profile in the cord blood represented the infant compartment. We also evaluated the differences between SFAs profile in maternal and infant compartments. Finally, we investigated whether the SFAs level in maternal and cord blood affect infant adiposity indicators in the early postpartum period.

METHODS

Design, location and time

This research was a longitudinal study conducted between May and December 2018 in Bogor City, West Java Province, Indonesia. Some of the data used were taken from a BASF South East Asia grant study entitled "Association of Maternal Dietary Intake and Blood Level of Long Chain Poly-Unsaturated Fatty Acids in Pregnancy and Newborn Body Composition" by IPB's SEAFAST CENTER team. The subject recruitment took place at the North Bogor and Tanah Sareal Public Health Center (PHC). In these two PHCs, the number of pregnant women who visited to receive Antenatal Care (ANC) services was the largest in Bogor. The blood's SFAs profile assessment took place at the DKI Jakarta Regional Health Laboratory. Ethical approval was granted by the Research Ethics Committee of LPPM IPB University (041/IT3. KEPMSM-IPB/SK/2018). Permission to conduct this study was obtained from the district health office of Bogor City, Indonesia.

Sampling

The study population was pregnant women aged 18–45 years in Bogor City with a gestational age between 32–40 weeks. The research subjects were third-trimester pregnant women who attended ANC service at the North Bogor and Tanah Sareal PHC from May to September 2018. The inclusion criteria were mothers with singleton pregnancies and had a pre-pregnancy BMI of more than or equal to 18.5 kg/m^2. As many as 142 pregnant women who met the criteria agreed to participate in the study and provide informed consent. Out of 142 subjects recruited, only 99 complete data were available for analysis. The remaining 43 maternal-infant dyad could not be included in the analysis due to unavailable maternal blood samples (17 subjects), lost to follow up (11 subjects), self-withdrawal (7 subjects), gave birth at different hospital (4 subjects), and cord blood hemolysis (4 subjects).

Data collection

The data used in this study consisted of maternal characteristics (age, parity, pre-

pregnancy BMI, Mid-upper Arm Circumference/ MUAC), maternal and cord blood SFA levels, infant characteristics and nutritional status (sex, birth weight, and triceps, subscapular and thigh skinfolds). Interviews and infant skinfolds measurement was carried out at the PHC or the subject's homes by trained enumerators. The laboratory staff collected maternal blood samples, while the midwives collected the cord blood samples. The laboratory staff performed the preparation of all blood samples in the PHC. Data on maternal (MUAC, pre-pregnancy weight, height) and infant Birth Weight (BW) and birth history were obtained from the PHC birth register book and the maternal and child health (KIA) book.

The enumerators, midwives, and laboratory staff received a set of training program before the data collection. The enumerator training consisted of recruitment procedures, interviews, and infant anthropometric measurement techniques. The training courses for laboratory staff and midwives consisted of the screening process of potential subjects, maternal and cord blood drawing, and blood preparation techniques.

Data collection consisted of two stages. The first stage was subjects recruitment. After receiving the signed informed consent, trained enumerators collected data on the characteristics and nutritional status of the subject through interviews using a structured questionnaire. The second stage was a home visit by enumerators on 5–14 days postpartum to collect data on birth history and skinfolds of the infants. The BW measurement of the baby were recorded in the PHC's birth registers book where the baby's delivery process took place. A trained midwife took this measurement right after the baby was born. The infant's triceps, subscapular and thigh skinfold was measured directly by trained enumerators at the subject's house at 5–14 days postpartum using Lange® body caliper. The skinfolds thickness measurement were taken twice at each site to ensure the data quality.

The cubital venous blood samples of the subjects were taken at the first visit or after the recruitment process, while the venous cord blood was right after the delivery process. The blood sample was stored in an EDTA tube and centrifuged within 12 hours after blood withdrawal. The erythrocyte portion was taken and stored in a labeled plastic container at -80^{o}C until further analysis. The fatty acids in erythrocytes were extracted and analyzed using the modified Folch *et al.* (1957) method. The fatty acids profile was measured using Gas Chromatography-Flame Ionization Detector (GC-FID) by professional laboratory staff at the DKI Jakarta Regional Health Laboratory.

Data analysis

Pre-pregnancy Body Mass Index (BMI) was calculated based on maternal pre-pregnancy weight and height data. We used the following equation (Aris *et al.* 2013) to calculate the newborn FM:

$$FM=-0.022+(0.307^{*}W)-(0.077^{*}G)-(0.019^{*}GA)+(0.028^{*}SSF)$$

FM: Fat Mass (kg)
GA: Gestational Age (weeks)
G: Gender (1=male; 0=female)
SSF: Subscapular Skin Fold (mm)
W: Birth Weight (kg)

The data processing and analysis were conducted using Microsoft Excel 2019 and SPSS version 25.0 software. Continuous data were presented descriptively as mean and standard for normally distributed data. Median and Interquartile Range (IQR) information was also added for non-normally distributed data. Paired sample t-test was employed to assess the mean difference between maternal and cord blood profile. We used Pearson correlation test to identify correlation of maternal and cord blood SFA profile with infant weight, triceps, subscapular, and thigh skinfolds, and FM percentage. The significant level was set at $p<0.05$ for all tests.

RESULTS AND DISCUSSION

Maternal and infant's characteristics

Table 1 shows the maternal and infant characteristics. Subjects were pregnant women between 20 and 45 years old. Most of the subjects have normal nutrition status (BMI 18.5–24.9 kg/ m^2) before their pregnancy (76.8%). The average gestational age at delivery was 39.23±1.20 weeks. The proportion of female and male infants are quite similar (50.5%) for female and (49.5%) male respectively. The average BW of the infants were 3168.83±341.64 g. The FM percentage in this study (9.39±3.52%) was lower than the reported FM of infant from Asian mothers (Wiechers *et al.* 2019).

Table 1. Maternal and infant characteristics (n=99)

Variables	n (%)	Mean±SD
Maternal characteristics		
Age (years)		29.62±5.84
20–29	51 (51.5)	
30–39	42 (42.4)	
40–45	6 (6)	
PP-BMI (kg/m^2)		22.87±3.90
18.5–24.9	76 (76.8)	
≥25	23 (23.2)	
MUAC (cm)	99 (100)	
Gestational age at delivery (weeks)		27.01±2.96
Infant characteristics		39.23±1.20
Gender		
Female	50 (50.5)	
Male	49 (49.5)	
Birth weight (g)		3168.83±341.64
Tricep skinfold (mm)		4.87±1.55
Subscapula skinfold (mm)		4.99±1.69
Tigh skinfold (mm)		5.57±1.63
Fat mass (%)		9.39±3.52

PP-BMI: Pre-Pregnancy Body Mass Index; MUAC: Mid-Upper Arm Circumference

Maternal and cord blood' SFA profile

Table 2 shows total SFA and ten individual SFAs measured in maternal and umbilical cord red blood cell samples. Total SFAs and palmitic acid (C16:0) concentration in maternal blood were significantly higher than cord blood (p=0.001). On the contrary, lignoceric acid (C24:0) concentration in cord blood was notably higher than maternal blood (p=0.000). It indicates that maternal blood SFA profile might not be similar to cord blood. Placenta, which connects the maternal and infant compartment, regulates fatty acids transfer to the fetus. Evidence suggests that compared to SFAs, placental plasma membrane binding sites have a strong preference for Long-Chain Polyunsaturated Fatty Acids (LCPUFAs), such as arachidonic acid, docosahexaenoic acid, and eicosapentaenoic acid (Duttoray & Bassak 2020). It may explain the decrease of total SFAs and palmitic acid (C16:0) concentration in cord blood.

Lignoceric acid (C24:0) is a long-chain saturated fatty acid, which can be found throughout the human body. It is one of the most common fatty acids bonded into the ceramide backbone to form sphingolipid. The lignoceric-acid-containing-sphingolipid mainly appears in axons of neuron cells in the liver, kidney, pancreas, and brown and white adipose tissue (Sassa & Kihara 2014). Increased lignoceric acid (C24:0) concentration in cord blood might suggest that this component came from maternal circulation and placenta. The placenta can produce its SFAs through the de novo synthesis process (Chavan-Gautam *et al.* 2018).

Palmitic and stearic acid universally are found in natural fats. In this study, palmitic acid (C16:0) concentration was the highest among other SFAs in maternal and cord blood (Table 2). These findings are in line with previous studies which stated that palmitic acid is the principal constituent of fatty acids found in human tissues, such as in serum (Liu *et al.* 2017; Yammine *et al.* 2018), red blood cell (Aktas *et al.* 2016), adipose tissue (Shramko *et al.* 2020) and other human tissues in general (Ruiz-Nunez *et al.* 2016). This pattern remains consistent when compared to Spanish vegetarian population (Salvador *et al.* 2019), and Italian or Tibetan population (Rise *et al.* 2008) whose main dietary oil is olive, sunflower, mustard, canola, and corn oil. It might also important to note that the human body also synthesizes SFAs through de novo synthesis, which its main products is palmitic (C16:0) and stearic acid (C18:0) (Chauvan-Gautam *et al.* 2018).

Association between maternal and cord blood SFA and infant adiposity

The correlation between high blood levels of SFAs and increased adiposity was well documented in a previous study (Yammine *et al.* 2018). Table 3 shows the association of maternal SFAs concentration with infant adiposity indicator (BW, FM percentage, tricep, subscapular, and thigh skinfolds). Lauric acid (C12:0) concentration was positively associated with all adiposity indicators ($p<0.05$). Caproic acid (C6:0) concentration was positively associated with tricep, subscapular, and thigh skinfolds ($p<0.05$). Capric acid (C10:0) was positively associated with tricep and thigh skinfolds.

Increasing evidence suggests that increased infant adiposity highly correlates with maternal

Tabel 2. Maternal and Cord Blood' SFA profile (n=99)

Fatty acid	Maternal blood (g/100 g fatty acid)		Cord blood (g/100 g fatty acid)		p
	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)	
Total SFA	24.78±13.27	28.29 (14.45–33.26)	21.09±16.16	23.74 (3.06–33.43)	0.001*
C6:0	2.93±5.65	0.55 (0.17–2.07)	3.08±6.30	0.44 (0.08–2.27)	0.364
C8:0	0.13±0.32	0.02 (0.00–0.07)	0.30±0.70	0.06 (0.01–0.23)	0.532
C10:0	0.38±0.85	0.06 (0.03–0.23)	0.41±0.79	0.10 (0.02–0.32)	0.584
C12:0	0.44±1.01	0.10 (0.03–0.28)	0.34±0.68	0.09 (0.02–0.24)	0.536
C14:0	0.31±0.24	0.31 (0.10–0.44)	0.93±1.99	0.28 (0.07–0.62)	0.526
C16:0	23.72±13.06	26.03 (14.30–30.99)	19.57±15.71	22.46 (1.29–31.99)	0.001*
C18:0	0.29±0.65	0.09 (0.01–0.19)	0.47±1.77	0.11 (0.01–0.29)	0.744
C20:0	0.23±0.59	0.00 (0.00–0.05)	0.67±2.18	0.03 (0.00–0.09)	0.482
C22:0	0.40±0.90	0.15 (0.01–0.34)	0.17±0.35	0.02 (0.01–0.26)	0.982
C24:0	0.15±0.48	0.00 (0.00–0.01)	0.21±0.69	0.01 (0.00–0.03)	0.000*

C6:0: Caproic Acid; C8:0: Caprylic Acid; C10:0: Capric Acid; C12:0: Lauric Acid; C14:0: Myristic Acid; C16:0: Palmitic Acid; C18:0: Stearic Acid; C20:0: Arachidic Acid; C22:0: Behenic Acid; C24:0: Lignoceric Acid; IQR: Inter Quartile Range (quartile 1–quartile 3); SFA: Saturated Fatty Acid; SD: Standard Deviation

*$p<0.05$ shows significant mean difference between maternal and cord blood samples

free fatty acids and their triglyceride sources (Barbour & Hernandez 2018). Lauric acid (C12:0), one of the long-chain SFA, is less prone to β-oxidation than the shorter chain SFAs. It also can increase all cholesterol fractions, including triglyceride (Shramko *et al.* 2020). It may explain the positive association of lauric acid level with infant adiposity indicators.

Compared to long-chain SFAs, medium-chain SFAs (6–12 carbons) is a preferred substrate of β-oxidation. Medium-chain SFAs are mainly oxidized in the liver, resulting in lower fat deposition in adipose tissue (Ruiz-Nunez *et al.* 2016). A study in mice also confirmed that compared to long-chain SFAs, medium-chain SFAs increase the mitochondrial intrinsic respiratory capacity without increasing the oxidative stress (Montgomery *et al.* 2013). However, in this study, we found that increased caproic acid (C6:0) and capric acid (C10:0) correlated with increased infants' skinfolds (Table 3).

The correlation between cord blood SFAs level and infant adiposity is presented in Table 4. Cord blood lauric acid (C12:0) is positively associated with tricep skinfold. This result contradicts previous study which stated that medium-chain SFAs prevent the lipid deposition in adipose tissue tissue (Ruiz-Nunez *et al.* 2016). Cord blood myristic acid (C14:0) is positively associated with subscapular, thigh skinfolds, and FM percentage. Myristic acid is a long-chain SFAs, which was reported to pose a greater obesogenic effect than other medium and short-chain SFAs (Sergi & Williams 2020). Long chain fatty acid is one of various nutrients that can easily reach the brain and induce cellular stress or inflammatory responses, mainly via Toll-like receptor 4 (TLR4) during the development of obesity (Mullins *et al.* 2020).

Total SFAs, palmitic acid (C16:0), stearic acid (C18:0), and behenic acid (C22:0) in cord blood are negatively correlated with infant adiposity indicators (Table 4). These findings are unexpected and inconsistent with previous studies, which suggest that SFAs intake, specifically long-chain SFAs, are associated with obesity (Yammine *et al.* 2018; Sergi & Williams 2020). This data, however, was in line with another study which shows that total SFAs, palmitic acid, and stearic acid intake of lean children was significantly higher than that of overweight or obese children (Jauregibeitia *et al.* 2020).

Table 3. Association between maternal blood SFAs and infant adiposity (n=99)

Saturated fatty acid	Birth weight	Skinfolds			% Fat mass
		Tricep	Subscapula	Tigh	
Total SFAs					
r	0.156	-0.136	-0.041	-0.118	0.061
p	0.123	0.181	0.687	0.244	0.549
Caproic acid (C6:0)					
r	0.100	0.263	0.224	0.256	0.157
p	0.323	0.009*	0.026*	0.011*	0.122
Caprylic acid (C8:0)					
r	0.008	0.142	0.126	0.102	0.069
p	0.934	0.162	0.214	0.314	0.500
Capric acid (C10:0)					
r	0.070	0.256	0.191	0.253	0.130
p	0.488	0.011*	0.058	0.015*	0.199
Lauric acid (C12:0)					
r	0.221	0.345	0.229	0.244	0.244
p	0.028*	0.000*	0.023*	0.015*	0.015*
Myristic acid (C14:0)					
r	0.010	-0.076	-0.044	-0.113	-0.021
p	0.919	0.452	0.665	0.267	0.833
Palmitic acid (C16:0)					
r	0.165	-0.129	-0.032	-0.102	0.164
p	0.104	0.205	0.751	0.316	0.105
Stearic acid (C18:0)					
r	-0.127	-0.087	-0.057	-0.173	-0.121
p	0.210	0.391	0.577	0.087	0.234
Arachidic acid (C20:0)					
r	0.097	0.011	-0.078	-0.002	0.029
p	0.337	0.916	0.443	0.983	0.774
Behenic acid (C22:0)					
r	0.013	-0.037	0.017	-0.080	0.074
p	0.901	0.713	0.870	0.432	0.469
Lignoceric acid (C24:0)					
r	-0.136	-0.075	-0.115	-0.107	-0.137
p	0.180	0.459	0.256	0.291	0.177

*$p<0.05$ shows significant association of SFAs concentration and infant adiposity indicators; SFA: Saturated Fatty Acid

This study confirmed that several SFAs paramater in maternal blood was associated with infant birth weight, skinfold thickness, and percentage of fat mass. However, it could not explain the mechanism underlying this association. Recent hypothesis suggested that maternal fatty acids enters the fetal circulation through placenta, and they either converted into acyl-CoA in the liver and used there for the synthesis of triaylglicerol or may be taken

Table 4. Association between cord blood SFAs and infant adiposity

Saturated fatty acid	Birth weight	Skinfolds			% Fat mass
		Tricep	Subscapula	Tigh	
Total SFAs					
r	-0.074	-0.164	-0.126	-0.230	-0.082
p	0.468	0.104	0.215	0.022*	0.419
Caproic acid (C6:0)					
r	0.001	-0.068	0.010	-0.037	0.014
p	0.995	0.506	0.925	0.714	0.888
Caprylic acid (C8:0)					
r	-0.027	-0.057	0.030	-0.003	-0.016
p	0.792	0.575	0.769	0.979	0.873
Capric acid (C10:0)					
r	-0.018	-0.133	0.007	-0.050	0.041
p	0.858	0.189	0.944	0.625	0.684
Lauric acid (C12:0)					
r	-0.029	0.264	0.019	-0.067	-0.009
p	0.777	0.008*	0.851	0.508	0.931
Myristic acid (C14:0)					
r	0.058	-0.141	0.258	0.207	0.209
p	0.569	0.164	0.010*	0.040*	0.038*
Palmitic acid (C16:0)					
r	-0.038	-0.145	-0.098	-0.214	-0.047
p	0.710	0.153	0.335	0.034*	0.648
Stearic acid (C18:0)					
r	-.251*	-0.145	-0.138	-0.143	-.238*
p	0.012	0.153	0.174	0.157	0.018
Arachidic acid (C20:0)					
r	-0.055	-0.015	-0.033	0.025	-0.040
p	0.587	0.885	0.746	0.803	0.692
Behenic acid (C22:0)					
r	0.088	-0.171	-0.165	-0.228	-0.014
p	0.386	0.090	0.103	0.023*	0.893
Lignoceric acid (C24:0)					
r	-0.094	-0.133	-0.170	-0.117	-0.118
p	0.353	0.189	0.092	0.247	0.244

*$p<0.05$ shows significant association of SFAs concentration and infant adiposity indicators: SFA: Saturated Fatty Acid

up directly by adipocytes (Desoye & Herrera 2021). It was also hypothesized that the higher maternal fatty acid transfers to fetal circulation, the higher adypocyte generation converted from the mesenchymal stem cells (Szabo 2019). Reviews on animal studies (Mennitti *et al.* 2013) concluded that intake of diet rich in SFAs during pregnancy and/or lactation mediated the high proinflammatory cytokines production through the TLR4 pathway activation. This TLR4-

mediated inflammation acts in the pathogenesis of obesity, which represented by increased body mass, visceral fat and adipocyte hypertrophy.

It has been well understood that infancy is a rapid growth and development period in which massive multiplication of body cells happens. In this process, fatty acids in general serves as energy sources, building blocks of membrane cells, cell division, differentiation and death, cell signaling, etc (Carvalho & Caramujo 2018). As an energy sources, fat (9 kcal/g) provides a higher energy than carbohydrate and protein (4 kcal/g). SFAs could be found as a major component of phospolipid or glycerophospolipid in cellular membranes. Very-long-chain SFA such as lignoceric acid (C24:0) is the most common fatty acids component of sphingomyelin, an important lipid molecules in cell division and differentiation (Sassa & Kihara 2014; Carvalho & Caramujo 2018).

SFAs compose a big portion in our dietary fats, especially the palmitic and stearic acids. Moreover, unlike the essential omega-3 and omega-6 fatty acids, our body has the ability to synthesize SFAs endogenously. It also attributed to the negative impact of SFAs to human health, such as increased of inflammatory response cardiovascular disease (Ruiz-Nunez *et al.* 2016), and adiposity as also concluded in the current study. It might be the main reason why the general recommendation in nutritional guidelines is to restrict dietary SFAs intake.

CONCLUSION

We found a higher total SFAs and lauric acid concentration in maternal blood during the third trimester of pregnancy than in cord blood. Our findings also contribute to the growing body of evidence on the role of SFAs during pregnancy on infant adiposity. Increased maternal caproic, capric, and lauric acids are associated with higher infant adiposity. Elevated lauric and myristic acids in cord blood contribute to greater adiposity. Conversely, increased total SFAs, palmitic, stearic, and behenic acids in cord blood are associated with lower infant adiposity. Further studies exploring the contribution of maternal and infant dietary SFAs are needed to establish a more comprehensive view on the role of SFAs to infant adiposity.

ACKNOWLEDGEMENT

We thanked SEAFAST Center IPB University and BASF South East Asia for allowing us to use part of the data from their research.

AUTHOR DISCLOSURES

The authors have no conflict of interest in preparing this manuscript.

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