MALAYSIAN JOURNAL OF

NUTRITION

VOL. 24 NO.4 DECEMBER 2018

PP18053/02/2013 (033331)

ISSN 1394-035X

Official Publication of the
PERSATUAN PEMAKANAN MALAYSIA
NUTRITION SOCIETY OF MALAYSIA

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

MALAYSIAN JOURNAL OF NUTRITION

Peer-reviewed Journal of the Nutrition Society of Malaysia
(http//www.nutriweb.org.my)

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Malaysian Journal of Nutrition
Vol. 24 No. 4, 2018

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Nutritional Status, Dietary Intake and Body Composition

Mal J Nutr 24(4): 475-492, 2018

Effects of micronutrient powder and complementary food blend on growth and micronutrient status of Filipino rural children: a randomised controlled trial

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ABSTRACT

Introduction: This study aimed to evaluate the effects of micronutrient powders (MNP) containing 15 versus nine nutrients, with or without complementary food blend (*BigMo*), on the nutritional status of rural young children in the Philippines. **Methods:** The study was conducted for 6 months among 126 rural children aged 6-17 months in four villages selected by cluster randomisation. Children were randomised into four groups: VitaMix with 15 micronutrients plus Bigas Mongo (*BigMo*) (*n*=31); VitaMix without *BigMo* (*n*=31); Micronutrient Growth Mix (MGM) with nine micronutrients plus *BigMo* (*n*=29); and MGM without *BigMo* (*n*=31). Blood samples were collected at baseline and endline to determine haemoglobin, ferritin, retinol, and zinc concentrations. Intervention compliance, weight, length, and dietary intakes were collected every month. One-way ANOVA was used to compare changes in the mean estimates across groups. McNemar and Pearson's x^2 tests were used to compare changes in the proportion estimates within groups and across groups, respectively. **Results:** Both VitaMix and MGM with or without *BigMo* improved haemoglobin concentrations and reduced anaemia (Hb<11g/dL). However, only VitaMix and MGM combined with *BigMo* had effects in reducing moderate anaemia (Hb<10g/dL), compared to groups without *BigMo*. Only MGM+*BigMo* group demonstrated significant reduction in the prevalence of moderate non-iron deficiency anaemia (IDA) (Hb<10g/dL and ferritin>12ug/L). A significant increase in the length-for-age z-scores was noted in the MGM with and without *BigMo* groups. **Conclusion:** Daily supplementation of MGM with nine micronutrients combined with complementary food blend may have a greater potential than MNP with 15 micronutrients in improving the nutritional status of young children.

Keywords: Micronutrient powder, complementary food blend, micronutrient deficiency, anaemia

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INTRODUCTION

The majority of Filipino young children aged 6-23 months do not meet the minimum acceptable diet, indicating poor dietary diversity and quality, coupled with inadequacy of energy, protein, iron and vitamin A in their diets (FNRI-DOST, 2016). One of the causes identified for the marked increase in undernutrition during the first two years of life was sub-optimal practices of exclusive breastfeeding and complementary feeding (FNRI-DOST, 2015a). In addition, anaemia was highly prevalent, at 39.4% among infants 6-12 months, 24.6% among 1 year old children and 14.0% in 2 years old children (FNRI-DOST, 2015b). Among children 6 months to 5 years, anaemia prevalence was highest in the poorest income group, which commonly resides in rural areas (FNRI-DOST, 2015b).

Provision of micronutrients through micronutrient powders (MNP) has been recognised as an effective way to combat infant and childhood micronutrient deficiencies (De-Regil *et al.*, 2011). The World Health Organization (WHO, 2011) highly recommends MNP fortification in countries where anaemia prevalence is 20% or higher among children under 2 years old. Since the Philippines meets this WHO criteria, the Department of Health (DOH) issued Department Memorandum No. 2011-0303 in October 2011 to replace iron syrup with MNP supplementation for all children aged 6-23 months (DOH Philippines, 2011). Despite the implementation of nationwide home fortification using MNP since 2011, there has been limited success in reducing the prevalence of anaemia among children under 2 years old. Hence, anaemia in young children remains a public health concern.

On this basis, plus the knowledge that complementary foods provided to Filipino young children are often low in energy and protein density, and that increasing micronutrient intake through MNP would not necessarily result in adequate energy and protein intake, this study hypothesised that the combination of MNP along with the complementary food blend known as *BigMo* was more effective in improving dietary quality and adequacy. Children who received the combined supplementation would therefore have better nutritional status than children who only received MNP. This study explored how combined nutrition strategies might effectively combat undernutrition and micronutrient deficiency.

The Philippines, like several other countries, has adopted the use of Vitamix containing 15 micronutrients based on the recommendations of WHO, World Food Program (WFP), and United Nations Children's Funds (UNICEF), as well as experiences from the disaster response to typhoon "*Ondoy*" and "*Pepeng*" in 2009. In 2016, the Department of Science and Technology-Food and Nutrition Research Institute (DOST-FNRI) developed Micronutrient Growth Mix (MGM), an MNP containing nine micronutrients aimed at improving the diet quality of young children. The micronutrients added to MGM are considered most problematic among young Filipino children based on the results of national nutrition surveys.

As these two different MNP formulations are currently available in the country, there is a need to evaluate their effects in addressing anaemia and micronutrient deficiency in young children. The findings may provide evidence in determining the appropriate types and levels of micronutrients to optimise MNP supplementation programmes in the Philippines. Thus, this study aimed to evaluate the effects of the two MNP formulations (15 versus nine micronutrients) with or without complementary food blend on the

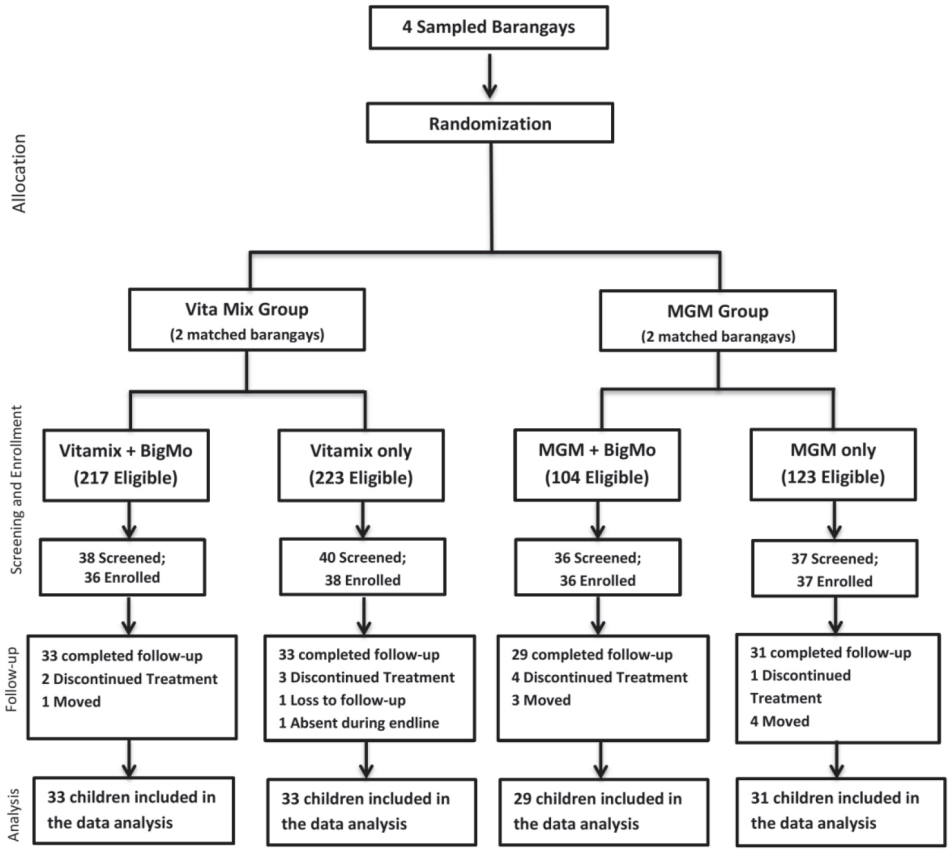

Figure 1. Intervention study participant flow chart

micronutrient status and anthropometric growth of Filipino children aged 6-23 months.

MATERIALS AND METHODS

Study area, design and randomisation

The study was conducted in the municipality of Calauan, an agricultural area with a mix of rice fields and upland areas in the southern part of Laguna province in the island of Luzon. The study design used was cluster randomised controlled non-blinded trial study. The village served as the unit of randomisation since it is the main access point for the delivery of health and nutrition services. Four barangays (villages) were randomly selected based on high prevalence of undernutrition among children aged 0-71 months. Three villages are located on the east of *poblacion* (Dayap, Sto. Tomas and Lamot II) and one barangay (Perez) is located on the west of *poblacion.* These four selected villages have respectively 217, 223, 123 and 104 children aged 6-17 months, providing a total of 667 children. The villages were matched based on their characteristics in terms of population size and location, and further randomised by a statistician into four intervention groups, as shown in Figure 1. Blinding of field staff and mothers involved was not possible in the implementation, but persons responsible for the laboratory and data analysis were blinded to the group assignment.

Sample size, sampling and subjects

With 90% level of confidence to detect anticipated mean difference in the haemoglobin level of 5.0 g/dL, a design effect of 1.5 and a 10% attrition rate to compensate loss to follow-up, the calculated minimum sample size of children was 144.

Out of the total of 667 children 6-17 months old from the four barangays, 151 were screened at baseline. A final total of 147 children who fulfilled the study's criteria were enrolled. Inclusion criteria were: 1) apparently healthy children between 6-17 months of age at the time of recruitment, who had no feeding problems/inborn/congenital/ severe illness, and were not suffering from disease as assessed by a physician; 2) without severe anaemia (Hb<7 g/dl) and without severe acute malnutrition (weight-for-height *z*-score <-3SD); 3) already consuming semi or solid food in addition to milk or breastmilk before the beginning of the study; 4) permanent residents in the barangay or municipality for the past 6 months; and 6) mothers who were willing to participate in the study with signed informed consent.

Each intervention group comprised 36-38 children who were selected based on stratified random sampling selection process using the list of eligible children per group. Of the 147 children enrolled to take part in this study, only 126 (85.7%) were able to complete the 6-month intervention period.

Type of intervention groups

1. *VitaMix and BigMo group*. Children received 12 sachets of VitaMix along with 20 sachets of *BigMo* every month. Mothers/caregivers were advised to add one sachet of MNP into 2-3 tablespoons or approximately 30-45 g of cooked *BigMo*. Giving of MNP along with *BigMo* to the child was done on Mondays, Wednesdays and Fridays. The remaining two sachets of *BigMo* were given on Tuesdays and Thursdays.
2. *VitaMix alone group*. Children received 12 sachets of VitaMix every month. Mothers/caregivers were advised to add one sachet of MNP on Mondays, Wednesdays and Fridays into a small portion of the child's meal before feeding the child.
3. *MGM and BigMo group*. Children received 30 sachets of MGM in addition to 20 sachets of *BigMo* every month. Mothers/caregivers were advised to add the sachet of MNP into 2-3 tablespoons or approximately 30-45 g of cooked *BigMo* on a daily basis from Monday until Sunday.
4. *MGM alone group*. Children received 30 sachets of MGM every month. Mothers/caregivers were advised to add one sachet of MNP daily into a small portion of the child's meal to ensure the entire consumption of the whole dose of MGM.

MNP formulations and composition

VitaMix is manufactured by Nutri Foods Corporation, a subsidiary of the Nutrition Center of the Philippines (NCP). The VitaMix formula contained 15 micronutrients including zinc and selenium in a single-dose sachet weighing 1 g/sachet. Each sachet of VitaMix provides 100% of the recommended nutrient intake (RNI) of each micronutrient for a child 6-24 months old, as shown in Table 1. The thrice weekly frequency of VitaMix provided an average of 40% of the RNI of each micronutrient for ages 6-24 months.

On the other hand, the nine nutrients added in the 2 g/sachet of MGM provides lower daily RNI for each nutrient (Table 1), ranging from 12.0-69.0% of the daily recommended amounts for the added micronutrients. These nine micronutrients are inadequately present

Table 1. Vitamin and mineral contents of VitaMix and MGM and the corresponding percent contribution of one sachet supplement to the Recommended Nutrient Intake (RNI) for ages 6-24 months

Micronutrients	*RNI*[†]		*VITAMIX (1 g) 15 micronutrients thrice weekly*		*MGM (2 g) 9 micronutrients daily frequency*	
	6-11 months	*12-24 months*	*Amount*	*% RNI*	*Amount*	*% RNI/ day*
Calcium (mg)	400	500			60	12
Vitamin A [µg(RE)]	400	400	400	100	239	60
Vitamin C (mg)	40	45	45	100	18.8	42
Vitamin D (µg)	5	5	5	100		
Vitamin E (IU)	4	4	5	100		
Vitamin B_1 (mg)	M: 0.4 F: 0.3	M: 0.5 F: 0.4	0.5	100	0.23	46
Vitamin B_2 (mg)	M: 0.4 F: 0.3	M: 0.5 F: 0.4	0.5	100		
Vitamin B_3 (mg)	5	6	6.0	100		
Vitamin B_6 (mg)	M: 0.2 F: 0.3	0.5	0.5	100		
Vitamin B_{12} (µg)	0.4	M: 0.9 F: 1.0	0.9	100	0.62	69
Folic Acid (µg)	M: 80 F: 70	150	150	100	55	34
Iron (mg)	M: 10 F: 9	8	10	100	2	28
Zinc (mg)	M: 4.2 F: 3.7	M: 4.1 F: 4.0	4.1	100	2	50
Iodine (µg)	90	90	90	100	16	17
Copper (µg)	-	-	0.56	-		
Selenium (µg)	M: 10 F: 9	M: 17 F: 16	17	100		

[†]Recommended Nutrient Intake based on 2015 Philippine Dietary Reference Intake (PDRI)
Abbreviation: M, male; F, female

in typical diets in developing countries, thus, it was presumed to be the optimal micronutrient composition to address anaemia.

The complementary food blend Bigas Mongo known as *BigMo* is a processed blend developed by extrusion cooking method and is ready-to-eat by adding hot water intended for infants and pre-school aged children. *BigMo* was first field tested for its effectiveness by the DOST-FNRI in selected Philippine provinces with high prevalence of underweight children aged 0-5 years, through the programme coined DOST-Pinoy in 2011-2012. The daily portion size of uncooked *BigMo* powder blend (30 g) provides 120 kcal or approximately, or 17.8% and 12.5% of the daily recommendation for calories for children aged 6-11 months and 12-23 months, respectively. For these age groups, the blend also contained 4 g of protein to meet 25.0% and 22.8% respectively of the daily recommendation

for protein. Mothers/caregivers were instructed to prepare the powder blend by adding one sachet or just the amount that the child can consume at a time to one cup of previously boiled water, stirring it well before adding it to the normal diet of the child. This is to ensure that children consume the entire dose of VitaMix and MGM, regardless of whichever group they were assigned to.

Data collection

Household, maternal and child's characteristics at baseline

Quantitative information at baseline was collected using structured questionnaire to assess the general background information on socio-demographic characteristics of the family including household water and sanitation, maternal health practices (during pregnancy and after giving birth), and the child's characteristics. The questionnaire was pretested among similar respondents before use in the study.

Intervention compliance

The mean percentage consumption of MNP and *BigMo* over the expected number of sachets to be consumed was determined among children who completed the study. Mothers or caregivers were instructed to fulfil the compliance form daily by recording the feeding schedule, amount consumed by the child, and perceived side effects of the respective intervention.

Anthropometric measurements

All children were weighed and measured at baseline during recruitment, midline period at 3 months, and after 6 months of receiving intervention. Children were weighed in kilograms (with accuracy of 0.01 kg) using an electronic scale (SECA 874, Hamburg, Germany) without shoes, slippers, or diapers. Recumbent or supine length was measured in centimetres (with an accuracy of 0.1 cm) using a medical plastic infantometer (SECA 417, Hamburg Germany) without shoes or slippers. Two measurements of weight and recumbent length were taken and recorded. The mean of two measurements was used. The same researchers measured weight and length of the children throughout the 6 months following the same procedure.

Blood samples

A non-fasting blood sample (1.5 ml) was collected from each child at baseline and endline to determine haemoglobin (Hb), serum ferritin (SF), retinol (vitamin A), zinc and C-reactive protein (CRP) concentrations. Blood samples were collected in the morning only at the field data collection centre through the finger prick method by three registered medical technologists. Blood samples were collected into three separate trace-element-free microtainer tubes (ferritin, retinol and zinc), which were immediately wrapped in dark cloth and placed in a cool box to allow blood to clot. The whole blood sample was centrifuged at 3,000 rpm for 10 min within 2 h of collection using an electric centrifuge (Beckman Allegra model) at the field data collection centre. Serum was aliquoted into plain microtainer tubes using a disposable pipette. The microtainer tubes were stored in the freezer. At the end of data collection, the aliquoted serum was transported to the FNRI laboratory in frozen gel within 2 h and stored at -20^{0}C in a freezer until analysis.

Determination of Hb, SF, retinol and zinc were undertaken at the FNRI laboratory. The FNRI laboratory personnel who performed blood analysis were blinded to the child's group assignment. The Hb level was measured immediately in the field through cyanmethemoglobin method using a portable spectrophotometer (DR2800, Hach Australia). Serum ferritin was

determined by immune-radiometric assay technique (Riakey Ferritin IRMA Kit, 2007) using wizard 2 gamma counter machine (PerkinElmer, IL USA). Serum retinol was measured based on isocratic elution high performance liquid chromatography (HPLC) method using a Hitachi model (China) automated clinical chemistry analyser. Serum zinc was analysed using flame atomic absorption spectrometer (Agilent 240 FS AA). C-reactive protein analysis was carried out using turbidimetry for specific proteins through a chemistry analyser (Cobas Integra 400 Plus). All the biochemical analyses were checked for accuracy using standard reference materials as control for Hb (Liquicheck Hematology™16), SF (Riakey Ferritin IRMA Kit, 2007), retinol (pooled serum), zinc (Seronorm Trace elements L1 and L2), and CRP (PreciControl ClinChem $Multi_1$ PCC_1).

In order to account for the effect of inflammation or infection in assessing SF and serum retinol, elevated CRP was used as inflammation biomarker in the study with levels greater than 5 mg/L taken as indicating the presence of infection. Among children with CRP>5 mg/L, a correction factor of 0.77 was applied for SF, 1.13 for retinol, and 1.20 for zinc based on Thurnham *et al.* method (2015 and 2010).

Dietary assessment

Dietary data collection was conducted using a 24-h dietary recall method at baseline, every month during the monthly visits and at endline. Calibrated measuring tools (e.g. tablespoon, teaspoon, cups, glass with gradation, matchbox and different sizes of circles) were used to assist the respondents in estimating accurate quantities of foods and beverages consumed by the child. The respondent for the dietary data collection was the person in charge of feeding the child during the previous day. All solid, semi-solid and liquid food consumed by children were assessed including snacks in between, upon waking up in the morning, at lunch, during afternoon snacks, at dinner and during the night.

Data analysis

All analyses of data were performed using SPSS version 10.0 for Windows software packages (Chicago, IL, USA). Descriptive statistics (percentages, means, and standard deviation) were calculated to describe the characteristics of the study children, their households and mothers including compliance to the intervention. A *p*-value of 0.10 was considered significant for all tests performed.

Anthropometric *z*-scores (weight-for-age, length-for-age, and weight-for-length) were computed using the WHO Child Growth Standards software (2006) based on the collected weight and length measurements in order to determine the nutritional status of the children.

Food intakes were converted into weight in grams by trained dietary editors. These food weights were later translated to as purchased values using appropriate conversion factors and were finally transformed into nutrient values through the use of individual dietary evaluation system (IDES) software that includes the updated Philippine food composition table (FNRI-DOST, 2015c). Nutrient-based adequacy was determined by comparing the percentage of consumption to the recommended energy intake (REI) and estimated average requirement (EAR) for specific micronutrients based on the 2015 Philippine Dietary Reference Intakes (FNRI-DOST, 2015d).

The effects of interventions on growth *z*-scores, Hb, SF, retinol and zinc concentrations were investigated. Significant differences in the baseline, midline (third month), and endline (sixth

month) mean values of growth z-scores across groups; baseline and endline mean values of Hb, SF, retinol and zinc across groups were examined using one-way analysis of variance (ANOVA). Blood samples were only collected at baseline and endline. On the other hand, significant differences in the baseline and endline prevalence of anaemia and micronutrient deficiency across groups were examined based on Pearson's x^2 test.

The baseline-endline differences within groups were also calculated. Significant changes in the mean growth z-scores, Hb, SF, retinol and zinc concentrations within groups were reported based on paired t-test, while significant changes in the proportions of these variables were examined based on the McNemar test. If significant difference was detected in the mean estimates across groups at $p<0.10$, pairwise test using Least Square Difference (LSD) was further performed.

Ethical approval

This study was approved by the FNRI Institutional Ethics Review Committee on 16 December 2016. The Informed Consent Form with *Tagalog* translation explained the rationale of the study, the selection process of participants, data collection procedure, the nutrient composition of MNP and *BigMo* blend, the voluntary participation and benefits of participation, possible risks and side effects, right to withdrawal or termination and maintaining subject privacy and confidentiality.

RESULTS

Profile of study participants

A total of 147 children enrolled to take part in this study, but only 126 (85.7%) completed the six-month intervention. Some reasons for not completing were due to dropping out of the intervention (6.8%), moved residence (5.4%), loss to follow-up (0.7%), and absence during endline assessment (1.4%).

The parental characteristics did not differ significantly between groups in terms of age, educational level, and household average monthly income (Table 2). No significant difference was found in the monthly income and number of households living below the food poverty threshold across intervention groups. Most (87.0%) respondents were from non-food poor families.

Children enrolled in the study did not differ significantly in age, sex, feeding practices, important birth and health characteristics (type of gestation, birth weight, received vitamin A capsule status, type of feeding, and morbidity status) including anthropometric status as shown in Table 2.

Compliance to the intervention

Compliance to the respective MNP supplementation was not significantly different between the VitaMix with and without *BigMo* (78.8% and 85.1%, respectively; $p>0.05$) and between the MGM with and without *BigMo* (70.3% and 64.7%, respectively; $p>0.05$). However, children in the VitaMix groups (with and without *BigMo*) had significantly higher percentage consumption relative to the expected amount of intake than those in the MGM groups (with and without). The mean *BigMo* consumption represents 61.3% of the expected intake among those who received the complementary food blend. No significant difference in mean percent consumption of *BigMo* between groups was noted.

Effects of intervention on haemoglobin and micronutrient status

The effects of intervention on changes in the mean concentrations and percentage of deficiency with respect to Hb, SF, retinol, zinc are shown in Table 3. After 6

Table 2. Baseline characteristic of study population by intervention group

Characteristics	*VITAMIX+BIGMO (n=33)*	*VITAMIX (n=33)*	*MGM+BIGMO (n=29)*	*MGM (n=31)*	*p**
	Mean ± SD				
Maternal age (years)	29.3±8.6	26.8±6.7	26.5±6.7	28.4±8.3	0.338
Maternal Education					0.574
No education	-	-	-	-	
Elementary education (%)	2.8	8.0	11.1	10.8	
High School education (%)	75.0	65.8	77.8	73.0	
College and above (%)	22.2	26.3	11.1	16.2	
Mothers working outside (%)	30.6	23.7	11.1	18.9	0.085
Household size	6.6±2.3	5.9±2.9	4.9±1.2	5.8±2.4	0.009
Household monthly income Mean ±SD (Php)	12,517.14±8,442.45	11,297.37±6,103.72	10,138.71±10,896.74	10,776.93±4,003.50	0.258
Household above threshold† (%)	88.9	86.8	75.0	96.8	0.381
Improved source of water (%)	100.0	94.7	100.0	100.0	0.656
Water-sealed toilet facility (%)	77.8	78.9	80.6	78.4	0.808
Age in months	10.0±2.7	9.3±2.9	10.1±3.2	9.9±2.8	
Age group, months					0.626
6-8	30.6	31.6	33.4	35.2	
9-11	33.3	28.9	33.3	32.4	
12-15	36.1	28.9	33.3	32.4	
Male children (%)	44.4	55.3	44.4	64.9	0.235
Full term babies (%)	97.2	100.0	97.2	97.3	0.316
Birth weight (g)	2,839±435.8	2,987±462.3	2,955±387.7	2,996±431.8	0.338
Received Vitamin A capsule (%)	90.1	96.0	91.0	94.0	0.432
With episode of illness in the past week (%)	61.1	65.8	50.0	51.4	0.449
Breastfed infants (%)	52.8	78.9	72.2	67.7	0.101
Bottle fed infants (%)	41.7	26.4	25.0	35.1	
Consuming complementary foods (%)	98.6	97.3	100	97.3	
Weight (kg)	7.87±1.1	8.27±0.9	8.17±1.4	8.04±1.4	0.568
Length (cm)	70.08±3.35	71.23±3.65	69.89±4.47	70.05±4.26	0.519
Weight-for-age (WAZ)	-0.97 ±1.0	-0.60±0.78	-0.70 ±1.1	-0.70 ±0.98	0.497
Weight-for-length (WLZ)	-0.66±1.04	-0.48±0.87	-0.19 ±1.09	-0.46 ±1.06	0.349

*Significant difference at <0.05; based on chi-square test (categorical variables) and student t-test (continuous variables)

†Amount in Philippine Peso (Php); above food threshold or non-food poor families are ≥ 4,869.00; food poor families are < 4,869.00

Table 3. Intervention effects on mean haemoglobin, micronutrient concentrations, prevalence of anaemia and micronutrient deficiencies among children

Primary Outcomes	*VITAMIX +BIGMO (n=33)*	*VITAMIX only (n=33)*	*MGM+BIGMO (n=29)*	*MGM only (n=31)*	*p*
Haemoglobin (Hb, g/dL)†					
Baseline	10.7±0.8	10.6±0.8	10.4±0.8	10.6±1.0	0.572
Endline	11.3±0.8	11.1±0.7	11.3±0.9	11.2±0.9	0.675
Change	0.6±0.6*	0.5±0.9*	0.9±0.7*	0.6±0.9*	0.205
Adjusted change¶	0.6±0.6	0.5±0.7	0.8±0.7	0.6±0.7	0.281
Ferritin (µg/dL)†					
Baseline	26.4±38.9	37.1±65.2	24.6±34.3	33.1±29.1	0.656
Endline	22.5±18.1	25.4±25.0	20.1±24.1	27.1±21.8	0.628
Change	-3.9±28.2	-11.8±67.4	-4.543.2	-6.0±34.6	0.899
Adjusted change¶	-7.3±22.6	-5.6±22.4	-9.7±22.5	-3.3±23.3	0.743
Retinol (µg/dL)†					
Baseline	26.5±3.6	27.4±4.8	29.4±17.5	26.6±4.9	0.400
Endline	28.1±4.8	27.6±6.1	25.8±4.4	27.7±6.7	0.389
Change	1.6±5.2*a	0.2±7.2a	-4.3±7.4b	1.1±6.4a	0.094
Adjusted change¶	0.6±5.6	-0.1±5.6	-2.0±5.6	-0.1±5.8	0.344
Adjusted zinc (µg/dL)†					
Baseline	88.6±19.0a	88.4±20.1 a	82.3±3.9 ab	79.4±13.1b	0.083
Endline	82.2±13.4	83.7±16.9	83.6±17.1	87.1±17.6	0.674
Change	-6.5 ±18.4*a	-4.7±3.9a	1.2±16.1 ab	7.7±22.8*b	0.025
Adjusted change¶	-3.5±2.8	-2.0±2.7	-1.6±2.9	3.9±2.9	0.306
Anaemia (Hb <11g/dL), n(%)‡					
Baseline	17 (51.5)	22 (66.7)	21 (72.4)	18 (58.1)	0.343
Endline	11 (33.3)	16 (48.5)	11 (37.9)	7 (22.6)	0.189
Reduction, %††	18.2*	18.2**	34.5*	35.5*	
Moderate anaemia (Hb<10g/dL), n(%)‡					
Baseline	7 (21.1)	4 (12.1)	8 (27.5)	5 (16.1)	0.453
Endline	1 (3.0)	2 (6.1)	2 (6.9)	3 (9.7)	0.757
Reduction, %††	18.1*	6.1	20.7*	6.4	
Moderate non-IDA (Hb<10 g/dL and Ferritin >12 µg/dL), n(%)‡					
Baseline	3 (9.1)	1 (3.0)	5 (17.2)	2 (6.4)	0.242
Endline	1 (3.0)	0 (0.0)	0 (0.0)	1 (3.2)	0.578
Reduction, %††	6.1	3.0	17.2**	3.2	
Low ferritin (< 12µg/dL), n (%)‡					
Baseline	9 (27.3)	14 (42.4)	13 (44.8)	11 (35.5)	0.469
Endline	8 (24.2)	10 (21.2)	10 (34.5)	7 (22.6)	0.122
Reduction, %††	3.0	12.1	10.3	12.9	
Iron Deficiency Anaemia (Hb <11 g/dL and Ferritin < 12µg/dL), n(%)‡					
Baseline	4 (12.1)	3 (9.1)	3 (10.3)	3 (9.7)	0.980
Endline	0 (0.0)	2 (6.1)	2 (6.9)	2 (6.4)	0.519
Reduction, %††	12.1	3.0	3.4	3.3	

Vitamin A deficiency (< 20µg/dL), n(%)§					
Baseline	0 (0.0)	3 (9.1)	0 (0.0)	1 (3.2)	0.378
Endline	0 (0.0)	3 (9.1)	0 (0.0)	1 (3.2)	0.322
Reduction, %††	-	0.0	-	0.0	
Zinc deficiency (< 65 µg/dL), n(%)§					
Baseline	1 (3.0)	4 (12.1)	4 (13.8)	4 (12.9)	0.455
Endline	4 (12.1)[a]	4 (12.1)[a]	2 (6.9)[b]	1 (3.2)[b]	0.021
Reduction, %††	-9.1	0.0	6.9	9.7	

†Values are means (±SD); with across groups significant difference at $p<0.10$ based on ANOVA. Ferritin, retinol and zinc values adjusted by a correction factor of 0.77, 1.13 and 1.20, respectively to remove the effect of infection (CRP > 5mg/L).

‡Values are proportion of children who had anaemia (< 11 g/dL), moderate anaemia (< 10 g/dL), moderate non-IDA (Hb < 10 g/dL and low ferritin > 12 ug/dL) with across groups significant difference at $p<0.10$ based on Pearson's x^2 test.

§Values are proportion of children who had marginal vitamin A (< 20µg/dL) and had low zinc (< 65µg/dL), with across groups significant difference at $p<0.10$ based on Pearson's x^2 test; within group significant difference at $p<0.10$ based on McNemar's test.

¶Adjusted for age and baseline value.

††Values are change in percentage points (PP); within group significant change from first measure to final value using McNemar's chi-square.

*Significant at $p<0.05$; within treatment change is significant at $p<0.05$ using paired t-test.

**Significant at $p<0.10$; across groups significant difference at $p<0.10$ based on Pearson's x^2 test.

[a,b]Values in the same row with different superscript are significantly different based on Least Square Difference $p<0.10$

months, the mean Hb concentration in all intervention groups significantly increased but did not differ significantly between groups. There was insignificant decrease in the mean SF concentrations for all the groups. Similarly, the change in the mean retinol level within group between baseline and endline periods were not statistically significant in all groups. The baseline mean zinc concentrations were similar among three groups, except for the MGM group without *BigMo*, which had significantly lower mean serum zinc at 79.4ug/L. A significant improvement in the mean zinc level among children in the MGM-only group was observed (+7.7±22.8 µg/dL). However, after adjusting for age and baseline zinc values, the differences in the mean change of zinc level between groups were no longer significant.

The prevalence of anaemia was significantly reduced after 6 months by 18.2%, 18.2%, 34.5% and 35.5% in the Vitamix with *BigMo*, Vitamix without *BigMo*, MGM with *BigMo* and MGM without *BigMo*, respectively. Moderate anaemia (Hb <10 g/dL) was significantly reduced by 18.1% and 20.7% only in the VitaMix with *BigMo* and MGM with *BigMo* groups, respectively. In the Vitamix only and MGM only groups, the reductions between baseline and endline period were not significant. Only the MGM with *BigMo* group demonstrated a significant reduction in the prevalence of moderate non-iron deficiency anaemia (IDA) (Hb <10 µg/dL and ferritin >12 ug/dL) by 17.2% between baseline and endline periods.

The prevalence of low storage iron as measured by ferritin level <12 µg/dL,

corrected by infection based on elevated CRP was not significantly reduced between baseline and endline period in all groups.

Similarly, the prevalence of zinc deficiency did not significantly change between baseline and endline period in all groups. However, MGM with *BigMo* (6.9%) and MGM without *BigMo* (3.2%) demonstrated significantly lower proportions of children with zinc deficiency (<65 µg/dL), compared to the VitaMix with *BigMo* (12.1%) and Vitamix without *BigMo* (12.1%) groups.

Effects of intervention on nutritional status

The mean weight-for-age z-scores (WAZ) significantly decreased between baseline and midline periods in all groups, while a significant improvement by 0.2 was noted in the MGM without *BigMo* group between midline and endline periods (Table 4). Length-for-age z-scores (LAZ) showed no improvement in the VitaMix groups (with and without *BigMo*). Conversely, a significant increment of 0.3 was noted in the MGM with *BigMo* group between baseline and midline,

Table 4. Change in anthropometric z-scores and proportion of children meeting energy and nutrient adequacy during the study period, by intervention groups

Measurements	*VITAMIX+ BIGMO (n=33)*	*VITAMIX ONLY (n=33)*	*MGM+ BIGMO (n=29)*	*MGM only (n=31)*	*p*†
Mean WAZ					
Baseline(Month 0)	-0.97±1.0	-0.72±1.0	-0.71±1.1	-0.60±0.8	0.497
Midline (Month 3)	-1.24±1.0	-0.95±1.1	-0.95±1.1	-0.96±0.8	0.469
Endline (Month 6)	-1.13±1.0	-0.91±1.0	-0.90±1.1	-0.72±0.8	0.394
Change in WAZ					
Midline-Baseline difference	-0.3	-0.2	-0.2	-0.4	0.485
p-value	0.003	0.012	0.014	0.000	
Endline-Midline difference	0.0	-0.0	0.1	0.2	0.054
p-value	0.769	0.749	0.520	0.001	
Endline-Baseline difference	-0.3	-0.2	-0.2	-0.1	0.959
p-value	0.110	0.097	0.143	0.167	
Mean LAZ					
Baseline (Month 0)	-0.9±0.9	-0.7±0.9	-1.0±1.2	-0.4±0.8	0.079
Midline (Month 3)	-0.9±0.9	-0.7±1.0	-0.8±1.1[b]	-0.4±0.9	0.232
Endline (Month 6)	-1.0±1.0[a]	-0.7±1.0[a]	-0.9±0.9[a]	-0.2±1.0[b]	<0.001
Change in LAZ					
Midline-Baseline difference	-0.1	0.0	0.3	0.0	0.135
p-value	0.738	0.998	0.010	0.980	
Endline-Midline difference	-0.0[a]	-0.1[a]	-0.1[a]	0.2[b]	<0.001
p-value	0.838	0.375	0.051	0.020	
Endline-Baseline difference	-0.1[a]	-0.1[a]	0.1[a]	0.2[b]	0.004
p-value	0.663	0.510	0.250	0.020	
Mean WLZ					

Baseline(Month 0)	-0.7±1.0	-0.5±1.1	-0.2±1.1	-0.5±0.9	0.350
Midline (Month 3)	-1.1±1.0	-0.8±1.1	-0.8±1.1	-1.0±0.8	0.457
Endline (Month 6)	-0.9±0.8	-0.8±0.9	-0.6±1.1	-1.0±0.8	0.454
Change in WLZ					
Midline-Baseline difference	-0.4	-0.3	-0.6	-0.5	0.216
p-value	< 0.001	< 0.001	0.000	0.025	
Endline-Midline difference	0.2	0.0	0.2	0.0	0.352
p-value	0.040	0.852	0.163	0.854	
Endline-Baseline difference	-0.3	-0.3	-0.4	-0.5	0.499
p-value	0.039	0.030	0.004	< 0.001	
% Meeting the REI/EAR‡					*P*§
Energy (kcal)					
Baseline (Month 0)	10.5 (0.6-20.5)	10.0 (0.5-19.5)	11.1 (0.6-21.6)	11.6 (8.1-35.2)	0.305
Intervention (Month 1-6)	27.1 (4.4-29.9)	25.4 (3.8-27.0)	24.3 (2.4-26.2)	26.2 (4.1-28.4)	0.990
Difference	16.6*	15.4*	13.2*	14.6*	0.707
p-value	0.041	0.047	0.045	0.055	
Protein (g)					
Baseline (Month 0)	25.3 (39.1-71.4)	27.5 (13.4-41.6)	19.4 (6.2-32.7)	35.1 (19.4-50.7)	0.115
Intervention (Month 1-6)	49.4 (34.5-68.4)	48.7 (32.7-64.7)	48.6 (31.6-65.5)	70.3 (55.2-85.3)	0.182
Difference	24.1*[a]	21.2*[a]	29.1*[b]	35.1*[b]	0.070
p-value	0.050	0.052	0.005	0.002	
Iron (mg)					
Baseline (Month 0)	26.3 (12.0-40.6)	17.5 (5.5-29.5)	16.7 (4.2-29.1)	35.1 (19.4-50.9)	0.143
Intervention (Month 1-6)	65.7 (49.6-81.8)[a]	84.6 (73.1-96.2)[a]	42.9 (26.1-59.6)[b]	62.2 (46.2-78.1)[a]	0.003
Difference	39.4*[a]	67.1*[a]	26.2*[b]	27.0*[b]	0.030
p-value	<0.001	<0.001	0.008	0.020	
Vitamin A (mcg RE)					
Baseline (Month 0)	42.1 (26.1-58.1)	25.0 (11.3-38.7)	25.0 (10.5-39.5)	35.1 (19.4-50.9)	0.116
Intervention (Month 1-6)	97.1 (91.5-103)[a]	100.0[a]	71.4 (56.1-86.7)[b]	91.9 (82.9-100.8)[a]	<0.001
Difference	55.0*	75.0*	46.4*	56.8*	0.239
p-value	<0.001	<0.001	<0.001	<0.001	

†Values are based on ANOVA, with significant difference across groups at *p*-value <0.10.
‡Values are estimated proportion, % (95% CI) of children meeting the energy and nutrient intakes based on age-specific recommended energy intake (REI) and estimated average requirement (EAR), respectively.
§Values are based on Pearson's x^2 test, with significant difference at *p*-value <0.10.
*Change is significant within intervention group at *p*-value <0.10 in independent t-test.
[a,b]Values in the same row with different superscript are significantly different based on Least Square Difference *p*<0.10

as well as in the MGM without *BigMo* (by 0.2) between midline and endline periods. The increase in length-for-age was significantly greater in the MGM-only group than in the remaining three groups. On the other hand, the weight-for-length z-scores (WLZ) significantly decreased between baseline and midline, as well as between baseline and endline periods in all groups, with the decreases similar across groups.

Effects of intervention on children's dietary intake

The proportion of children meeting the recommended energy intake significantly increased from 10.5% to 27.1% in VitaMix with *BigMo*, from 10.0% to 25.4% in VitaMix without *BigMo*, from 11.1% to 24.3% in MGM with *BigMo* and from 11.6% to 26.2% in MGM without *BigMo* over the six-month study period. However, majority of children in VitaMix with *BigMo* (72.9%), VitaMix without *BigMo* (74.6%), MGM with *BigMo* (75.7%) and MGM without *BigMo* (73.8%) had inadequate intake in energy, with no significant differences noted between groups (Table 4).

Significant increase in the proportion of children meeting the protein requirement was also observed over the 6 months intervention in VitaMix with *BigMo* (25.3% to 49.4%), VitaMix without *BigMo* (27.5% to 48.7%), MGM with *BigMo* (19.5% to 48.6%), and MGM without *BigMo* (35.1% to 70.3%). However, a significantly greater number of children met the age-specific protein requirement in the MGM group (with: 29.1% and without *BigMo*: 35.1%) than in the VitaMix group (with: 24.1% and without *BigMo*: 21.1%).

Overall, adding MNP to the complementary food of children 6-23 months of age significantly increased intake of vitamin A and iron during the whole study period in all groups as shown in Table 4. But the increase in the proportion of children meeting iron requirement between baseline and during the intervention period was significantly higher in the Vitamix with (39.4%) and without *BigMo* (67.1%) than in the MGM with (26.2%) and without *BigMo* (27.0%). It was also noted that during intervention period, there was significantly higher proportion of children meeting the Vitamin A requirement in Vitamix with (55.0%) and without *BigMo* (75.0%) than in the MGM with (46.4%) and without *BigMo* (56.8%). However, no significant difference was noted in the increase in proportion of children meeting vitamin A requirement across groups.

DISCUSSION

Analysis on the compliance of the supplementation showed that Vitamix groups (with and without *BigMo*) had significantly greater compliance than MGM groups (with and without *BigMo*). Significantly higher mean consumption of the supplements in the VitaMix groups than in the MGM groups might be attributed to the generally less frequent supplementation in the VitaMix groups at only thrice a week compared to the daily supplementation of MNP in the MGM group (with and without *BigMo*), which explains the lower compliance rate in the daily MNP supplementation. These findings are consistent with the study by Kounnavong *et al.* (2011) in Lao PDR where children under twice-weekly supplementation yielded a higher compliance rate than those in the daily MNP supplementation. Likewise, highest adherence was observed in trials wherein children received MNP on an intermittent basis (Ip *et al.*, 2009), which may be explained by mothers or caregivers perceiving the intermittent supplementation (e.g. 2-3 times weekly) as causing less mental pressure and anxiety among mothers or caregivers.

However, both types of MNP (VitaMix and MGM) showed a similar effect in increasing Hb levels among the study children 6-23 months of age. This finding is consistent with the Cochrane review of trials on home fortification with MNP containing at least iron, zinc, and vitamin A in children under 2 years old (De-Regil *et al.*, 2011; Jack *et al.*, 2012; Suchdev *et al.*, 2012; Kounnavong *et al.*, 2011; Adu-Afarwuah *et al.*, 2008; Mennon *et al.*, 2007). This result can be attributed to the following reasons. First, both formulations contain several nutrients, including B_{12}, folic acid, vitamin A, zinc, and vitamin C, which, together with iron, enhance Hb synthesis, iron transport, or absorption in young children. Our results agree with research conducted in Mexico (Rosado *et al.*, 2010), which concluded that micronutrient supplementation with smaller doses of iron is as effective as larger doses to increase Hb level and to reduce anaemia among children 6-42 months old. Second, giving VitaMix intermittently (thrice a week) and daily supplementation with MGM but with lower dose of iron per sachet resulted in similar increases in Hb concentrations, consistent with the findings in the cluster randomised trial conducted by Ip *et al.* (2009) among children aged 6-24 months. Third, all intervention groups received infant and young child feeding education and promotion activities through home visits and mothers' classes to encourage MNP compliance and adoption of appropriate feeding practices. However, only VitaMix and MGM along with *BigMo* blend were effective in reducing moderate anaemia, compared to VitaMix and MGM use without complementary food blend, indicating that MNP usage was insufficient unless supported by adequate food intake.

With regards to SF levels, both MNP formulations, with or without *BigMo* failed to significantly improve ferritin concentration among young children in the study area within 6 months of supplementation. The high proportion of anaemia incidence (baseline: 62.0%, endline: 37.8%) among the study children could not be attributed to iron deficiency anaemia based on low Hb and low SF levels (baseline: 10.3%, endline: 4.0%), suggesting that other nutritional and non-nutritional factors were the underlying causes of anaemia, such as infection, presence of genetic Hb disorders, or other micronutrient deficiencies such as vitamin B_{12} and folate. The result of this study is consistent with the results of the meta-analysis study of Salam *et al.* (2013) on the effectiveness of MNP among children in reducing anaemia and improving Hb level but not on SF deficiency.

The retinol level of vitamin A deficiency prevalence was the same over the intervention period of 6 months, indicating that MNP supplementation likely contributed to the maintenance of normal levels of stored retinol. The very low prevalence of vitamin A deficiency among the study children at baseline and at endline periods may be attributed to the bi-annual, high-dose vitamin A supplementation (VAS) targeting children 9 months old and above employed by the national *Garantisadong Pambata* programme of the DOH Philippines, coupled with regular MNP supplementation. A small and insignificant effect of MNP on serum zinc and reduction of zinc deficiency were noted in the MGM group (with and without *BigMo*), while no effect was observed in the VitaMix group (with and without *BigMo*). This finding was more likely attributable to the finding of a significantly greater increase in the percentage of children meeting the recommended protein intake were recorded in the MGM group (with and without *BigMo*) than in the VitaMix group (with and without *BigMo*), highlighting the importance of protein-rich foods

not only in improving dietary diversity but also in improving micronutrient status such as zinc. The enhancing effect of animal protein on dietary zinc absorption has been reported for beef, pork, chicken, and fish (Tontisirin, Nantel & Bhattacharjee, 2002).

The study showed that daily supplementation of low iron-MGM with and without *BigMo* blend was more effective in improving linear growth than VitaMix with 100% RNI for most nutrients. The significant improvement in the Z- scores for length-for-age may be related to the greater increase in the percentage of children meeting the protein recommendation in the MGM group and meeting the minimum acceptable diet, including the small but insignificant improvement in zinc intake, in the MGM with *BigMo* group relative to the VitaMix group. This underscores that increased intakes of food from animal sources containing high-quality protein and zinc are beneficial to child growth.

Furthermore, the result of this study showed that when MNP supplements were added to complementary food significantly improved the children's intake of micronutrients, thereby, contributing to children meeting their age-specific requirements for micronutrients. Nonetheless, the majority of children's intake remained inadequate in energy and protein, which resembles the national estimates in the Philippines. Unfortunately, adequate micronutrient intake alone, without sufficient macronutrient intake, cannot optimize children's health and growth.

While combining MNP with *BigMo* blend was hypothesised to be effective in improving the dietary adequacy of children, our results did not show that children who received *BigMo* had higher energy intake than children who did not receive, in both the VitaMix and MGM groups. The lack of significant difference in energy intake could be mainly due to the low mean consumption (61.3%) of *BigMo* by the children. Only 37.0% of children who received *BigMo* showed 80.0% compliance, indicating that the product was not well accepted. Similar findings were reported by three efficacy studies (Owino *et al.*, 2007; Hossain, Wahed & Ahmed, 2005; Mamiro *et al.*, 2004), whereby complementary food supplementation (fortified or unfortified) aimed at increasing energy intake showed no significant increase in all intervention groups.

CONCLUSION AND RECOMMENDATIONS

After evaluating the effects of MNP containing 15 versus 9 nutrients, with or without complementary food blend on the nutritional status of rural young children for 6 months, it can be concluded from this study that daily supplementation of MNP containing less than 100% of the RNI combined with complementary food blend and education may have a greater potential effect in addressing anaemia, zinc deficiency, and improving linear growth of young children than MNP containing 100% of the RNI. Aside from supplementation, appropriate education strategies such as home visits or mothers' classes within the behavioural change perspective should be put in place. This is to ensure that requirements for additional energy and micronutrient intakes from complementary foods, specifically the consumption of protein rich foods such as meat, fish, poultry, and eggs as sources of high-quality protein and micronutrients, alongside breastfeeding promotion be delivered among mothers and caregivers of young children.

Acknowledgement

The authors wish to thank all the mothers, children and community health workers for the patience they showed in responding to all questionnaires and for giving consent to participate in this study.

The study was funded by various organizations, namely: Neys van Hoogstraten Foundation (NHF) of The Hague, The Netherlands, the Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA), and the Department of Science and Technology through the Human Resource Development Program (HRDP) and the Science and Technology Human Resource Development Program (ASTHRDP).

Authors' contributions

GEA, carried out the field work, conceived the manuscript, drafted and revised the manuscript; BCVC, conceived the manuscript; TMTM, drafted and revised the manuscript; PMM, conceived the manuscript; RAC, conceived the manuscript; TNA, analysed and interpreted the data; all authors read and approved the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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Mal J Nutr 24(4): 493-506, 2018

Development and validation of a dietary iron score for screening populations at risk for inadequate iron intake

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ABSTRACT

Introduction: Inadequate iron intake is a determinant of iron deficiency. A simplified tool for dietary assessment is needed. This study aimed to develop a dietary iron scoring system (DISS) and to determine the validity of the dietary iron score (DIS) for screening populations at risk for inadequate iron intake. **Methods:** A three-step process was undertaken to develop the DISS, namely (1) iron score (IS) for each food was constructed based on its iron content per 100 g, adjusted for heme content equivalence; (2) the predicted modifying effect (PME) was formulated based on either enhancing or inhibiting effects of dietary constituents; (3) the DIS of a meal was obtained by multiplying the total IS and the PME of that meal. The validity of the DIS for screening populations at risk for inadequate iron intake was determined against absorbable iron calculated by the Hallberg & Hulthen algorithm. A probability of adequacy of absorbable iron intake of 0.75 was used as a cutoff in defining the population at risk. **Results:** There was a significant correlation between the absorbable iron and DIS (r=0.34, p<0.001). Using the Receiver Operating Characteristic (ROC) curve , three cutoffs of DIS, namely 5, 6 and 7, had comparable results. However, sensitivity (82.9%) and specificity (50.0%) was the best for DIS cutoff of 7. **Conclusion:** The proposed DISS is potentially a field-friendly tool for screening populations at risk for inadequate iron intake. Further verifications are needed, using more complete dietary data.

Keywords: Iron, dietary iron score, screening tool

INTRODUCTION

Iron deficiency has been recognised as one of the most significant public health problems in the world. The World Health Organization (WHO) estimated that 42%, 49% and 50% of children under 5 years, non-pregnant and pregnant women, respectively were anaemic (WHO, 2015). About half of the anaemic cases in developing countries were associated with iron deficiency (Erick *et al.*, 2009). Poor iron status is associated with reduced work capacity, lowered immunity, and reduced cognition (WHO, 2001). Much effort has been made to prevent and control this nutritional problem throughout the world.

A key element for programmes to alleviate nutrient deficiency is to have an appropriate assessment tool, which is simple, practical and low cost. Determination of iron intake is useful

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for assessing the adequacy of dietary iron to meet iron requirements. The critical issue concerning diet and iron status is not the total amount of iron ingested but rather, the amount of iron available for absorption. Importantly, absorption of iron is highly variable, depending not only on the iron status of the individual, but also on other factors present in the diet that enhance or inhibit its absorption. Iron absorption can vary more than ten-folds at fixed iron content (Hallberg & Hulthen, 2000). Thus, the amount of bioavailable iron is most relevant for determining whether or not iron requirements are met in a population.

Twenty-four hours recall or record of food intake for one or more days, or a food frequency questionnaire is commonly used in investigating the risk or etiology of micronutrient deficiencies. Even though these methods provide detailed data, they are time consuming and require skilled workers. In addition, data processing to estimate the amounts of nutrient intake is a complicated and tedious procedure. Consequently, the dietary diversity score (DDS) was introduced as a simplified dietary assessment tool for non-nutritionists and other lay users. It involves a summation of the number of food items or food groups consumed over a specified period of time (1-7 days or up to 15 days) (Ruel, 2003). It was found that it was not complicated to train field staffs to obtain information on dietary diversity; it was not invasive and burdensome for the respondents, and not time consuming (FANTA, 2002). Several studies consistently showed positive correlations between DDS and micronutrient adequacy (Foote *et al.*, 2004; Kennedy *et al.*, 2007; Moursi *et al.*, 2008; Savy *et al.*, 2008; Steyn *et al.*, 2006). Therefore, the DDS concept is an appealing approach for assessing micronutrient adequacy. However, the correlation coefficient between DDS and micronutrient adequacy has been found quite low (r=0.30-0.40), and presently there is no consensus on the measurement components of DDS, such as classification of food groups, scoring system, minimum portion size of specific foods for inclusion, and DDS cutoffs (Ruel, 2003). Moreover, DDS does not take into account the bioavailability of micronutrients in aggregating food groups and performing the scoring system. This may lead to uncertainty in assessing adequacy of micronutrients, especially iron, zinc and vitamin A. Therefore, development of a dietary iron scoring system which considers iron bioavailability would provide a more reliable dietary assessment tool to identify populations at risk for iron deficiency.

This study was designed to develop and validate a dietary iron scoring system, which is an intentionally simplified dietary assessment method for screening adequacy of iron intake. The proposed tool also takes into account enhancers and inhibitors of iron absorption.

MATERIALS AND METHODS

Development of dietary iron scoring system

The development of a dietary score for iron was based on the principle that bioavailability of iron from foods depends on the form of iron (heme or non-heme) and other constituents present in foods. While heme iron is readily absorbable, non-heme iron absorption is determined by the presence of other food constituents. Dietary constituents which modify the absorption of iron fall into two main groups – iron absorption enhancers and inhibitors. The development of the proposed dietary iron scoring system (DISS) consisted of three main parts, namely (1) adjusting the scores for iron contents for heme/

non-heme iron contents (iron score; IS), (2) deriving scores for iron absorption modifying effects (modifying score; MS) and (3) calculating scores for availability of dietary iron in a meal (dietary iron score; DIS).

Iron score (IS)

Heme iron is present in animal food sources, including red meat, organ meat, poultry and fish in varying proportions. The higher the heme iron content, the higher the iron bioavailability. The rest of the iron in these foods is in the non-heme form. Although classified as animal food sources, iron in eggs and milk are non-heme iron and less bioavailable than the iron in meat sources (Callender, Marney & Warner, 1970). In plant sources, iron is in various chemical forms and collectively referred to as non-heme iron. Therefore, iron score in this study is based on the total iron content adjusted for its availability based on the proportion of heme in the food.

Scores for iron content

Eight hundred and nine food items listed in INMUCAL-Nutrient 4.1 database (Institute of Nutrition, 2007) were classified into eight groups, namely meat, milk, egg, cereals, tubers, legumes & nuts, vegetables and fruits. Foods having similar iron contents were then aggregated into 56 subgroups. The median value of iron content of each group/subgroup was computed. The minimum score of 1 was set for iron content below the tenth percentile (corresponds to an iron content of 0.44 mg/100 g, rounding to 0.5 mg). Since the average Thai recommended dietary intake (MOPH Thailand, 2003) of iron from the age of 6 years upwards is on average of 10 mg, and the bioavailability of habitual Thai diets is 10% of total iron (Hallberg *et al.*, 1974), thus, a score of 1 was given for each increment of 1 mg iron (Table 1).

Table 1. Iron scores (IS) according to amount of iron in foods

Iron score (IS)	*Iron content in foods, mg/100 g*
1	< 0.50
2	0.51-1.50
3	1.51-2.50
4	2.51-3.50
5	3.51-4.50
6	4.51-5.50
7	5.51-6.50
8	6.51-7.50
9	7.51-8.50
10	8.51-9.50
11	9.51-10.50
12	10.51-11.50
13	11.51-12.50
14	12.51-13.50
15	13.51-14.50
16	14.51-15.50
17	15.51-16.50
18	16.51-17.50
19	17.51-18.50
20	18.51-19.50
21	19.51-20.50

Adjusting for heme content in foods by weighting scores

Absorption of both heme and non-heme iron depends on the iron status of individuals. Based on the regression equations for heme and non-heme iron absorption related to iron status (Hallberg, Hulthen & Gramatkovski, 1997), the ratio of iron absorption of heme iron to non-heme iron at an average iron store of 500 mg (the average of estimated iron store based on serum ferritin level in Thai school children) was set at 3.5:1. This ratio was used as basis for deriving a weighting score for conversion of heme iron to non-heme iron. Then, the weighting score for adjusted heme content in foods was calculated by the derived equation of [(2.5 × % heme) + 100]/100. Due to its wide content range, heme iron contents in animal food sources were sub-grouped according to the percentage of heme iron based on literature values (Napatthalung, 2000). In this way, a calculated weighting was determined for each subgroup. Table 2 presents the calculated weightings and accordingly, the assigned weighting

score after adjusting for heme and non-heme iron contents. These weighting scores were used to multiply the iron contents in foods to derive the final iron score for various foods.

Modifying score (MS) and predicted modifying effect (PME)

The total absorbable iron in a meal is the net result of interaction between the form of iron and iron absorption modifiers in a meal. It is not known whether these effects are additive or multiplicative. Therefore, the following steps were taken to obtain the MS for each food item. The net modifying effects of a meal were derived based on a linear regression of MS on calculated modifying effects of the meal, that were derived by using the Hallberg & Hulthen algorithm of all possible combinations of foods (Hallberg & Hulthen, 2000). This prediction equation, called predicted modifying effect (PME) was then used to derive the dietary iron scores of meals. Details of these steps are as follow:

I. Compilation of contents of vitamin C, phytate, calcium, and tannin

Since the extent of modifying effect of inhibitors/enhancers is associated with the amount ingested, and as consumption size varies according to age, it is more accurate to derive the MS according to the target age group. In this study, school age children were chosen. Lists of foods commonly consumed by children aged 6-12 years were identified from the Thai Food Consumption Survey 2003-2004 (National Bureau of Agricultural Commodity and Food Standards, 2007), followed by reduction to only food items for which the percentage of consumption was more than 50.0%. Contents of vitamin C, phytate, calcium and tannin were obtained from INMUCAL-Nutrient 4.1 and other sources (Chansuwan, 2005; Charoensiri & Kongkachuichai, 2008; Harland & Harland, 1980; Ma *et al.*, 2005; Ravindran, Ravindran & Sivalogan, 1994; Reddy, 2002; Somsub *et al.*, 2008; Suttikomin, 2002). When data on these contents were not available, the values were estimated from other foods that have similar characteristics.

II. Calculation of the inhibiting or enhancing effect of dietary modifiers by food item and portion size

Since the absorption of non-heme iron depends on the co-presence of factors that enhance or inhibit iron absorption, the score for the net modifying effect associated with each food item was required. Hallberg and Hulthen (2000) provided algorithms for calculating absorption ratio (AR) for various food constituents (factors). The AR value was derived directly from the measured absorption value when the factor is present, otherwise, the absorption was estimated as follows:

Table 2. Weighting scores for adjusting heme iron content in foods to non-heme iron

Foods by percentage of heme iron	*Calculated weighting*	*Weighting score*
Plant, egg and milk	0.00	1.0
Animal sources:		
<10% heme	< 1.25	1.0
10-30%	1.25-1.75	1.5
31-50%	1.75-2.25	2.0
51-70%	2.25-2.75	2.5
71-90%	2.75-3.25	3.0

Note: Calculated weighting was derived by using the formula; [(2.5 × % heme) + 100]/100

Median weighting score is derived from calculating the weighting score for each range of heme percentage

Vitamin C-factor
$= 1 + (0.01 \times \text{Vit C}) + \log(\text{phytate-P} + 1) \times 0.01 \times 10^{0.8875 \times \log(\text{Vit C}+1)}$

Meat, fish poultry (MFP)-factor
$= (1 + 0.01\text{MFP}) \times 10^{0.4515-[0.715-0.1825 \times \log(1+\text{Vit C})] \times \log(1+\text{Tannin})}$

Phytate-factor
$= 10^{(-0.30 \times \log(1+\text{mg phytate-P}))}$

Calcium-factor
$= 0.4081 + (0.5919/1 + 10^{-[2.022-\log(\text{Calcium}+1)] \times 2.919})$; where calcium is ≤ 50 mg, calcium-factor is assumed to be 1.

Tannin-factor
$= (1 + 0.01\text{MFP}) \times 10^{0.4515-[0.715-0.1825 \times \log(1+\text{Vit C})] \times \log(1+\text{Tannin})}$; the factor should be ≤ 1, corrected to 1 if it is not.

Since the inhibiting or enhancing effect is dependent on the amount of the specific food eaten, the portion size of each food item consumed was required. The median values for portion size were obtained from the Thai Food Consumption Survey 2003-2004 (National Bureau of Agricultural Commodity and Food Standards, 2007), based on dietary intake data of children aged 6-12 years. The portion size used for estimating the modifying effect of dietary factors were: small = median/2; medium size = median; and large size = median × 2.

When more than one enhancing or inhibiting factor is present in a food item, the AR for that food is the product of AR for each of the factors estimated separately for enhancing and inhibiting factors. AR for enhancers (AR-Enhancer) included vitamin C-factor and MFP-factor, while AR for inhibitors (AR-Inhibitor) included phytate-factor, calcium-factor and tannin-factor. Finally, the MS for each food item by portion size was calculated as follows:

$$\text{MS} = [(\text{AR-Enhancer} - 1) + (\text{AR-Inhibitor} - 1)] \times 10^{*}$$

*This is an arbitary number to enable easy counting of the score

III. Determination of the PME of dietary factors in a meal pattern

Although it is known that when dietary factors in individual foods are combined in a meal leading to changes in iron bioavailability, there is no generally accepted model for estimating the results from such interactions. Therefore, summing up the modifying scores of enhancers and inhibitors is practical, but will not be meaningful. This study used a regression approach to obtain the predicted modifying effects of iron modifiers in a meal by adding the sum modifying scores of a meal.

In order to determine the PME, hypothetical meals based on all known dietary patterns were formulated. These meals represent various combinations of the quantity and extent of modifying effects of food items present in a meal. The foods selected in designing the hypothetical meals were grouped according to habitual dietary patterns of Thai diets, as follows:

1) Rice: Rice represents the staple food of the dietary pattern in Thailand.
2) Iron source foods: Six food characteristics representing levels of iron content, percent heme and concurrent modifiers were chosen (e.g. extremely high iron & high heme content, high iron & moderate heme content, moderate iron & high heme content, low iron & moderate heme content, non-heme iron with inhibitor, non-heme iron without inhibitor).
3) Vegetables: There are three types of vegetables defined according to the AR-Enhancer and AR-Inhibitor estimations covering the range of vegetables commonly consumed in Thai diets (e.g. low/no enhancer & high inhibitor, low/no enhancer & moderate inhibitor, moderate enhancer & low inhibitor).
4) Fruits: Similarly, four characteristics of fruits were chosen according to the AR-Enhancer and AR-Inhibitor values in fruits commonly consumed

Table 3. Comparison of correlation coefficients for linear relationships derived from four meal patterns

Meal pattern	*n*	*Constant*	*Slope*	R^2
Rice-based meal with iron source	204	0.703	0.035	0.71
Meals without rice	198	0.831	0.057	0.75
Rice-based meal without iron source	204	0.611	0.030	0.79
All meals pooled	600	0.764	0.042	0.72

(e.g. high enhancer & moderate inhibitor, moderate/low inhibitor & moderate inhibitor, moderate enhancer & less/no inhibitor, low enhancer & low inhibitor).

5) Milk: Due to the calcium content in milk and the fact that milk is now commonly consumed, especially among school-aged children, hypothetical meals were designed to include those that are taken with or without milk.

Meal combinations were then formulated to represent all known combinations of the food groups mentioned above. Nine groups of combinations were designed as following:

a. Rice + one iron source + one vegetable + one fruit (72 meal combinations)
b. Rice + one iron source + one vegetable + one fruit + milk (72 meal combinations)
c. Rice + one iron source (6 meal combinations)
d. Rice + one iron source + one vegetable (24 meal combinations)
e. Rice + one iron source + one fruit (18 meal combinations)
f. Rice + one iron source + milk (6 meal combinations)
g. One iron source + milk (6 meal combinations)
h. All meal in group a-f excluded rice (198 meal combinations)
i. All meal in group a-g excluded iron source (204 meal combinations)

Finally, 600 hypothetical meals were designed to derive the prediction equation for iron modifying effects in meals.

IV. Deriving the prediction equations of modifying effects for different types of meals

According the 600 hypothetical meals, four different sets of meal patterns were examined, namely, (1) rice-based meals with iron source, (2) meals without rice, (3) rice-based meal without iron source, and (4) all meals. The AR of each meal was calculated by using the Hallberg & Hulthen algorithm (Hallberg & Hulthen, 2000) and the modifying score of each food item in a meal as given in step II above. The comparison of the various regression parameters using different meal compositions as described above are shown in Table 3. Since the slope and correlation coefficient of the four regression lines were similar, it was decided that the equation for the pooling of all meals could be used for any meal patterns. Thus, the equation for the PME is:

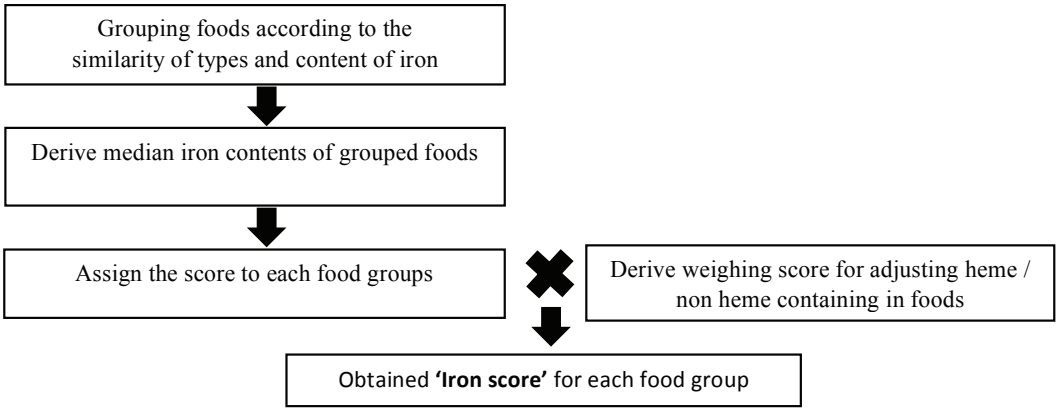
$$PME = 0.764 + (0.042 \times \text{sum MS})$$

Deriving total DIS for a meal and per day

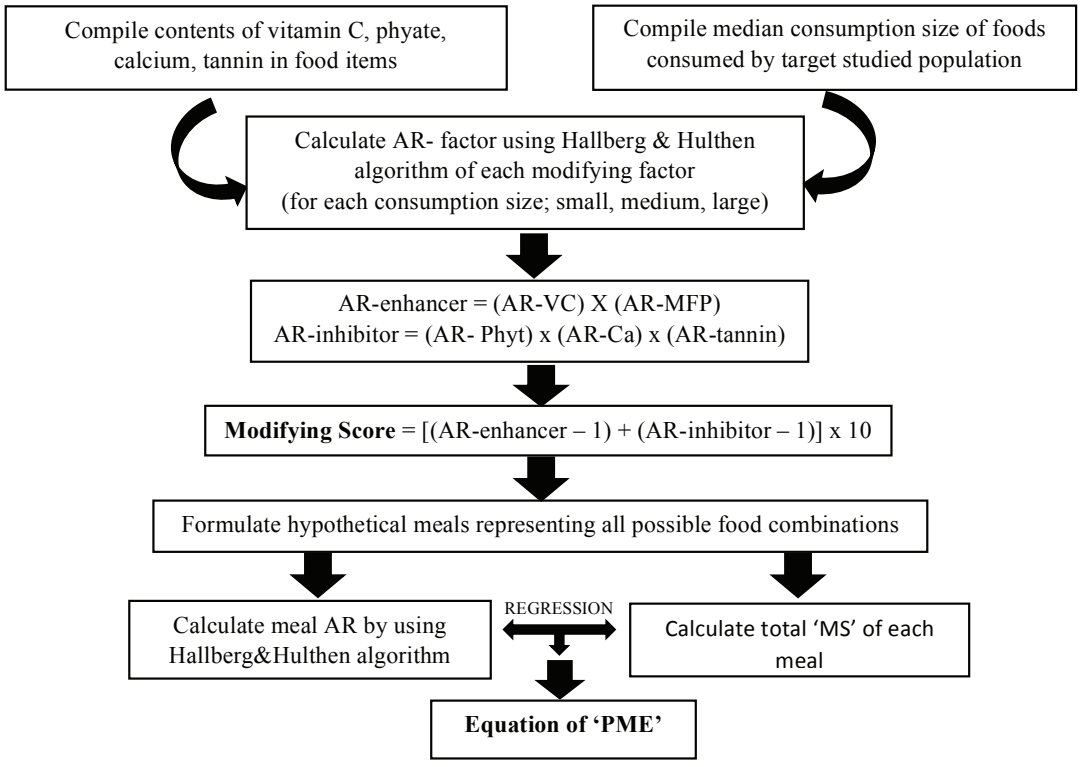
The final DIS for each meal was derived by multiplying the IS and PME. For comparison of DIS of individuals, DIS of all meals consumed in a day was summed to obtain the dietary score per day.

A summary of the steps involved in developing the DISS is shown in the schematic below.

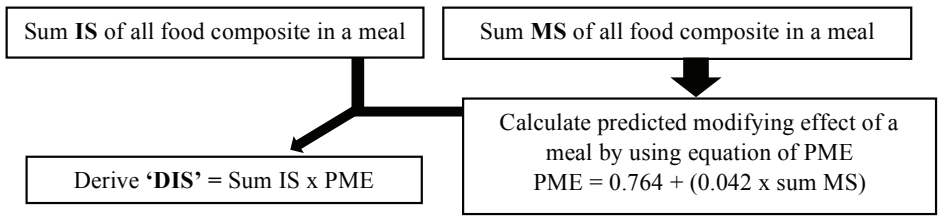
Step 1: Derived 'Iron score' of individual food items



Step 2: Derived 'Modifying score' of each food item for calculating 'Predicted modifying effect' of a meal



Step 3: Finally calculating 'Dietary iron score' for a meal



Validation of the developed DIS with Hallberg & Hulthen algorithm and its use in assessing the risk of inadequate iron intake

Dietary data used in the present study were from the Project entitled 'Efficacy of multiple micronutrients fortified soup-based instant noodles in school children, Northeast Thailand', conducted between 2003-2004 (Winichagoon *et al.*, 2006). Briefly, this is a randomised placebo-controlled trial comparing biochemical parameters, growth, morbidity and cognition of children receiving micronutrient-fortified (vitamin A, iron, iodine and zinc) vs non-fortified lunch in school for 32 weeks. Dietary intakes of stunted and non-stunted (ratio 1:4) children aged 6-12 years (n=230) were assessed using one 24-hour recall by trained interviewers. Attempt was made to include both week-day and week-ends in the dietary assessment.

Determination of probability of adequacy of iron intake

Probability of adequacy (PA) is the probability that an individual's usual intake is greater than the nutrient requirement. This is determined by dividing the difference between the estimated nutrient intake and estimated average requirement (EAR) with the standard deviation of the reference requirements. Since the frequency distribution of iron requirement is not a normal pattern, the concept of PA could not be applied directly (Institute of Medicine, 2000). The Institute of Medicine (IOM) suggested using the table of probability of inadequacy (PI). A matrix for probability of inadequate (PI) iron intake for children aged 4-8 years and 9-13 years was then constructed. The recommended iron intakes in these tables were based on iron absorption of 18.0%. Therefore, the intake values were converted to absorbed iron by multiplying the recommended intakes by 0.18. Finally, the PI was transformed to PA by subtracting the values from one.

Absorbable iron from the dietary intake data of school children was calculated using the Hallberg & Hulthen algorithm (2000) and transformed to PA according to this guideline. For validation of the DIS, this PA was used for comparison of its performance in identifying populations at risk for iron inadequacy.

Validation of the 'DIS' against the 'absorbable iron' algorithm (Hallberg & Hulthen, 2000) as a reference method

The agreement of the two methods in classifying a population by the adequacy of iron intake was performed using Kappa statistics. The Receiver Operating Curve (ROC) was used to derive the cutoff for the DIS for defining risk of inadequacy of iron intake. Sensitivity and specificity were calculated for different cutoffs of dietary iron score to identify populations at risk for iron inadequacy using PA cutoffs at 0.75.

RESULTS

Assessing risk of inadequacy of iron intake using DIS versus absorbable iron derived by the Hallberg & Hulthen algorithm

Absorbable iron derived using the Hallberg & Hulthen algorithm was used as a reference method for validation of the DIS. First, the risk of inadequacy of iron intake was determined by the PA approach. A PA of '0' indicates 100% risk of inadequacy of iron intake, while PA of '1' indicates zero risk of inadequate iron intake. In other words, the higher the PA, the lower is the risk of inadequacy. Applying this approach using the data of Winichagoon *et al.* (2006), it was found that about 63.0% of the school children were likely to have inadequate iron intake, i.e., PA = 0 (expressed as absorbable iron). Cumulatively, only about eight percent of them had PA of iron intake above 0.55.

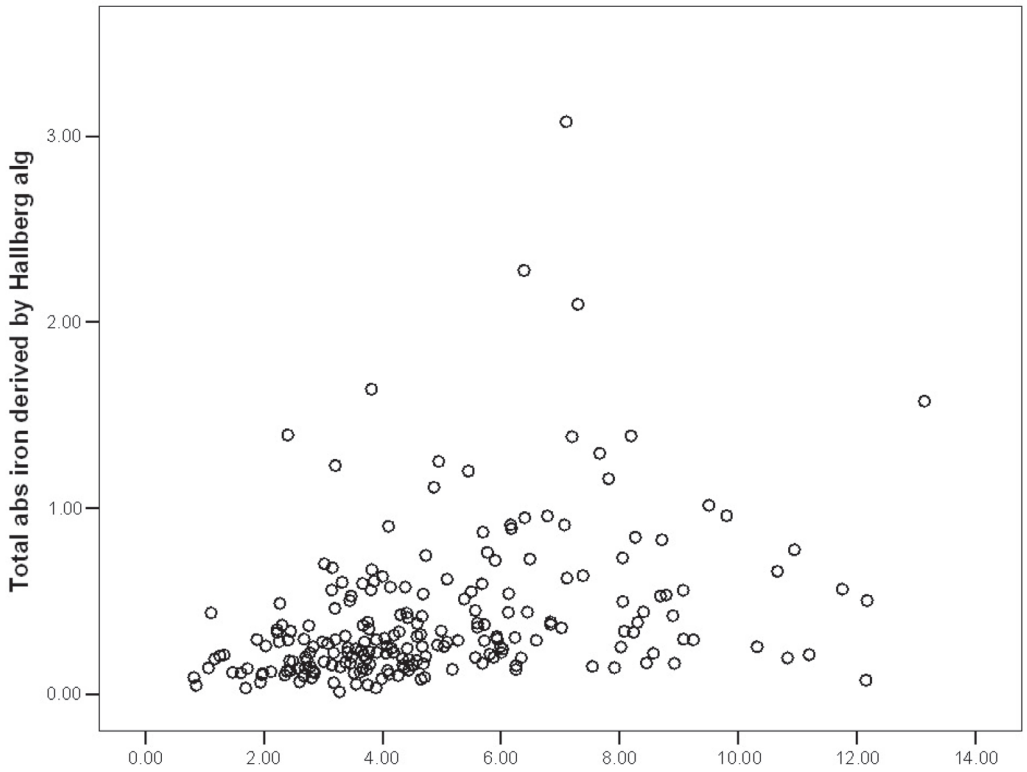


Figure 1. Scatter plot of absorbable iron calculated by the Hallberg & Hulthen algorithm and dietary iron score (r=0.34, p<0.001)

Correlation between dietary iron score and absorbable iron

Dietary iron score and absorbable iron estimated by the Hallberg & Hulthen algorithm were determined. Figure 1 shows the scatter plot between DIS and absorbable iron calculated from the Hallberg & Hulthen algorithm. The correlation between absorbable iron and DIS was found to be significant (r= 0.34, p<0.001).

Determination of the cutoffs for DIS to identify populations at risk of inadequate iron intakes

The cutoff of PA of 0.50, was recommended for use to define individuals at risk of nutrient inadequacy (Institute of Medicine, 2000) while PA 0.75 had been used as an alternative (Kennedy *et al.*, 2007). Theoretically, a risk population identified by using PA cutoff of 0.50 is more likely to have a higher risk of iron deficiency than those identified by using PA cutoff of 0.75. Different cutoffs of dietary iron score ranging from 1-12 were applied and compared to the PA derived from absorbable iron intake to identify populations at risk of iron inadequacy (PA<0.75). The ROCs of 12 DIS cutoffs, including ranges of values (1-12), were examined. The three DIS cutoffs of 5, 6, and 7 were considerably higher than the rest of the 12 cutoffs (Figure 2). Based on the area under the curve (AUC), this result indicates that the cutoff of 5 was good while the other two, namely 6 and 7 were fair (Tape, 2010). While these three cutoffs were considered reasonable cutoffs, DIS cutoff at 7 provides the optimal performance based on sensitivity 82.9% and specificity 50.0% (Table 4).

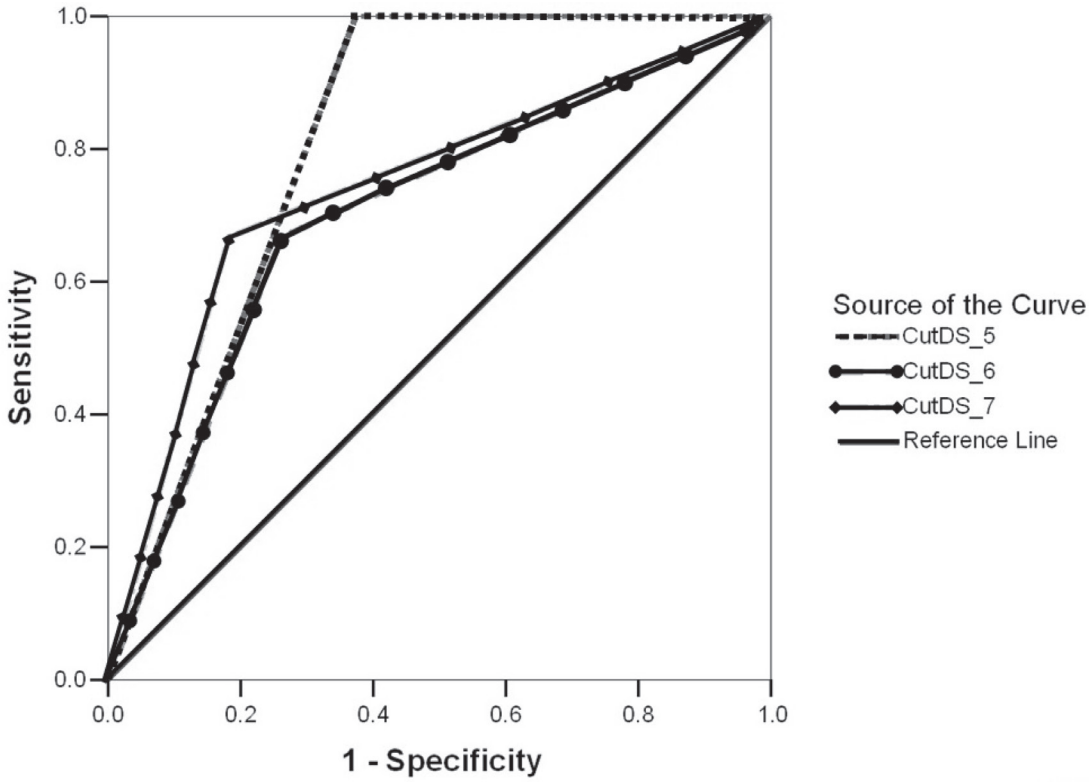


Figure 2. ROC of different cutoffs, i.e. 5, 6, 7 of dietary iron score against the probability of adequacy (PA) cutoff of 0.75; AUCs were 0.81, 0.70, 0.74, respectively

Table 4. Comparison of sensitivity and specificity of the three selected DIS cutoffs

DIS cutoff	*Sensitivity (%)*	*Specificity (%)*
5	62.9	41.7
6	75.1	41.7
7	82.9	50.0

DISCUSSION

Development of DISS

In developing a DIS, availability of quality data and accuracy of databases are critical components. The INMUCAL–Nutrient 4.1, was reviewed and iron content in foods on a dry basis were compared, e.g. cooked and raw forms of the same food item. Where there was discrepancy or unusual values, it was re-checked with another database, such as the USDA database. The scoring system developed here was based on the nutrient contents of foods which are ready to eat, not in the raw state. Therefore, for ranking the iron scores, iron content of food in the state that it is consumed (raw or cooked) was used. If the iron content of the cooked state is not available in the database, adjustment for percent loss was performed using available published information.

According to the current database (INMUCAL-Nutrient 4.1) for calculating nutrients from Thai diets (Institute of Nutrition Mahidol University, unpublished data on phytate and tannin

contents were limited. Borrowing data from other sources was inevitable, and was done adjusting for moisture contents of the foods. It is also recognised that maturity of plants, length and manner of storage, and different milling fractions result in wide variations of the phytate content in foods or food products formulated from phytate-containing raw materials (Gibson & Ferguson, 1999). These factors should be considered in adjusting the final phytate values. However, only moisture content was used in adjusting for borrowed data because of limited data on other factors. Another crucial problem encountered was the use of different analytical methods to quantify phytate contents. The high-performance liquid chromatography (HPLC) technique provides more specific, sensitive and accurate phytate contents. It is the inositol phosphate, not the total phosphate, which exerts inhibitory effect on iron absorption. Unfortunately, it is not always available in food composition databases and literature. Thus, the total phytate-P value was used for this study. In this way, the modifying effect of a meal which include food items with phytate might be underestimated.

In the development of the DISS, it was first thought that summation of inhibiting and enhancing scores derived from the absorption ratios of various individual foods would be valid. However, when the MS of foods were summed for all food items contained in a meal, it was found to be impractical, since the net modifying effects in a meal is the final result of interactions between food components, which is not quantifiable from the calculation of absorption ratios of individual foods, as was obtained in estimating MS. Comparatively, the absorbable iron derived using the Hallberg & Hulthen algorithm (Hallberg & Hulthen, 2000) seems advantageous since it includes various possible key enhancers and inhibitors of iron absorption known to date. Hypothetical meals which provided the absorbable values for all possible combinations of foods in the habitual diets were formulated and the net modifying effects of all dietary modifiers contained in meals were calculated as AR. Among the hypothetical meals, the range (minimum to maximum) of vitamin C, MFP, phytate, calcium, tannin, and egg were 0-212 mg, 0-39 g, 0-114 mg, 3-289 mg, 0-158 mg, and 0-1 egg, respectively**.** These maximum amounts of inhibitors (i.e. phytate, calcium, and tannin) corresponded to almost the maximum threshold of inhibiting effect (Hallberg & Hulthen, 2000). The maximum threshold effect of vitamin C was difficult to establish since it varied according to the molar ratio of iron and concurrent phytate and tannin contents (Teucher, Olivares & Cori, 2004). However, up to 500 mg vitamin C added to the meal showed significantly increased enhancing effect (Siegenberg *et al.*, 1991). In terms of portion size, in order to make sure that the PME equation is applicable for the population, it has to be verified that the increase in size of foods composed in a meal may change the modifying effects. Thus, that the same relationship (slope and validity) holds when higher vitamin C (>212 mg) and MFP (>39 g) were consumed, e.g., in a larger consumption size of adults.

The MS was constructed based on the amounts of dietary modifiers corresponding to the consumption size. In the present study, portion size of school children was used. Hence, this modifying score may not be appropriate if it is applied to an adult's dietary intake. Verification may be needed to examine whether the final modifying scores, when they are constructed based on the adult's dietary patterns and consumption sizes, will concur with the results obtained from intakes of school children in this study.

Before further application of DISS, it is advisable that the following conditions are verified:

1) Dietary patterns of the target population: Since this DISS was derived based on specific sets of meal combinations common in Thai diets, testing DISS with other habitual dietary patterns which are both more and less monotonous should be performed.
2) Since the summed MS is dependent on portion size consumed, further investigations whether there is any threshold level when consumption size of a particular food items is larger than that used in this study should also be performed.

Validation of DISS against absorbable iron derived by the Hallberg & Hulthen algorithm

The performance of the DISS in screening populations at risk for iron inadequacy was tested with the secondary dietary data. The correlation between DIS and absorbable iron calculated by the Hallberg & Hulthen algorithm was significant (r=0.34, p<0.001) (Figure 1). It was stronger than the correlation between DDS (counting numbers of food groups consumed) and estimated absorbable iron (r=0.11) reported by Kennedy *et al.* (2007). This suggested that including the influences of dietary iron modifiers in formulating dietary score for iron may result in an improved predictive ability of simplified dietary scoring.

In establishing the cutoff for DIS to classify people at risk of inadequate iron intake, the three cutoffs were found to be reasonable according to the high AUC of ROCs. Among the cutoffs tested, 5, 6 and 7, the best sensitivity (Se) and specificity (Sp) was for cutoff 7 (Se=82.9%, Sp=50.0%) (Figure 2). The Kappa statistic, however, was rather low for all cutoffs used (Kappa = 0.06, 0.12 and 0.15 for the dietary iron cutoff of 5, 6 and 7, respectively).

Limitations of study

The present study has several limitations that must be considered in validating or improving this scoring system, specifically including several days of dietary intake data, consumption data of other age/population groups to account for wider range of iron intakes and portion size. These will need to be verified before the developed DISS can be recommended for use. In addition for testing intake adequacy using DISS, the relationship between DISS and biochemical parameters such as ferritin will be useful to strengthen the validity of this newly developed scoring system.

Interpretation of the findings include the point that the data from one day intake of most of the children (92.0%) in this study showed rather low iron intakes, and hence, high probability to have inadequacy. In fact, efforts were made in some steps, e.g. iron score, to include foods which might not be consumed by children in this data set, but which are known to be common in the habitual diets, however, there seems to be other issues that need empirical testing. Validation may be repeated with a population's consumption when the intake distribution covers a wider range of iron intake. In addition, the absorbable iron calculation was based on nutrient contents of raw foods that may result in overestimating the amount of vitamin C. Hence, the enhancing effect of vitamin C leads to overestimated absorbable iron.

CONCLUSION

To the best of our knowledge, this is the first study that takes into consideration the interactions among dietary factors affecting bioavailability of dietary iron to develop a simplified DIS. It was intended to be used as a field-friendly tool for screening populations at risk of iron inadequacy. Although more verification and validation study may still be needed, the DIS is potentially useful for monitoring iron adequacy

in a programmatic context. Further simplification may still be needed to reduce the burden of calculation.

List of Abbreviations

AR: Absorption ratio; AR-Enhancer: Absorption ratio for enhancers; AR-Ca: Absorption ratio of calcium; AR-Inhibitor: Absorption ratio for inhibitors; AR-MFP: Absorption ratio for meat-fish-poultry; AR-Phyt: Absorption ratio for phytate; AR-tannin: Absorption ratio for tannin; AR-VC: Absorption ratio for vitamin C; AUC: Area under the curve; DDS: Dietary diversity score; DIS: Dietary iron score; DISS: Dietary iron scoring system; EAR: Estimated average requirement; IS: Iron score; MFP-factor: Meat fish poultry factor; MS: Modifying score; PA: Probability of adequacy; PI: Probability of inadequacy; PME: Predicted modifying effect; ROC: Receiver operating curve; Se: Sensitivity; Sp: Specificity; VitC: Vitamin C

Acknowledgement

Mrs. Orapin Banjong and Mrs. Chayanit Wanichagoon are gratefully acknowledged for their technical assistance in editing the food composition database used in this study.

Authors' contribution

LC, PW, NP and EW planned the study; LC wrote the manuscript with advice and editing by PW and inputs from the other authors.

Conflict of interest

The authors declare having no conflicts of interest. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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Mal J Nutr 24(4): 507-513, 2018

Association between serum B_{12} and folate levels and manifestations of oral lesions in HIV adult patients

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ABSTRACT

Introduction: Micronutrient deficiencies are common in Human Immunodeficiency Virus (HIV) infection. The long-term side effects of antiretroviral therapy (ART), specifically Azidothymine (AZT), include low serum levels of vitamin B_{12} and folate, which in turn, lead to megaloblastic anaemia and oral lesions. **Methods:** A cross-sectional study was conducted to determine the associations between manifestation of oral lesions and serum vitamin B_{12} and folate levels in HIV-adult patients with or without receiving ART therapy. Oral lesions were determined based on the EC-Clearinghouse diagnostic criteria. Serum vitamin B_{12} and folate were assessed by electrochemiluminescence immunoassay (ECLIA). Sixty participants (48 males and 12 females) aged 20 to 51 years were recruited from a private hospital in Bandung, Indonesia. **Results:** Subnormal levels of serum vitamin B_{12} and folate were found in 16.6% and 6.7% HIV patients, respectively. Significantly lower serum levels of vitamin B_{12} and folate were shown in HIV patients receiving ART than those without ART. Oral lesions were found in all the participants with subnormal levels of serum vitamin B_{12} and folate. Presence of oral lesions was significantly associated with low levels of serum vitamin B_{12} in HIV patients with ART, but not with low folate levels. **Conclusion:** Low levels of serum vitamin B_{12} and folate were shown in HIV patients, indicating the need for early nutritional intervention to ensure optimal nutritional status and prevention of oral lesions in HIV patients.

Keywords: Serum folate and B_{12}, HIV patients, oral lesions

INTRODUCTION

Micronutrient deficiencies are common in Human Immunodeficiency Virus (HIV) infection and occur at all stages of immunodeficiency, including asymptomatic infection. Reduced serum levels of micronutrients are associated with higher transmission of opportunistic infections, immunodeficiency, rapid disease progression and mortality (Carter *et al.*, 2015). HIV-associated anaemia was reported common (49.6%) among HIV-infected patients in Indonesia (Wisaksana *et al.*, 2011) at higher levels (18-37%) than found in other countries (Hoffmann *et al.*, 2008; Mata-Marín *et al.*, 2010; Takuva *et al.*, 2013). Several observational studies have reported low levels of serum vitamin B_{12} and folate in HIV patients (Remacha *et al.*, 2003; Semeere *et al.*, 2012). A deficiency of either vitamin can occur due to HIV infection itself or as a side effect of antiretroviral therapy (ART), specifically zidovudine (AZT), which clinically manifest as megaloblastic anaemia or

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neutropenia (Volberding *et al.*, 2004). Haematinic deficiency (vitamin B_{12}, folate, and iron), either as predisposing or etiological factor, can produce oral mucosal diseases including glossitis, recurrent aphthous stomatitis (RAS), angular cheilitis, or oral candidiasis (OC) (Adeyemo *et al.*, 2011).

Oral manifestations of HIV infection vary depending on the populations studied, geographical locations, clinical diversity, as well as the impact of ART (Leão *et al.*, 2009; Sharma *et al.*, 2015). Oral candidiasis, oral hairy leukoplakia (OHL), and oral hypermelanosis were predominantly found in oral lesions of HIV/Acquired Immune Deficiency Syndrome (AIDS) in Asia (Sharma *et al.*, 2015). Oral lesions play an important role as an early clinical indicator and progression predictor of HIV infection. Their occurrences, mainly OC and OHL, are strongly associated with a low cluster of differentiation 4 (CD4) count and a higher plasma viral load (Shiboski *et al.*, 2015). There have been conflicting results regarding reduction in prevalence of specific oral lesions even though expanded access to ART has significantly contributed to reducing new HIV cases, mortality, and morbidity of HIV-positive patients (Leão *et al.*, 2009; Sharma *et al.*, 2015; Shiboski *et al.*, 2015).

Long-term side effects of ART, specifically AZT, include low levels of serum vitamin B_{12} and folate, which lead to megaloblastic anaemia and oral manifestations. Several observational studies in Indonesia have focused on the occurrence of OC and its variants (Sumintarti & Rasdiana, 2014), or OHL associated with a low CD4 count (Parmadiati *et al.*, 2017). Researchers have addressed the issue of oral manifestations and HIV-associated anaemia without receiving ART in adults (Hidayat *et al.*, 2017), and children (Lugito *et al.*, 2016). Little is known on the relationship between onset of oral lesions, vitamin B_{12} and folate deficiency, and HIV-patients with or without ART.

The aim of our research was to determine the correlations between serum levels of vitamin B_{12} and folate among HIV-adult patients with or without receiving either ART or AZT. This study also aimed to determine the association between the manifestation of oral lesions in HIV-adult patients with or without receiving ART. To the best of our knowledge, this observational study is the first of its type to be conducted in Indonesia.

MATERIALS AND METHODS

Study design and population

In preparing this report, we adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement guidelines for reporting observational studies. This cross-sectional study was conducted from November 2016 to January 2017 at Teratai outpatient clinic of Dr. Hasan Sadikin Hospital, Bandung, Indonesia.

This study was conducted in accordance with the Declaration of Helsinki and independently reviewed and approved by the Human Research Ethics Committee of the Faculty of Medicine Universitas Padjadjaran, Dr. Hasan Sadikin Hospital, number LB.02.01/C02/L4702/Z/2016.

The sample size of the participants was calculated based on the formula for comparing two independent groups. Assuming that 5% of Indonesian adults are vitamin B_{12} and folate deficient, a sample size of 30.2 per group was calculated based on a confidence level of 80% with the confidence limits as 5%. Accounting for dropouts, an additional 30 per group was computed, giving a required total of 60 patients for the trial.

The participants were recruited based on the consecutive sampling method in which every patient who meets the inclusion criteria is selected. The inclusion criteria, include the patient's agreement to sign the informed consent,

aged ≥ 19 years, CD4 count ≤ 200 cells/mm^3, and receiving ART duration for ≥ 6 months. We excluded HIV-positive patients who did not adhere to ART therapy. The patients were divided into two groups, namely patients receiving ART, and those not on ART. Informed consent was taken from all patients. Intraoral examinations were performed to collect the data on oral lesions based on the EC-Clearinghouse diagnostic criteria.

Biochemistry analysis

Five ml of participant's blood samples were collected from the median cubital vein with aseptic precautions at the Clinical Pathology Department. Serum levels of vitamin B_{12} and folate were measured using electrochemiluminescence immunoassay (ECLIA). The normal range for serum B_{12} is 100-400 pg/ml and levels <100pg/ml were considered as subnormal for this study. As for serum folate, its normal range is 3-15 ng/ml and subnormal level was taken as <3 ng/ml.

Statistical analyses

Descriptive statistics were used to summarise the categorical data of demographic characteristics. Shapiro-wilk test was used to analyse numeric data which were not normally distributed and presented as median. Bivariate analysis was used to test the hypothesis, including Mann-Whitney test, for analysing differences in serum levels of vitamin B_{12} and folate in HIV-patients with or without ART, and in HIV-patients with or without AZT. Fisher's exact test was used to analyse the correlation between oral lesions and serum levels of vitamin B_{12} and folate. *P*-value of ≤0.05 was used to determine the statistical significance in the bivariate analysis.

RESULTS

The study included 60 HIV-adult patients with an equal number with or without

Table 1. Characteristics of HIV-adult patients with or without receiving ART therapy

Characteristics	*ART*		*Non-ART*		*Total*	
	n	*%*	*n*	*%*	*n*	*%*
Gender						
Male	25	41.7	23	38.3	48	80.0
Female	5	8.3	7	11.7	12	20.0
Age (years)						
20-29	17	28.3	15	25.0	32	53.3
30-39	12	20.0	7	11.7	19	31.7
40-49	3	5.0	4	6.6	7	11.6
≥ 50	1	1.7	1	1.7	2	3.4
CD4 count (cells/mm^3)						
≤50	5	8.3	19	31.7	24	40.0
51-100	7	11.7	3	5.0	10	16.7
101-200	18	30.0	8	13.3	26	43.3
Serum vitamin B-12 level (pg/ml)†						
Subnormal	8	13.3	2	3.3	10	16.6
Normal	22	36.7	28	46.7	50	83.4
Serum Folate (ng/ml)‡						
Subnormal	4	6.7	0	0.0	4	6.7
Normal	26	43.3	30	50.0	56	93.3

†Normal range serum vitamin B_{12} is 100-400 pg/ml and subnormal <100 pg/ml
‡Normal range serum folate is 3-15 ng/ml and subnormal <3 ng/ml

Table 2. Serum levels of vitamin B_{12} and folate in HIV-adult patients with or without receiving ART and AZT therapy

Serum level	*ART (n=30) Median (Range)*	*Non-ART (n=30) Median (Range)*	*p-value*
Vitamin B_{12} (pg/ml)	176.5 (30.0 – 844.2)	296.5 (77.3 – 1211.0)	0.006
Folate (ng/ml)	6.39 (2.22 – 11.92)	9.22 (3.80 – 20.00)	0.006
	AZT (n=17) Median (Range)	*Non-AZT (n=13) Median (Range)*	
Vitamin B_{12} (pg/ml)	147.6 (30.0 - 407.7)	258.5(114.6 - 844.2)	0.035
Folate (ng/ml)	6.07 (2.22 - 10.95)	6.50 (5.27 - 11.92)	0.174

receiving ART. There were 48 (80.0%) male and 12 (20.0%) female patients. The distribution of characteristics, such as age and sex, were largely similar for both ART and non-ART categories. In terms of CD4 count, the proportion of patients was highest among those in the 101-200 cells/mm^3 group (30.0%) receiving ART, while the lowest proportion was among those in the ≤50 cells/mm^3 group (31.7%) without receiving ART. None of the HIV-adult patients without ART had subnormal serum folate levels (Table 1).

Statistically significant differences were observed between serum levels of vitamin B_{12} and folate in HIV-adult patients with or without receiving ART (p=0.006) (Table 2). As for HIV-adult patients with or without receiving AZT, only serum B_{12}, and not serum folate, showed a significant difference between these two groups (p=0.035).

A statistically significant association was only found between serum levels of vitamin B_{12} and manifestation of oral lesions in HIV-adult patients receiving ART therapy (p=0.014) (Table 3).

In terms of oral lesions related to either low serum levels of vitamin B_{12}, the most prevalent oral lesion was exfoliative cheilitis (38.3%) and the least prevalent was angular cheilitis (5.0%) (Table 4).

Table 3. Correlations between serum levels of vitamin B_{12} and folic acid and oral lesions in HIV-adult patients with or without receiving ART therapy

HIV patients	*Serum level*	*With oral lesions*		*Without oral lesions*		*p-value*
		n	*%*	*n*	*%*	
Receiving ART	Vitamin B_{12}†					
	Subnormal	8	100.0	0	0.0	0.014
	Normal	11	50.0	11	50.0	
	Folate‡					
	Subnormal	4	100.0	0	0.0	0.268
	Normal	15	57.7	11	42.3	
Non-ART	*Vitamin B_{12}†*					
	Subnormal	2	100.0	0	0.0	1.000
	Normal	20	71.4	8	28.6	
	Folate‡					
	Subnormal	-	-	-	-	
	Normal	22	73.3	8	26.7	

†Normal range serum vitamin B_{12} is 100-400 pg/ml and subnormal <100 pg/ml
‡Normal range serum folate is 3-15 ng/ml and subnormal <3 ng/ml

Table 4. Oral lesions related to low serum levels of either vitamin B_{12} and folate

Oral lesions	*n*	*%*
Exfoliative cheilitis	23	38.3
Oral candidiasis	15	25.0
Recurrent aphthous stomatitis	12	20.0
Glossitis	4	6.7
Median rhomboid glossitis	3	5.0
Angular cheilitis	3	5.0

DISCUSSION

A higher prevalence of male (80.0%) than females, and also a higher percentage of those aged 20-29 years were observed in this study. The 2016 data from Ministry of Health of the Republic of Indonesia for the cumulative cases of all reported HIV/AIDS in Indonesia showed that the 20-29 years age group was the most highly affected. It was also reported the cases included 55% of male, 31% of female, and 14% of those who did not report their sex. HIV primarily affects those in their most productive years; about a third of new infections are among young people (ages 15-24). Globally, an estimated 35.3 million people were living with HIV at the end of 2012; of these, 2.1 million were adolescents aged 10-19 years, of which the majority was girls (56%) (Idele *et al.*, 2014).

The degree of immunosuppression by the CD4 cell count testing in this study was found somewhat higher among patients in the 101-200 cells/mm^3 group (43.3%) compared to those in the ≤50 cells/mm^3 group (40.0%), irrespective of receiving ART or not. Low CD4 cell count (<200 cells/mL) is an independent risk factor for megaloblastic anaemia due to acquired deficiency in vitamin B_{12} and folate, as it appears to be the most common haematological complication in HIV-infected adults. Although there were differences in the cut-off points for defining anaemia, its prevalence was greater in HIV patients and those with lower CD4 cell counts. The pathogenesis of anaemia in HIV-infected adults, although multifactorial, relates primarily to a reduced production of erythrocytes. This reduction is influenced by several aetiological factors, including infection and neoplasms, use of drugs such as AZT, a direct effect of HIV on erythropoiesis, and a blunted response to erythropoietin and nutritional deficiencies (Calis *et al.*, 2008). Micronutrient deficiencies that have been associated with HIV infection leading to anaemia are iron, folate, vitamin B_{12}, vitamin A, and zinc. Folate and vitamin B_{12} are essential for cellular proliferation and erythropoiesis, and their deficiency can depress cell-mediated immunity (Volberding *et al*, 2004). Hidayat *et al.* (2017) in a study of 40 HIV-adult patients without receiving ART in Teratai outpatient clinic RSHS Bandung reported a correlation between oral manifestations of anaemia and CD4 count ≤200 cells/mm^3. A cohort study in Uganda by Semere *et al.* (2012) reported that CD4 count ≤350 cells/mm^3 was found in low serum levels of vitamin B_{12}, which was suspected to be due to the increase in HIV replication.

The study of Remacha *et al.* (2003) reported reduced serum levels of vitamin B_{12} (10.3%) and folate (11.1%) in patients after receiving ART. This reduction was attributed to HIV infection resulting in changes in plasma protein binding capability with lymphocytes and neutrophils (Semeere *et al.*, 2012; Tang *et al.*, 1997).

Kaiser *et al.* (2006) showed that micronutrient supplementation, which included vitamin B_{12} and folic acid twice daily for 12 weeks, significantly increased absolute CD4 cell count and the mean change in CD4 cell count from baseline in HIV-infected patients on ART therapy. Vitamin B_{12} supplementation in HIV-infected patients on ART might be of clinical importance, since vitamin B_{12} can inhibit nitric oxide synthase and modulate cellular immunity, particularly T lymphocytes (Kaiser *et al.*, 2006; Tang *et al.*, 1997). Nevertheless, larger trials

are needed to conclude this clinically important effect (Visser *et al.*, 2017).

In this study, serum vitamin B_{12} and folate levels in HIV-AIDS patients with ART were found lower than those without ART. The distribution of ART, specifically AZT, can lead to mitochondria deoxyribonucleic acid (DNA) synthesis interference, which leads to haematology interference in the form of reduction of blood vitamin B_{12} and folate levels (Shiboski *et al.*, 2015).

This study shows that various types of oral lesions were associated with vitamin B_{12} deficiency and folic acid in HIV-AIDS patients. Manifestation of exfoliative cheilitis was highest (38.3%), followed by oral candidiasis (25.0%), recurrent aphtous stomatitis (RAS) (20.0%), glossitis (6.7%), median rhomboid glossitis and angular cheilitis (5.0%). Deficiencies of vitamin B_{12} and folate are known to lead to the oral manifestations of glossitis, angular cheilitis, recurrent oral ulcer, oral candidiasis, and diffuse erythematous mucositis (Reynolds, 2006; Pontes *et al.*, 2009).

Reichart *et al.* (1997) showed that exfoliative cheilitis occurred in 28.5% of their study HIV-AIDS patients. Hidayat *et al.* (2017) reported at the Teratai outpatient clinic that there were 94.8% HIV-AIDS patients with anaemia had oral candidiasis with CD4 ≤200 cells/mm^3 . HIV-AIDS patients with CD4 ≤200 cells/mm^3 are known to be susceptible to opportunistic infections, such as candida, thus the anaemia condition will worsen the oral candidiasis state.

While the deficiency mechanisms of both vitamin B_{12} and folate leading to oral lesions have not yet been established, both vitamins serve as coenzymes in hematopoiesis and erythropoiesis (Kozlak *et al.*, 2010). Macrocytosis, as a result of vitamin B_{12} and folate deficiency, is a direct effect of ineffective erythropoiesis. This process leads to changes of erythrocytes into erythroblasts with poikilocytosis, which is characteristic of megaloblastic anaemia with such clinical manifestations oral lesions (stomatitis, cheilitis, and glossitis), malabsorption and gastrointestinal lesions, as well as diarrhoea.

CONCLUSION

The current study shows significantly lower levels of serum vitamin B_{12} and folate in HIV-adult patients with ART therapy, compared to those without ART. Oral lesions were found significantly associated with low levels of vitamin B_{12}, but not with folate in HIV-adult patients with ART. Further large-scale studies are needed to confirm these findings and to establish whether low level of serum vitamin B_{12} and folate in HIV patients are long-term side effects of ART therapy, before recommending routine supplementation of vitamin B_{12} and folate among HIV patients under antiretroviral therapy.

Acknowledgement

We would like to thank the staff members of Teratai Clinic and the Clinical Pathology Laboratory of Dr. Hasan Sadikin Hospital Bandung Indonesia for their technical assistance. This project was funded by the Internal Research Grant of Universitas Padjadjaran.

Authors' contributions:

IS, principal investigor, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; DP, conducted the study, data analysis and interpretation, prepared the draft of the manuscript; DMDH, data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript.

Conflict of interest

The authors declared no conflict of interest.

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Mal J Nutr 24(4): 515-528, 2018

Construct validity of an adapted Radimer/Cornell measure of food insecurity in the Philippines

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ABSTRACT

Introduction: Measuring hunger and food insecurity has always been a challenge given the various tools available to provide estimates both at the macro (sufficiency in staple stock) and micro (household food security) levels. In the Philippines, estimates of food insecurity have been provided by the Food and Nutrition Research Institute (FNRI) starting 2001 using an adaptation of the Radimer/Cornell (1992) measures of hunger and food insecurity. The tool has been found to be reliable using the 2003 data extracted from the sixth National Nutrition Survey (NNS), Food Security module but was recommended for further exploratory factor analysis to test for efficiency of items. **Methods:** This study assessed the construct validity of the adapted Radimer/Cornell instrument for measuring household food insecurity using principal component analysis with varimax rotation based on the 2003 NNS data. **Results:** The results revealed the prevalence of food insecurity was higher at the mother's level (33.7%) compared to the child (21.0%), indicative of "managed process" or coping with food insecurity at the households. "Altered eating" emerged (factor 1) at the individual level of food insecurity, while "anxiety over quantity and quality of food" was (factor 2) at the household level, that explained 44.0% and 23.2% of the total variance, respectively. Thus, a high cumulative variance (67.2%) was generated for these two factors, implying sufficient variance was obtained to justify the derivation of these two factors from the dataset. **Conclusion:** The food security items in the adapted Radimer/Cornell instrument contained valid indicators for assessing food insecurity in Filipino households.

Keywords: Radimer/Cornell, food insecurity, Philippines, Filipino households

INTRODUCTION

Global hunger and food insecurity remain high. In 2017, chronic food deprivation or undernourishment was estimated to affect 821 million people comprising 10.9% of the world population of which 770 million experienced this at higher severity of food insecurity (FAO, IFAD, UNICEF, WFP & WHO, 2018).

The Food and Agriculture Organization (FAO) measures hunger as undernourishment referred to as the "proportion of the population whose dietary energy consumption is less than a pre-determined caloric threshold, the minimum that most people require to live a healthy and productive life" (FAO, 2008). The mean per capita calorie intake of 69% of Filipino households were below 100% of its dietary energy requirements in 2015 (FNRI-DOST, 2016).

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Studies on food security and related issues in the Philippines are limited. Some studies examined households' response to economic and social shocks, as these affect daily food access and consumption at home and the changes in household food and non-food expenditure and consumption pattern (Castañeda *et al.,* 2006; Villavieja *et al.*, 1985; Valdecañas *et al.*, 1984). In 2001, a survey on food security was undertaken by the Department of Science and Technology's Food and Nutrition Research Institute (DOST-FNRI) to determine food security status of Filipino households (Molano *et al.,* 2003) using an adaptation of the Radimer/Cornell measures of hunger and food insecurity (Radimer *et al.*, 1992).

Radimer/Cornell measures of hunger and food insecurity

The origins and use of the Radimer/Cornell measures of hunger and food insecurity stemmed from the need to evaluate the impact of the United States' targeted food programmes amidst arguments that "hunger is a construct that is hard to measure." The tool was developed in two phases: Phase 1 that extracted perceptions of hunger as these are experienced among 32 rural women using grounded theory and Phase 2 that focused on the development of hunger items and subjecting these to face and construct validation.

According to Radimer *et al.* (1992), hunger is manifested in eight concepts that were experienced at the individual and household levels. The concepts at the individual level are: (a) insufficient intake, (b) nutritional inadequacy, (c) lack of choice and feeling of deprivation and (d) disrupted eating pattern. At the household level, these are: (e) food depletion, (f) unsuitable food, (g) food anxiety and (h) food acquisition in socially acceptable way. These concepts are further described as quantitative (a and e), qualitative (b and f), psychological (c and g) and social (d and h).

Five out of these eight concepts were validated as Phase 2 by Radimer *et al.* (1992) among a convenience sample of 189 women. These concepts were: "insufficient intake", "nutritional adequacy" and "disrupted eating pattern" for the individual level assessment; and "food depletion" and "food anxiety" for household level assessment. Further construct validation of the tool using principal component factor analysis yielded 12 hunger measures (out of the 30 items extracted) that were included in the final Radimer/Cornell instrument. Detailed descriptions of the procedures in developing these items and scale measurements as well as the list of the 30 items are described in by Radimer *et al.* (1992).

Hunger was defined by Radimer *et al.* (1992) as "the inability to acquire or consume an adequate quality or sufficient quantity of food in socially acceptable ways, or the uncertainty that one will be able to do so". This concept was reported to be a valid measure of hunger and food insecurity among homogenous (Radimer *et al.*, 1992) and diverse populations (Kendall, Olson & Frongillo, 1995) within rural (Leyna *et al.*, 2007; Frongillo *et al.*, 1997) and urban settings (Shoae *et al.*, 2007; Zalilah & Ang, 2001) in developed and developing countries, and even in situations described to be suffering from the impact of the global economic crisis such as in Java, Indonesia (Studdert, Frongillo & Valois, 2001). The results of our survey of countries that have measured hunger and food insecurity by adapting the Radimer/Cornell tool is shown in Table 1.

The Radimer/Cornell measure of food insecurity adapted for use by the DOST-FNRI as a component of the national and regional surveys of the Institute was found to be reliable (Cronbach's α=0.81 to 0.89) and valid using criterion-related validity (Molano, Gulles & Tarrayo, 2007). However, there are arguments to the use of criterion-related validation. Criterion-

Table 1. Summary of studies that assessed the reliability and validity of the Radimer/Cornell instrument

Country	*Author*	*Items/Subjects*	*Statistical analysis*	*Findings*
Tanzania	Leyna *et al.* (2007)	9-items Radimer/ Cornell questionnaire excluding 1 item	Construct validity using factor analysis	Results revealed two factors: 1) altered eating pattern at household level with loadings ranging from 0.67-0.86; and 2) altered eating pattern at child level with loadings 0.74-0.89.
		530 women aged 15-44 years with children under 5 years old	Internal consistency using Cronbach's α	Cronbach's α = 0.85 (household subscale) and 0.78 (child subscale)
			Criterion-related validity using χ^2 *test*	Food insecurity was significantly associated with age and marital status, while, food insecurity worsened among women without formal education and who are involved in farming.
Tehran, Iran	Shoae *et al.* (2007)	12-item Radimer/ Cornell questionnaire was modified and 6 questions were added	Construct validity using factor analysis	Analysis resulted in the extraction of the following factors: 1) contained items on food anxiety and food depletion; 2) contained items about food intake inadequacy of adults and children and food intake insufficiency of adults; and 3) composed of items about food intake insufficiency of children.
		250 poor urban households with at least one child aged 1-18 years old and non-pregnant and non-lactating woman of childbearing age	Internal consistency using Cronbach's α	Cronbach's α = 0.89, 0.82 and 0.80 for household security, individual insecure and child hunger scales, respectively.
			Criterion-related validity using χ^2 *test*	Adult food insecurity and child hunger were inversely associated with income, parent's education and father's occupation, but directly associated with household size
				Household insecurity inversely associated with household size but directly associated with parent's education, father's occupation and income
Philippines	Molano *et al.* (2007)	Radimer/Cornell hunger and food inseurity items adapted into 10	Internal consistency using Cronbach's α	Cronbach's α = 0.81 (mother); 0.84 (child); 0.89 (household)
			Criterion-related validity	Food secure households, mother and children have higher mean energy and nutrient adequacy level than food insecure households.
		3,568 households with 0-10 years old children		Prevalence of undernutrition is lower for the food secure group compared to the food insecure group

Malaysia	Zalilah & Ang (2001)	Low income, urban poor households *n*=137 preschool children, 4-6 years old	Descriptive statistics	34.3% food insecure; 65.7% with some degree of food insecurity, where 27.7% as household food insecurity, 10.9% as individual food insecurity and 27.0% with child hunger
			Odds ratio	Significant risk factors to food insecurity: larger household size (*OR*=1.418); lower education of fathers (*OR*=0.802); lower education of mothers (*OR*=0.749)
			One-way ANOVA	
			Multinomial logistic regression	No significant differences in nutritional status across categories of food insecurity
Indonesia	Studdert *et al.* (2001)	1,423 mothers with children < 5 years old	Correlation of tool with the Radimer/Cornell and Hamelin measure	Categories created: food security; uncertainty about food; insecurity for family; insecurity for adults; severe insecurity for children or adults
			Graphical representation of consistency of affirmative responses	Concept of household food insecurity: decreased food intake; compromised diets; and changes in food stores
			Criterion validity	High correlation of percent of affirmative responses: food insecure households had both lower food and total expenditures; households with more severe food insecurity were more likely to have had a decline in income, food stores and rice stores
Russia	Welch & Mock (1998)	12 Radimer/ Cornell hunger items to measure hunger of households, women and children	Criterion-related validity	Household socio-economic and demographic characteristics were highly related to hunger where children were least likely classified as hungry, while, mothers and their households were very likely to be considered hungry.
United States of America	Kendall *et al.* (1995)	10-items Radimer/ Cornell question	Construct validity using factor analysis with varimax and oblique rotation	Progression in the severity of hunger and food insecurity, from household level food insecurity to adult level food insecurity to child hunger.
		Random sample survey of 193 households with women and children using 1993 survey data	Internal consistency using Cronbach's α	Cronbach's α = 0.84 (household insecure measure), 0.86 (individual insecure measure); 0.85 (child hunger measure)
			Criterion-related validity by comparing demographic and dietary characteristics	

related validation studies assume the availability of a criterion measure or a close approximation or "gold standard" of the construct of interest (here, food security). However, the criterion measures often used in studies of this nature are "proxy, indirect and derived" which may yield inconclusive results or whether an approximate measure of the construct is indeed provided (Webb *et al.*, 2006; Wolfe & Frongillo, 2001). Criterion measures used in these studies were socio-economic and food and nutrition-related variables.

This study is aimed at assessing the construct validity of an adaptation of the Radimer/Cornell measure of food insecurity by providing a factor model of the food security construct to better characterise the experience among Filipino households. This study is envisioned to contribute to the growing body of evidence on valid measures of assessing food security at different levels of estimates and in different country settings.

MATERIALS AND METHODS

Data set

The study used the Food Security component of the FNRI sixth National Nutrition Survey (NNS) as secondary data. This is a cross-sectional survey undertaken from July through December 2003 that covered 17 regions and 79 provinces and 5,533 households of the Philippines (Molano *et al.*, 2007). The data files on food security were merged with data files on household energy adequacy and nutritional status. Household energy adequacy levels were derived from household food consumption data gathered using food weighing from 25.0% of one replicate of the sample households. Households with children 0-10 years old (n=3,568) and with available data for nutritional status (n=3,535) were included for this analysis. Height, weight and recumbent length of children were measured using standardised techniques. Height/length-for-age and weight-for-age z-scores <-2SD are considered as stunted and underweight, respectively. Accounting for missing data, final sample size included for the analysis of food insecurity measures are household and individuals/mothers (n=3568); children (n=3,525); households with children for questions 9 and 10 (n=3,535).

The FNRI adapted Radimer/Cornell questionnaire

The DOST-FNRI questionnaire asked the respondents whether or not they have experienced specific situations pertaining to food insecurity during the past six months as reference period. Respondents who replied affirmatively to these situations were also asked the frequency of occurrence of the particular experience or food security item (Table 2).

The questionnaire employs ten food insecurity items adapted from the Radimer/Cornell measurement of hunger and food insecurity translated into Filipino. Specifically, four of these items were adapted from the final 12-item Radimer/Cornell measure and six from the full 30-item Radimer/Cornell measure.

Six items addressed to mothers and children were framed as questions and the four items on "knowledge of situation" (denoting household level) were framed as statements. Period of recall was past six months. The FNRI questionnaire used a "yes" or "no" response choice for each frequency of experience for items framed as questions, and "not true", "true, often" and "true, sometimes" for items framed as statements.

The Radimer/Cornell tool did not use a reference period of recall of the hunger experience, and a score were assigned to each response choice to denote scale of responses. The team of Radimer saw fit that response choices covers "periodic and episodic types of

Table 2. Ten-item FNRI Food Security Survey Questionnaire

Food security items		*Frequency of occurrence*
Knowledge of self (in the last 6 months)		
1	Did you skip eating or miss meals/food, because there was no food or no money to buy food?	0 – never 1 – yes, once during the past 6 months 2 – yes, > once during the past 6 months
2	Did you ever not eat for a whole day because there was no food or money to buy food?	
3	Were you ever hungry but did not eat because there was no food or money to buy food?	
Knowledge of child/children		
4	Did your child/children skip eating or miss meals/food, because there was no food or no money to buy food?	0 – never 1 – yes, once during the past 6 months 2 – yes, > once during the past 6 months
5	Did your child/children ever not eat for a whole day because there was no food or money to buy food?	
6	Was/were your child/children ever hungry but did not eat because there was no food or money to buy food?	
Knowledge of situation (statement form)		
7	"I worried that our food would run out before we got money to buy more"	0 – not true 1 – true, often 2 – true, sometimes
8	"The food we bought did not last and we did not have enough money to get more"	
9	"The children were not eating enough because we did not have enough food and we could not afford to buy more"	
10	"We could not feed the children nutritionally adequate meals because we do not have enough food and enough money to buy food more"	

hunger, thus avoiding specific time-referenced response choices and including more generic ones" such as "never", "sometimes" and "often" for items framed as questions and "not true", "true, sometimes" and "true, often" for items framed as statements.

Four items used in the questionnaire were adapted from the Radimer/Cornell tool, one each from the mother and child level. These items were related to "insufficiency of intake":

- Were you ever hungry but did not eat because there was no food or money to buy food? (mother; quantitative, intake insufficiency)
- Was/were your child/children ever hungry but did not eat because there was no food or money to buy food? (child; quantitative, intake insufficiency)

The two statements that pertained to "knowledge of the situation" or household level was related to "food depletion" and "food anxiety" in the household:

- "The food we bought did not last and

we do not have enough money to get more" (quantitative, food depletion)
- "I worried that our food would run out before we got money to buy more" (psychological, food anxiety)

Two statements, namely "children were not eating enough" and "we could not feed the children a nutritionally-adequate meal" that were used to assess "knowledge of the situation" are items intended to measure child hunger in the initial 30-item list of the Radimer/Cornell instrument.

At the individual level, the Radimer/Cornell tool focused on food insecurity experiences in terms of "inadequacy and insufficiency of diets" (qualitative and quantitative domains) compared to the adapted questionnaire used by the DOST-FNRI which focused on "disrupted eating patterns" as measures of the food insecurity experience. "Disrupted eating pattern" was initially included in Radimer's analysis, but was eventually excluded in the final tool as this did not figure significantly in the statistical analysis. Based on this, Radimer's group refrained from recommending the "use of 'disrupted eating pattern' as hunger indicators (alone) since these are very specific indicators of intake quantity and that more general items are more widely applicable and preferable" according to Radimer, Olson & Campbell (1990). A major adaptation by the DOST-FNRI was the use of "disrupted eating pattern" as a concept of food insecurity in assessing the situation of mothers and children, specifically "skipped eating or missed meals" and "hungry but did not eat".

Financial constraint is the conditional phrase used in administering the Radimer tool: "there was no food or no money to buy food". The social support network embedded in the Filipino culture as well as the practice of engaging in home food production activities were considerations in adapting the questionnaire for use in the Philippine setting. These were viewed as "coping mechanisms" to access "food on the table" (for the household), thereby giving some form of assurance or security. Nevertheless, the conditional phrase "there was no food or no money to buy food" was still used.

Pretesting of the questionnaire had been previously undertaken prior to the start of the 2001 regional nutrition survey. The tool was used for the first time in DOST-FNRI's 2001 updating of the Nutritional Status of the Filipino Children at the Regional Level, after which it became part of the NNS as its food security component from 2003 to 2011.

Construct validation

Construct validation of the data entailed exploratory factor analysis that "focuses on finding structures (patterns) of correlation in the data" (Vogt, 2007). Construct validity of the FNRI food security questionnaire was assessed using principal component analysis with varimax rotation (SPSS 16 for Windows). The FNRI tool containing ten items were subjected to factor analysis at three levels (mother, child, household). The analysis extracted two factors after the first run had complied with data requirements.

The components collectively explained more than 60 percent of the variance in the set of included variables (total Eigenvalue is 67.2%).

a. The derived components explained 50 percent or more of the variance in each of the variables, i.e., have a communality greater than 0.500 (0.550 - 0.757).
b. None of the variables had loadings (or correlations) of 0.400 or higher for more than one component, i.e., did not have a complex structure.
c. None of the components consisted of only one variable.

RESULTS

Characteristics of the study households and children are presented in Table 3.

Table 3. Percent distribution of selected characteristics of Filipino households and children: food security component of the National Nutrition Survey, 2003

Variable	*n*	*%*
Household	3568	
Household size (*M*=6.04, *SD*=2.30)		
1 – 3	364	10.2
4 – 6	1945	54.5
7 – 9	947	26.5
10 – 12	259	7.3
≥ 13	53	1.5
Children	3535	
Sex		
Male	1858	52.8
Female	1677	47.2
Age		
0 – < 1 year	656	18.1
1 – 3 years	1914	54.4
4 - 6 years	843	24.8
7 - 9 years	115	3.5
10 years	7	0.2
Weight-for-age†		
Normal	2605	74.2
Underweight	879	24.2
Overweight	51	1.6
Height-for-age‡		
Normal	2549	72.7
Stunted/short	966	26.7
Above average/tall	20	0.6

†Underweight: <-2SD, Normal: -2SD to +2SD, Overweight: >+2SD
‡Stunted/short: <-2SD, Normal: -2SD to +2SD, Above average/tall: >+2SD

Mean household size was five, while 31% had 4-6 members.

Half of the children were male (52.8%), and were mostly young, within the ages of infancy (18.1%), toddlers (54.4%) and preschool age (24.8%). Less than 5% were school-aged children (7 – 10 years old). Prevalence of underweight and stunting were high at 24.2% and 26.7%, respectively.

Food security situation among women, children and households

In terms of specific food security items, 71.0% mothers claimed that they themselves did not experience "skipping of meals", "going hungry for a day" (87.8%) or "not eating even when hungry" (75.6%), suggesting food security at her own level (Table 4). Lower proportions of mothers responded "yes, more than once" to these questions (14.3%, 4.6% and 11.5%, respectively), indicating the presence of serious food insecurity faced by the mothers themselves.

High proportions of the mothers answered "never" to questions that indicate child facing food insecurity – "skipping meals" (82.0%), "not eating the whole day" (91.8%), or "not eating even when hungry" (84.9%). This suggests that more mothers considered their children did not experience food insecurity.

Household food insecurity was reported whereby, while 27.2% did not "worry that food would run out", 44.8% affirmed that this experience was "true,

Table 4. Percentage distribution of responses by food security items, Philippines, 2003

Food security items	*n*	*Never (%)*	*Yes, once (%)*	*Yes, more than once (%)*	*Total* † *(%)*
Knowledge of self (in the last 6 months)					
1 Did you skip eating or miss meals/food, because there was no food or no money to buy food?	3568	71.0	14.7	14.3	100
2 Did you ever not eat for a whole day because there was no food or money to buy food?	3568	87.8	7.6	4.6	100
3 Were you ever hungry but did not eat because there was no food or money to buy food?	3568	75.6	13.0	11.5	100
Knowledge of child/children					
4 Did your child/children skip eating or miss meals/food, because there was no food or no money to buy food?	3525	82.0	9.6	8.3	100
5 Did your child/children ever not eat for a whole day because there was no food or money to buy food?	3525	91.8	5.0	3.3	100
6 Was/were your child/children ever hungry but did not eat because there was no food or money to buy food?	3525	84.9	8.2	6.9	100
Knowledge of situation (statement form)		Not true	True – sometimes	True-often	
7 "I worried that our food would run out before we got money to buy more"	3568	27.2	44.8	28.1	100
8 "The food we bought did not last and we did not have enough money to get more"	3568	38.4	38.1	23.5	100
9 "The children were not eating enough because we did not have enough food and we could not afford to buy more"	3535	48.2	31.5	20.3	100
10 "We could not feed the children nutritionally adequate meals because we do not have enough food and enough money to buy food more"	3535	42.9	34.4	22.7	100

†Figures may not add up to 100% due to rounding

sometimes" and 28.1% said that this was "true, often". While 38.4% did not experience that "the food bought did not last", the same proportion affirmed that they did experience this "sometimes", while the remaining 23.5% experienced this "often". More than 40.0% (42.9%) of households did not experience "not feeding the children nutritionally-adequate meals" nor perceived that their "children were not eating enough" (48.2%). However, 31.5% and 20.3% of the households experienced this type of food insecurity "sometimes" and "often", respectively.

Construct validity

Two factors emerged from the rotated principal component analysis of the adapted Radimer/Cornell food insecurity items. These two factors were at two levels, namely individual (mothers and children) and household. These are components of "altered eating" (factor 1)

Table 5. Distribution of affirmative responses and rotated factor loadings of the adapted Radimer/Cornell food security items as assessed in Filipino households, 2003

	Food security item[†]	*%*[‡]	*Factor loadings*
	Altered eating (Individual)		Factor 1[§]
1	Did you skip eating or miss meals/food, because there was no food or no money to buy food?	29.0	0.766
2	Did you ever not eat for a whole day because there was no food or money to buy food?	12.2	0.763
3	Did you ever not eat for a whole day because there was no food or money to buy food?	24.4	0.808
4	Did your child/children skip eating or miss meals/ food, because there was no food or no money to buy food?	18.0	0.827
5	Did your child/children ever not eat for a whole day because there was no food or money to buy food?	8.2	0.784
6	Was/were your child/children ever hungry but did not eat because there was no food or money to buy food?	15.1	0.841
	Anxiety over quantity and quality of food (Household)		Factor 2[¶]
7	"I worried that our food would run out before we got money to buy more"	72.8	0.741
8	"The food we bought did not last and we did not have enough money to get more"	61.6	0.853
9	"The children were not eating enough because we did not have enough food and we could not afford to buy more"	51.8	0.845
10	"We could not feed the children nutritionally adequate meals because we do not have enough food and enough money to buy food more"	57.1	0.859

[†]Source (table format): Leyna *et al.* (2007)

[‡]Responding as "yes, once" or "yes, more than once" and "true, sometimes" or "true, often" to the food insecurity items

[§]Factor 1 explained 44.0% of the total variance

[¶]Factor 2 explained 23.2% of the total variance

and "anxiety over quantity and quality of food" (factor 2), that explained 44.0% and 23.2% of the total variance, respectively (Table 5). The results showed high cumulative variance (67.2%) for these two factors which implies that sufficient variance was obtained to justify the two components or factors derived from the dataset.

Food security items 1 to 6 loads highly for the first component with factor loadings ranging from 0.763-0.841. Food security items 7 to 10 load highly for the second component with factor loadings ranging from 0.741-0.859. A higher proportion of mothers "skipped meals" (29.0%) with a factor loading of 0.766 compared to "not eating" (12.2%) or "going hungry" (24.4%). Less than 20.0% of children "skipped meals" (18.0%), "went hungry" (15.1%) and "did not eat" (8.2%). Majority of households "worried that food would run out" (72.8%) and "food bought will not last" (61.6%). Anxiety over their inability to feed their children "nutritionally-adequate meals" or "they were not eating enough" were experienced by 57.1% and 51.8% of the households, respectively.

High internal consistency across the items in the tool were found with Cronbach's α at 0.84 for all items and 0.88 and 0.86 at the individual and household levels, respectively.

DISCUSSION

The Radimer/Cornell measure of hunger and food insecurity as adapted in different country settings (Leyna *et al.*, 2007; Shoae *et al.*, 2007; Molano *et al.*, 2007) has been reported to be reliable with Cronbach's α coefficient ranging from 0.85-0.89 (household level), 0.78-0.84 (child level) and 0.81-0.82 (individual level). This study revealed a range of severity with prevalence of food insecurity higher at the mother's level (33.7%) compared to the child (21.0%). This is similar to the study conducted by Castañeda *et al.* (2006) where 57.0%, 31.6%, and 27.6% of Filipino mothers, fathers or both, respectively, reportedly "skipped meals", while only 11.4% of their children was reported to have done so.

The study also indicates some forms of "managed process" or coping with food insecurity as described in Radimer *et al.* (1992) being practised among the study households. For example, "altered eating" among mothers and children contributed to 44.0% of the total variance of the construct. "Skipping" and "missing out" on meals characterise individual food insecurity experience among Filipino households. Castañeda *et al, (*2006) reported that among marginalised Filipino communities in Baguio, Dumaguete and Davao City, adjustments of food quantity and quality preceded cutting down on number of meals as a form of coping mechanism. They reported that 86.6% of the households "eliminated or sacrificed food items" and 70.2% "reduced quantity of foods served". "Skipping of meals" and "cutting down on the number of meals" were observed among 57.5% and 32.2% of 210 households with preschool and school children, respectively.

Conversely, "having three meals a day" was perceived to contribute to a sense of food security among women-respondents in a study by Balatibat (2004) who examined the linkages between food and nutrition security in lowland and coastal villages in the Philippines. Gender differences in the perception of food security was noted wherein "security of income base" dominated the men-respondents' perception of food security being the usual breadwinner.

In this study, "altered eating" emerged as the first factor at the individual level of food insecurity, similar to the validation study of Leyna *et al.* (2007) among 530 women with children under 5 years in rural Tanzania. However, the "altered eating pattern" at the child level reported by Leyna *et al.* (2007) pertained

to economic constraints in the quantity and quality of available food as well as hunger experience of the children, whereas "altered eating" in the present study pertained more to "skipping" and "missing out" on meals, termed as "disrupted eating pattern" by Radimer *et al.* (1992).

"Anxiety over quantity and quality of food" emerged as the second factor at the household level in this study. A similar finding was cited by Shoae *et al.* (2007), in a study among 250 poor urban households with at least one child aged 1-18 years. Their finding may be expected among low socio-economic status of the subjects.

According to Radimer's hypothesis, mothers tend to sacrifice "their" own food needs for their children as a form of coping mechanism. It should be cautioned that biased reporting of food insecurity experiences may confound findings where a greater proportion of children experienced food insecurity (27.0%) compared with individual/ mother experience (10.9%) as seen in the study by Zalilah and Ang (2001).

Food security continuum as defined by the FAO (2008) encompasses both quantitative and qualitative aspects of food accessibility and availability. In this study, the qualitative aspect of food security pertained to item ten in the adapted FNRI questionnaire used for households with children: "we could not feed the children nutritionally adequate meals because we do not have enough food and enough money to buy food more". Kendall *et al.* (1995) recommended the "inclusion of items assessing diet quality especially in a more socio-economically diverse population in order to accurately estimate the prevalence of individual-level food insecurity".

Based on the high factor loadings derived for each food insecurity item, the use of some questions from the individual and the household level measures are suggested below for further investigations.

1. While any item from the individual level can be used for this purpose, regardless of the derived factor loading, addressed either to the respondent (mother, caregiver) or the child as reference individual, items one and four appear to be more plausible given the high percentage of affirmative answer for these items.
   a. Question 1: Did you skip eating or miss meals/food, because there was no food or money to buy food?
   b. Question 4: Did your child/ children skip eating or miss meals/ food, because there was no food or money to buy food?
2. For the household level, item seven or eight can be used since this does not require a child to be present in the household before they can be assessed for food insecurity.
   a. Question 7: "I worried that our food would run out before we get money to buy more."
   b. Question 8: "The food we bought did not last and we did not have enough money to get more."

Rapid assessment of food insecurity can be used to document transient food insecurity which may now become more apparent with the compounding effects of disaster and climate change-related incidents.

Limitations of study

The strength of this validation study lies in characterising hunger and food insecurity as experienced among Filipino households. The gold standard of providing a real picture of the phenomenon via the use of criterion-related validity remain elusive, hence, proxy indicators have been used (socio-economic variables and food and nutrition-related variables).

As suggested in the interpretation of the Radimer/Cornell measure and as

used by the DOST-FNRI, a response in at least one of these items presupposes the food insecurity experience that is used to record prevalence of food insecurity. This interpretation, however, could mask the more specific experiences of individuals and households.

CONCLUSION

The adapted Radimer/Cornell measure of food insecurity contains valid indicators of food insecurity. "Altered eating" characterises the individual level of food insecurity while "anxiety over quantity and quality of food" characterises the household level of food insecurity. This study indicates the feasibility of the FNRI adaptation of the Radimer/Cornell tool to detect food insecurity at the individual and household levels.

Acknowledgement

The technical expertise of Maria Paz N. Marquez, Associate Professor IV of the Population Institute, University of the Philippines Diliman campus as Trainor-Consultant on Factor Analysis, is greatly appreciated. Gratitude is also extended to Ms. Charmaine A. Duante and Mr. Glen Melvin Gironella, Supervising Science Research Specialist and Senior Science Research Specialist, respectively, of the Nutrition Assessment and Monitoring Division of the DOST-FNRI for facilitating the use of the 2003 National Nutrition Survey data set and assisting in the analysis. More importantly, the senior author would like to acknowledge Dr. Cecilia A. Florencio, Professor Emeritus of the Department of Food Science and Nutrition at the College of Home Economics, University of the Philippines Diliman, for helping plant and nurture the seed of this research endeavor.

Authors' contributions

RMARM, conceptualised, conducted analysis and interpretation of results, prepared the draft of the manuscript; VRV, reviewed related literature, conducted factor analysis and drafting of manuscript; AGP, reviewed related literature, conducted factor analysis and interpretation of factor model.

Conflict of interest

The authors declare that they have no competing interests in whatever form.

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Mal J Nutr 24(4): 529-538, 2018

Household food insecurity and undernutrition in children below 5 years living in different geographical areas in East Java, Indonesia

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ABSTRACT

Introduction: Geographical conditions may be linked with food insecurity and growth retardation in young children. This research assessed household food insecurity status and undernutrition in different geographical areas. **Methods:** A cross-sectional study was conducted in four different types of geographical areas: coastal, limestone, agricultural and municipality, which were purposely selected in East Java Province. The samples were households with children aged below 5 years. A total of 736 households that fulfilled the inclusion criteria were recruited. Household food security was assessed using the Household Food Security Supplement Measure (US-HFSSM) adapted for developing countries. Nutritional status of children was determined and classified according to World Health Organization Growth Standard (2006). **Results:** Prevalence and severity of household food insecurity differed significantly among the different geographical areas. Almost half (44.8%) of the households were categorised as "food insecure without hunger". Prevalence of "hunger" was highest in coastal (7.2%) and limestone areas (5.3%). Highest prevalence of stunting was in coastal areas (11.6%), whereas highest prevalence of wasting (6.2%) and underweight (8.9%) were in limestone areas. Prevalence of undernutrition was relatively low among children living in municipalities. The differences in the distribution of undernutrition of young children and household food insecurity status were statistically associated with the types of geographical areas. **Conclusion:** Prevalence of household food insecurity differed according to the types of geographical areas in East Java. Prevalence of household food insecurity and young child undernutrition were greater for households in the coastal and limestone areas, compared to those in the agricultural and municipality areas.

Keywords: Household food insecurity, underweight, wasting, stunting, geographical areas

INTRODUCTION

"Food insecurity exists whenever the availability of nutritionally adequate, safe foods or the ability to acquire personally acceptable foods in socially acceptable ways is limited or uncertain" (FAO, IFAD & WFP, 2015). In developing countries, food insecurity is linked with negative nutrition outcome, such as low birth weight, child's underweight, wasting and stunting. A study of adolescents in Ethiopia showed that food insecurity

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was negatively associated with linear growth among girls (Belachew *et al.*, 2013a). Low dietary diversity as a proxy for food insecurity was positively related with child stunting, according to National Food Consumption Surveys in South Africa (Belachew *et al.,* 2013b). Household food security was found to be positively associated with greater subsequent infant weight gain in a cohort study in Bangladesh (Saha *et al.*, 2009). Economic inequalities at a provincial level in Ecuador, which reflected food insecurity in terms of food purchasing ability, were also positively associated with child's stunting (Larrea & Kawachi, 2005)

In Indonesia, food insecurity remains a major concern despite achievement of economic growth since the economic crisis of 1998 (Yusdja & Soeparno, 2011). Increasing food insecurity among households has been reported as correlating with increase in food insufficiency and decreased dietary intake (Indonesian Ministry of Health, 2013). This condition results in increasing prevalence of undernourished children in both rural and urban areas. Based on the National Research data (Indonesian Ministry of Health, 2013), the prevalence of wasting in East Java increased from 13.7% in 2007 to 14.1% in 2010, while stunting increased from 34.8% to 35.8%. This prevalence of stunting is similar to the national figure of 35.6% (Indonesian Ministry of Health, 2013). Hence, it has been suggested that East Java Province is a miniature Indonesia in terms of child nutritional status.

Physical environmental and geographical conditions, and climate change can affect food availability, which in turn, can affect household food insecurity (Lysenko, Squires & Verheye, 2010; Butler, 2009; Gross, 2013). This study assessed household food insecurity and prevalence of undernutrition among children under 5 years of age living in different geographical areas in East Java Province.

MATERIALS AND METHODS

A cross-sectional study was conducted in four different types of geographical areas: coastal, limestone or dry, lowland agricultural and urban or municipality, which were purposely selected in East Java Province. Coastal areas selected were Pasuruan and Lamongan; limestone areas selected were Gresik and Pamekasan, lowland agricultural areas selected were Ngawi and Banyuwangi Districts; while urban areas were represented by Madiun and Blitar Municipalities. The study samples were households with at least one child aged below 5 years.

The sample size was determined by using multi-stage cluster random sampling. The census block determined by Statistics Indonesia (2010) was used as clusters. Sampling was carried out as follows: three sub-districts were randomly selected from each district, followed by random selection of two villages from each sub-district. There were 8-10 census blocks in each village, in which a census block consisted of 30 households. Two census blocks were randomly selected in each of the selected village. Finally, eight households were randomly selected in every census block. Thus, a total of 96 households was selected in each district or municipality. Out of 768 households initially identified from all the districts/municipalities, a final sample size of 736 household who fulfilled the inclusion criteria were included in the study.

Characteristics of the households were assessed using a questionnaire, and these include food expenditure, source of drinking water, maternal characteristics, household food security and nutritional status of children under 5 years old. Nutritional status of children was determined and classified as underweight, wasting

and stunting, according to weight-for-age z-score (WAZ), weight-for-height z-score (WHZ), and height-for-age z-score (HAZ) respectively (WHO, 2006). The children's weight was measured by using standardised electronic scale to the nearest 0.1 kg (Tanita HS302). Standing height was measured by using a microtoise tape to the nearest 0.1 cm.

Household food security was assessed using the Household Food Security Supplement Measure (US-HFSSM) adapted for developing countries (Bickel *et al.*, 2000). This measure has been shown to provide valid results in several developing countries including Peru (Chaparo & Estrada, 2012), Ecuador (Weigel *et al.*, 2016), Bolivia, Burkina Faso, Philippines (Melgar-Quinonez *et al.*, 2006) and Indonesia (Usfar Fahmida & Februhartanty, 2007).

The US-HFSSM method simplifies the food insecurity scale into small set of categories, each one representing a meaningful range of severity. The questionnaire consists of 18 standardised questions and corresponding scales. Three sequences of screening questions serve as preliminary testing followed by 18 questions as the core scale. Four categories describe the range of food severity: (i) Food secure [FS] for scores <2.32, (ii) Food insecure without hunger [FIWH], when the score is between 2.32 and <4.56; (iii) Food insecure with moderate hunger [FIMH], for scores between 4.56 and <6.53; and (iv) Food insecure with severe hunger [FISH], when the score is ≥6.53 (Bickel *et al.*, 2000).

Appendix 1 shows the questions asked of the household member, particularly the mother. A range of questions depict the severity of food access problems ranging from household that might run out of food, to children not having food a whole day. The mothers' answers were coded into 18 items, with affirmative response (1) given for "often" or "sometimes", while "never" was coded as negative (0). For response to the other questions, affirmative (1) was given for "yes" answers and negative (0) was given for "no". For "how often?" questions, responses, "almost every month" and "some months" were regarded as affirmative (1) and response of "only 1 or 2 months" was deemed as negative response (0) (Appendix 1).

Maternal attributes included mid-upper arm circumference (MUAC), and breastfeeding status. Socio-demographic covariates included total monthly expenditure, food expenditure, and source of drinking water, number of children under 5 years and number of household members.

Breast feeding status is categorised as exclusive breastfeeding for up to 6 months, while predominant breastfeeding is the practice in which only non-milk fluids given in addition to breast milk during the first six months; meanwhile, partial breastfeeding is breastfeeding combined with other milks and/or solid foods (WHO, 1991; Greiner, 2014).

SPSS software version 17.0 (SPSS Inc. Chicago, IL) was used for data entry and statistical analyses. Association between household food security status and child nutritional status (wasting, stunting and underweight) from various geographical areas was determined, with significance set at p-value <0.05.

Ethical approval was obtained from Ethical Committee of Faculty of Public Health Universitas Airlangga No. 190-KEPK. Consent was obtained from each subject in writing, after information regarding the purpose of the study was explained to them. The identity of all subjects was kept confidential.

RESULTS

Maternal characteristics

Overall, 14.7% of mothers had MUAC of less than 23.5 cm, indicating low fat deposit. In comparison, only 1.8% of the mothers from the municipalities showed unsatisfactory MUAC (Table 1).

Less than 7.0% of the mothers from all areas practised exclusive breastfeeding for 6 months. The lowest rate of exclusive breastfeeding was reported for the limestone areas (0.5%). Majority of the mothers (73.6%) did not breastfeed their children until two years of age. Mothers in limestone areas were also found to show relatively low prevalence of predominant breastfeeding and partial breastfeeding, whereas comparatively higher prevalence of these breastfeeding practices were reported among mothers in the coastal areas.

Household and environmental characteristics

More than half (55.5%) of the study households consist of nuclear families consisting of 1-4 members. Households that use the well for drinking water were mostly found in agricultural areas (23.6%), while the lowest percentage was in the municipalities (14.9%). The latter had the highest percentage of households (8.8%) that use drinking water supplied by a public water company. Some households, particularly in agricultural areas (2.2%) also used rivers as their drinking water source.

Approximately 64.5% of the households had one person as income earner. Household economic status was determined by using the proxy indicators of monthly total and food expenditures. The average household monthly total expenditure was US$47.71 – 275.14, and monthly food expenditure was US$32.79 – 225.50. Households having the average total and food expenditures were mostly found in municipality areas (8.7%). Majority of the households in the study locations were categorised as having poor economic status, in which the monthly total expenditure was <US$47.71, and monthly food expenditure <US$32.79. Poor economic status was highest in the agricultural areas (18.9%).

Nutritional status of children and household food security status

Using the 2006 WHO Growth Standard (WHO, 2006) the overall prevalence of stunting, wasting and underweight was 39.4%, 18.4%, and 25.0%, respectively (Table 2). The coastal areas had the highest prevalence of stunted children (11.6%). Meanwhile, the limestone area had the highest prevalence of wasting and underweight (6.2% and 8.9%, respectively).

Overall, 34.4% of the households were classified as “FS”. However, 44.8% of the households were found as “FIWH”, with the limestone areas having the highest proportion (12.9%). Both “moderate hunger (FIMH)” and “severe hunger (FISH)” were reported mostly in households from the coastal 7.2%) and limestone areas (5.3%).

Prevalence of stunting, wasting and underweight were relatively higher in the coastal and limestone areas. Stunting in the coastal and limestone areas was 11.6% and 10.9% respectively, compared to 9.6% in the agricultural areas and 7.3% in the municipalities. Wasting was also relatively higher in the coastal and limestone areas (5.7% and 6.2%, respectively), compared to the agricultural areas and municipalities (3.2% and 3.3%, respectively). Statistically, Table 2 shows that these differences in the distribution of undernutrition of young children and household food insecurity status were statistically associated with the studied geographical areas.

DISCUSSION

The overall high prevalence of underweight (25.0%) and stunting (39.4%) among children under 5 years in the study areas indicate that East Java Province as having a serious public health problem (WHO, 2000). Meanwhile wasting prevalence (overall 18.4%) exceeding the WHO’s cut-off point of

Table 1. Maternal and social economic characteristics in various geographical areas

Characteristics	*Geographical Area*				*Total*
	Agriculture n (%)	*Coastal* n (%)	*Limestone* n (%)	*Municipal* n (%)	n (%)
Mother's MUAC (*n*=736)					
<23.5 cm	36 (4.9)	27 (3.7)	32 (4.3)	13 (1.8)	108 (14.7)
≥23.5 cm	156 (21.2)	165 (22.4)	144 (19.6)	163 (22.1)	628 (85.3)
Breastfeeding practice (*n*=734)					
Breastfeeding for 2 years	81 (11.1)	48 (6.5)	29 (3.9)	36 (4.9)	194 (26.5)
Do not breastfeed for 2 years	111 (15.1)	143 (19.5)	146 (19.9)	140 (19.1)	540 (73.5)
Breastfeeding status (*n*=734)					
Exclusive breastfeeding for 6 months	24 (3.3)	12 (1.6)	4 (0.5)	6 (0.8)	46 (6.3)
Predominantly breastfeeding	57 (7.8)	36 (4.9)	25 (3.4)	30 (4.1)	148 (20.2)
Partially breastfeeding	111 (15.1)	143 (19.5)	146 (19.9)	140 (19.1)	540 (73.5)
Type of family (*n*=736)					
Nuclear Family	119 (16.2)	106 (14.4)	92 (12.5)	91 (12.4)	408 (55.4)
Extended Family	73 (9.9)	86 (11.7)	84 (11.4)	85 (11.5)	328 (44.6)
Family member employed (*n*=736)					
≤1	107 (14.5)	144 (19.6)	115 (15.6)	109 (14.8)	475 (64.5)
2	62 (8.4)	35 (4.8)	46 (6.3)	49 (6.7)	192 (26.1)
≥3	23 (3.1)	13 (1.7)	15 (2.1)	18 (2.4)	69 (9.4)
Source of drinking water (*n*=736)					
River	16 (2.2)	1 (0.1)	1 (0.1)	1 (0.1)	19 (2.6)
Well	174 (23.6)	132 (17.9)	165 (22.4)	110 (14.9)	581 (78.9)
Public water company	2 (0.3)	25 (3.4)	6 (0.8)	65 (8.8)	98 (13.3)
Others	0 (0.0)	34 (4.6)	4 (0.5)	0 (0.0)	38 (5.2)
Economic status (*n*=736)[†]					
Poor	139 (18.9)	135 (18.3)	138 (18.8)	112 (15.2)	524 (71.2)
Average	53 (7.2)	57 (7.7)	38 (5.2)	64 (8.7)	212 (28.8)
Monthly expenditure (Mean in US$) (*n*=736)[‡]					
Low (< US$47.71)	142 (19.3)	101 (13.7)	107 (14.5)	98 (13.3)	448 (60.9)
Average (US$47.71 – 275.14)	50 (6.8)	91 (12.4)	69 (9.4)	78 (10.6)	288 (39.1)
Monthly food expenditure (Mean in US$) (*n*=736)[§]					
Low (< US$32.79)	131 (17.8)	97 (13.2)	95 (12.9)	100 (13.6)	423 (57.5)
Average (US$32.79 – 225.50)	61 (8.3)	95 (12.9)	81 (11.0)	76 (10.3)	313 (42.5)

[†]Range for economic status classification: poor = <US$1/day, average = US$1-5/day

[‡]Range for monthly total expenditure: low = < US$47.71/month, average = US$47.71–275.14/month

[§]Range for monthly food expenditure: low = <US$32.79/month, average = US$32.79 – 225.5 /month.

Table 2. Prevalence of underweight, wasting, stunting among children below 5 years, and household food security status in various geographical areas

Variables	*Geographical Area*				*Total n (%)*	*p-value*
	Agriculture n (%)	*Coastal n (%)*	*Limestone n (%)*	*Municipal n (%)*		
Child's height-for-age z-score (n=731)†						0.008**
Not Stunted (≥ -2 SD)	122 (16.7)	106 (14.5)	94 (12.9)	121 (16.6)	443 (60.6)	
Stunted (< -2 SD)	70 (9.6)	85 (11.6)	80 (10.9)	53 (7.3)	288 (39.4)	
Child's weight-for-height z-score (n=721)†						0.030*
Not Wasted (≥ -2 SD)	167 (23.2)	149 (20.7)	129 (17.9)	143 (19.8)	588 (81.6)	
Wasted (< -2 SD)	23 (3.2)	41 (5.7)	45 (6.2)	24 (3.3)	133 (18.4)	
Child's weight-for-age z-score (n=732)†						0.005**
Not Underweight (≥ -2 SD)	157 (21.4)	134 (18.3)	111 (15.2)	147 (20.1)	549 (75.0)	
Underweight (< -2 SD)	34 (4.6)	57 (7.8)	65 (8.9)	27 (3.7)	183 (25.0)	
Household Food Security Status (n=736)‡						0.000***
Food Secure	71 (9.6)	56 (7.6)	42 (5.7)	85 (11.5)	254 (34.5)	
Food Insecurity without Hunger	93 (12.6)	83 (11.3)	95 (12.9)	59 (8.0)	330 (44.8)	
Food Insecurity with Moderate Hunger	26 (3.5)	43 (5.8)	31 (4.2)	29 (3.9)	129 (17.5)	
Food Insecurity with Severe Hunger	2 (0.3)	10 (1.4)	8 (1.1)	3 (0.4)	23 (3.2)	

†Cut-off point for stunting was height-for-age z-score (HAZ) of < -2 SD; cut-off point for wasting was weight-for-height z-score (WHZ) of < -2 SD; cut-off point for underweight was weight-for-age z-score (WAZ) of < -2 SD.
‡Household Food Security Classification was based on the US-HFSSM; Food secure [FS], when the score <2.32; Food insecure without hunger [FIWH], when the score 2.32≤FIWH<4.56; Food insecure with moderate hunger [FIMH], when the score 4.56≤FIMH<6.53, and Food insecure with severe hunger [FISH], when the score ≥6.53 (Bickel *et al.*, 2000).
*Significant at p<0.05; **significant at p<0.01; *** significant at p<0.001, using chi-square

acute malnutrition (>15.0%), suggests the study areas as having a critical public health problem. The nutritional status of the young children in East Java is worse than the national prevalence of underweight, wasting and stunting rates of 19.6%, 12.1% and 37.2%, respectively (Indonesian Ministry of Health, 2013). These data are useful to alert the regional and national public authorities to address the undernutrition problems in East Java.

In this study, the limestone area has the highest rate of underweight and wasting among the under-five children. The high rates of acute malnutrition in this geographic area might due to low prevalence of breastfeeding, including exclusive, predominant or continued breastfeeding until 2 years. Stunting rate was high in the coastal and limestone areas, with the prevalence of 11.6% and 10.9%, respectively.

Various definitions of hunger have been suggested, including the "state of a strong desire of food where it's lacking" (Barquera *et al.*, 2007). Mutisya *et al.* (2015) described the three indicators of hunger: the prevalence of undernourishment (food deprivation), the prevalence of critical food poverty (income deprivation) and the prevalence of child's underweight. This study found that being food insecure either with or without hunger was reported in all types of geographical areas. Based on the US-HFSSM (Bickel *et al.*, 2000), a total of 65.5% of households was categorised as "food insecure", majority of whom were FIWH. This finding is similar to another study in Indonesia, which identified 77.0% households in urban areas and 84.0% in rural settings as "food insecure" (Usfar *et al.*, 2007).

The total prevalence of food insecure in our study was close to that among low social economic households in Quebec, Canada (62.8%) (Carter *et al.*, 2012). Thus, food insecurity is related to household income status, which was also reported in a study in Malaysia (Mohamadpour, Sharif & Keysami, 2012). While household income was shown to be a better predictor for dietary adequacy than food expenditure by Booth & Smith (2001), in this study, monthly expenditures appeared easier to compute in rural communities.

This study shows that geographical condition exerts an influence on household food security status and child nutritional status. Similar findings were reported in Western Australia, where geographic location contributes to food availability, and also has impact on food pricing (Pollard *et al.,* 2014). The coastal and limestone areas in East Java are relatively remote and encounter transportation challenges. Thus, households residing in these areas have limited access to food and are vulnerable to food deficits and hunger (Godrich *et al.,* 2017). Most people living in the coastal areas work as fisherman, and are vulnerable to climatic changes. The impact of climate change on food security was delineated among households in coastal areas in Paraty, Brazil (Hanazaki *et al.* 2013), as well as in Bangladesh (Shams & Shohel, 2016). Meanwhile, the limestone area is a dry place and the residents are dependent on rainfall for their livelihoods.

Food and nutrition security have complex linkages with agriculture and the environment (Hwalla, El Labban & Bahn, 2016). Household food insecurity is influenced in part by environmental factors (Carter *et al.*, 2012). Evidence shows that due to land degradation and climate change, rice yields have been decreasing among Malagasy farmers (Gross, 2013), which in turn threatens them to become food insecured.

CONCLUSION

Based on the US-HFSSM modified for developing countries, a high prevalence of household food insecurity was observed among the different types of

geographical areas. Household food insecurity with and without hunger was more prevalent in the coastal and limestone areas. Likewise, the prevalence of child undernutrition was higher in the coastal and limestone areas, compared to the municipalities. Studies on household food security and child nutritional status should take into consideration the potential influence of geographical conditions of the residents.

Acknowledgement

This study was funded by East Java Provincial Office of Food Security Board, Indonesia. We would like to acknowledge the support of the Head of Public Health Centres where the study was located, as well as our appreciation to all participants. The first draft of the manuscript was written during the summer session at Harvard School of Public Health with the financial support from the Higher Education Network Ring Initiative (HENRI) grant. The authors would like to express our sincere thanks to Calista Segalita for helping in the preparation of this manuscript.

Authors' contributions

SS, contributed to the design and concept of the study, revised the manuscript and helped in writing the discussion part, responsible for data collection and quality check for data input, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; SM, contributed to the research methodology, cleaning the data and carrying out the statistical analysis; TM, responsible for sectional scientific management, formulated research question, carried out the analysis, preparation of draft manuscript, and carried out revisions. All authors have given approval to the final manuscript.

Conflict of interest

Authors do not have any conflict of interest in this study.

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Appendix 1. List of food insecurity items adapted in the study

US Food Security Supplement Measure (US-HFSSM)	*Adapted Items in the Questionnaire*
1. Worries that food would run out	1. Mother worries that food would run out
2. Food bought just didn't last	2. Food bought not sufficient
3. Couldn't afford to eat balanced meals	3. Mother couldn't afford to serve a balanced diet
4. Few kinds of low cost food for children	4. Mother couldn't buy low cost food for children
5. Couldn't feed children a balanced meal	5. Mother couldn't feed a balanced meal for children
6. Children were not eating enough	6. Mother says that food to eat is not enough
7. Adult(s) cut or skipped meals	7. Mother or other adults decrease the meals
8. Adult(s) cut or skipped meals, 3+ months	8. Mother or other adults decrease the meals, 3+ months
9. You ate less than felt you should	9. Mother ate less because they run out of money
10. You were hungry but didn't eat	10. Mother felt hungry but didn't eat
11. You lost weight because not enough food	11. Mother lost weight because there was not enough food
12. Adult(s) not eat for a whole day	12. Mother or other adults did not eat for a whole day (except during the fasting month of Ramadhan)
13. Adult(s) not eat for a whole day, 3+ months	13. Mother or other adults did not eat for a whole day, 3+ months
14. Cut size of children's meals	14. Mother cuts size of children meals
15. Children had ever skipped meals	15. Mother gave only one meal a day for children
16. Children skip meals, 3+ months	16. Mother gave only one meal a day for children, 3+ months
17. Children ever hungry	17. Child hungry but mother did not feed
18. Children did not eat for a whole day	18. Children did not eat for a whole day because they ran out of money

Mal J Nutr 24(4): 539-550, 2018

Nutritional status and complementary feeding among Penan infants and young children in rural Sarawak, Malaysia

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ABSTRACT

Introduction: The Penan people are largely settled in rural and remote areas of Sarawak with high rates of undernutrition among the children. The study aimed to determine the nutritional status and infant and young child feeding (IYCF) practices of Penan children. **Methods**: Subjects consisted of 121 children, aged between 0-23 months, from 15 Penan longhouses in Belaga district. Malnutrition was assessed using anthropometric measurements of children and categorised according to the World Health Organization (WHO) Growth Standards (2006). Feeding practices were assessed using questionnaires based on WHO IYCF Indicators (2008). **Results**: The prevalence of underweight was 29.8%; stunting 43.0% and wasting 5.8%. Prevalence of exclusive breastfeeding under 6 months was 44.4%. About 86% of infants aged 6-8 months were already given solid, semi-solid and soft foods. The proportion of children aged 6-23 months achieving minimum dietary diversity (MDD) was 76.6%, while minimum meal frequency (MMF) was 83.0% (breastfed and non-breastfed). Among the children who achieved MDD, 64.9% of their meals were derived from 4-5 food groups. More than half (55.3%) of all subjects (breastfed and non-breastfed) received minimum acceptable diet (MAD). Achievement of iron-rich foods (IRF) indicator was 77.7%. There was no significant association between the IYCF indicators of MDD, MMF and MAD and underweight, stunting and wasting. **Conclusion**: A high prevalence of poor nutritional status was recorded among the Penan children. While the IYCF indicators apparently showed satisfactory complementary feeding practices, future studies should assess quantitatively the intake of complementary food among Penan infants and young children.

Keywords: Infant and young child feeding, nutritional status, Penan, Sarawak

INTRODUCTION

Global estimates show underweight (6.0%), stunting (22.9%) and wasting (7.7%) among children below 5 years old (UNICEF, WHO & World Bank Group, 2017). Undernutrition (underweight, stunting, wasting) is linked with increased morbidity and mortality (Black *et al.*, 2013); as well as non-communicable disease and productive capacity in adulthood (Stewart *et al.*, 2013).

Recent reports revealed that while global prevalence of stunting in children below 5 years has reduced from 37.2%

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in 2000 to 22.9% in 2016, nevertheless a total of 154.8 million stunted children were reported in 2016, and disparities still exist between different regions of the world (UNICEF/WHO/World Bank Group, 2017). A systematic review addressing child health inequities revealed that income and economic status were the structural determinants of child health inequities. The reported child health determinants include shortcomings within the rural health care system and repercussions of food poverty with low health literacy among parents (Schröders *et al.*, 2015).

The national prevalence of stunting among children below 5 years in Malaysia was 20.7% in 2016, with Sarawak having a high rate at 23.1% compared to the other states. Overall, the prevalence of stunting was higher in rural localities (23.3%) than urban areas (19.2%). In terms of ethnicity, "Other Bumiputera"[1] recorded the highest prevalence of stunting at 24.5% (IPH, 2016).

A cross-sectional study in Serian District, Sarawak reported 20.9% underweight, 10.2% wasting and 11.9% stunting among children below 5 years (Eunice, Cheah & Lee, 2014). The study also revealed only 64.4% of subjects had been given complementary foods starting from the age of 6 months and above. In order to reduce child undernutrition and stunting, effective nutrition-specific interventions such as breastfeeding and appropriate complementary feeding of children need to be scaled up, especially in children below 2 years. It has been estimated that nutrition-specific interventions such as infant and young child feeding packages could save 221,000 lives when implemented together with other nutrition interventions at 90% coverage (Bhutta *et al.*, 2013).

The National Health and Morbidity Survey (NHMS) (2016) reported the prevalence of exclusive breastfeeding amongst infants below 6 months in Malaysia at 47.1% (IPH, 2016). This is a substantial increase compared to the prevalence of 14.5% in 2006 (IPH, 2008). Meanwhile the national prevalence of timely complementary feeding among children aged 6-10 months in 2006 was 41.5% (Khor *et al.*, 2009).

A study conducted in Klang District, Malaysia reported 43.1% prevalence of exclusive breastfeeding among infants aged 1-6 months (Tan, 2011). A study conducted among 173 "Orang Asli"[2] children (*Temuan and Mah Meri*) in Sepang district and Carey Island, Selangor reported that 33% of the mothers stopped breastfeeding before their infants were 6 months old, while 31% breastfed their children for more than one year (Wan Norlida *et al.*, 2007). The authors also found insufficient mean number of servings for all food groups and high under-nutrition rates among these Orang Asli children aged 1-3 years.

The Penan community is part of the Dayak group in Sarawak, Brunei and Kalimantan (Sercombe, 2008). Sarawak is home to an estimated 16,000 tribal Penans, living in remote areas of Baram and Belaga districts, with 77% living in permanent settlements, 20% leading a semi-nomadic lifestyle, while another 3% live as nomads. Almost two thirds of the Penan population live in Baram, Tutoh and Limbang areas and have been referred to as Eastern Penans. Meanwhile, Western Penans live mostly in the vicinity of Bintulu, and Belaga district (Lyndon *et al.*, 2013).

Primary health care is delivered to the rural community through various modes, such as static health clinics, mobile health teams and flying doctor service. Furthermore, to address malnutrition among Penan children aged below 6 years, two community feeding programmes were implemented in Sarawak from 2013 (Ministry of Health Malaysia, 2014). The community feeding programmes comprise supplementary feeding, provision of complementary foods, nutrition education and efforts

[1] "Other Bumiputera" meaning native tribes of Sarawak and Sabah, including Dayak
[2] "Orang Asli" meaning aboriginal tribes of Peninsular Malaysia.

to improve personal hygiene and environmental sanitation.

The Third National Plan of Action for Nutrition Malaysia has identified specified targets to improve infant and young child feeding practices and nutritional status of children (NCCFN, 2016). There is a lack of data on the quality of complementary feeding in Malaysia (NCCFN, 2011).

Despite high undernutrition rates, studies on infant and young child feeding practices amongst Penan children, especially complementary feeding practices, have not been extensively carried out. Two previous studies that included assessment of nutritional status amongst children were undertaken in the 1970-1980s (Anderson, 2015; Chen, 1984).

This study focused on complementary feeding in children aged below 24 months, based on the World Health Organization (WHO) infant and young child feeding (IYCF) indicators of minimum dietary diversity (MDD), minimum meal frequency (MMF) and minimum adequate diet (MAD). The objective of this study was to determine the nutritional status and infant and young child feeding practices in a sample of Penan children in Belaga district, Sarawak.

MATERIALS AND METHODS

Study design

This is a cross-sectional study which was carried out in Belaga district, Sarawak. Period of data collection was between August-December 2016.

Participants

Written permission was obtained from the Belaga District Office in May 2016 before carrying out the study. Following that, verbal permission was also obtained from each Penan community leader or *Tuai Rumah* before the study was carried out in each locality.

A total of 121 Penan infants (n=52) and young children aged 12.0-23.9 months (n=69) were recruited based on the following inclusion criteria: i) Penan; ii) aged below 24 months; iii) free of physical disability and known diseases. Written consent was obtained from mothers and caregivers of the Penan children who participated in the study.

Data collection

Anthropometric measurements including weight and length measurements of the children were taken. Recumbent length measurements of children were performed using SECA 210 measuring mat and recorded to the nearest 0.1 cm. Children's weight measurements were carried out using SECA 803 weighing scale. Weight measurement was recorded to the nearest 0.01 kg. Infants were weighed together with their mothers, and after that, their mothers were weighed, with the difference being recorded as the child's weight. Body length and weight measurements consisted of two readings for each child, and the average value was then recorded.

Nutritional status of children was categorised according to the WHO Child Growth Standards (WHO, 2006), based on three indicators namely, weight-for age z-scores (WAZ), length/height-for-age z-scores (HAZ) and weight-for-height z-scores (WHZ), which were calculated using WHO *Anthro* software (version 3.2.2). For WAZ, HAZ and WHZ, a cut-off value of below 2 standard deviations (SD) from the median value for the reference population was used to classify underweight, stunting and wasting, respectively.

The Penan mothers or caregivers were interviewed using a pre-tested questionnaire based on the WHO IYCF Indicators (WHO, 2008). The questionnaire consisted of two sections, i) socio-demography and health information of the children, and ii) IYCF practices. Data on the following core IYCF indicators was collected: exclusive breastfeeding under 6 months, continued breastfeeding at 1 year of

age, timely complementary feeding at 6 months, MDD, MMF, MAD and consumption of iron-rich or iron-fortified foods (IRF). MDD was calculated using the food group score (based on seven food groups) for both breastfed and non-breastfed children, and this indicator is considered as achieved if the child had been given foods from at least four food groups the previous day. MMF refers to the minimum number of times the child was given solid, semi-solid or soft foods the previous day, specific for their age range. MMF was calculated for breastfed and non-breastfed children. MAD indicator achievement was calculated based upon the child achieving both the MDD and MMF indicators during the previous day. MAD was calculated for breastfed and non-breastfed subjects. Optional indicators for infant and young child feeding practices were also collected: continued breastfeeding at 2 years and bottle-feeding practices.

Data analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software version 20. Descriptive statistics including mean and standard deviation was used. Independent Chi-square test and logistic regression analysis were used to determine association between factors contributing to malnutrition and nutritional status of children aged below 2 years.

Ethics approval

This study was approved by the Medical Research and Ethical Committee of Universiti Kebangsaan Malaysia (UKM/PPI/111/8/JEP-2016-381).

RESULTS

A total of 121 Penan infants and children participated in this study (Table 1). There were 22.3% aged below 5.9 months, 20.7% aged 6.0-11.9 months and 57.0% aged 12.0-23.9 months. Available birth records obtained from home-based child health cards showed 14.9% low birth weight (<2500g) among the study subjects. Their mean birth weight was 2.78±0.40 kg, ranging from 1.30-4.00 kg.

The mean age of their mothers was 24.6±7.0 years. With regards to mother's education status, 54.5% were without any formal schooling, while 34.7% and 10.7% had attended primary and secondary school respectively.

The mean household size was 7.3±3.9, ranging from 3-25. A total of 52.1% reported household size of ≥ 7. Almost all (96.5%) the mothers reported having household income level below the poverty line income (EPU, 2014).

Table 2 shows the nutritional indices of the children by age group and sex. There was no significant difference between the mean weight of boys and girls for each age group. For all age groups (0.0-5.9 months; 6.0-11.9 months; 12.0-23.9 months), there was no significant difference in mean length-for-age z-scores (LAZ) between boys and girls.

In terms of weight-for-age z-scores (WAZ), 70.2% of subjects had normal WAZ, while 29.8% were underweight (WAZ < -2SD). Among underweight children, 3.3% of children were severely underweight (WAZ < -3SD).

Stunting prevalence (LAZ < -2SD) among the children was 43.0%, while the rest achieved normal length-for-age status (57.0%).

A total of 94.2% of children had normal weight-for-height status, while wasting prevalence was 5.8% (WLZ < -2SD). Among subjects categorised as wasting, 0.8% suffered from severe wasting (WLZ < -3SD).

Overall, there was no statistically significant association between sex and all three nutritional status indicators of underweight, stunting and wasting (p>0.05). Also, there was no significant association between child age group and underweight, stunting and wasting (p>0.05).

Table 1. Socio-demographic characteristics of study sample (*n*=121)

Characteristics	*n*	*Percentage (%)*
Sex of child		
Male	64	52.9
Female	57	47.1
Age of child		
0.0-5.9 months	27	22.3
6.0-11.9 months	25	20.7
12.0- 23.9 months	69	57.0
Birth weight		
< 2500 g	18	14.9
≥ 2500 g	103	85.1
Number of children in the household		
< 3	72	59.5
≥ 3	49	40.5
Age of mother		
< 25 years	70	57.9
25-34 years	35	28.9
35-44 years	15	12.4
≥ 45 years	1	0.8
Mother's education		
No schooling	66	54.5
Primary school	42	34.7
Secondary school	13	10.7
Household size		
< 7	58	47.9
≥ 7	63	52.1
Household income[†]		
Household income ≤ RM 610 (extremely poor)	105	93.8
RM610 < Household income ≤ RM 920 (poor)	3	2.7
RM 920 < Household income < RM 2000 (medium)	4	3.6

[†]*n*=112, 9 respondents did not report household income; the poverty line income classification used was based upon recent guidelines (Economic Planning Unit Prime Minister's Department, 2014).

Table 3 shows the IYCF practices among the Penans. A total of 65.3% were breastfed (had been given breast milk the previous day). Out of 27 infants aged < 6 months, exclusive breastfeeding prevalence was 44.4%. Prevalence of continued breastfeeding at age of one year was 66.7% while 38.2% continued breastfeeding up to two years. The prevalence of bottle feeding was 46.3% among children aged 0.0-23.9 months. Milk feeds given included formula milk and sweetened condensed milk.

In terms of timing of introduction of complementary feeding, 85.7% infants aged 6.0-8.9 months had been given complementary foods (Table 3). Common staple foods fed as complementary foods included rice porridge, rice and sago (soft paste made up of sago flour and water).

The proportion of children (6.0-23.9 months) achieving MDD was 76.6%. The study shows that 41.5% were fed at least four food groups, while another 23.4% consumed five food groups. Nonetheless,

Table 2. Mean weight (kg), mean length (cm) and nutritional status by age group and sex (n=121)

Variable	*Age group (months)*	n (%)	*Boys (n=64)*			*Girls (n=57)*			*p-value between sexes*[†]
			n	*Mean±SD*	*(95% CI)*	*n*	*Mean±SD*	*(95% CI)*	
Weight (kg)	0.0 – 5.9		14	5.71 ± 0.84	(5.23 – 6.20)	13	4.92 ± 1.37	(4.10 – 5.75)	0.080
	6.0 – 11.9		14	7.49 ± 0.91	(6.97 – 8.02)	11	7.07 ± 0.94	(6.44 – 7.70)	0.270
	12.0 – 23.9		36	9.46 ± 1.15	(9.07 – 9.85)	33	8.89 ± 1.43	(8.38 – 9.40)	0.165[‡]
	0.0 – 23.9		64	8.21 ± 1.85	(7.70 – 8.70)	57	7.64 ± 2.10	(7.10 – 8.20)	
Length (cm)	0.0 – 5.9		14	58.9 ± 3.6	(56.8 – 60.9)	13	56.6 ± 4.9	(53.7– 59.6)	0.325[‡]
	6.0 – 11.9		14	67.4 ± 4.1	(65.1 – 69.8)	11	66.8 ± 2.8	(64.9 – 68.7)	0.691
	12.0 – 23.9		36	77.9 ± 4.8	(76.3 – 79.6)	33	75.9 ± 5.5	(73.9 – 77.9)	0.108
	0.0 – 23.9		64	71.5 ± 9.0	(69.2 – 73.7)	57	69.8 ± 9.4	(67.3 – 72.2)	
WAZ	0.0 – 5.9		14	-1.26 ± 1.00	(-1.84 – -0.68)	13	-1.11 ± 0.85	(-1.63 – -0.60)	0.688
	6.0 – 11.9		14	-1.34 ± 0.94	(-1.89 – -0.80)	11	-1.48 ± 0.99	(-2.14 – -0.81)	0.727
	12.0 – 23.9		36	-1.57 ± 0.91	(-1.88 – -1.26)	33	-1.37 ± 1.00	(-1.72 – -1.02)	0.383
Underweight (WAZ < -2SD)		36 (29.8)							
LAZ	0.0 – 5.9		14	-1.72 ± 1.16	(-2.39 – -1.05)	13	-1.19 ± 1.18	(-1.90 – -0.48)	0.254
	6.0 – 11.9		14	-1.53 ± 1.24	(-2.25 – -0.82)	11	-1.74 ± 0.83	(-2.30 – -1.18)	0.572[‡]
	12.0 – 23.9		36	-2.03 ± 1.18	(-2.43 – -1.63)	33	-1.83 ± 1.23	(-2.27 – -1.39)	0.500
Stunting (LAZ < -2SD)		52 (43.0)							
WLZ	0.0 – 5.9		14	0.17 ± 0.80	(-0.29 – 0.64)	13	-0.38 ± 1.17	(-1.09 – 0.32)	0.158
	6.0 – 11.9		14	-0.53 ± 1.20	(-1.21 – -0.17)	11	-0.69 ± 1.07	(-1.40 – 0.03)	0.729
	12.0 – 23.9		36	-0.80 ± 0.96	(-1.12 – -0.47)	33	-0.61 ± 0.98	(-0.95 – -0.26)	0.244[‡]
Wasting (WLZ < -2SD)		7 (5.8)							

[†]independent t–test
[‡]independent Mann-Whitney U test

Table 3. Infant and young child feeding practices

Achievement of IYCF indicators	*n*	*Percentage (%)*	*Criteria for IYCF indicator*
Breastfeeding	79	65.3	Proportion of children 0-23 months of age breastfed
Exclusive breastfeeding under 6 months	12	44.4	Proportion of infants 0-5 months of age fed exclusively with breast milk
Continued breastfeeding at 1 year	12	66.7	Proportion of children 12-15 months of age who are fed breast milk
Continued breastfeeding at 2 years	13	38.2	Proportion of children 20-23 months of age who are fed breast milk
Introduction of solid, semi-solid and soft foods	12	85.7	Proportion of infants 6-8 months of age who receive solid, semi-solid or soft foods
Minimum dietary diversity (MDD)	72	76.6	Proportion of children 6-23 months of age who receive foods from 4 or more food groups
Minimum meal frequency (MMF)	78	83.0	Proportion of breastfed and non-breastfed children 6-23 months of age who receive solid, semi-solid or soft foods (but also including milk feeds for non-breastfed children) the minimum number of times or more
Minimum acceptable diet (MAD)	52	55.3	Proportion of breastfed and non-breastfed children 6-23 months of age who receive a MAD
Iron-rich or iron-fortified foods (IRF)	73	77.7	Proportion of children 6-23 months of age who receive an IRF
Number of food groups given			
0	2	2.1	
1	2	2.1	
2	9	9.6	
3	9	9.6	
4	39	41.5	
5	22	23.4	
6	9	9.6	
7	2	2.1	
Food groups given			
Grains, roots and tubers	91	96.8	
Legumes and nuts	5	5.3	
Dairy products	45	47.9	
Flesh foods	73	77.7	
Eggs	52	55.3	
Vitamin-A rich fruits and vegetables	80	85.1	
Other fruits and vegetables	35	37.2	
Children who received iron-rich foods (IRF)			
6.0-11.9 months (n=25)	16	64.0	
12.0-23.9 months (n=69)	57	82.6	
Total	73	77.7	

Table 4. Infant and young child feeding indicators by nutritional status

Indicator	*Weight-for-age (WAZ)*					*Length-for-age (LAZ)*					*Weight-for-length (WLZ)*				
	Under-weight (n=32)		*Not under-weight (n=62)*		x^2 *(p-value)*[†]	*Stunting (n=43)*		*Not stunting (n=51)*		x^2 *(p-value)*[†]	*Wasting (n=7)*		*Not wasting (n=87)*		x^2 *(p-value)*[‡]
	n	*%*	*n*	*%*		*n*	*%*	*n*	*%*		*n*	*%*	*n*	*%*	
MDD															
Yes	25	34.7	47	65.3	0.063	33	45.8	39	54.2	0.001	4	5.6	68	94.4	
No	7	31.8	15	68.2	(0.801)	10	45.5	12	54.5	(0.975)	3	13.6	19	86.4	(0.348)[‡]
MMF															
Yes	28	35.9	50	64.1	0.702	39	50.0	39	50.0	3.343	6	7.7	72	92.3	
No	4	25.0	12	75.0	(0.402)	4	25.0	12	75.0	(0.067)	1	6.2	15	93.8	(1.000)[‡]
MAD															
Yes	21	40.4	31	59.6	2.085	26	50.0	26	50.0	0.849	2	3.8	50	96.2	
No	11	26.2	31	73.8	(0.149)	17	40.5	25	59.5	(0.357)	5	11.9	37	88.1	(0.236)[‡]

[†]Independent chi-square test
[‡]Fisher's exact test
Abbreviation: MDD, minimum dietary diversity; MMF, minimum meal frequency; MAD, minimum acceptable diet

23.4% were given less than four food groups. For the type of complementary foods given, almost all (96.8%) had been given grains, roots and tubers. While 77.7% were given animal foods (meat, fish, poultry, liver or organ meats), 85.1% children received vitamin-A rich foods and vegetables. Almost half (47.9%) had been given dairy products and 55.3% had consumed eggs. However, the proportion of children fed fruits and vegetables was low (37.2%). Consumption of legumes and nuts was also low at 5.3%.

Achievement of MMF was high at 83.0% (overall for both breastfed and non-breastfed children). The study also revealed that 55.3% had achieved MAD indicator (overall for both breastfed and non-breastfed children). Consumption of IRF was high at 77.7%.

None of the indicators, MDD, MMF and MAD were significantly associated with underweight, stunting and wasting. Children who had achieved MDD showed wasting prevalence of 5.6%, compared with those who did not achieve MDD (13.6%) (Table 4). Children who did not achieve MAD had 11.9% prevalence of wasting compared to 3.8% for those who achieved MAD.

DISCUSSION

Achieving optimal nutritional status of children is important for healthy growth, development and has long term consequences on adult health. Effort to improve nutritional status of children is of global importance, as reflected in global targets, which include 40% stunting reduction in children below five, 30% reduction in low birth weight, reduction of childhood wasting to 5% and also 50% increase in breastfeeding. Targets to increase breastfeeding rates and reduce stunting and wasting prevalence in children below five are also found in the Sustainable Development Goals (GBD 2015 SDG Collaborators, 2016).

Global low birth weight prevalence has been reported at 15% in 2013 (UNICEF, 2013). A previous study reporting that small-for-gestational-age (SGA) and pre-term babies was associated with 4.5 times higher risk of stunting, points to the need for early intervention in order to address foetal growth restriction (FGR) and reduce childhood stunting (Christian *et al.*, 2013). Another study identified FGR (defined as being term and SGA) as the leading individual risk factor contributing to stunting in developing countries (Danaei *et al.*, 2016).

This study showed the Penan community having a high prevalence of low birth weight of 14.9%. Higher low birth weight rates of 18.1% and 16.2% was reported by Eunice *et al.* (2014) in Serian District, Sarawak, and Sarawak state, respectively.

The prevalence of stunting reported in this study was 43.0%, out of which 14.0% were severely stunted (HAZ < -3SD). Higher stunting rates were seen in older children, 46.3% amongst ages 12.0-23.9 months compared to 33.3% in ages 0.0-5.9 months. Prevalence of stunting among the Penans is double the stunting prevalence for children under 5 years in Malaysia (20.7%), and that reported in NHMS 2016 for Sarawak state (IPH, 2016). This study also found 5.8% wasting which is lower than that for Malaysia in general (11.5%) and Sarawak state (12.0%) (IPH, 2016). Underweight prevalence in this study was 29.8%, which is higher compared to Malaysian children below 5 years (13.7%) (IPH, 2016). Higher underweight and stunting prevalence in young Penan children may be attributed to high poverty and food insecurity in the Penans community.

Exclusive breastfeeding prevalence under 6 months in this study (44.4%) is slightly lower than the national prevalence of 47.1% (IPH, 2016). Generally higher exclusive breastfeeding rates were reported in South East Asia region, as 11.0% in Myanmar, 38.9% in

Indonesia, 33.7% in Vietnam and 60.0% in Cambodia (Dibley, Senarath & Agho, 2010). Continued breastfeeding among the Penans decreased from 66.7% at 1 year of age to 38.2% at 2 years old. This finding is in agreement with the global breastfeeding prevalence, which also showed a similar downward trend from 74% at 1 year of age to 46.3% at 2 years old (White *et al.*, 2017).

Recent reports on global estimates for complementary feeding practices indicated a third of infants 4-5 months old were already fed solid foods; whereas 20% of 10-11 months old had not started solid foods (White *et al.*, 2017). This report noted that achievement of MDD was low (28.2%) and suggested that continuous effort is needed to ensure more children benefit from optimal complementary feeding.

Achievement of introduction to solid, semi-solid and soft foods and minimum meal frequency indicators among the study subjects was high at 85.7% and 83.0% respectively. Previous studies conducted in Kuala Lumpur and Putrajaya also reported that 97.9% of infants were given timely introduction to solid food and 95.2% achieved MMF (Khor *et al.*, 2016). Minimum dietary diversity achievement for the Penan children was satisfactory at 76.6%, similar to that for Sarawak state (76.4%) as reported in NHMS 2016, whilst the national prevalence of MDD was 66.4% (IPH, 2016). Achievement of MAD was lower at 55.3% and slightly higher compared with MAD achievement for Malaysia which is 53.1% (IPH, 2016). Consumption of IRF among the Penans children was satisfactory at 77.7%. Overall, the qualitative aspects of complementary feeding of young Penan children appear satisfactory based on the use of the WHO (2008) indicators.

This study did not find significant associations between the IYCF indicators and nutritional status. Besides IYCF practices, several other factors may cause undernutrition in children (Jones *et al.*, 2014). Furthermore, use of the IYCF indicators might not reflect usual intake (Saaka *et al.*, 2015) and does not capture portion sizes or amounts of food given or fat intake of children. Furthermore, poor physical and environmental sanitation are contributing factors to malnutrition in children (Schmidt, 2014; Bentley *et al.*, 2015).

Nutrition education is one of the components of the community feeding programme for Penan children. In light of current findings, namely higher prevalence of malnutrition among infants and young children, nutrition education should be strengthened. Nutrition education of the Penans needs to be context-specific, taking into consideration the current IYCF practices and availability of local complementary foods. Further studies on environmental factors and household food insecurity of the Penan community are needed to identify factors associated with malnutrition among the young children.

Limitations of study

A major limitation encountered in this study is the language barrier, as interviews with the Penan mothers had to be conducted in the Malay language. However, whenever necessary, Kayan language was used if the mothers had difficulty in understanding Malay. Translation in the process may have altered the meaning of some local words or terms. The indicators used depended on the mother's ability to recall and this posed challenges in some instances.

CONCLUSION

A high prevalence of poor nutritional status was recorded among the Penan children. While the IYCF indicators apparently showed satisfactory complementary feeding practices, future studies should assess quantitatively the intake of complementary food among Penan infants and young children. In light of the high rate of low birth weight in

the Penan community (14.9%), nutrition education is needed for pregnant women to ensure healthy weight gain of both mother and child. Further studies to be conducted among Penan children should include assessment of household food insecurity and evaluation of environmental factors which may contribute to child malnutrition.

Acknowledgements

The authors express their deepest gratitude to all mothers, and their children who participated in this study, Belaga District Office and Ministry of Health Malaysia.

Author's contributions

BMW, conceptualised and designed the study, conducted the study, conducted data collection, data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript; NAK, conceptualised and designed the study, conducted the study, conducted data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript; IMN, conceptualised and designed the study, conducted the study, conducted data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript.

Conflict of Interest

The authors declare no conflict of interest in undertaking this study.

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Mal J Nutr 24(4): 551-558, 2018

High prevalence of undernutrition among preschool children in Pattani Province, southern Thailand

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ABSTRACT

Introduction: Malnutrition is one of the leading causes of childhood death. In the southern provinces of Thailand that are encountering a civil conflict, the extent of malnutrition among young children has not been adequately reported. **Methods:** A cross-sectional study was undertaken to assess the nutritional status of children aged 2-5 years enrolled in early childhood development centres (ECDC) in Pattani Province. A total of 112 ECDCs were randomly selected and 871 children who met the inclusion criteria were recruited. Anthropometric measurements were taken and nutritional status determined according to World Health Organization (2006) growth standards. Statistical analyses of nutritional indicators, namely wasting, stunting, underweight and Composite Index of Anthropometric Failure (CIAF) across socio-demographic variables were carried out. Binary logistic regression models, based on the different nutritional indicators, were used to determine the relationships between undernutrition status and associated factors. **Results:** Prevalence of wasting (7.7%), stunting (19.6%) and underweight (16.8%) were determined. Based on CIAF, 27.4% of the children were undernourished. Logistic regression odds showed that prevalence of all forms of undernutrition was statistically independent of sex and place of residence. Religion was associated with all four indicators of undernutrition. Stunting was associated with child's age and religion, while underweight and CIAF were associated with the child's age, religion and mother's occupation. **Conclusion:** Prevalence of undernutrition was estimated to be high in comparison with the national average figure. The study findings highlighted the need for more effective nutrition promotion activities to alleviate undernutrition problems among young children in Pattani Province.

Keywords: Nutrition status, preschool children, southern Thailand

INTRODUCTION

People who are well nourished and cared for from early childhood enjoy optimal growth, health, and wellbeing (Wang & Stewart, 2013). Undernutrition is one of the leading underlying causes of childhood illness and death (Rice *et al.*, 2000). Food choices and eating patterns developed during early childhood can help evade undernutrition, restrained growth, short-term nutrition problems, along with preventing non-communicable diseases (Wang & Stewart, 2013).

The burden of malnutrition (undernutrition or overnutrition) are of public health concern in Thailand

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(Winichagoon, 2013). In the southern border provinces, the population is predominantly Muslim, in a largely Buddhist dominated nation. Since 2004, low-level separatist violence had occurred in the region (UNICEF, 2006). As a result, socio-economic, health services and education have been adversely affected. Maternal and child health status lags behind the national average, contributing to higher maternal and infant mortality rates (UNICEF, 2006). About 74.3% of the children in Pattani province attend early childhood development centres (ECDC) (NSO & UNICEF, 2017). However, there is a lack of data on the nutritional status of children aged 2 to 5 years.

For the measurement of the overall prevalence of undernutrition, Svedberg (2000) suggested an aggregate indicator, namely the Composite Index of Anthropometric Failure (CIAF). The CIAF is made up of typical anthropometric indicators, including wasting, stunting, underweight, and their combinations into seven categories. The CIAF provides an additional measure for assessing malnutrition and serves as an alternative to assessing wasting, stunting and underweight as separate measures (Nandy *et al.*, 2005; Svedberg, 2000). Savanur & Ghugre (2015) found that CIAF could identify more undernourished children in the slums of Mumbai city than the conventional indices.

The aim of this study was to assess the nutrition status of children aged 2-5 years in Pattani Province. Both conventional anthropometric indices as well as the CIAF were used.

MATERIALS AND METHODS

Study design

The study design was cross-sectional with random cluster sampling. Each of the early childhood development centres (ECDCs) under the local administrative office was a cluster unit.

Study population

The study area was Pattani Province, Thailand, which is one of the southern provinces adjacent to the Thai-Malaysian border. The study population was children attending ECDC in Pattani Province. A total of 165 ECDCs with 9,520 children was enrolled in the 2017-2018 academic year. Children with physical disability and children below 2 years or above 5 years of age were excluded.

Sample

The sample size of the study was calculated using 16.7% prevalence of stunting (NSO, 2012) with relative desired precision of 0.03 and 1.3 design effect. Adding a 15.0% non-response rate, the sample size (n) was calculated to be 889. Cluster sampling was used to determine an equal number of children from each ECDC, serving as a cluster. The lowest number of children in the ECDC of Pattani province was eight. The number of clusters, was determined by dividing the total sample size (889) by the lowest number of children in one ECDC (8). In this way, a total of 112 ECDCs was computed and they were randomly selected without replacement. Eight children in each selected centre were selected randomly using a random number table.

Ethics approval

The ethical committee of Prince of Songkla University, Pattani campus, approved the study. Certificate of approval letter was received on 8th June 2017 with record number psu. pn 1-020/60. Prior to the field visit, the heads of the ECDCs were informed about the purpose of the study and procedures of data collection. Written consent was obtained from the head of each ECDC for the measurement of the selected children.

Data collection

Anthropometric measurements were taken by trained enumerators using calibrated instruments. Standardisation of measurements among the enumerators was conducted for accurate and precise measurements. Tools used for the measurements were weighing scale (Seca weighing scale, capacity: 2.0-150.0 kg, precision: 0.1 kg), child height-length measurement board (one-side tape, precision: 0.1 cm). Measurements were made to the nearest 0.1 cm and 0.1 kg for height and weight, respectively. Working in a group, two enumerators followed standard procedures in taking height and weight measurements. Due to socio-religious reasons, all the children were measured wearing similar light clothing. In order to account for the clothing, 0.1 kg was subtracted from all the weight measurements during data analysis. Date of birth was obtained from the ECDC register.

Outcome measures

The World Health Organization (WHO) child growth standard 2006 was used for the classification of anthropometric indices. Wasting reflects acute undernutrition with weight-for-height z-score value < -2 standard deviations (SD) (< -3SD as severe wasting). Overweight is weight-for-height > 2SD. Stunting reflects chronic undernutrition with height-for-age z-score < -2SD (< -3SD as severe stunting). Underweight reflects weight-for-age < -2SD (de Onis, 2006; WHO, 2006). CIAF excludes those children without any forms of undernutrition (Table 1, Group A) and includes all wasted, stunted, or underweight cases, and their combinations (Table 1, Group names B-F and Y) (Nandy *et al.,* 2005).

Data analysis

Anthropometric data were converted to z-scores using WHO Anthro 3.2.2 software and statistical analyses were undertaken using R program 3.4.0. Statistical analyses of four nutritional indicators (wasting, stunting, underweight and CIAF) across age, sex, religion, residence and mother's occupation were carried out. Cross tabulations with Pearson's chi-square test were performed. Logistic regression was used to assess the effects of candidate factors on the wasting, stunting, underweight and CIAF separately.

RESULTS

Out of a total of 889 children initially included, 2.2% were excluded. The WHO flagged these cases as outliers with > ±5SD for wasting, ±6SD for stunting and < -6SD, and > +5SD for underweight. Among the remaining 871 children, 417 (47.9%) were males and 454 (52.1%) females, giving a sex ratio of 0.92. The mean age of the children was 38.8±7.2 months (ranging from 24.0-59.9

Table 1. Classification of children based on Composite Index of Anthropometric Failure (CIAF)

Category	*Description*	*Wasting*	*Stunting*	*Underweight*
A	No failure	No	No	No
B	Wasting only	Yes	No	No
C	Wasting and underweight	Yes	No	Yes
D	Wasting, stunting and underweight	Yes	Yes	Yes
E	Stunting and underweight	No	Yes	Yes
F	Stunting only	No	Yes	No
Y	Underweight only	No	No	Yes

Table 2. Nutrition status of children according to socio-economic factors

Factors	*N (%)*	*Wasting (%)*†		*Stunting (%)*†		*Underweight (%)*†		*CIAF (%) Failures*
		<-3SD	*<-2SD*	*<-3SD*	*<-2SD*	*<-3SD*	*<-2SD*	
Overall	871 (100.0)	1.4	7.7	4.1	19.6	3.0	16.8	27.4
Sex								
Male	417 (47.9)	1.9	8.2	4.6	19.4	2.9	15.6	26.4
Female	454 (52.1)	0.9	7.3	3.7	19.8	3.1	17.8	28.4
		p=0.717, df=1		p=0.95, df=1		p=0.424, df=1		p=0.551, df=1
Age of child‡								
2-3 Years	347 (39.8)	0.9	7.2	3.5	15.9	1.4	14.1	24.8
3-4 Years	420 (48.2)	1.4	6.9	2.6	18.8	2.4	16.2	26.4
4-5 Years	104 (11.9)	2.9	12.5	12.5	35.6	10.6	27.9	40.4
		p=0.145, df=2		p<0.001*, df=2		p=0.004*, df=2		p=0.006*, df=2
Religion								
Buddhist	73 (8.4)	0.0	0.0	0.0	8.2	0.0	4.1	11.0
Muslim	798 (91.6)	1.5	8.4	4.5	20.7	3.3	17.9	28.9
		p<0.019*, df=1		p=0.016*, df=1		p=0.004*, df=1		p=0.002*, df=1
Residence								
Urban	94 (10.8)	1.1	7.4	5.3	14.9	1.1	13.8	24.5
Rural	777 (89.2)	1.4	7.7	4.0	20.2	3.2	17.1	27.8
		p=1.000, df=1		p=0.277, df=1		p=0.509, df=1		p=0.575, df=1
Mother's occupation								
Government	88 (10.1)	2.3	4.5	2.3	11.4	1.1	5.7	14.8
Agriculture	76 (8.7)	3.9	11.8	3.9	28.9	5.3	23.7	38.2
Business	157 (18.4)	1.2	5.0	2.5	19.4	1.9	15.6	25.6
Private	321 (36.7)	0.6	7.2	5.0	19.7	3.1	17.2	27.2
Others	229 (26.1)	1.3	10.1	4.8	19.8	3.5	18.9	30.4
		p=0.151, df=4		p=0.091, df=4		p=0.023*, df=4		p=0.013*, df=4

*statistically significant difference at p<0.05; p-values are calculated using Chi-square test
†Children <–2SD include those with <–3SD
‡Mean age of children 38.8 months with SD 7.2

months). Almost all the children (91.6%) were Muslim. Most of the children (89.2%) were from rural areas. Table 2 summarises the distribution of the study subjects.

It was observed that 7.7% of the children were wasted, out of which 1.4% were severe cases. Stunting was observed in 19.6%, among whom 4.1% were severely stunted. Prevalence of underweight children was 16.8%, among them 3.0% were severely underweight. Based on CIAF, the total prevalence of undernutrition was 27.4%. Within this category, 13.5% children had a single anthropometric failure and 13.9% had multiple anthropometric failures. Overweight was observed in 2.5% children. Similarly, 2.9% children had weight-for-height and/or weight-for-age greater than +2 z-scores. Hence, the total prevalence of any form of malnutrition (both undernutrition and overnutrition) among the pre-school children was 30.3%.

Prevalence of all forms of malnutrition was statistically independent of sex or place of residence. The age group of children was associated with stunting (p<0.001), underweight (p=0.004) and

Table 3. Logistic regression for predicting nutrition status: undernourished vs. normal

Factors	*Wasting OR†(95% CI)*	*Stunting OR†(95% CI)*	*Underweight OR†(95% CI)*	*CIAF OR†(95% CI)*
Years				
2-3 Years (ref)	1.00	1.00	1.00	1.00
3-4 Years	0.95 (0.54, 1.67)	1.24 (0.85, 1.83)	1.17 (0.78, 1.75)	1.08 (0.78, 1.51)
4-5 Years	1.89 (0.92, 3.90)	3.09 (1.86, 5.12)*	2.42 (1.41, 4.14)*	2.13 (1.33, 3.43)*
Religion				
Buddhist (ref)	1.00	1.00	1.00	1.00
Muslim	-	3.38 (1.42, 8.03)*	5.79 (1.78, 18.8)*	3.69 (1.73, 7.89)*
Occupation				
Government (ref)	1.00	1.00	1.00	1.00
Agriculture	2.75 (0.80, 9.42)	3.04 (1.32, 7.01)*	5.00 (1.74, 14.35)*	3.53 (1.62, 7.53)*
Business	1.07 (0.31, 3.68)	1.78 (0.82, 3.86)	2.95 (1.08, 8.05)*	1.94 (0.97, 3.88)
Private	1.62 (0.54, 4.84)	1.83 (0.89, 3.75)	3.38 (1.30, 8.75)*	2.13 (1.12, 4.05)*
Others	2.32 (0.77, 6.97)	1.77 (0.84, 3.73)	3.68 (1.40, 9.69)*	2.44 (1.26, 4.73)*

†OR represents adjusted odds ratio
*Statistically significant difference at $p<0.05$; p-values are calculated using Wald's test

CIAF (p=0.006). Religion was associated with all four indicators of undernutrition. Mother's occupation was associated with underweight (p=0.023) and CIAF (p=0.013) only. Table 2 shows the details of the nutritional status of the study children.

Based on CIAF, a binary logistic analysis showed that the Muslim children was more vulnerable to undernutrition (OR: 3.69, 95% CI: 1.73, 7.88) than Buddhist children. Compared to 2-3 years old children, children aged 3-4 years (OR: 1.08, 95% CI: 0.78, 1.51) and 4-5 years (OR: 2.13, 95% CI: 1.33, 3.43) were more likely to be undernourished based on the CIAF. The possibility of undernutrition was low among children having their mother working in the government service, as opposed to being involved in agricultural occupations (OR: 3.53, 95% CI: 1.62, 7.53). Table 3 shows the details of logistic regression results of socio-economic factors on wasting, stunting, underweight and CIAF separately.

DISCUSSION

Prevalence of malnutrition

Prevalence of wasting, stunting and underweight was estimated to be 7.7%, 19.6% and 16.8%, respectively. Wasting is often due to insufficient feeding or illness during a recent period, while stunting is generally associated with inadequate feeding and/or frequent illnesses over a prolonged period of time. Underweight reflects both acute and/or chronic undernutrition (Nandy *et al.,* 2005).

The prevalence of wasting, stunting and underweight were higher in the studied area than the national average of 5.4%, 10.5% and 6.7% respectively (MICS, 2017). This finding may be attributed to the fact that almost all the health and developmental indicators in the southern provinces were worse than the national average figures (UNICEF, 2006).

Based on CIAF, the total prevalence of undernutrition was 27.4%, which is relatively higher than the individual

malnutrition indicators. Similar findings for relatively higher CIAF results were reported in surveys undertaken in different parts of low middle income and low income countries like India (Goswami, 2016; Gupta, Sharma & Choudhary, 2017), Bangladesh (Khan & Raza, 2016) and Ethiopia (Endris, Asefa & Dube, 2017).

In our study, no significant difference in prevalence of any form of undernutrition was related to the sex of the children. In a civil conflict environment, such as in the southern Thai provinces, male children have been reported to be more vulnerable, at higher risk of morbidity and more likely to exhibit effects of prolonged undernourishment (Asfaw *et al.*, 2015; Pei, Ren & Yan, 2014). In contrast, in some places where patriarchal cultural practices are prevalent, female children might be more vulnerable (Gangadharan, 2011). However, the young children of both sexes in this study suffer from poor nutritional status.

Significant positive associations were found between age and prevalence of stunting, underweight and CIAF. These children face increased likelihood of undernutrition as they grow older. Similar results were reported in west China (Pei *et al.*, 2014), Bangladesh (Khan & Raza, 2014) and Ethiopia (Endris *et al.*, 2017). These findings suggest poorer care and provision of adequate food among older children.

The majority of people in the southern Thai provinces are Muslim. Our study observed that religion was significantly associated with all four indices of undernutrition. Muslim children were found to be more vulnerable to undernutrition than those of the Buddhist faith. A similar finding was reported by a study in Bangladesh (Chowdhury *et al.*, 2016). Socio-economic, educational and cultural factors may influence feeding practices of young children in affecting their nutritional status.

In our study, place of residence was not found to be associated with any form of undernutrition. This might be because most of the children were from rural areas. Also, there are no significant differences in the infrastructure, family income, socio-culture, and the lifestyle of the population in the urban and rural areas in Pattani Province. In contrast, a study in Iran reported a higher prevalence of undernutrition in urban areas than in rural areas, because of the rapid pace of urbanisation, high poverty and hunger rates (Kavosi *et al.,* 2014).

While no significant association was found between prevalence of undernutrition (wasting and stunting) and mother's occupation, the CIAF showed significant association with mother's occupation. Children from an agricultural background were found to be more vulnerable to undernutrition. This may be attributed to generally low earnings associated with agricultural occupations.

Limitations of study

The study offers a snapshot of the study population and therefore does not provide sufficient data for an understanding of underlying causes and mechanisms of undernutrition. The study focussed on pre-school children in the ECDC facilities. Information on variables which might affect the nutritional status of the children, like eating patterns in households, care, socio-economic conditions and water sanitation and hygiene conditions were not assessed. The strength of this study is that the findings from this effort could contribute to a better understanding of the magnitude of undernutrition in the unrest provinces in southern Thailand.

CONCLUSION

The estimated prevalence of undernutrition (especially stunting and underweight) was high among ages 2-5 years in Pattani Province in

comparison with the national average figures. It is recommended that the local administration, health institutions and school authorities of the province include routine monitoring of the nutritional status of young children. Nutrition promotion should be implemented with greater focus on vulnerable Muslim children.

Acknowledgements

The authors would like to express their gratitude to the Prince of Songkla University, Faculty of Science and Technology, for financing this study. We also appreciate the contributions of all the data collectors and respondents of the study.

Authors' contributions

SS, principal investigator, conceptualised and designed the study, led the overall data collection, prepared the draft of the manuscript and reviewed the manuscript; LC, conceptualised and planned the study, advised on the study design, data collection, analysis and interpretation, and reviewed the manuscript; AL, advised on the data analysis and interpretation and reviewed the manuscript.

Conflict of interest

Authors have no conflict of interest.

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Mal J Nutr 24(4): 559-566, 2018

Maternal factors associated with vitamin A concentration in colostrum of postpartum mothers in South Sulawesi Province, Indonesia

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ABSTRACT

Introduction: Newborns have low vitamin A reserves and rely on breastmilk to meet their vitamin A needs. This study aimed to determine factors associated with vitamin A concentration in colostrum of rural postpartum mothers. **Methods**: Out of 180 postpartum women interviewed at seven Community Health Centers in South Sulawesi Province, 160 who met the inclusion criteria were included in the study. Breastmilk (colostrum) samples were obtained shortly after delivery from the breast that was not full and not fed to the infant for at least 30 min. Breastmilk samples of 3-5 mls were collected into sterile plastic tubes using a manual pump. The samples were immediately placed in an icebox and transported to the laboratory within six hours after collection. Vitamin A concentrations were determined using HPLC method. Socioeconomic characteristics, anthropometric measurements, gestational age, and dietary intake of mothers (24-hour recall) were obtained. Bivariate and multiple linear regression analysis were undertaken to determine factors associated with vitamin A concentration in colostrum. **Results:** Dietary assessment showed unsatisfactory intake levels of calories, fat, protein, vitamin A, iron and zinc compared to the recommendations for Indonesia. Mean vitamin A concentration in colostrum was 58.2 μg/dl, and about 81.2% of the participants had normal colostrum vitamin A concentration. Iron intake and gestational age were significantly associated with colostrum vitamin A concentrations. **Conclusion:** Majority of the postpartum mothers had normal colostrum vitamin A concentration. Maternal dietary intake including iron is important to ensure adequacy of vitamin A in breastmilk.

Keywords: Breastmilk vitamin A, colostrum, postpartum mother

INTRODUCTION

Vitamin A deficiency remains a public health problem in developing countries affecting women of reproductive age, children and pregnant women (WHO 2011; Ayah *et al.,* 2007). More than 7.2 million pregnant women in developing countries are considered vitamin A deficient, whereas 13.5 million are considered to have a low vitamin A status (Gogia & Sachdev, 2010).

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Vitamin A plays an important role in vision, growth, physical development and immune functions (Fujita *et al.,* 2011). Vitamin A deficiency increases the risk of night blindness and other vision disorders, such as xerophthalmia. Vitamin A also helps maintain maternal health during pregnancy and lactation (Bahl *et al.,* 2002). There is a close relationship between vitamin A deficiency and an increase in the occurrence of diarrhoea as well as death in children. Furthermore, a low vitamin A status is related to high incidences of other diseases, such as dysentery, measles and acute respiratory infections (Imdad *et al.*, 2016).

Infants born to vitamin A-deficient mothers have an increased risk of vitamin A deficiency later in life (Klemm *et al.,* 2008; Rotondi & Khobzi, 2010). The infant is protected from vitamin A deficiency through breastfeeding when the vitamin A level in breastmilk is adequate. Vitamin A concentration in breast milk reaches its optimum level in the first 21 days postpartum, namely in the colostrum in the first 4–6 days and in the transitional milk thereafter (WHO, 2011).

In Indonesia, several studies have shown that the mean vitamin A concentration in breastmilk is relatively low according to World Health Organization (WHO) standards (< 20.0 µg/dl) (Dijkhuizen *et al.,* 2001; Permaesih & Rosmalina, 2008; Permaesih, 2009; Permaesih, Rosmalina & Tanumihardjo, 2014). Dietary intake of vitamin A among Indonesian women was only one-third of the recommendation (Cahyanto & Roosita, 2013). The Indonesian Basic Health Research in 2010 reported that one out of two post-partum women received vitamin A supplementation (Kemenkes, 2010), which was lower than the coverage of vitamin A supplementation of infants.

Various factors, such as food intake, maternal nutritional status, age, parity and pregnancy duration are known to modulate the secretion of vitamin A in breastmilk (Campoos, Paixao & Ferraz, 2007; Mello-Neto *et al.,* 2009). Moreover, micronutrient interactions, such as between iron and zinc, may also modify the levels of vitamin A in the body. Research shows that iron deficiency is associated with low plasma retinol levels and increased hepatic vitamin A (Oliveira *et al.,* 2008). The purpose of the present study was to determine maternal factors associated with vitamin A concentrations in colostrum.

MATERIALS AND METHODS

This cross-sectional study was conducted in seven Community Health Centers (Puskesmas) in Gowa District between July and November 2017. Gowa is about 20 km from Makassar, the capital city of the South Sulawesi Province.

At the screening stage, 187 pregnant women were interviewed, out of whom, 160 who met the inclusion criteria were included in the study. These criteria were delivery with singleton pregnancy between 37 and 40 weeks of gestation, normal birth weight (≥ 2500 g), vaginal delivery and maximum parity of three. Meanwhile, exclusion criteria were diagnosed human immunodeficiency virus (HIV) infection, diabetes mellitus, hypertension and severe mental disorder. The information about postpartum disease (especially related to diseases in the exclusion criteria) was obtained by asking the mother directly or based on previous diagnosis by health personnel.

A questionnaire was developed to obtain information regarding socio-economic characteristics, pregnancy history, and lactation duration among the participants. Dietary intake of the mothers was assessed using a 24-hour recall questionnaire. Dietary intake data was then analysed using Nutrisurvey

2007 software and the findings were compared with the recommended dietary allowance (RDA) for Indonesia (Kemenkes, 2013). Anthropometric data and breastmilk samples were collected after the interview. Body weight was measured using a SECA digital weighing scale (Model SECA 813, Hamburg, Germany), while a microtoise tape was used to measure height (GEA medical SH-2A, Germany), following which body mass index (BMI) was computed. The anthropometric measurements were performed on lightly clothed barefooted participants.

Breastmilk samples were obtained a few days after delivery, as it is known that colostrum reaches a maximum level by the third day. Before taking breastmilk, the participant was told to clean the breasts with a clean wet cloth. Milk was taken from the breast that was not full and was not used for feeding the infant for at least 30 min for sampling consistency. Breastmilk of 3-5 ml was collected from each mother using a manual pump between 9am-12pm. The breastmilk samples were collected into sterile plastic tubes, which were immediately placed in an icebox and transported to the laboratory within six hours of collection. In the laboratory, the samples were stored at –20°C. Breastmilk vitamin A analysis was performed using high-performance liquid chromatography (HPLC) method (Esposito *et al.,* 2017) at the Integrated Nutrition Laboratory of the Health Research and Development Agency in Bogor, Indonesia.

Univariate data analysis was performed to describe the distribution of each research variable. Bivariate analysis was conducted using Pearson correlation test and multiple linear regression analysis was performed to determine the factors associated with colostrum vitamin A concentrations. A *p*-value of <0.05 was considered significant.

This study was approved by the Human Research Ethics Committee under the Research and Community Service Institute of Bogor Agriculture University (No. 01/IT3.KEPMSM-IPB/SK/2017). Informed consent was obtained from the participating women before the commencement of the study.

RESULTS

Mean maternal age of the mothers was 26.9 years, with more than one-third aged 20–25 years (43.1%) (Table 1). The average duration of education was 9.9 years with about half of them (48.8%) having completed formal education for at least 12 years.

Most of the participants were housewives (91.9%). On average, mothers had parity of 2.1 and majority (72.5%) were in the multiparous category (parity ≥ 2). Most of the infants were born at 37 weeks of gestation (78.0%). Mean BMI of the mothers was 23.1 kg/m^2, with 17.5% overweight, 13.8% obese and 9.4% underweight.

Mean vitamin A concentration in colostrum was 58.2 µg/dl. More than two-thirds (81.2%) had normal colostrum vitamin A concentration with 18.8% having a low concentration.

Dietary intake assessment showed mean fat and protein intakes were low, at 41.1 g and 56.7 g per day, or achieving respectively 52.3% and 74.1% of the RDA for Indonesia. Mean calorie intake was 1448 kcal or about 57.2% of the RDA. As for micronutrient intake, mean vitamin A intake per day was 508.0 µg or 59.7% of the RDA. Maternal vitamin A intake was shown to be higher than that for zinc and iron. Mean zinc and iron intakes were 5.8 µg and 6.3 mg per day, or 38.6% and 19.7% of the RDA, respectively.

Bivariate analysis showed that only iron intake of the mothers was significantly related to vitamin A concentrations in the breastmilk samples (Table 3). Intake of vitamin A

Table 1. Characteristics of postpartum mothers

Maternal characteristics	*n*	*%*	*Mean±SD*
Age (years)			26.9±4.9
20 – 25	69	43.1	
26 – 30	48	30.0	
31 – 35	43	26.9	
Education (years)			9.9±3.3
< 9	46	28.7	
9 – 11	36	22.5	
≥ 12	78	48.8	
Occupation status			NA
Unemployed / housewife	147	91.9	
Work	13	8.1	
Parity (number)			2.1±0.7
1	44	27.5	
2	64	40.0	
3	52	32.5	
Gestational age (weeks)			37.5±1.1
37	126	78.0	
38	5	3.1	
39	7	4.4	
40	22	13.8	
Body mass index (kg/m^2)			23.1±3.5
Underweight (<18.5)	15	9.4	
Normal (18.5 – 24.9)	95	59.4	
Overweight (25.0 – 26.9)	28	17.5	
Obese (≥27.0)	22	13.8	
Breastmilk vitamin A (µg/dl)			58.2±44.8
Low (≤ 30.0)	30	18.8	
Normal (>30.0)	130	81.2	

Table 2. Dietary intake of vitamin A, zinc, fat and protein among postpartum mothers

Intake per day	*Mean*	*S.E*	*Minimum - Maximum*	*%RDA*[†]
Calorie intake (kcal)	1448.2±402.2	31.79	701.7 -2196.3	57.2
Fat intake (g)	41.1±23.5	1.85	6.3 – 89.6	52.3
Protein intake (g)	56.7±18.8	1.49	19.5 – 95.0	74.1
Vitamin A intake(µg)	508.0±286.0	22.61	12.2 – 989.4	59.7
Zinc intake (µg)	5.8±2.3	0.18	2.1 – 18.0	38.6
Fe intake (mg)	6.3±2.9	0.23	2.4 – 20.9	19.7

[†]RDA = recommended dietary allowance (Kemenkes, 2013)

and zinc, as well as fat and protein intake did not show significant correlation with colostrum vitamin A concentration.

Multiple linear regression analysis showed that, among the studied factors, only maternal iron intake (coefficient beta=3.091; p=0.048) and gestational age (coefficient beta= –6.994; p=0.046) were significantly associated with colostrum vitamin A concentrations.

Table 3. Bivariate analysis of factors related to vitamin A concentrations in colostrum

Factors	*r*	*p*
Body mass index	-0.007	0.926
Vitamin A intake	-0.047	0.554
Zinc intake	-0.043	0.591
Iron intake	0.163	0.040*
Fat intake	-0.128	0.108
Protein intake	-0.049	0.540
Maternal age	0.096	0.226
Parity	0.047	0.552
Gestational age	-0.115	0.147

*Significant at $p<0.05$,
r: coefficient correlation, *p*: significance level

Table 4. Multiple linear regression analysis of factors related to vitamin A levels in colostrum

Factors	*Coefficients Beta*	*S.E*	*95% CI*	*p*
Fe intake	3.091	2.81	-2.478 to 8.662	0.048*
Gestational age	-6.994	3.45	-13.778 to -0.111	0.046*
Constant	322.104	139.49	46.468 to 597.74	0.022*

*Significant at $p<0.05$, $R^2=0.075$

The latter negative finding indicates that as gestation age prolongs, vitamin A concentration in breast milk decreases. Moreover, as the R^2 value obtained in the study was low at 0.075, indicating that only 7.5% of the variations in the colostrum vitamin A concentration could be explained in a linear manner by the study model, which includes the factors shown in Table 3. Thus, there are several other factors that could potentially influence colostrum vitamin A concentration of the study participants.

DISCUSSION

In the studied sample of mothers, iron intake and gestational age at delivery were significantly related to vitamin A concentrations in colostrum. The result indicates the importance of adequate maternal dietary intake of iron, and possibly other macro- and micronutrients in influencing vitamin A concentrations in breast milk. Weekly iron supplementation of Indonesian mothers during pregnancy increased vitamin A concentration in breastmilk (Muslimatun *et al.,* 2001). Iron deficiency can alter the metabolism of vitamin A, leading to a decrease in the activity of retinyl ester hydrolases, or an increase in retinol sequestration to the liver (Oliveira *et al.,* 2008).

There was no association between maternal age and vitamin A concentration in breastmilk. However, Mello-Neto *et al.* (2009) had previously reported a positive association between maternal age and vitamin A levels in breastmilk. This difference in findings could be because of a wider age range among postpartum mothers in the previous study (16–41 years) than in the present study of 20–35 years.

High parity has been shown to be associated with low fat levels in breastmilk that can ultimately affect vitamin A levels in breastmilk (Muslimatun *et al.,* 2001). However, there was no observed relationship between parity and vitamin A levels in breastmilk in this study. This finding is in agreement with the study of Panpanich *et al.* (2002), which reported that parity was not associated with

vitamin A concentrations in serum or breastmilk.

Generally, vitamin A concentration in breastmilk is quite high in the first 21 days after delivery (colostrum breastmilk for 4–6 days and transition breastmilk for 7–21 days) (WHO, 2011). The current study found that the mean vitamin A concentration in colostrum was 58.2 µg/dl. This value is slightly lower than that among Brazilian mothers reported by by Lira *et al.* (2011) (60.0 µg/dl), but higher than that of another study in Brazil (46.8 µg/dl) (Grilo *et al.,* 2015).

Vitamin A intake is essential during pregnancy and throughout the breastfeeding period, and it plays a vital role in the healthy development of the foetus and new born, particularly lung development and maturation (Strobel, Tinz & Biesalski, 2007). In this study, there was no observed association between vitamin A or zinc intake and vitamin A levels in breastmilk. This is in line with a research conducted by Deminice *et al.* (2018) which found no correlation between maternal vitamin A intake and levels of vitamin A in breast milk and blood serum. In contrast, we found that low iron intake was associated with low vitamin A levels in breastmilk. Mothers in the study showed iron intake that was about one-fifth of the RDA. Iron deficiency has been shown to be associated with reduced serum vitamin A levels and increased hepatic vitamin A levels (Oliveira *et al.,* 2008).

The percentage of body fat in breastfeeding mothers may influence the vitamin A levels in breastmilk, considering that fat is required for the transport of vitamin A and other fat-soluble vitamins. However, excess body fat may negatively affect body vitamin A concentration. Adipose tissue in obesity is reported to synthesise retinol binding protein (RBP) that is released into circulation not bound to retinol (Mills, Furr & Tanumihardjo, 2008).

In the present study, BMI was measured instead of body fat composition, and this may be one of the study limitations. Other limitations were that several factors related to vitamin A levels in breastmilk were not assessed in this study, owing to limited resources to conduct this study.

CONCLUSION

Most postpartum mothers in this study had normal colostrum vitamin A concentrations. Future studies should include more factors, including dietary intake of micronutrients, and body fatness, for a better understanding of the influencing variables on micronutrients in breast milk.

Acknowledgement

Researchers would like to thank those who have contributed in this research: SEAMEO RECFON Jakarta and Indonesian Ministry of Research, Technology and Higher Education for their financial support.

Authors' contribution

AS, designed and conceptualised the study, conducted analysis and interpretation of data, drafted the article, and approved final version to be published; DB, designed, conceptualised the study and drafted the article; DM, designed, conceptualised the study and drafted the article; ART, designed and conceptualised the study, conducted analysis and interpretation of data; AIA, conducted analysis and interpretation of data and conceptualized the content; LAAW, conceptualised the content.

Conflict of interest

All authors declared no conflict of interest in conducting this study.

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Mal J Nutr 24(4): 567-574, 2018

Fruit consumption and associated determinants in a sample of young urban Malaysian adults

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ABSTRACT

Introduction: Adequacy of fruit intake contributes to an individual's health including reducing the risk of non-communicable disease. This study aimed to assess consumption of fruits in various forms and to determine associated factors and barriers. **Methods:** In this cross-sectional study, a total of 300 adults aged 20-39 years were purposely recruited from several urban locations in the Klang Valley. Consumption of fruits in the past 12 months was assessed using a self-administered food frequency questionnaire (FFQ), assisted with pictures of serving size of fruits. Anthropometric measurements were taken and body mass index and waist circumference computed. **Results:** Fruit intake among young adults was lower (1.6±1.0 servings/day) than the Malaysian Dietary Guideline 2010 of ≥ 2 servings/day. Only 32.3% consumed fruits as recommended, with women consuming significantly more fruits than men. Preferred fresh fruits were red apple, banana and papaya. Consumption of fruit juice was associated with increase in waist circumference (R^2=0.261, p=0.027) after adjustment for age, sex, ethnicity, education level and marital status. Fruit intake showed no significant association with other anthropometric measurements. Sensory appeal, perceived health benefit, easy to prepare and influence of family were the main determinants of fruit intake, whilst affordability and availability were the major barriers. **Conclusion:** Fruit consumption among young adults in this study was lower than the recommendation for daily fruit intake. Studies with larger sample size are suggested to verify the finding of significant association between fruit juice consumption and risk of abdominal obesity.

Keywords: Fruit, fruit juice, abdominal obesity, barriers, young adults

INTRODUCTION

Low consumption of fruits is among the top five leading risk factors of global burden of diseases worldwide (Lim *et al.*, 2013). The Malaysian Dietary Guidelines 2010 recommended at least 2 servings of fruit (160g) daily (NCCFN, 2010). This practice reduces the risk of major non-communicable diseases (NCD) such as cardiovascular disease and certain types of cancer (WHO, 2014), and likely reduces body weight (Slavin & Lloyd 2012). Adequacy of fruit intake is associated with optimal

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intake of micronutrients, dietary fibre and phytochemicals which are vital for health. Despite these evidence of health benefits associated with regular fruit consumption, fruit intake among Malaysian adults has been reported to be low (IPH, 2015).

According to the National Health and Morbidity Survey (NHMS) 2015, 90.1% of Malaysian adults did not meet the recommendation of daily fruit intake, and the highest prevalence of inadequate intake was among men and young adults (IPH, 2015). Lifestyle habits of young adults during their transitional stage of life (including pattern of food intake) may have long term health implications (Spanos & Hankey, 2010). Adequate consumption of fruits may contribute toward reducing the risk of chronic diseases at a later stage of life.

Apart from fresh fruits, fruit juice, dried and preserved fruits are also included in guidelines for fruit intake. The French Dietary Guidelines 2008 and American Dietary Guidelines 2010 recommended that fruit juice should be consumed at no more than half of the total recommended daily fruit servings (Drewnowski & Rehn, 2015; USDA & HHS, 2010).

Socio-demographic factors such as age, sex, ethnicity, level of education and income are important predictors of fruit consumption (Yen & Tan, 2012). In Nepal, individuals from high socio-economic status were more engaged with healthy and nutritionally balanced diets than those from low socio-economic status (Nepal, Bohara & Gawande, 2011). Following education on the importance of fruit intake, subjects showed a modest increase in fruit intake (Wagner *et al.* 2016). However, other studies reported that health campaigns and interventions on increasing fruit intake were insufficient to bring about sustainable change in fruit consumption (Duthie *et al.*, 2017). Interactions of factors influencing fruit consumption should be determined (Krølner *et al.*, 2011).

This study was aimed at assessing consumption of fruits in different forms, associated factors and barriers in a sample of Malaysian young adults.

MATERIALS AND METHODS

This cross-sectional study was conducted with enrolment of subjects based on ethnicity of Malaysian population distribution 2016. Young Malaysian adults aged 20-39 years were invited to participate. Excluded from the study were pregnant women, ages outside 20-39 years, and individuals participating in intervention programmes that may alter their habitual diet. Recruitment made use of print advertising, social media engagement and professional networking. Six institutions, including private and government organisations, from several urban locations in the Klang Valley were randomly selected. The study was conducted from June 2016 to February 2017.

A validated questionnaire consisting of socio-demographic data, medical history, fruit consumption pattern and food frequency questionnaire (FFQ) (107 items) for fruit intake was self-administered by the subjects. Measurement of fruit intake for the past 12 months, either as fresh, dried or juice using the FFQ was recorded according to the amount consumed and the average frequency of consumption, ranging from daily, weekly, monthly and annual consumption. Subjects were assisted with pictures of serving size of fruits. The original portion size of each fresh, juice and dried fruit was translated into recommended serving size according to the Malaysian Dietary Guidelines (NCCFN, 2010) and aided by the Atlas of Food Exchanges & Portion Sizes (Shahar *et al.*, 2009). The total amount

consumed per day was then calculated by multiplying the number of servings by the frequency of fruit intake.

Blood pressure, body weight and height for computation of body mass index (BMI), waist circumference (WC) and body fat percentage were determined by trained enumerators. Waist circumference was categorized according to World Health Organisation, WHO (2008). Body fat percentage was measured using Body Composition Analyzer (Tanita TBF 300, Germany) and categorized based on WHO (1995).

Data were analysed using IBM SPSS Statistics for Windows version 21.0 (IBM Corporation, New York, NY, USA). Descriptive statistics, independent sample t-test, one-way ANOVA and multiple linear regressions were used for data analysis. Significant value was set at $p<0.05$.

This study was approved by the Universiti Kebangsaan Malaysia Research Ethics committee (NN-2016-032). Informed consent was obtained from all subjects before commencement of the study.

RESULTS

Majority of subjects were female (62.7%), with a mean age of 26±5 years. The subjects were predominantly Malay (68.0%) followed by Chinese (23.4%), Indian (7.3%) and others (1.3%). Most of them were single (72.3%). Their highest education level was STPM/A-level/ foundation (pre-university) with mean monthly income of ≤ RM1,000 (Table 1). More than half reported monthly earnings of ≤ RM2,000.

Mean fruit intake was 1.6±1.0 serving per day, with 32.3% consuming fruits ≥ 2 servings/day. Female subjects consumed significantly more fruits (1.7±1.0 serving/day) than men (1.5±1.0 serving/day). Among the ethnic groups, highest mean intake of fruits was among Malay subjects (1.6±1.0 serving/day). In general, the subjects preferred to consume fresh fruits (1.3±0.8 serving/ day) compared to fruit juices (0.2±0.3 serving/day) and dried fruits (0.1±0.3 serving/day). Frequently consumed fresh fruits were red apple, banana and papaya, whilst the most preferred dried fruits were dates, raisin and mango. Orange, mango and watermelon juices were preferred by the subjects.

Body mass index, WC and body fat percentage showed no significant associations with mean daily intake of fruits (Table 2). However, consumption of fruit juice had a significant association with WC, wherein an increase of one unit of fruit juice consumption significantly increased 0.126 unit of WC. No significant association was found between consumption of fruit juice with BMI and percentage of body fat (Table 3).

Most subjects liked to eat fruits due to the sensory appeal (taste) (97.3%), for health benefits (96.3%), and to keep a healthy bowel movement (90.0%). Apart from that, more than half of the subjects (61.0%) reported avoiding consuming certain fruits. Common barriers for consuming fruits include costs (expensive) (57.9%), limited choices available (48.1%) and storage problems (45.9%). However, none of the barriers mentioned were found to have a significant influence on fruit intake (Table 4).

DISCUSSION

National surveys have reported a reduction in fruit consumption among Malaysian adults, decreasing from 14.6% in 2011 to 9.9% in 2015 (IPH, 2015). This study found the mean intake of fruits among young adults was lower than the recommendation of ≥ 2 servings/day by Malaysian Dietary Guidelines 2010 (NCCFN, 2010). About 67.7% of the subjects failed to meet the

Table 1. Consumption pattern of fruits (fresh, dried and juice) according to demographic profile of participants

Demographic factor	*Number of subjects, n(%)*	*Total fruit intake (servings/day), Mean±SD*
Age (mean=26±5 years)		1.6±1.0
Sex		
Male	112(37.3)	1.5±1.0*
Female	188(62.7)	1.7±1.0
Ethnicity		
Malay	204(68.0)	1.6±1.0[a]
Chinese	70(23.4)	1.5±0.9
Indian	22(7.3)	1.8±1.2
Others	4(1.3)	1.2±0.2[b]
Marital status		
Single	217(72.3)	1.6±1.0
Married	80(26.7)	1.7±1.0
Widowed/Divorced	3(1.0)	2.0±1.1
Education level		
Secondary school	21(7.0)	1.8±1.3
STPM/A-level/Foundation	112(37.3)	1.5±0.9
Diploma	49(16.3)	1.7±1.1
Degree	101(33.7)	1.6±1.0
Postgraduate	17(5.7)	1.9±0.9
Working status		
Worker	144(48.0)	1.7±1.1
Student	117(39.0)	1.5±1.0
Not working	10(3.3)	1.9±1.0
Income†		
<RM1000	131(43.7)	1.5±1.0
RM1001-2000	38(12.7)	1.7±0.2
RM2001-3000	55(18.3)	1.8±1.2
RM3001-4000	40(13.3)	1.5±0.9
>RM4000	36(12.0)	1.6±1.0

*Mean difference using independent t-test significant at $p<0.05$

[a,b] Different alphabets within the same column for the same factor indicate significant difference (one-way ANOVA) at $p<0.05$

†RM1.00 = USD4.10

Abbreviation: STPM, *Sijil Tinggi Pelajaran Malaysia*

daily recommendation of fruit intake. This result is consistent with the finding of the NHMS 2015, whereby 90.1% of adults daily consumed inadequate amounts of fruits (IPH, 2015).

Consistent with the findings by Yen & Tan (2012), female subjects in this study consumed more fruits than the males. This might be due to social and cultural norms, in which most women have a greater interest in healthy diet as compared to men (Othman *et al.*, 2012).

Studies have reported that an increase in fruit consumption was associated with a reduction in adiposity among obese adults after 8 weeks of consumption of blueberries (Basu *et al.*, 2010). Weerts & Amoran (2011) reported significant weight loss within 3 months among overweight adults after increasing fruit consumption. While this study found no association between total fruit intake

Table 2. Consumption of fruits (fresh, dried and juice) based on anthropometric indicators

Anthropometric indicators	*Mean total of fruit intake (servings/day), Mean±SD*	*p-value*[†]
Body Mass Index (BMI), kg/m^2		
Underweight	1.5±0.7	0.808
Normal	1.6±1.0	
Overweight	1.6±1.1	
Obese	1.5±1.0	
Waist circumference (WC)[‡], cm		
Normal	1.6±1.0	0.497
Abdominal obesity	1.5±1.0	
Percentage of body fat[§], %		
Normal	1.6±1.0	0.862
Obese	1.6±1.0	

[†]Mean difference was calculated using one-way ANOVA and Games-Howell post hoc analysis for BMI and independent sample *t*-test for WC and percentage of body fat
[‡]Waist circumference cut-offs: ≥90 cm in men, ≥80 cm in women
[§]Body fat percentage cut offs: ≥ 25% in men, ≥ 35% in women

Table 3. Association between consumption of fruit juice and adiposity

Indicators	*Adjusted b (95%CI)*[†]	*t*	*p*	R^2
Body mass index BMI), kg/m^2	0.103 (-0.284, 3.733)	1.691	0.092	0.107
Waist circumference (WC), cm	0.126 (0.625, 10.201)	2.226	0.027[*]	0.261
Percentage of body fat, %	0.077 (-1.010, 6.047)	1.405	0.161	0.295

[*]Significant at $p<0.05$, multiple linear regression
[†]Adjusted for age, sex, ethnicity, education level, marital status

and BMI, WC and percentage of body fat, however, a significant association between fruit juice consumption and WC was shown, indicating that higher fruit juice consumption was related to greater WC. A similar finding was reported by Clemens *et al.* (2015). Naturally present sugar in fruit juice is absorbed and converted into fat, which builds up in the abdominal area (Walker, Dumke & Goran, 2014; Tappy *et al.*, 2010).

Both USDA & HHS (2010) and Malaysian Dietary Guidelines 2010 (NCCFN, 2010) advised that fruit juice consumption should be limited to one serving per day. Fresh fruits have been recommended as the best choice as it contains more nutritional value compared to fruit juice (Clemens *et al.*, 2015) and dried fruits (Holzwarth *et al.*, 2012), as their nutritional value may be degraded due to processing or thawing procedure.

Various factors including sensory appeal influence food choices (Saba *et al.*, 2010). Sensory appeal is a source of pleasure and also an important determinant of a person's food choices (Stewart-Knox *et al.*, 2015). In this study, individuals who enjoy eating fruits believe fruits help "keep a healthy bowel". Subjects who face less time constraints were more likely to eat more fruits. Time constraint among young adults with a busy lifestyle poses a barrier, and hence fresh fruit especially cut fruits was preferred by the subjects, as less preparation time is needed. Familiarity with the type of fruit tend to favour fruit consumption. Influence

Table 4. Determinants and barriers of fruit intake (fresh, dried and juice) among young adults

Factors	*Score*[†]	*Number of subjects, n(%)*	*Mean fruit intake (serving/ day)*	*p-value*
Determinants:				
Sensory appeal (taste)	1	3(1.0)	0.2±0.2[a]	0.049[*]
	2	5(1.7)	1.7±1.1	
	3	291(97.3)	1.6±1.0[b]	
Health benefits	1	3(1.0)	0.5±0.4	0.062
	2	8(2.7)	1.2±0.9	
	3	288(96.3)	1.6±1.0	
To keep a healthy bowel	1	8(2.7)	0.7±0.5[a]	0.009[*]
	2	22(7.3)	1.3±1.0	
	3	269(90.0)	1.7±1.0[b]	
Less preparation effort	1	22(7.4)	1.1±0.9[a]	0.019[*]
	2	32(10.7)	1.5±1.0	
	3	245(81.9)	1.7±1.0[b]	
Availability	1	36(12.0)	1.3±0.9	0.075
	2	35(11.7)	1.5±0.9	
	3	228(76.3)	1.7±1.0	
Influence of family	1	48(16.1)	1.3±1.0[a]	0.007[*]
	2	43(14.4)	1.4±0.8	
	3	207(69.5)	1.7±1.0[b]	
Cost (cheaper price)	1	81(27.1)	1.6±0.9	0.422
	2	70(23.4)	1.5±0.9	
	3	148(49.5)	1.6±1.1	
Health promotion campaign	1	104(34.8)	1.5±0.9	0.231
	2	91(30.4)	1.5±0.9	
	3	104(34.8)	1.7±1.1	
Barriers:				
Cost (expensive price)	1	50(27.3)	1.3±0.9	0.407
	2	27(14.8)	1.4±0.9	
	3	106(57.9)	1.6±1.0	
Storage	1	75(41.0)	1.6±1.0	0.538
	2	24(13.1)	1.5±1.1	
	3	84(45.9)	1.4±0.9	
Limited choice	1	68(37.2)	1.5±1.0	0.535
	2	27(14.8)	1.6±1.0	
	3	88(48.1)	1.4±0.1	
Eating outside	1	83(45.4)	1.6±1.0	0.161
	2	25(13.6)	1.5±0.8	
	3	75(41.0)	1.3±0.9	
Sensory appeal (taste)	1	93(50.8)	1.5±1.0	0.711
	2	24(13.1)	1.3±0.8	
	3	66(36.1)	1.5±0.9	
Not commonly consume by family	1	105(57.4)	1.5±0.9	0.523
	2	21(11.5)	1.5±0.9	
	3	57(31.1)	1.4±1.0	
Bloating	1	119(65.0)	1.6±0.9	0.056
	2	31(17.0)	1.1±0.8	
	3	33(18.0)	1.5±1.0	
Feeling cold	1	112(61.2)	1.5±1.0	0.441
	2	48(26.2)	1.3±0.9	
	3	23(12.6)	1.6±1.1	

[†]Score: 1= Disagree, 2= Neither agree nor disagree, 3= Agree

[a,b] Different alphabet denotes significant difference ($p<0.05$) using one-way ANOVA and Games-Howell post hoc analysis

[*] $p<0.05$

of family for food habits may lead a person to develop preferences or dislikes for foods including fruits (Giacalone & Jaeger, 2016). Factors such as attitude, habit, social influences and availability of fruits are factors that significantly affect the intention of individuals to consume fruits (Othman *et al.*, 2012). Recognising existing barriers of fruit consumption, such as affordability and availability, is key to enabling strategies and policies in encouraging fruit intake. An increase in fruit intake can also be achieved by a variety of approaches such as social marketing, approaches based on behavioural economics, and technology-based behaviour change models (Thomson & Ravia, 2011).

Limitations of study

Estimation of fruit intake was based on self-reported intake which depends on memory recall leading to bias and inaccuracies (Yaroch *et al.*, 2012). The nature of this cross-sectional study does not permit inference of causation. A strength of this present study is the inclusion of different forms of fruit, such as fresh fruit, fruit juice and dried fruit, all of which contribute toward daily servings of fruit intake.

CONCLUSION

Fruit consumption among young urban Malaysian adults was unsatisfactory, being lower than the recommended guidelines. Research should be undertaken to verify this study's finding of a significant association between fruit juice consumption and risk of abdominal obesity. It is also suggested that future studies include determining the link between fruit intake and associated blood biomarkers.

Acknowledgement

The authors would like to thank to all of the subjects and enumerators for their participation in this study. This study was funded by a grant from Universiti Kebangsaan Malaysia (Grant Number: GUP-2014-88).

Authors' contributions

All the authors involved in conceptualised and designed the study; BNAH, principal investigator, led the data collection in Klang Valley, analysing the data and prepared the draft of the manuscript and reviewed the manuscript; HMY, SS & ZAM assisted in drafting and reviewed the manuscript.

Conflict of interest

The authors do not have any conflict of interest to declare.

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Mal J Nutr 24(4): 575-585, 2018

Delivery of healthy lunch to worksites: a two weeks pilot study in a sample of working adults in Selangor, Malaysia

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ABSTRACT

Introduction: Lack of healthy food choices at the worksite is associated with unhealthy eating habits and poor diet quality. This study aimed to conduct a two weeks pilot study to assess the diet quality of lunch-meals delivered to worksites. **Methods:** Using a crossover study design, a total of 50 adults were purposely recruited from among university and hospital staffs in Selangor. Participants were randomised into two groups, whereby in week one, Group A was provided with the study's "healthy lunch-meals" (RD4U©) for 3 work days/week, while Group B consumed their usual lunch. In week two, the groups switched over with Group B receiving RD4U© lunch-meals for three days. Diet quality of the lunch meals was assessed using the Healthy Eating Index for Malaysian (HEI-M). Participant satisfaction for RD4U© service was determined using SERVQUAL. **Results:** Mean protein consumption was significantly higher (27±4 g/day) while fat consumption was significantly lower (18±5 g/day) for the RD4U© lunch-meals, compared to the respective levels (24±11 g/day; 22±12 g/day) for the usual lunch meals. Total HEI score of the RD4U© meals (61.9±9.2) was higher than that for the usual lunch meals (56.1±11.2). Nonetheless, the overall diet quality scores indicate that both RD4U© and usual lunch meals were in the "need improvement" category. Generally, participants were satisfied with the RD4U© lunch-meal service. **Conclusion:** The RD4U© lunch-meals showed potential in delivering healthy lunch to worksites, and feasibility studies to expand the RD4U© delivery service is recommended.

Keyword: Healthy food delivery service, diet quality, working adults

INTRODUCTION

Consumption of food away from home has become a norm in today's busy lifestyles. The demand for eating outside food has escalated with increased participation of women in the work force. Long hours of work and travelling time have also limited the time to prepare home meals (Bezerra & Sichieri, 2009). This phenomenon has led to increase

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in the availability and accessibility of outside food such as kiosks, hawkers' stalls and restaurants, to cater for growing consumer demand, especially in the urban population.

The Malaysian Food Barometer showed that approximately 64.1% Malaysians consume food away from home at least one meal per day (Poulain *et al.,* 2014). The Ministry of Domestic Trade, Co-operatives and Consumerism (2017) reported that the average household monthly expenditure on eating outside the home has increased to RM470 in 2016, compared to RM194 in 2009. In China, owing to economic changes and urbanisation since the 1970s, eating away from home has increased rapidly (Dong & Hu, 2010). Studies have shown that eating outside is associated with poor diet quality (Todd, Mancino & Lin 2010).

In Malaysia, the National Health Morbidity Survey (NHMS) (IPH, 2015) reported that nutrition-related problems such as obesity, cardiovascular disease, hypertension, hypercholesterolemia and diabetes affect almost half of Malaysian adults from all socio-demographic sectors, including civil servants. As reported by Soon *et al.* (2013), the prevalence of overweight and obesity among civil servants was 50.2%. Obesity reduces work productivity, quality of life, and also increases medical costs (Shrestha *et al.,* 2016).

The Malaysian Adult Nutrition Survey (MANS) highlighted that, among the underlying factors of increased risk of obesity, are lack of time to prepare meals, and tiredness after work to prepare meals (IPH, 2014). In addition, Ng & Suzana (2011) have also reported that adult workers generally chose to eat out during lunch at least five times a week. Most of the meals were high in sugar, fat, salt, oil and low in fibre (Todd *et al.* 2010). Although there are existing policies such Food Act 1983 (Act 281) and Food Regulations (1985) that need to be complied by food operators (i.e. providing clean and safe food, use of nutrition labeling and nutritional content) to protect the public from unhealthy nutritional practices, its effectiveness is questionable because the trend of providing unhealthy food menu still dominates the market.

While there is a positive inclination towards healthy food services, this tends to be confined to cities where the choices for healthy food may be limited (Lim, Esther & David, 2018). There are limited studies that evaluate the implementation of healthy food service at workplaces (Maes *et al.,* 2012). Thus, this study is aimed at determining the diet quality of lunch-meals delivered to workplaces and participant's satisfaction for such food delivery.

MATERIALS AND METHODS

Study design and data collection

An intervention study was conducted to determine the acceptability of a "healthy food" delivery service for lunch at selected workplaces using a cross-over intervention design. A total of 52 working adults were screened by purposive sampling at three locations selected by convenience in Bangi and Kajang, namely Universiti Kebangsaan Malaysia, Hospital Serdang and Kolej Universiti Islam Selangor (KUIS). The inclusion criteria were working adults aged 18-59 years, having lunch daily at the workplace, no known food allergies and agreed to purchase the lunch-meals provided by the researcher. Eligible participants were also required to complete a food diary during the study period. Those who did not complete the food diary were excluded from the study. Fifty participants completed the study. Information sheets of the study were

distributed and the signed consent forms were obtained from all the participants prior to this study.

The participants were randomised into two groups. In week one, Group A was provided with the study's "healthy lunch", named Right Diet For You (RD4U©) lunch-meals on Monday to Wednesday, while Group B was told to consume their usual lunch. In the following week, the groups switched over such that Group B received the RD4U© lunch-meals on Monday to Wednesday, whereas Group A consumed their usual lunch. Every participant was offered a choice of three sets out of six of the RD4U© lunch-meals. The RD4U© lunch-meals offered choices from these food groups: cereals/grains, meat/fish, vegetables, fruits and mineral water. Three sets of the RD4U© lunch-meal cost RM50.00 (about US$12.25). Each RD4U© meal was freshly prepared and delivered to the participants at their work place.

A food diary was given to every participant to self-record his/her daily lunch-meal intake on Monday to Wednesday each week during the 2-weeks study period. The participants were asked to estimate their food intake using household measures such as plates, cups, glass, bowls, ladles and spoons. The completed food diaries were collected on the last day of the study. The researchers discussed the returned food diaries with the participants through face to face interviews to ensure the reports given were clear and accurate.

Food intake data was analysed using the Nutritionist Pro™ (Version 4.0) by Axxya System which can produce energy and macronutrients based on the Malaysia Food Composition Table (Tee *et al.,* 1997). All food and beverages data were then evaluated using the Healthy Eating Index (HEI). The study was conducted between September 2016 to April 2017.

Anthropometric assessment

Weight of the participants was measured using the calibrated TANITA (Model TBF-300, Japan) digital weighing scale, recorded to the nearest 0.1 kg. Height was obtained using SECA Stadiometer (Model SECA 213, Germany) scale, measured to the nearest 0.1 cm. The Body Mass Index (BMI) of the participants was calculated using the formula weight (kg) divided by height squared (m^2).

Recruitment of healthy food delivery operators

A module called Right Diet For You (RD4U©) was developed and patented by the researcher as a guide for use in study. The module consisted of 10 chapters (namely introduction, healthy eating messages, food safety, hygiene, handling and delivery operation, healthy food preparation, food labelling and packaging, online business, product marketing, customer satisfaction and financial management). The contents of the module were contributed by eight professionals from four fields involving nutrition and dietetic, food quality and safety, foodservice management and food entrepreneurship.

Eight food delivery operators (known as RD4U© food operator) comprising housewives, low income individuals and single mothers aged 30-49 years were selected and trained by professional dietitians and chefs. The training of two weeks duration included a comprehensive theoretical and practical aspects of food handling, food quality and safety, nutrition labels, entrepreneurship and healthy food preparation of healthy lunch-meal packages. The training was conducted in a central community kitchen in Bangi, Selangor.

Healthy lunch-meals intervention

Six types of "healthy lunch-meals"

were developed based on the Malaysia Healthy Plate Model (MOH, 2016), which recommended 500-600 kcal/day for lunch or 28% of total daily calorie intake, and appropriate intake from healthful food groups. The meals were prepared and delivered by the trained operators.

Diet quality assessment

Diet quality for each participant was assessed using the Healthy Eating Index for Malaysian (HEI-M) that was developed and validated by Lee, Norimah & Safiah (2011) for adults in Malaysia. The HEI-M consists of a Food Group and a Nutrient Group. The former comprises seven components, namely cereals and grains, vegetables, fruits, milk and dairy products, meat, poultry and eggs, fish and legumes. The Nutrient Group consists of two components, namely total fat and total sodium (Table 1). Each component is given a score ranging from 0-10, which indicates the extent of compliance with recommendations. The HEI provides a composite score of 100, obtained by the formula (total score of nine components / 9 x 10) (Lee *et al.*, 2011). Overall score of the HEI was classified into three categories of diet quality, namely <51 (poor), 51-80 (needs improvement) and >80 (good).

Participants' satisfaction assessment

The participants' satisfaction with the RD4U© meals, was determined using a SERVQUAL questionnaire adapted and validated by Joung *et al.* (2011). This questionnaire consisted of 18 questions, whereby questions 1 to 16 were about participants' satisfaction towards the food and service offered (e.g. presentation, overall taste, texture, amount of vegetables and meat/fish, food temperature, meal diversity, portion size, menu variety, service satisfaction, appearance, smile/kindness, attitude, timeliness of delivery, helpfulness and overall satisfaction). Each aspect involves a 5-point scale (1-very dissatisfied to 5-very satisfied). As for questions 17 and 18, participants were asked about when was the food consumed after the meals were received, and whether they had intention to continue with the delivery service.

Statistical analysis

Data analysis was carried out with IBM© Statistical Package for Social Sciences (SPSS©) Statistic version 20.0 software (SPSS, Inc. Chicago, IL, USA). Significance was interpreted at $p<0.05$. Mean, standard deviation and percentage were used for descriptive data on socio-

Table 1. Healthy Eating Index for Malaysian adults based on 2000 kcal/day†

HEI Components	*Range of score*	*Maximum score 10*	*Minimum score 0*
Food Groups			
Cereals and grains	0 - 10	6 servings/day	0 serving
Vegetables	0 - 10	3 servings/day	0 serving
Fruits	0 - 10	2 servings/day	0 serving
Milk and dairy products	0 - 10	2 servings/day	0 serving
Meat, poultry and egg	0 - 10	1 serving/day	0 serving
Fish	0 - 10	1 serving/day	0 serving
Legumes	0 - 10	1 serving/day	0 serving
Nutrient Groups			
Total fat	0 - 10	≤ 30% total energy intake	≥ 35% total energy intake
Sodium	0 - 10	≤ 2000 mg	≥ 4200 mg

†Source: Lee *et al.* (2011)

demographic characteristics, energy and macronutrient intake, HEI score and service quality. The comparison in total energy and macronutrient intake were performed using paired *t*-test between (usual meals) and (RD4U© meals). Whilst, HEI scores were performed using paired Wilcoxon test as distribution was not normally distributed. The one-way ANOVA test was used to determine the relationship between HEI scores and BMI status.

This study was approved by the research ethics committee, Universiti Kebangsaan Malaysia (UKM 1.21.3 / 244 / NN-2016-062).

RESULTS

Socio-demographic characteristics of participants

A total of 50 working adults participated in this study. Majority of the participants were Malay (98%) with a mean age of 39.4±9.7 years, with tertiary education (84%), working in the government sector (94%), and 38% earning monthly income of more than RM5000 per month. About

Table 2. Sociodemograpic characteristics of participants

Characteristic	*Participants (n=50)*	
	n	*%*
Gender		
Male	10	20
Female	40	80
Age (years)		
18 – 39	24	48
40 – 59	26	52
Race		
Malay	49	98
Chinese	1	2
Religion		
Muslim	49	98
Buddhist	1	2
Marital Status		
Single	18	36
Married	32	64
Educational Status		
Secondary School	8	16
Tertiary (Diploma and above)	42	84
Employment		
Government institutions	47	94
Private institutions	1	2
Others	2	4
Income (monthly)		
≤ RM 3000	14	28
RM 3001 - 5000	17	34
> RM 5000	19	38
BMI†		
Underweight/Normal	19	38
Overweight	21	42
Obese	10	20

†BMI classification: <24.9 kg/m^2 (Underweight/Normal), 25 – 29.9 kg/m^2 (Overweight), >30 kg/m^2 (Obese)

two-thirds (62%) of them were overweight and obese (Table 2).

Lunch intake of calories and macronutrients

The mean energy intake from the healthy lunch-meals (RD4U©) was 528±86 kcal/day, while that from the participants' usual meals was 537±217 kcal/day respectively (Table 3). Protein consumption was significantly higher (27±4 g/day vs 24±11 g/day), while fat consumption was significantly lower (p<0.05) for the RD4U© meals (18±5 g/day), compared to the usual lunch meals (22±12 g/day).

Lunch diet quality

The total HEI score of the RD4U© meals (61.9±9.2) was higher than that for the usual lunch meals (56.1±11.2) (p<0.001). Nonetheless, these overall diet quality scores indicate that both the intervention and usual lunch meals were in the "need improvement" category.

The mean score of the vegetable component was higher (8.1±1.5) for the RD4U© meals as compared to usual lunch meals (4.8±.7) (p<0.001). Likewise, the mean scores of the meat (p<0.05) and fish components for the RD4U© meals were significantly higher than for the usual lunch meals. Consumption of dairy products showed the lowest score followed by the legume components, whereby both these components did not meet the dietary recommendations of two and one serving per day, respectively.

The diet quality of the lunch-meals was also compared according to the BMI status of the participants (Table 4). Participants in the normal BMI category recorded a significant higher (p<0.05) total mean HEI for the RD4U© meals. Likewise, for all categories of BMI, the participants showed a significantly higher HEI score for the vegetable component (p<0.001) from the RD4U© lunch meals, than from the usual lunch meals.

Table 3. Energy and macronutrients from usual lunch and *RD4U©* lunch meals

Components	*Lunch Intake*		*p-value*	*Recommended Nutrient Intake, RNI (%)*
	Usual Meals (n=50) (M±SD)	*RD4U© Meals (n=50) (M±SD)*		
Macronutrients				
Energy (kcal/day)	537±217	528±86	0.973	
% energy†	29.8±12.1	29.3±4.8	0.786	100%
Carbohydrate (g)	60±26	66±12	0.076	
% energy	46.0±10.9	50.3±4.5	0.015*	50 – 65%
Protein (g)	24±11	27±4	0.031*	
% energy	17.8±4.5	20.8±1.6	0.001***	10 – 20%
Fat (g)	22±12	18±5	0.042*	
% energy	35.7±9.3	29.8±5.8	0.001***	25 – 30%

†Based on 1800 kcal/day

*p<0.05, **p<0.01, ***p<0.001 significant using paired t-test

Table 4. Comparison of total HEI scores and components between usual lunch meals and RD4U© meals according to BMI categories

Components	Total (n=50)		Underweight/ Normal (n=19)			Overweight (n=21)			Obese (n=10)			p-value§
	M±SD	p-value	M±SD	p-value	Mean Diff (M±SD)	M±SD	p-value	Mean Diff (M±SD)	M±SD	p-value	Mean Diff (M±SD)	
Total HEI Score†												
Usual lunch meals	56.1±11.2	<0.001***	52.8±13.3	0.017*	7.7±12.8	55.6±8.8	0.056	5.5±12.5	63.4±8.5	0.377	2.4±8.2	0.519
RD4U© Meals	61.9±9.2		60.6±9.4			61.1±9.4			65.8±8.0			
Cereals and grains												
Usual lunch meals	7.8±1.9	0.856	7.9±2.1	1.000	1.2±1.0	7.9±1.9	0.983	1.1±1.1	7.4±2.0	0.646	2.1±1.6	0.060
RD4U© Meals	7.9±1.8		7.9±1.7			7.9±2.0			7.8±1.8			
Vegetables‡												
Usual lunch meals	4.8±2.7	<0.001***	3.7±2.9	<0.001***	4.4±2.3	5.2±2.3	<0.001***	2.9±1.9	5.9±2.9	<0.021*	3.5±2.7	0.114
RD4U© Meals	8.1±1.5		8.0±1.5			7.7±1.7			9.1±1.1			
Fruits												
Usual lunch meals	6.7±4.0	0.320	5.8±4.1	0.795	2.9±1.9	6.6±4.1	0.632	3.5±2.9	8.5±3.4	0.128	3.5±2.7	0.741
RD4U© Meals	6.2±2.5		5.9±2.3			6.3±2.5			6.7±3.1			
Milk and dairy products												
Usual lunch meals	1.4±2.3	0.454	1.4±2.7	0.379	1.1±1.7	1.1±1.8	0.379	1.5±1.7	1.9±2.4	0.345	1.2±1.9	0.718
RD4U© Meals	1.3±1.9		1.4±2.1			1.2±1.7			1.1±2.2			
Meat, poultry and egg‡												
Usual lunch meals	9.0±2.6	0.033*	9.1±2.5	0.144	1.0±2.5	8.5±3.2	0.066	1.5±3.2	9.9±0.2	0.285	0.5±1.0	0.656
RD4U© Meals	9.9±0.6		9.9±0.5			10.0±0.0			9.5±1.0			
Fish‡												
Usual lunch meals	8.2±3.0	<0.001***	8.1±3.3	0.080	1.1±2.5	8.0±3.0	0.017*	1.8±2.9	8.5±2.4	0.102	1.5±2.4	0.712
RD4U© Meals	9.6±1.8		9.2±2.5			9.7±1.4			10.0±0.0			
Legumes												
Usual lunch meals	3.8±4.1	0.706	3.4±3.9	0.878	3.0±3.8	3.8±4.3	0.436	4.3±4.1	4.4±4.1	0.944	6.2±4.0	0.136
RD4U© Meals	3.4±4.3		3.2±4.2			2.8±3.8			5.0±5.3			
Total fat												
Usual lunch meals	2.7±3.6	0.139	2.2±3.4	0.221	1.4±4.9	3.0±4.1	0.381	0.7±4.6	3.2±3.4	0.779	0.6±5.7	0.879
RD4U© Meals	3.7±4.2		3.6±4.1			3.8±4.3			3.8±4.7			
Sodium												
Usual lunch meals	6.1±3.4	0.278	5.9±3.4	0.433	0.6±3.5	5.8±3.5	0.811	0.2±3.1	7.2±3.3	0.173	1.1±3.0	0.764
RD4U© Meals	5.6±3.0		5.4±2.4			5.6±3.5			6.2±3.2			

†Paired *t*-test analysis with significant at *p<0.05, **p<0.01, ***p<0.001
‡Paired Wilcoxon test analysis with significant at *p<0.05, **p<0.01, ***p<0.001
§One-way Anova test analysis

Participant satisfaction for the RD4U© lunch-meals and delivery service

The mean scores for all the service quality attributes were above 4, except for temperature (3.78±0.62) (Table 5). Personality attributes of the delivery staff, such as smile and kindness expressed, showed the highest mean score of 4.62±0.49. The second highest score was for attitude of delivery staff and food presentation (4.58±0.50). Overall, the results indicated that, participants were highly satisfied with the overall taste, service and satisfaction of the healthy lunch-meal delivery services (RD4U©) with scores of 4.32±0.55, 4.52±0.54 and 4.56±0.50 respectively.

Almost all the participants (92%) were willing to continue to receive this service. Only 8% of participants said 'no' because of their own impending retirement plans, However, half of the participants (56%) said 'no' to immediately consuming the meal upon receiving it. This was due to work barriers and for the fact that the meals were delivered an hour earlier before lunch time.

Table 5. Service Quality Attributes†

Attributes	*Service Quality Score‡ (M±SD)*
Food Quality	
Presentation	4.58±0.54
Texture	4.18±0.48
Vegetables	4.34±0.66
Meat/Fish	4.46±0.58
Temperature	3.78±0.62
Meals diversity	4.38±0.53
Portion Size	4.24±0.55
Is operator offered menu variety	4.18±0.56
Delivery staff	
Appearance	4.48±0.54
Smile and kindness	4.62±0.49
Attitude	4.58±0.50
Responsiveness	
Does food delivered on-time	4.42±0.61
Do deliverer help you (e.g. bring food to your place)	4.52±0.61
Satisfaction	
Overall taste	4.32±0.55
Overall service	4.52±0.54
Overall satisfaction	4.56±0.50
Behavioral intention	*n* (%)
Will you return to buy this product in the future? (yes/no)	
Yes	46 (92%)
No	4 (8%)
Do you eat immediately after the meal received?	
Yes	22 (44%)
No	28 (56%)

†Adapted: Joung *et al.* (2011)

‡Range of scores for the level of service quality from 1 to 5, where; 1 (very dissatisfied), 2 (dissatisfaction), 3 (moderate), 4 (satisfied), 5 (very satisfied)

DISCUSSION

Office workers are often associated with sedentary lifestyle, long working hours with heavy workload and restricted time constraint, leading to consumption of fast food and convenience food at the workplace. Office workers require better quality diet to support their work productivity, quality of life and to prevent non-communicable disease (NCDs). Thus, accessibility to healthy food at the workplace is a way for office workers to gain access to better diet quality.

More women than men expressed interest to subscribe to the healthy food delivery offered in this study. This may be due to women being generally more conscious about eating healthy, which is consistent with findings from other studies (Arganini *et al.,* 2012 & Glorioso *et al.,* 2018). In addition, lack of time during work day prevents them to prepare lunch for work (Raulio *et al.*, 2008).

While the overall quality of the RD4U© lunch meals was found in the "need improvement" category based on the HEI, significantly higher scores were shown for the RD4U© lunch meals than for the usual lunch meals, especially for the vegetables and fish components. Therefore, healthy lunch delivery may be a solution to increase the consumption of fruits and vegetables among office workers. However, the intake of fruits and vegetables among the workers in this study is still below the recommended intake of two and three servings per day respectively as recommended by Malaysian Food Pyramid (NCCFN 2010). These findings show similarities to the MANS study that reported that fruit and vegetable consumption among Malaysian adult population needs to be improved (IPH, 2014).

Convenience and ready-to-eat foods are most preferred among workers leading to poor diet quality, especially intake of micronutrients (Blanck *et al.*, 2009; Neckerman, 2014). A comprehensive review found that delivery meals improved diet quality and increased nutrient intake among older adults (Zhu & An, 2013).

The RD4U© lunch meals contain less fat due to the use of healthier meal preparation methods, including use of healthy ingredients (i.e. less oil, low-fat of dairy products, and less fat from meat) and cooking by baking, grilling and steaming.

Intake of dairy products and legume components were found least satisfactory among the HEI food groups, as they did not meet the recommended servings. Incorporating dairy and legumes in the main and side dishes in the RD4U© lunch meals may increase workers consumption of these food components.

This present study showed that most participants were highly satisfied with the RD4U© food delivery service, except for the temperature of the packed food. This indicates that the participants preferred to receive their lunch meals warm. Temperature control is an important aspect in the delivery process. The use of food warmer helps to maintain the meal temperature. Food operators should give more attention and effort in controlling meal temperatures during delivery, planning routes and delivery times, and also providing clear usage instructions on the food labels to the consumers.

Limitations of the study

The limitations of this study were that there were fewer male participants and almost all participants were Malay. Thus, the HEI data could not be compared among male and female workers. Similarly, because of the small number of other main ethnic groups (i.e. Chinese and Indian), we were not able to compare the findings among the different ethnic groups. Caution should

be exercised in the interpretation of the HEI-M results as the tool is designed to calculate the entire day's diet quality based on Malaysian Food Pyramid and Malaysian Dietary Guidelines.

CONCLUSION

The RD4U© lunch-meals showed potential in delivering healthy lunch to worksites to promote healthy dietary habits and in improving diet quality among the workers. Feasibility studies to expand the RD4U© delivery service is recommended.

Acknowledgement

The researcher would like to thank all the participants, staff of Community Rehabilitation and Ageing Research Centre (HCARE) Universiti Kebangsaan Malaysia, Bandar Seri Putra Residents Association and Ultimate Modern Mum's Initiative (UMMI), Bangi who were involved in this study and to the enumerators who helped in data collection. This study was supported by the internal fund of Universiti Kebangsaan Malaysia with the Code No: UKM 1.21.3 / 244 / NN-2016-062.

Authors' contribution

MKNK, conducted the study, drafted and reviewed the manuscript; ZAM, supervised the study, advised on the data analysis and interpretation; and reviewed the manuscript; SS, supervised the study, advised on the data analysis and interpretation; and reviewed the manuscript; AFML, supervised the study, advised on the data analysis and interpretation and reviewed the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Mal J Nutr 24(4): 587-596, 2018

Food consumption and dietary diversity of women in transmigrant area Buol, Central Sulawesi and original location Demak, Central Java, Indonesia

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ABSTRACT

Introduction: The transmigration scheme of the Indonesian government was aimed at easing overpopulation in Java by moving people to less populated areas. This study investigated food consumption and dietary diversity of women from the original location and transmigration rice farming areas. **Methods:** Food intake using a single 24-hour dietary recall was determined among women of reproductive age in Demak, Central Java (original location) and Buol, Central Sulawesi (transmigrant area). Food taboos were investigated using focus group discussions (FGDs). Dietary diversity and its score were obtained from 387 and 121 women in Demak and Buol, respectively, while 38 women from both areas participated in four FGDs on food taboos. **Results:** On average, women from both study areas had low dietary diversity scores, especially among lactation mothers. Rice, swamp cabbage, spinach and *tempeh* were popular in both areas. Fruits and vegetables from own cultivation or collected as wild foods were consumed in Buol, while women in Demak consumed comparatively more purchased foods (fruits and vegetables, legumes, meat and fish). Fewer types of food was avoided during pregnancy and lactation in Buol. **Conclusion:** While some food intake behaviour was similar in both study areas, differences were observed as influenced by economic and environment factors. Food intake of Demak women was influenced by household purchasing power, while Buol women depended on own grown food and gatherings from nearby forest. As dietary diversity scores in both areas was low, nutrition interventions are suggested to improve maternal food intake during pregnancy and lactation.

Keywords: Transmigration, women, dietary diversity

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INTRODUCTION

Nutrition plays an important role in maternal and child health, whereby optimum nutrition in early life is the foundation for future health. Maternal nutritional requirements increase during pregnancy and lactation period, not only for her own well-being, but also for healthy growth and development of the child (Black *et al.*, 2008). One of the indicators of maternal dietary adequacy is dietary diversity (Kennedy, Ballard & Dop, 2011). During pregnancy and lactating period, many nutritious foods are restricted because of traditional beliefs globally, including Asia (Banu *et al.*, 2016; Gao *et al.*, 2013; Mohamad & Ling, 2016). Food taboos tend to limit women's dietary intake (Asi & Teri, 2016; Gadegbeku *et al.*, 2013; Mohamad & Ling, 2016) and may result in inadequate weight gain of the pregnant women.

Transmigration in Indonesia refers to the movement of state-sponsored migrants since 1969 from densely inhabited regions to dispersed periphery settlements of the country (Arndt, 1983). Transmigration provides dietary acculturation affecting local food habits. Migrants, including transmigrants are known to retain intake of their traditional foods, and/or adopt the diets of the local people (Satia-Abouta, 2003; Rosenmöller *et al.*, 2011; Novotny & Rumalatu, 1995).

This study aimed to obtain information on food consumption and dietary diversity of Javanese women, living in a transmigrated area as second generation (Buol, Central Sulawesi), and Javanese women living in the original location of the transmigrans (Demak, Central Java).

MATERIAL AND METHODS

This report was based on data from two cross-sectional survey projects targeting Javanese women farmers. The first study was carried out among women living in Demak, Central Java in December 2014, while the second study was conducted among women farmers in Buol, Central Sulawesi, a transmigrated intervention area of World Agroforestry Centre (also known as International Centre for Research in Agroforestry, ICRAF) in December 2015.

Study sites and subjects

Prior to the study, we identified that the most vulnerable group as reported by the Indonesian Ministry of Health (MoHRI) was farmers, with the highest proportion of malnourished women and children, based on adult body mass index (BMI) and stunting, respectively (MoHRI, 2013). Demak has high rice productivity (the sixth highest in 2013 in Central Java province, and one of the Indonesian rice buffer areas), but it had the seventh highest prevalence of child malnutrition in the province. The inhabitants of Demak are mostly Javanese and comprised mostly rice-farming households. Eight villages in Karanganyar, seven villages in Dempet and one village in Gajah were categorised as rice surplus areas and were chosen as sites for quantitative data collection (Purwestri *et al.*, 2017).

A high prevalence of stunting, wasting and underweight was reported among children in Central Sulawesi according to the 'Food Security and Vulnerability Atlas of Indonesia 2015' of the World Food Programme (WFP) (WFP, 2015). Despite its high rate of child malnutrition, Buol district in Central Sulawesi province was also considered as a rice-stock production area due to the high production of rice. In Buol, several villages were identified as rice growing transmigrant areas, including Boilan and Kokobuka villages. Residents in these two villages were mostly transmigrant families comprising mostly Javanese and Balinese, as well as some Lomboknese

and Sundanese. These ethnic groups are well known for their rice farming background and hardworking character. They had moved to these villages through the transmigration programme of the government of Indonesia.

A calculated minimum sample size of 330 mothers in Demak was determined based on stunting prevalence of 31.1% in Central Java (MoHRI, 2013), with a confidence level of 95% and a power of 0.8. Meanwhile in Buol, a minimum samples size of 95 mothers was calculated based on wasting prevalence of 6.4% in Central Sulawesi (MoHRI, 2010), and with a confidence level of 95%. In this way, mothers of malnourished young children were selected for this study of dietary behaviour before and after transmigration. All women of reproductive age from rice farming households in Demak and Buol were invited to participate in the study.

Prior to the qualitative research in each study site, an observational study was carried out in each district, in order to select the villages based on socio-economic and demography characteristics. Lists of focus group discussion (FGD) respondents (about six to maximum ten persons per group) were provided by midwives, voluntary workers (cadres) in both study sites. A total of 38 participants were purposively selected and interviewed. All of them voluntarily joined this study and agreed to be recorded.

Data on selected general and socio-demographic characteristics, dietary diversity, and consumed foods among women were collected using a one day 24-hour recall (Murphy, 2003; Arimond & Ruel, 2004). Dietary intake data of the women obtained from the one day 24-hour recall were grouped, based on the Women Dietary Diversity Score (WDDS), as follows (1) starchy staples (combination of cereal, white roots and tubers), (2) dark green leafy vegetables, (3) other vitamin A rich fruits/vegetables/tubers, (4) other fruits and vegetables, (5) organ meat, (6) meat and fish, (7) eggs, (8) legumes, nuts, and seeds, (9) milk and milk products (Kennedy *et al.*, 2011). If a food group was consumed, it was coded as 1 (one), but if not consumed, it was coded as 0 (zero). Dietary diversity score was derived by summing all codes in each food group and categorised based on low (equal and below food groups) and high dietary diversity (above four food groups). Median dietary diversity score was further determined.

Focus group discussions (FGDs) among the women in Demak were carried out in Kedungwaru Kidul village (Karanganyar subdistrict) and Dempet village (Dempet subdistrict). In Buol, FGDs were conducted among women farmers in two villages, namely Kokobuka (highland) and Boilan (lowland) from Tiloan subdistrict. The FGDs gathered information on food avoidance/taboos and beliefs during pregnancy and lactating period, including socio-cultural factors and reasons behind these beliefs.

All procedures performed in the study involving human subjects were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The objectives of the study were explained to the participants of the FGDs, followed by a verbal consent prior to the interviews and recordings. The participants were informed that their names were not revealed. A written consent was obtained from the respondents before the women were interviewed regarding their socio-demographic situation and dietary intake using a structured questionnaire. The study protocol in Demak was approved by the Ethics Committee of the Faculty of Medicine, University Brawijaya, Malang-Indonesia (No. 10.7.50.444). Ethics Committee of the Health Polytechnic

Ujung Pandang-Indonesia approved the study protocol in Buol (No. 078/KEPK-SE/XI/2015).

Data analysis

Data from FGDs were recorded and transcribed. The second author (RCP) listened to the recording interviews multiple times in order to list all important keywords and coded them in Microsoft Excel. Thematic keywords were gathered, grouped and analysed based on the study objectives. In the case of unclear information, the author checked with the local field officer and voluntary workers. The first draft of FGD results was shared with the local field-officers or voluntary workers for correctness of the interpretation.

The quantitative data were checked graphically for normality and homogeneous variance. The Mann-Whitney test was used for comparing non-parametric means of dietary diversity scores. Categorical data were analysed using generalised linear model and Fisher's exact test. Statistical analysis was performed using IBM SPSS Statistics Version 22 and statistical significance is indicated by p-value<0.05.

RESULTS

Food intake and dietary diversity scores

In total, 387 and 121 eligible women in Demak and Buol, respectively were included for data analysis. Out of these participants, there were a few pregnant and several breastfeeding women in both study areas during the surveys. Based on 24-hour dietary recall, among the top three commonly consumed food items in both Buol and Demak were rice and dark green leafy vegetables (mostly swamp cabbage, spinach), followed by legumes (tofu/tempeh, long beans). All women in both areas consumed rice as their staple food.

Food consumption data and dietary diversity scores are shown separately for pregnant, lactating and all women (Table 1). While there was no significant difference in intake of dark green leafy vegetables between the women (overall) in the two areas, women in Buol showed significantly higher consumption of vitamin A rich fruits and vegetables (e.g. mango, longbeans and okra), gathered mostly from their own gardens or nearby forest. Also, the women in Buol consumed significantly more dairy products than women in Demak. Overall, the women in Demak had higher intake of other types of fruits and vegetables that were mostly purchased (e.g. banana, sapodilla, cabbage). Other purchased foodstuffs consumed in Demak were meat and fish (chicken, catfish), legumes (tempeh/tofu).

More than half (57.7%) of the women in Demak had a low dietary diversity score (consumed less than four food groups), which was significantly lower than that in Buol (73.6%). Consumption of tofu/tempeh, meat and other protein food sources was reported as only 'sometimes', based on FGDs in both Buol and Demak. They agreed that consumption of protein foods was dependent on household purchasing power. Some women in both study sites reared chickens for meat and eggs.

Based on the women's 24-hour recall of food intake, pregnant women in Demak mostly consumed rice and vegetables (cabbage, swamp cabbage, spinach), along with a side-dish, either meat/fish (usually fried cat fish) and legumes (tofu/tempeh). In Buol, pregnant women mainly consumed rice and vegetables (swamp cabbage, spinach), followed by either legumes (tofu/tempeh) or meat (chicken). The results also showed that the lactating women in both study sites had lower dietary diversity scores (median: 3-4, range 1-7) than the non-pregnant -non lactating women (median:

Table 1. Food consumption and dietary diversity scores among women from Demak (original location) and Buol (transmigran area)

Food groups[†]	*Women in Demak*				*Women in Buol*				*All women in Demak vs. in Buol*
	Pregnant (n=12)	*Lactating (n=250)*	*NP-NL (n=125)*	*Total (n=387)*	*Pregnant (n=9)*	*Lactating (n=35)*	*NP-NL (n=77)*	*Total (n=121)*	*p-value*[‡]
Staple food (rice), *% (n)*	100.0 (12)	100.0 (250)	100.0 (125)	100.0 (387)	100.0 (9)	100.0 (35)	100.0 (77)	100.0 (121)	-
Dark green leafy vegetables, *% (n)*	75.0 (9)	64.8 (162)	56.0 (70)	62.3 (241)	44.4 (4)	65.7 (23)	63.6 (49)	62.8 (76)	0.915
Other vitamin A rich fruits and vegetables, *% (n)*	16.7 (2)	28.4 (71)	26.4 (33)	27.4 (106)	44.4 (4)	42.9 (15)	55.8 (43)	51.2 (62)	0.000
Other fruits and vegetables, *% (n)*	83.3 (10)	70.4 (176)	59.2 (74)	67.2 (260)	33.3 (3)	34.3 (12)	23.4 (18)	27.3 (33)	0.000
Organ meat, *% (n)*	0.0 (0)	0.8 (2)	3.2 (4)	1.6 (6)	0.0 (0)	0.0 (0)	1.3 (1)	0.8 (1)	1.000
Meat and fish, *% (n)*	83.3 (10)	64.0 (160)	65.6 (82)	65.1 (252)	33.3 (3)	42.9 (15)	45.5 (35)	43.8 (53)	0.000
Eggs, *% (n)*	33.3 (4)	32.4 (81)	40.8(51)	35.1 (136)	22.2 (2)	37.1 (13)	31.2 (24)	32.2 (39)	0.555
Legumes, nuts and seeds, *% (n)*	75.0 (9)	79.2 (198)	76.8 (96)	78.3 (303)	55.6 (5)	34.3 (12)	48.1 (37)	44.6 (54)	0.000
Dairy products, *% (n)*	2.0) 16.7)	3.2 (8)	0.0 (0)	2.6 (10)	33.3 (3)	17.1 (6)	18.2 (14)	19.0 (23)	0.000
Median of women dietary diversity score (minimum, maximum)	5 (3,6)	4 (1,7)	4 (2,7)	4 (1,7)	4 (2,6)	3 (2,7)	4 (2,7)	4 (2,7)	0.000
Low dietary diversity score (≤ 4), *% (n)*	25.0 (3)	51.6 (129)	(72) 57.6	52.7 (204)	66.7 (6)	71.4 (25)	75.3 (58)	73.6 (89)	0.000

[†]Data are presented as relative value (absolute count) (for categorical data), or median (minimum, maximum)
[‡]Data were analysed using a generalised linear model or Fischer exact test (for categorical data) and using the Mann-Whitney test (dietary diversity score)

4, range 2-7). During the FGDs, the women informed that during pregnancy and lactation, they were prohibited from consuming certain foods, as instructed by their mothers or mothers-in-law.

Food beliefs during pregnancy and lactation

Women from both areas believed that foods that they craved for during pregnancy should be consumed somehow "to prevent over-production of saliva ('*ngeces*') after the baby is born". Unlike during pregnancy, lactating women were advised to consume more soup made from spinach and *katuk* leaves (*Sauropus androgynus* or *daun katuk*). The list of prohibited foods and reasons for not allowing women to take those foods during pregnancy and lactation is shown in Table 2.

The women in Demak reported that traditional herbal medicine or *'jamu'* was often recommended by older women, traditional healers (*dukun*) or traditional birth attendants (*dukun bayi*) to improve health and breastmilk production. '*Jamu*' is the local term for local herbal medicines usually made from leaves, bark, roots and flowers (Afdhal & Welsch, 1988; Romuli & Romuli, 2015). In Demak, herbal medicines were part of a series of traditional health care, including counseling and massage, provided by traditional birth attendants. Consuming the herbs after delivery (so called '*jamu selapan*') was prescribed for 40 days for maintaining the "health" of the uterus.

Although not all the women followed all the foods restrictions, most of them agreed that young woman, especially those giving birth for the first time, and those who lived close to their mother or mother in-law, were more likely to comply with the taboos and beliefs. Women with higher education levels (senior high school and above) were more likely to refuse adhering to local food taboos, especially if the foods are known to cause allergic reactions to her or to her child.

DISCUSSION

The types of food consumed and dietary diversity of Javanese women in the original residential location (Demak, Central Java) and transmigrant area (Buol, Central Sulawesi) showed significant differences, as influenced by their economic status and habitats. Dietary modifications occurred among women in the transmigrant area in Buol.

Women from rice farming transmigrant families in Buol maintained their previous food habits of eating rice, tempeh and tofu. Similar findings were reported among transmigrants from western parts of Indonesia, including Central Java, whereby they retained their food habits of eating rice although relocated to a sago-based staple food area in Moluccas (Novotny & Rumalatu, 1995). The native Buol had gradually abandoned sago as their staple food, and considered consuming sago only in a food insecure situation. The women also believed that eating rice is healthier than sago. It is also interesting to note that tempeh and tofu were not available before the arrival of the Javanese transmigrants in Buol. Currently, these foods can be found sold daily by the local vendors. There are also some home industry of tempeh/tofu making in Buol, indicating that the Javanese have introduced these foods after relocation. Residents in Demak usually substitute beef with other meat, e.g. carabeef due to the historical influence of Hindu, which was the religion of ancient Demak inhabitants.

This study found the median dietary diversity score of the women in Demak and Buol critically low (equal and below four) (USAID, AED, UNICEF & IFPRI, 2008), especially among lactating women.

Table 2. Food taboos beliefs of pregnant and lactating women in Demak and Buol based on focus group discussions

Phase	*Food taboos*		*Reasons*
	In Demak	*In Buol*	
Pregnancy	Eggplants		Pregnant mother will be lacking internal strength and go limp ("lemes")
	Pineapple, jackfruits, carbonate drinks, or ginger		Increase the womb temperature and stimulate spontaneous miscarriage during early pregnancy
	Too much sugar		Cause nausea, vomit
	Squid		Fetus will be lacking internal strength and go limp
	Shrimp	Shrimp	Fetus will bend both legs to chest direction ("melungker") causing difficulty during delivery
	Fish (in general)	Chicken egg and fish	Prolong the bleeding and to avoid fishy smell after delivery
	Fish corks		The infant will be born too big, thus cause difficulty during delivery
	Consume plenty of carbohydrate-source of foods		The infant will be born too big, thus cause difficulty during delivery
	All vegetable soups with meat; or drink too much water		Delay in healing process of delivery wound
Lactation	Young jackfruits ("kluweh ")		Mother will get fever
	Sweet potato		Cause further bleeding for lactating mother
	Orange		Mother and baby will get respiratory infection (cough and runny nose)
	Sapodilla fruits ("sawo")		Mother will get fever
	Ice water		The baby will catch a cold
		Chicken egg and fish	To avoid fishy smell of breastmilk, and the breastfed children
	Chili		The baby will get diarrhea

Pregnant women in both locations showed a slightly higher median dietary diversity scores, and this could be attributed to public health programmes that provide pregnant women with iron supplements and health/nutrition education in both locations. Butte & King (2005) and Dewey (1997) emphasised the importance of increasing nutrient intake among pregnant and lactating women.

Food taboos mentioned by the FGD participants in Buol were significantly fewer than in Demak. As the second generation of the Javanese transmigrants, the cultural beliefs that they received might have been modified owing to influence of different food environments, e.g. some of the restricted foods were not locally available. In contrast, in Demak, the respondents living in close proximity with older women, traditional healers and birth attendants, continued to practise several food taboos during pregnancy and lactation.

Food taboos of pregnant women in Ethiopia (Zerfu, Umeta & Baye, 2016), Kenya (Kariuki *et al.*, 2016) and China (Lee *et al.*, 2009) were reportedly rather similar to the prohibited foods in this study. Restriction was mainly on consumption of high energy, protein-rich foods, selected fruits and vegetables. The major reasons for restricting food intake among pregnant women were fear of difficult delivery as a result of having a big baby, and consumption of certain foods perceived to cause the foetus to be in an abnormal position. These findings are similar to another study in Central Java (Hartini *et al.*, 2005) and in Malaysia (Mohamad & Ling, 2016). Other reasons include fear that certain foods might cause spontaneous miscarriage and the baby born with deformities. Some believe that eating shrimps could cause difficulty during labour because the foetus might be positioned like a shrimp (*melungker*). This study and that by Hartini *et al.* (2005) in Central Java, reported that chicken egg was prohibited during pregnancy and lactation as eating eggs could result in continued bleeding after delivery.

Food restrictions for lactating mothers (mostly vegetables and fruits) were common in both study locations. Main reasons for food avoidance during lactation were concern for the mother getting sick and producing inadequate and low-quality milk for the baby. Similar findings were also reported in Mexico (Santos-Torres & Vásquez-Garibay, 2003). Lactating women were also advised to consume *katuk* and spinach soup, which is believed to induce breastmilk production, and to give more energy to the mothers (especially spinach). Spinach soup was one of the most commonly consumed dark green leafy vegetable by the Javanese women in Demak, especially during lactating period. Traditional herbs (*jamu*) were also believed to improve health status and breastmilk production. Younger and women from lower income status tend to believe that they should follow food recommendations and restrictions.

Limitations of study

Consumption data were limited by a single 24-hour recall which is known for shortcomings including not being typical of usual intake. We did not perform in-depth interviews of older women, including traditional healers or birth attendants. They are known to exert influence on food beliefs in the community.

CONCLUSION

Javanese transmigrants in Buol maintained their food intake of rice, tofu and tempeh. Reasons for food taboos during pregnancy and lactating women were common in both study sites, indicating the persistence of

dietary restriction practices despite transmigration. It is suggested that nutrition information be provided to women on the shortcomings of avoiding foods during pregnancy and lactation, especially nutrient-rich foods that are important for the health of the mother and child.

Acknowledgement

The authors would like to thank the Neys van-Hoogstraten foundation (IN252) and the International Fund for Agricultural Development (IFAD) through the Climate-Smart, Tree-Based, Co-Investment in Adaptation and Mitigation in Asia (Smart Tree-Invest) project implemented by the World Agroforestry Centre (ICRAF) for the financial contributions. Dr. Purwestri was also funded by the Federal Ministry of Education and Research within the project Humboldt reloaded (01PL11003) at the University of Hohenheim. Both research projects were collaborative studies between the Faculty of Medicine, Nutrition Department, University of Brawijaya, Malang, Indonesia, and the Institute of Biological Chemistry and Nutrition, University of Hohenheim, Stuttgart, Germany.

Authors' contributions

RCP, planned the study design, implemented the study, performed data analysis and interpreted the data; BL, planned the study design, implemented the study, performed data analysis and interpreted the data; NNW, planned the study design, implemented the study, performed data analysis and interpreted the data; IF, implemented the study; ZB, performed data analysis and interpreted the data; JH, performed data analysis and interpreted the data; all authors drafted the article, gave valuable comments, contributed to the final version of the manuscript and approved the final manuscript.

Conflict of interest

The authors have declared that no competing interests exist.

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Mal J Nutr 24(4): 597-605, 2018

Microencapsulation of red palm oil and its stability during accelerated storage

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ABSTRACT

Introduction: Sensitivity of red palm oil (RPO) towards oxidation is known to result in degradation of nutritional value and organoleptic properties. This study aimed to determine the stability of microencapsulated RPO during accelerated storage at 65˚C for 24 days. **Methods:** Microencapsulated was undertaken by co-extrusion technology using sodium alginate with high methoxyl pectin, and calcium chloride solution enhanced with chitosan as cross-linking agent in the presence of Tween® 80 as surfactant. The encapsulated beads were freeze dried and the physical properties, antioxidant activities and total carotenoid content of dried powder were determined. Microencapsulated red palm oil (MRPO) was then subjected to accelerated storage at 65°C for 24 days. **Results:** Antioxidant activity of both RPO and MRPO measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was significantly decreased, with higher percentage loss in MRPO during accelerated storage. RPO and MRPO also experienced decreases in percentage inhibition with higher percentage of loss as measured by Azino-bis (3-ethylbenzothiazoline-6-sulpohnic acid) (ABTS) radical scavenging activity. Both RPO and MRPO showed decreasing trends in total carotenoid content, with higher content in MRPO than RPO at end of storage period. Antioxidant activities of RPO and MRPO correlated well with the carotenoid content, with best correlation coefficient in RPO between the ABTS assay and total carotenoid content measured by high performance liquid chromatography (r=0.952). Very strong association between DPPH and ABTS values (r=0.871) for the MRPO, and between DPPH and total carotenoid content (r=0.856) were noted. **Conclusion:** The study showed that microencapsulation effectively protected the carotenoid content in MRPO, but not its other natural antioxidants.

Keywords: Carotenoids, microencapsulation, co-extrusion, antioxidants, accelerated storage

INTRODUCTION

Crude palm oil extracted from the mesocarp of ripened fruit of oil palm tree, *Elaeis guineensis,* is a complex mixture consisting more than 99% glycerides. This oil is also known as red palm oil (RPO). It differs from other plant and animal oils in that it contains 50% of saturated fatty acids, 40% unsaturated fatty acids and 10% polyunsaturated fatty acids. Palm oil and its products play prominent roles in several manufacturing industries and are beneficial to human and animal diets. They are widely consumed and

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exported in many forms, including refined, bleached and deodorized (RBD).

Red palm oil contains carotenoids, of which 80-90% are α- and β- carotene, as well as vitamin E (tocopherols and tocotrienols) (Sadiq, Rasool & Sharif, 2006; Andreu-Sevilla *et al.*, 2009). These compounds are essential as antioxidants and may contribute to the oxidative stability of the oil. The oil also contains appreciable amounts of components, such as sterols, phospholipids, glycolipids, ubiquinones and squalene, which are nutritious and provide benefits to human health (Alyas, Abdullah & Idris, 2006; Atawodi *et al.*, 2011; Bakry *et al.*, 2015). However, RPO as a cooking oil is not favoured by consumers due to its red colour, Thus, RPO has been modified resulting in the oil having a light golden color without the carotenes (NorAini *et al.*, 1998; Okonkwo, 2012).

Microencapsulation technology has been applied in the food industry for various benefits (Estevinho *et al.*, 2013). Encapsulation is a process to entrap a substance within another substance resulting in the production of small particles with diameters ranging from few micrometers to few millimeters. The inner substance is known as the core material, while the outer substance is called the wall material or encapsulant (Zuidam & Nedović, 2010). Microencapsulation allows the encasement of micron-sized particles of either solid, droplet of liquids or gasses in inert shell, which isolate and protect them from the external environment. Therefore, microencapsulation is able to protect the sensitive ingredients and organoleptic properties including colour, taste and odour from external effects (Jyothi *et al.*, 2010). In addition, microencapsulation techniques are regarded as an alternative method to control the release of substances over prolonged periods under specific conditions (Estevinho *et al.*, 2013).

The selection of appropriate encapsulation technology, core material, wall material and capsule properties are critical in promoting its successful commercial application (Lim & Nyam, 2016). There are numerous techniques of microencapsulation of food compounds available, including spray drying, spray chilling, freeze-drying, coacervation and co-extrusion (Sagis, 2015; Chew & Nyam, 2016). The choice of microencapsulation techniques depends on the physical and chemical properties of the materials to be encapsulated (Jyothi *et al.*, 2012). Each technique has its own advantages and disadvantages.

MATERIALS AND METHODS

Red palm oil (Carotino, Johor) was purchased by convenience from a supermarket in Kuala Lumpur. Wall materials made from sodium alginate were acquired from R & M Marketing (Essex, UK), while high methoxyl pectin (HMP) was purchased from a local food ingredient supplier. All chemicals and reagent used in this study were of analytical grade (Merck, Darmstadt, Germany and Sigma-Aldrich, Germany).

Microencapsulation of RPO using co-extrusion technology

Sodium alginate solution (1.50% w/w) and HMP solution (1.50% w/w) were prepared and gently homogenised at 12000 rpm for 2 min and 7200 rpm for 1 minute (min), using digital Ultra-Turrax® homogenizer (T25, IKA, Germany). The HMP-alginate solution was mixed according to the method of Chew *et al.* (2015) at a volume ratio of 2:1 and stored overnight at 4°C.

The hardening solution made from calcium chloride solution (3.00% w/v) enhanced with chitosan coating (0.10% w/v) was freshly prepared. Tween® 80 (0.10% w/v) was added and mixed together until fully dissolved. The solution was adjusted to pH 5.0-5.5. Subsequently, any insoluble material was filtered out and the solution was topped up to 1 L. The chitosan solution

was incubated in the water bath set at 50°C until further usage (Lab Companion, Korea).

The microencapsulation of RPO by co-extrusion technology was carried out using Encapsulator B-390 (Buchi, Switzerland) with the concentric nozzle (150 µm for inner nozzle and 300 µm for outer nozzle). The air pressure was set at 600 mbar to give a core-shell fluid stream, which was sprayed out through the nozzle. The frequency was set at 300 Hz under amplitude of three with an additional electrostatic field of 1.5 kV between the nozzle and hardening solution to minimise the potential dissolution of core-shell droplets when approaching hardening solution surface.

Microcapsules obtained were hardened when dropped into the calcium chloride solution enhanced with chitosan coating and Tween® 80. The microcapsules were incubated for 10 min in the hardening solution with gentle stirring to avoid clumping of microcapsules. The microcapsules were next collected with nylon sieve, rinsed and drained until no further moisture was found. The microcapsules were spread thinly and evenly to increase the drying rate.

After forming, the microcapsules were dried using freeze dryer (SciQuip, United Kingdom) for 23 hours. All the dried microcapsules were collected in aluminum foil-wrapped Schott bottle, following which the bottle was flushed with 99.9% nitrogen to remove oxygen, and stored in a freezer (E388, Fisher and Paykel, Australia) at -20°C for later use.

Physical analysis

The morphology of fresh and dried microcapsules was observed using optical microscope under magnification of x4 (Nikon Instruments Inc., United State) and their size measured with the aid of a stage micrometer slide (Ladd Research, United State). The moisture content of the microcapsules was determined using oven, while the water activity of the microcapsules was analysed using water activity analyzer (Aqua Lab, United State) (Chew & Nyam 2016). The moisture content was calculated using the equation shown below:

$$\text{Misture content (\%)} = \frac{(W1 - W2)}{W1} \times 100\%$$

where W1 is the weight of the samples before drying (g) and W2 is the weight of the samples after drying in g.

Microencapsulation efficiency (MEE)

The MEE of MRPO after drying was determined according to Thamaket & Raviyan (2015) and MPOB P2.6: 2004 analytical method. The microencapsulation efficiency was determined using the following equation.

$$\text{MEE} = \frac{\text{Total carotenoids in microcapsules} - \text{Surface carotenoids}}{\text{Total carotenoids input}} \times 100\%$$

Total carotenoids in the microcapsules were obtained by dissolving 10 mg of microcapsules in 25 mL of sodium citrate solution with gentle agitation. Hexane (25 mL) was added and the mixture was shaken vigorously to facilitate the transfer of oil into the hexane layer. The obtained hexane layer was evaporated using a Multivapor P-6 (BÜCHI Labortechnik AG, Switzerland) at 40°C, 125 mbar for 30 min with speed six. The residual solvent was next removed by flushing with 99.90% nitrogen.

Total carotenoids input content was determined by dissolving 0.04 g of oil in 10 mL volumetric flask with hexane. It was then gently shaken until it was fully dissolved and measured at 446 nm using ultraviolet-visible (UV-Vis) spectrophotometer (Secomam, France) with hexane as the blank.

The surface carotenoids content was determined by adding 25 mL of hexane to 50 mg of microcapsules, followed by

agitating it using orbital shaking plate (Vision Scientific, Pakistan) for 15 seconds at 100 rpm. The supernatant collected was measured spectrophotometrically at 446 nm.

Total carotenoids in microcapsules, surface carotenoids and total carotenoids input content were then calculated using the formula as shown below:

$$\text{Total carotenoids content} = V \times \frac{383}{100W} \times (As - Ab)$$

where V is the volume used for analysis (mL), 383 is the extinction coefficient for carotenoids, W is weight of sample (gram, g), As is the absorbance of sample at 446 nm, and Ab is the absorbance of blank at 446 nm.

Storage stability

The microcapsules were subjected to an accelerated storage condition at 65°C for 24 days, whereby 1 day of storage reflected 1 month of storage at room temperature (Ng *et al.*, 2014). The antioxidant activity tests carried out were DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) and Azino-bis (3-ethylbenzothiazoline-6-sulpohnic acid) (ABTS$^{\bullet+}$) radical scavenging activities. The total carotenoid content in the RPO and MRPO were examined using high performance liquid chromatography (HPLC) (Agilent Technologies, United State) and UV-Vis spectrophotometer (Secomam, France) every 6 days. RPO was used as a control for comparison.

Assessment of antioxidant activities upon accelerated storage

DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging activity was determined according to the method of Cheong, Tan & Nyam (2016), Chew *et al.* (2015) and Chew *et al.* (2016). The antioxidant activity of the oil samples was expressed as mg Trolox equivalents (mg Teq/100 g oil) with calibration equation of y=61.633x–0.2148 (R^2=0.9889). The percentage of inhibition (%) was calculated based on the equation below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Azino-bis (3-ethylbenzothiazoline-6-sulpohnic acid) (ABTS$^{\bullet+}$) radical scavenging activity

The ABTS radical scavenging activity was performed according to Cheong, Tan & Nyam (2016) and Chew *et al.* (2015; 2016). The antioxidant activity of the oil samples was expressed as mg Trolox equivalents (mg Teq/100 g oil) with calibration equation of y=210.67x – 1.0596 (R^2=0.9861). The percentage of inhibition (%) was calculated based on the equation below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Assessment of total carotenoid content upon accelerated storage

High performance liquid chromatography (HPLC)

The total carotenoid contents in MRPO and RPO were determined according to the modified method of Jain *et al.* (2016). An aliquot (20 µL) of sample was injected and analysed by HPLC (Agilent Technologies 1200 series, United State). The carotenoid content was expressed as mg/kg. HPLC analysis was carried out isocratically at 35°C using C18 column (250x4 mm i.d., particle size 5 µm) (Merck, Germany). The mobile phase used was methanol/acetonitrile/ethyl acetate (80:10:10 v/v). The peaks were quantified at 446 nm.

UV-Vis spectrophotometer
The total carotenes content in MRPO and RPO was evaluated based on MPOB P2.6:2004 analytical test. The absorbance was measured at 446 nm using UV-Vis spectrophotometer.

Statistical analysis
The results obtained were subjected to statistical analysis utilising MINITAB 17 (Minitab Inc, Pennsylvania, United State). One-way analysis of variance (ANOVA) was performed and significant differences (p<0.05) were determined using Tukey Honestly Significant Differences (HSD) multiple comparison test. Independent t-test was conducted to compare the differences of antioxidant activities and total carotenoid contents between RPO and MRPO on the same day of storage. Pearson correlation was determined to quantify the relationship between the two data samples. The results were expressed as mean±standard deviation for both physical analysis and storage stability tests.

RESULTS AND DISCUSSION

Physical analysis
The RPO was successfully encapsulated within the wall materials forming a bulbous shape. The microcapsules had a consistent size with regular and smooth surface. After subjecting the microcapsules to freeze drying, the microcapsules shrunk and appeared to be slightly irregular with darker yellow colour. The surface of dried microcapsules was fibrous and not smooth, compared to freshly produced microcapsules (Figure 1). The average particle size of fresh MRPO (626.67±75.28 μm) was significantly higher compared to the dried MRPO (545.00±110.91 μm). This was expected as fresh MRPO contained water, which was removed during freeze drying, resulting in the smaller size of dried MRPO.

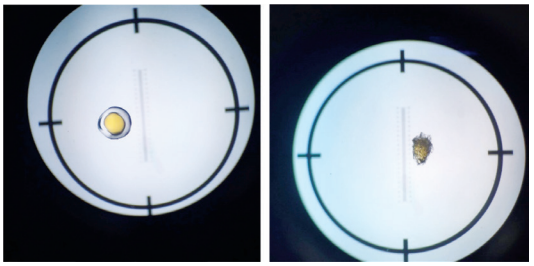

Figure 1. Microencapsulated red palm oil before drying (left) and after drying (right) under light microscope viewed with 4x magnification

The MRPO was found to have low moisture content (1.98±0.11%). According to Onwulata (2005), microencapsulation of fats is able to reduce the adhesiveness and improve the handling properties during storage, transport and blending with non-fat ingredients. Through water activity analysis, it was found that the MRPO is categorised as a low water activity product (0.36±0.02) as the water activity was less than 0.70 (Gurtler, Doyle & Kornacki, 2014). Due to its low water activity, dried MRPO had a lower risk of microbial spoilage and therefore able to prolong its shelf life by preventing the growth of microorganisms.

Microencapsulation efficiency (MEE)
Microencapsulation efficiency plays a significant role in this study because MEE refers to the proportion of oil that is surrounded by the shell wall matrix and less exposed to the surrounding environment and thus, having an effect on the oxidative stability of the product (Chew & Nyam, 2016). The MEE of MRPO was found to be 76.95±5.42%, which is higher than reported by Chew & Nyam (2016). Therefore, the wall materials selection and its proportions can be considered to be optimum in this study.

Analysis of DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of RPO and MRPO during accelerated storage

The percentage inhibition of DPPH value of both RPO and MRPO showed a decreasing trend from day 0 to day 24 with an initial value of 40.23±6.03% and 27.30±5.34 %, respectively (Figure 2). MRPO experienced a higher decrease compared to RPO starting from day 0. This could be due to oxidation as the oil had longer exposure to the oxygen and water during the microencapsulation. Results obtained indicated that the percentage of inhibition was significantly higher for RPO at day 0. The total decrease of DPPH values in RPO and MRPO were 77.50% and 81.21%, respectively. Therefore, the results indicated that microencapsulation could not protect and stabilise the natural antioxidants in RPO during storage.

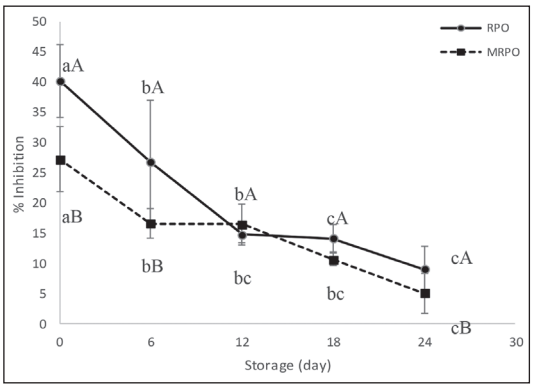

Figure 2. Total percentage of inhibition of DPPH in RPO and MRPO upon accelerated storage. Mean±standard deviation (n=4) with different superscript letters ^{abc} indicate significant differences (p<0.05) among different days of the same sample. Mean±standard deviation (n=4) with different superscript letters ^{AB} indicate significant differences (p<0.05) between two samples at the same day of storage

Analysis of Azino-bis (3-ethylbenzothiazoline-6-sulpohnic acid) (ABTS$^{\bullet+}$) radical scavenging activity of RPO and MRPO during accelerated storage

Percentage inhibition values of ABTS for both RPO and MRPO declined from day 0 to day 24 (Figure 3). The decreasing trend in ABTS might be due to the encapsulation process having an impact on the oil oxidation. The longer the exposure time of oil to the environment, the higher the likelihood of degradation of the antioxidants in the oil. This brought about a lowered capability in free radicals scavenging. At day 0, RPO samples showed significantly higher ABTS value compared to the other days, while the MRPO samples showed no significant difference with the ABTS value on day 6. The differences in % inhibition between both RPO and MRPO became smaller from day 12 of storage due to the degradation of antioxidants in RPO. In brief, the total percentage decrease in inhibition values for ABTS in RPO and MRPO were 88.07% and 73.54%, respectively.

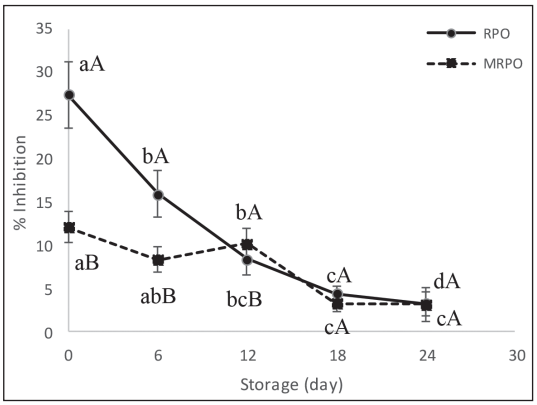

Figure 3. Total percentage of inhibition of ABTS in RPO and MRPO upon accelerated storage. Mean±standard deviation (n=4) with different superscript letters ^{abcd} indicate significant differences (p<0.05) among different days of the same sample. Mean±standard deviation (n=4) with different superscript letters ^{AB} indicate significant differences (p<0.05) between two samples at the same day of storage

Total carotenoid contents of RPO and MRPO during accelerated storage

Decreasing trends of the total carotenoid contents in RPO and MRPO were observed during accelerated storage. The initial total carotenoid content of RPO was higher than in MRPO and decreased continuously till a low value of about 10 mg/kg on day 24 (Figure 4). However, for MRPO, after an initial decline, the carotenoid content was observed to increase from 65.92±7.38 to 88.91±2.63 mg/kg between day 6 and day 12. The carotenoids showed considerable fluctuations depending on environmental conditions. After day 12, the carotenoid content in MPRO continued to decrease but the level at the end of storage (day 24) was higher than that of RPO.

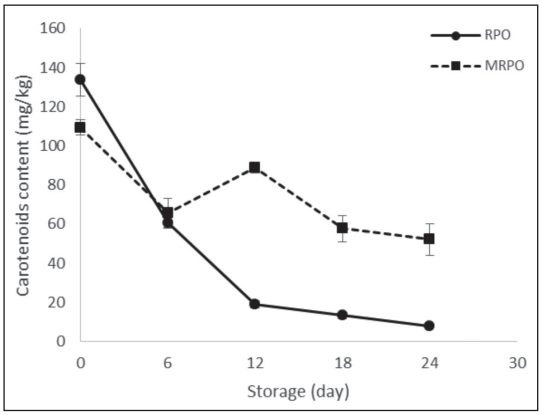

Figure 4. Total carotenoid content by HPLC in RPO and MRPO during accelerated storage. Mean±standard deviation (n=4) with different superscript letters ^{abcd} indicate significant differences (p<0.05) among different days of the same sample. Mean±standard deviation (n=4) with different superscript letters ^{AB} indicate significant differences (p<0.05) between two samples at the same day of storage

Similarly, the initial total carotenoid content of RPO was higher than MRPO. However, over the storage period, there was less decline in carotenoid content for MRPO and on the last day of storage (day 24), the carotenoids content in MRPO was significantly higher than that in RPO (Figure 5). It could be concluded that through microencapsulation, the carotenoids content in the oil in MRPO was comparatively better retained than RPO during storage.

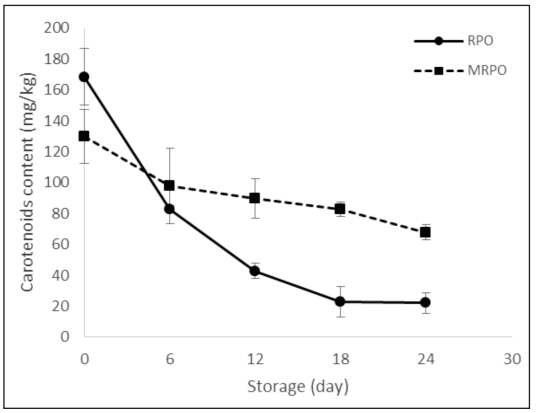

Figure 5. Total carotenoids content by UV-Vis spectrophotometer in RPO and MRPO during accelerated storage. Mean±standard deviation (n=4) with different superscript letters ^{abc} indicate significant differences (p<0.05) among different days of the same sample. Mean±standard deviation (n=4) with different superscript letters ^{AB} indicate significant differences (p<0.05) between two samples at the same day of storage

Correlation assessment among RPO traits

There was a significantly positive and strong association between the antioxidant activities, measured by DPPH and ABTS, and the total carotenoid content in RPO. The best correlation coefficient was found between the ABTS assay and total carotenoid content measured by HPLC (r=0.952). The total carotenoid content as measured by HPLC and UV-Vis spectrophotometer showed strong and significant positive relationship with one another. According to Martysiak-Żurowska & Wenta (2012), DPPH method has more limitations as characterised by a lower sensitivity and slower reaction with most antioxidants compared to ABTS assay.

Correlation assessment among MRPO traits

Pearson's correlation coefficients (r) among DPPH, ABTS and total carotenoids in MRPO showed significant positive relationships. Very strong association between DPPH and ABTS values (r=0.871), as well as between DPPH and total carotenoid content determined using HPLC (r=0.856) were noted from the results. This was in agreement with the study by Dudonné *et al.* (2009), where DPPH and ABTS assays showed a strong positive correlation. The lowest correlation was observed between ABTS value and total carotenoids measured spectrophotometrically (r=0.704). As the results demonstrated positive linear relationships, it can be interpreted that the antioxidant assays, (DPPH and ABTS) employed in this study were suitable and can be used to measure the antioxidant activity of both RPO and MRPO (Razmkhah *et al.*, 2013).

CONCLUSION

Red palm oil, which was micro-encapsulated by co-extrusion technology and dried using freeze drying method showed relatively low moisture content and water activity. This indicates that microencapsulated RPO (MRPO) has good stability and low risk of microbial contamination. The microencapsulation efficiency of MRPO was considered relatively high, hence, the combination of the wall materials utilised is considered suitable as coating agent. It is suggested that more advanced tests, such as Fourier transform infrared spectroscopy (FTIR) and immobilized horseradish peroxide assay be conducted along with DPPH and ABTS assays to quantify the actual amounts of scavenging activity.

Acknowledgement

Financial support of this work by internal funding from CERVIE UCSI University (Proj-In-FAS-053) is gratefully acknowledged.

Authors' contributions

KLN, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; FA, conducted the study, data analysis and interpretation, assisted in drafting of the manuscript.

Conflict of interest

The authors declared no conflict of interest

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Mal J Nutr 24(4): 607-615, 2018

Development of food products using fish maw (*Pangasius hypophthalmus*) and roasted sunflower kernel (*Helianthus annuus*) for branched-chain organic acidurias patients

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ABSTRACT

Introduction: Branched-chain organic acidurias include maple syrup urine disease (MSUD), isovaleric acidemia (IVA), propionic acidemia (PA), and methylmalonic acidemia (MMA). Long term management requires diets of adequate energy and protein with restriction of the offending amino acids. Standard commercial formulas are expensive and unaffordable to patients of low socio-economic status. **Methods:** This study aimed to develop food products for branched-chain organic acidurias children aged 4-15 years using locally available raw materials in Thailand. Fish maw (*Pangasius hypophthalmus*) and roasted sunflower kernel (*Helianthus annuus*) were selected as protein sources due to their low leucine contents. Five formulations were developed, namely (i) powder (low leucine, isoleucine, and valine for MSUD) for tube feeding preparation, (ii) – (v) rice sprinkle powder, bouillon cube, instant cocoa drink, and snack bar, respectively with low leucine for IVA; low valine, isoleucine, methionine and threonine for PA and MMA. **Results:** All five formulated products provide 500-600 kcal/100 g, adequate protein in which the offending amino acids were controlled at non-harmful levels. These products were shelf stable at room temperature (Aw = 0.3-0.5). **Conclusion:** The products that were formulated from fish maw and roasted sunflower kernel provide proteins of appropriate quality and quantity for long-term management of branched-chain organic acidurias. The developed products should be further tested for efficacy among patients in accordance with an adequately powered study design.

Keywords: Branched-chain organic acidurias, *Pangasius hypophthalmus*, *Helianthus annuus*, leucine, valine

INTRODUCTION

Branched-chain organic acidurias are a group of rare inborn errors of metabolism, caused by abnormality of specific enzymes that are primarily involved in the degradation of branched-chain amino acids (leucine, isoleucine and valine) and their derivative compounds. As a result

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of degradation, an accumulation of the aforementioned amino acids and/or associated organic acids occurs in blood, cerebrospinal fluid (CSF) and urine (de Baulny, Vici & Wendel, 2012). Rapid severe accumulation of these organic acids and branched-chain amino acids, specifically leucine, can lead to cerebral oedema and early death, if left untreated. The chronically elevated blood levels of these compounds adversely affect brain microstructure and functions, resulting in psychomotor retardation (Dionisi-Vici *et al.*, 2002; Strauss *et al.*, 2010) in patients with maple syrup urine disease (MSUD), isovaleric acidemia (IVA), propionic acidemia (PA), and methylmalonic acidemia (MMA) (Knerr *et al.*, 2012; de Baulny *et al.*, 2012). Patients of these rare diseases are found worldwide. The prevalence per 100,000 has been reported to be 3.69 in British Colombia, Canada; 4.67 in Italy; 12.56 in West Midlands, United Kingdom; and 135.14 in Saudi Arabia due to the high consanguinity rate among Arab populations (Rashed *et al.*, 1994; Applegarth, Toone & Lowry, 2000; Dionisi-Vici *et al.*, 2002; Sanderson *et al.*, 2006). The prevalence of these diseases in Thailand is 50-60 cases for the whole country, which is quite low as compared to many countries.

There are two modalities for the treatment of branched-chain organic acidurias patients in relation to the phase of the diseases, namely (i) acute-phase treatment and (ii) long-term management. The acute-phase treatment aims to quickly remove the toxic metabolites by dialysis or hemofiltration in order to prevent death or severe permanent brain damage (Morton *et al.*, 2002; Saudubray *et al.*, 2002; Zand *et al.*, 2008). For the long-term management, diets of low protein and adequate/high energy in combination with limiting the offending amino acids are recommended to support normal growth and neurodevelopment (Yannicelli, 2006; Knerr *et al.*, 2012). These diets are commercially available in powder form, which are categorised as medical products. The commercial products are freed of the offending amino acids, namely (i) leucine, isoleucine and valine in the case of MSUD, (ii) leucine in IVA, and (iii) valine, isoleucine, methionine and threonine in MMA and PA. These products are also enriched with vitamins and minerals. Since the number of patients worldwide is quite low, the production and availability of commercial formulas are costly, unaffordable and unavailable to low-income patients.

In Thailand, these commercial products are usually available through international donations, and patients occasionally encounter shortage problems, that may result in poor control of the disorder symptoms. Therefore, there is a need to develop products for these patients by using locally available raw materials to provide a sustainable supply. Based on available database on amino acid profiles, certain kinds of locally available foods can potentially be used as raw materials for preparing food products that are low in branched-chain amino acids. The purpose of this study was to formulate food products for treating young branched-chain organic acidurias patients. Different product forms would be developed using sustainable sources of raw materials in Thailand.

MATERIALS AND METHODS

This study formulated products to be taken by patients as supplements to their usual diet of three meals a day. Patients should be able to acquire adequate energy and protein to meet the requirements of their age group. In this study, the target age was 4-15 years. Energy requirements for patients were calculated by using the Holliday-Segar equation and nutritional

recommendation for severe IVA, MSUD, MMA, and PA (Wappner & Gibson, 2006; Barshop, 2006). The energy requirements of patients were expected to be met through (i) their normal diets, and (ii) the formulated food products (de Baulny *et al.*, 2012).

Total protein intake for patients aged 4-8 and 8-15 years were designed as 1.5-2.0 g/kg/day and 1.0-1.2 g/kg/day, respectively and also following individual baseline protein intake prior to entering the study (Wappner & Gibson, 2006). For MSUD and IVA, leucine was selected as the primary offending amino acid for calculation of amino acid allowance for daily intake. Total leucine intakes allowed were 500-750 mg/day for MSUD and 650-1500 mg/day for IVA. For MMA and PA patients, the intakes of valine, isoleucine, threonine and methionine were controlled at 700-1600 mg/day, 600-1300 mg/day, 500-1200 mg/day, and 250-800 mg/day, respectively (Wappner & Gibson, 2006; Barshop, 2006) and valine was chosen for calculation of the offending amino acid allowance for daily intake.

Selection of raw materials

Based on the Thai Foods database on amino acid profiles (MOPH Thailand, 2001), several food items were identified based on the ratio of leucine to protein content, and the amount of protein in the edible portion. Food items that contained reasonable protein content with minimum ratio of leucine to protein were considered ideal. Besides, they should be produced under systematic farming, which is able to maintain quality and minimise natural variations. Based on these criteria, two food items were selected, namely (i) fried fish maw and (ii) roasted sunflower kernel. Fish maw was obtained from a farmed fish called "Pla Swai" (*Pangasius hypophthalmus*), while sunflower kernel (*Helianthus annuus*) was harvested from a sunflower farm, followed by sorting and roasting. Amino acid profiles of both materials were determined, while information on their nutrient profiles was obtained from the suppliers. For food safety reasons, both materials were sent for analysis of heavy metal contamination, while information on aflatoxin contamination was obtained from the suppliers.

Additional carbohydrate and fat contents were added to improve energy density of the formulated products. Sucrose and maltodextrin were used as carbohydrate sources, while palm olein oil and non-dairy creamer provided additional fat.

Production

A total of five formulations were developed from fish maw and sunflower kernel. Formula I, low in leucine and prepared for MSUD patients, was in powder form, which could be orally consumed as a rice sprinkle. For MUSD patients, it was necessary to prepare formula I in the form of a tube-feeding diet by blending the product with selected food ingredients, such as cooked rice, soybean oil, fruits and vegetables. Formulas II - V, low in leucine and valine and prepared for IVA, PA and MMA patients, were presented in four different product forms, namely (i) rice sprinkle powder, (ii) bouillon cube, (iii) instant cocoa drink and (iv) snack bar.

Dried fish maw obtained from the farm was well mixed following which it was ground to approximately 2 mm size in an electric blender and then pasteurised in a hot air oven at 80°C for 30 min. The ground fish maw (5 kg per batch) was portioned into 1,000 g packs and vacuum-sealed in plastic laminated aluminum foil bags and stored at -20°C.

Sunflower kernel was mixed, portioned and similarly packed as for fish maw, but without the grinding step. The sunflower kernel was stored at -20°C before use.

The ground fish maw and sunflower kernel were mixed with other food

ingredients selected as sources of energy, as well as salt for flavouring, glucose syrup, baking chocolate and cocoa powder were used for preparing the five different food products as mentioned above. All the Formulas, except for Formula V, were similarly prepared by weighing and blending all the ingredients in an electric blender before packing. Formula V was prepared by blending the ingredients and mixing them with melted baking chocolate bar before spreading on a tray. The solidified bar was then cut into chewable sizes (2 x 2 cm) before packing. The packing size of these food products was based on the nutrient requirements per meal of each patient (based on individual weight) and providing for 2 meals a day. All the final products were packed under vacuum in a plastic-laminated aluminium foil bag. The products were analysed for macronutrients, amino acid profile, microbiological quality and water activity.

Quality analysis

Macronutrients

Moisture content was determined by drying in a hot air oven at 105°C for 3 h until a constant weight was obtained (AOAC International, 2012a). Protein content was determined by Kjeldahl method, in which food was digested with sulfuric acid in the presence of catalysts. Crude protein content of the food was determined by multiplying the nitrogen content with a factor of 6.25 (AOAC International, 2005; Chang, 2010). Fat content was determined using Soxhlet apparatus. Fat was extracted with a mixture of ethyl ether and petroleum ether in a Mojonnier flask, and the extracted fat was dried to a constant weight and expressed as percent fat by weight (AOAC International, 2012b; Min, 2010). Ash content was determined by dry-ashing in which the sample was incinerated in a muffle furnace at ≥ 525°C overnight. The sample was then cooled down in a desiccator prior to weighing (AOAC International, 2012c; Marshall, 2010).

Amino acid profile

Food sample was hydrolysed by 0.1 M hydrochloric acid before being analysed for amino acid profiles using high performance liquid chromatography (HPLC). The chromatographic system was Hewlett Packard 1090 Series II/M AminoQuant™ liquid chromatograph. Separation of amino acids was performed on a narrow bore C18 HP AminoAcid Analysis (200 X 2.1 mm) column, protected by a 15 X 2.1 mm guard column to which had been injected 8 ml of the derivatives. The separated amino acids were detected in a fluorescence detector at 340/450 nm for primary amino acids and 237/340 nm for secondary amino acids (Herbert *et al.*, 2000).

Water activity

Water activity was determined by using water activity meter (Novasina sensor™, Switzerland).

Microbial quality

Aerobic plate count, most probable number (MPN) E. coli, MPN coliforms, MPN fecal coliforms, yeast and mold, and V*ibrio parahaemolyticus* were analysed based on Bacteriological Analytical Manual (USFDA, 2001; USFDA, 2002; USFDA, 2004).

RESULTS

Quality of raw materials

Based on the database on amino acid profiles and protein contents of Thai foods (MOPH Thailand, 2001), the ratios of leucine to protein content were lowest in dried fish maw and sunflower seed (Table 1). The fish maw was commercially available in fried form, while the sunflower seed was in roasted form. Both of these raw materials had

the lowest amounts of leucine in their amino acid profiles as compared to those found in the other foods. Besides, they also contained reasonable contents of protein, which was required for the formulation.

In order to address the safety concern of the raw materials, the most suitable reference was the EC regulation No. 333/2007 on lead, cadmium, mercury, and inorganic tin in foods. The heavy metal contamination levels in both raw materials did not exceed the EC regulation (data not shown). No aflatoxin contamination was found in the roasted sunflower seed product (data not shown).

Formulated food products

In all formulas, fish maw and roasted sunflower kernel were used as the protein sources, but at different amounts, based on the need for leucine limitation. Formula I was developed for the MSUD patient since it needed to be a tube-feeding diet. Formulas II - V were developed for using as an instant drink (IV), snack (V) and ingredients for main dishes (II and III).

Sucrose was added to provide energy and sweet taste for most formulas except for Formula III, which did not require sweetness due to the nature of the formulation. Palm olein oil in Formula III (bouillon cube) functioned as binder and energy source. Baking chocolate was the main flavouring and structuring compound for Formula V as well as energy source. Other minor ingredients i.e. salt, monosodium glutamate (98% MSG + 1% Inosinate and 1% Guanylate), mushroom flavoured seasoning, glucose syrup, and cocoa powder were mixed into the food products to provide better flavouring (Table 2).

All the formulas provided 500-600 kcal/100 g. Energy sources were mainly from carbohydrate and fat, which were similar to the commercial products, BCAD 2® for MSUD and LMD® for IVA (Table 2). The macronutrient profiles of the developed products however were different from the commercial products, especially for the protein content. Since foods ingredients were used in the formulations, the amount of total protein was limited in order to control the blood level of undesirable branched chain amino acids at a non-harmful level (Wappner & Gibson, 2006; Barshop, 2006). Overall, Formula I had the lowest level of protein and fat but higher in carbohydrate content than Formulas II-

Table 1. Ratio of leucine to protein content based on 100 g edible portion of various protein sources

Protein source	*Per 100 g edible portion*		*Leucine:Protein ratio*
	Protein (g)	*Leucine (mg)*	
Sesame seed, black	20.3	1309	64.5
Mungbean, dried	24.4	1878	77.0
Soybean, dried	34.6	2549	73.7
Chicken, breast	25.6	1538	60.1
Pork, lean meat	20.4	1584	77.6
Pork, liver	20.4	1560	76.5
Hen egg, whole	13.1	860	65.6
Fish maw, fried	38.3	1443	37.7
Sunflower seed†, roasted	24.7	1244	50.4
Milk powder, non-fat	36.9	2927	79.3

†When sunflower seed is dehulled, the edible remainder is called the sunflower kernel
Source: The database on amino acid profiles and protein contents of Thai foods (MOPH Thailand, 2001)

Table 2. Contents of nutrients and branched-chain amino acids of the food formulations developed for patients of branched-chain organic acidurias, as compared to commercial products

Formula[†]	*Ingredients (g/ 100 g)*	*Nutrient composition per 100 g (% energy distribution)*			*Energy (kcal/ 100 g)*	*Branched-chain amino acid (mg/ 100 g)*		
		Protein	*Fat*	*Carbohydrate*		*Isoleucine*	*Leucine*	*Valine*
I	Fish maw (5), roasted sunflower kernel (30), non-dairy creamer (20), maltodextrin (30), sucrose (15), salt (1)	9.9 (7.8)	26.2 (46.1)	59.3 (46.2)	512	285	470	461
[‡]BCAD 2®	Corn syrup solids, amino acids, sugar, soy oil, modified corn starch (no percentages mentioned)	24.0 (23.4)	8.5 (18.7)	57.0 (55.6)	410	0	0	0
II	Fish maw (10), roasted sunflower kernel (25), non-dairy creamer (40), maltodextrin (12), sucrose (13), salt (1)	11.8 (8.6)	33.6 (55.4)	49.1 (36.0)	546	294	526	452
III	Fish maw (10), roasted sunflower kernel (25), non-dairy creamer (15), maltodextrin (30), palm oil (20), salt (1), MSG (1), mushroom flavwored seasoning (2)	12.3 (8.2)	45.0 (67.4)	36.7 (24.4)	601	268	489	516
IV	Fish maw (10), roasted sunflower kernel (25), non-dairy creamer (40), sucrose (25), salt (1), cocoa powder (1)	11.8 (8.6)	33.6 (55.4)	49.1 (36.0)	546	302	538	464
V	Fish maw (6.7), roasted sunflower kernel (16.8), non-dairy creamer (26.8), sucrose (16.8), baking chocolate (33), salt (1)	8.2 (6.2)	36.7 (58.3)	51.0 (36.0)	567	212	398	328
[‡]LMD®	Corn syrup solids, vegetable oil, amino acids, modified corn starch, sugar (no percentages mentioned)	16.2 (13.0)	26.0 (46.8)	51.0 (40.8)	580	580	0	630

[†]Formula I: Mixed powder for preparing tube-feeding diet; Formula II: Rice sprinkle powder; Formula III: Bouillon cube; Formula IV: Instant cocoa drink; Formula V: Snack bar

[‡]BCAD 2 and LMD: Commercial products for MSUD and IVA patients, respectively

Table 3. Water activity and microbiological quality of the food products developed for patients of branched-chain organic acidurias

Analysis	*Formula*†				
	I	*II*	*III*	*IV*	*V*
Water Activity (Aw)	0.3	0.4	0.4	0.4	0.5
Aerobic Plate Count, cfu/g	Not detected	Not detected	Not detected	Not detected	Not detected
MPN *E. coli* /g	< 3	< 3	< 3	< 3	< 3
MPN Coliforms /g	< 3	< 3	< 3	< 3	< 3
MPN Fecal coliforms /g	< 3	< 3	< 3	< 3	< 3
Yeast and mold, cfu/g	Not detected	Not detected	Not detected	Not detected	Not detected
Vibrio parahaemolyticus, cfu/25 g	Not detected	Not detected	Not detected	Not detected	Not detected

†Formula I: Mixed powder for preparing tube-feeding diet; Formula II: Rice sprinkle powder; Formula III: Bouillon cube; Formula IV: Instant cocoa drink; Formula V: Snack bar

V. Formula III (bouillon cube) contained slightly more energy and total fat than the others due to the added fat.

Amino acid profiles

Since branched-chain amino acids were the critical nutrients for the branched-chain organic acidurias patients, it was important to determine the amino acid profiles of the developed food products. The commercial products, LMD® and BCAD 2® did not contain the offending amino acids since they were formulated from pure amino acids. Table 2 shows that the developed formulas (Formulas I–V) contained the offending amino acids but at controlled levels that were not harmful for the IVA and MSUD patients. For example, an IVA patient of 36.5 kg weight who consumed 1,016 kcal from Formula II, III, IV or V plus 490 kcal from his/her regular low protein meal per day would obtain 1,433 mg of leucine, which was still under the maximum limit for the IVA patient of 1,500 mg/day.

Microbial quality and water activity

For safety reasons, the developed products were analysed for the microbiological quality based on the nature of the products. Table 3 indicates that no growth of the tested bacteria was detected, including mesophilic aerobic bacteria (aerobic plate count), pathogenic/sanitation indicator bacteria (MPN *E. coli*, MPN coliforms, MPN fecal coliforms), yeast and mold, and *Vibrio parahaemolyticus*. Water activities of the developed products were 0.3-0.5 which were not conducive for microbial growth.

DISCUSSION

After reviewing the locally available food items in Thailand, it was found that fish maw and sunflower kernel contained reasonable amounts of protein with limited content of branched chain amino acid. The selected fish maw was derived from farmed pangasius fish (*Pangasius hypophthalmus*), in which the variety and feeding environment were controlled. Sunflower kernel was also obtained from systematic cultivation of *Helianthus annus* farm. Under such conditions, both raw materials were more stable in their nutrient profiles. Obtaining the raw materials from farmed sources ensures continued availability of the study materials.

Both raw materials were preserved in dried/roasted and dried/fried forms,

which made them available all year round. These raw materials were used as the main sources of amino acids and at the same time they also provided energy from fat that is naturally found in sunflower kernel and from the cooking oil used in frying of fish maw. For safety reasons, fish maw was tested for contaminant heavy metals while sunflower kernels were tested for pesticide and aflatoxin. In the product development process, the products were treated as low water activity (Aw<0.85), which did not allow any growth of pathogens. However, the microbiological qualities of all products were also tested since the products were used with no further heating. By using the developed processes, the products passed the relevant microbial standards for the products (FAO/WHO, 1993).

For long term use, all forms of products should be made available as choices to the patients. As compared to the commercial products, our products still contained certain amounts of branch-chained amino acids, however the amount of leucine per day was 1,000–1,471 mg/day, which was at a level that is below the harmful level for patients (more than 1,500 mg/day).

Total cost of the locally available raw materials that were used for preparing these products ranged from US$ 0.42-0.51 per 100 g, while the costs of the commercial products i.e. BACD2 and LMD were up to US$ 15.6 and US$ 12.3 per 100 g, respectively.

CONCLUSION

It is feasible to formulate and produce food products that used locally available raw materials, namely fried fish maw and roasted sunflower kernel as alternatives to commercial products for branched-chain organic acidurias patients. Leucine in the developed products could be controlled to be less than 1,500 mg/day. The products were shelf-stable and could be easily controlled for safety. Several forms of products could be produced as choices for patients and parents. The products should be beneficial for patients in developing countries where the commercial disease-specific amino acid formulas are unaffordable and often unavailable.

Acknowledgements

This research work was supported by Research Assistant scholarship from the Faculty of Graduate Studies, Mahidol University Academic Year 2012 and grant from Faculty of Medicine Ramathibodi Hospital (directed to DW). DW is a recipient of the Research Career Development Awards from the Faculty of Medicine Ramathibodi Hospital, Mahidol University.

Authors' contributions

CS, conducted the study and wrote the manuscript; CV, designed the study, advised on food science and nutrition issues and wrote manuscript; WD, designed the study and advised on the medical issues; SU, designed the study and advised on medial issues; CN, advised on medical issues.

Conflict of interest

The authors declared no conflict of interest.

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Mal J Nutr 24(4): 617-625, 2018

Indigenous pigmented corn (*Zea mays* L.) flour as substitute for all-purpose flour to improve the sensory characteristics and nutrient content of crackers

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ABSTRACT

Introduction: There is growing interest in using indigenous crops as alternative food sources that can address food and nutrition insecurity in developing countries. This study aimed to evaluate the nutrient content and sensory characteristics of crackers developed from indigenous pigmented corn (*Zea mays* L.) called *camotes*. **Methods:** Eleven *camotes* and all-purpose flour (APF) combinations were made into crackers following the modified method of Manley (2001) at University of the Philippines Los Baños. Sensory evaluation was conducted using the linear scale of quality scoring based on standard methods. Proximate composition analysis, nutrient contents and phytochemical components were conducted using standard methods. Data from the sensory evaluation were analysed using non-parametric Analysis of Variance (ANOVA), while results from the chemical analysis were analysed using One-Way ANOVA and Tukey's honestly significant difference (HSD) test. **Results:** Sensory characteristics of the cracker containing 80% *camotes* flour were comparable with those of the 100% APF cracker except in colour. Crackers containing 100% and 80% *camotes* flour had significantly higher levels of protein, dietary fibre, lysine, tryptophan, zinc, antioxidant activity, phenols, and flavonoids than crackers made of 100% APF. **Conclusion:** Crackers formulated at 80:20 *camotes*:APF blend compares most favourably with that from APF crackers. Incorporation of *camotes* flour into making of crackers increases its nutrient content. Such crackers can serve as a nutrient-dense alternative food source to address the food and nutrition insecurity situation in the Philippines.

Keywords: Cracker, pigmented corn, all-purpose flour

INTRODUCTION

Snack foods play an important part in people's lives especially for individuals who need to increase their energy intake, maintain normal blood glucose levels, prevent emotional eating and control weight. However, many of the snack foods available nowadays are high in calories, fat, salt, or sugar (or all of these), as well as contain processed ingredients and additives. One of the most popular cereal-based snacks are crackers. Crackers represent one of the most important segments of the baking

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industry because consumers view these products as having lower energy density compared to sugar biscuits (Serna-Saldivar, 2012).

Crackers are formulated with higher-protein flours, often from a mixture of hard and soft wheats. While wheat-based bakery products are generally considered as ideal, however in countries where climatic conditions do not allow wheat cultivation, or imported wheat is unaffordable, production of bakery products from 100% wheat flour is prohibitive. Hence, the use of composite flours in making bakery products has been widely researched (McWatters *et al.*, 2003).

A popular crop in the Philippines is corn (*Zea mays* L.) or maize. It is well known as "poor man's nutricereal" due to the presence of high contents of carbohydrates, fats, proteins, and important vitamins and minerals at a cheaper cost than other cereals. The value-added products prepared from specialty corns include traditional foods, infant foods, health foods, snacks, as well as savoury and baked products. Corn has a wide range of kernel colours such as white, yellow, orange, red, purple and black. In addition to its attractive colours, pigmented corn is rich in phytochemicals and many secondary metabolites, such as phenolic compounds, carotenoids, and flavonoids (Zilic *et al.*, 2012). If corn flour from pigmented corn is to be used as a substitute for all-purpose flour (APF) in cracker production, a more nutritious cracker could be produced. It is with this aim that a study was undertaken to produce crackers of acceptable sensory, nutritional, and microbial quality by utilising locally grown and available corn.

The general objective of the study was to evaluate the nutrient content and sensory characteristics of crackers developed from indigenous pigmented corn (*Zea mays* L.) or *camotes* flour. Specifically, the study aimed to evaluate the sensory characteristics in terms of colour, taste, texture/mouthfeel and general acceptability of developed *camotes* crackers; determine the nutrient content, such as the proximate composition (moisture, fat, protein, total ash, and carbohydrates), total dietary fibre, mineral content (iron and zinc), as well as lysine and tryptophan contents of *camotes* flour, all-purpose flour (APF), APF crackers. The study also determined antioxidant activity and phytochemical components (phenols, tannins, flavonoids, saponins, and alkaloids) of *camotes* flour, APF, APF crackers, and the most acceptable cracker developed.

MATERIALS AND METHODS

The study was conducted from May 2015 to August 2015 at the Bio-assay Laboratory of the Institute of Human Nutrition and Food (IHNF) at the University of the Philippines Los Baños (UPLB), Laguna. The *camotes* flour was procured from the Institute of Plant Breeding (IPB) Los Baños, Laguna while APF was purchased from a conveniently chosen supermarket. Various proportions of *camotes* and APF were made into crackers and evaluated for acceptability and sensory characteristics. Crackers from 11 formulations, consisting of the following *camotes:* APF ratio: 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100 were prepared following the modified method of Manley (2001). Yeast was mixed with water (25°C) to form a suspension, to which the other ingredients were added and kneaded to form a smooth dough. The dough was stored for 2 hours in a refrigerator followed by sheeting to 1 mm thickness using a rolling pin. The dough was then cut into squares measuring 3cm x 3cm and baked at 350°F for 15 minutes.

Sensory evaluation was conducted to identify the cracker with the most acceptable *camotes*:APF ratio. A panel comprising of 30 graduate students from the International House in UPLB were

recruited to serve as judges. Each judge was served with six randomly arranged cracker samples for the first batch and five randomly arranged cracker samples for the second batch. The crackers were individually placed in identical small covered containers and coded with a three-digit number. Testing was done in two batches on the same day. The samples were evaluated using the 15-cm linear scale of quality scoring for colour, taste (saltiness), texture/mouthfeel (crispiness, hardness, grainy texture, cohesiveness of mass, and adhesiveness of mass), aftertaste, and general acceptability of the crackers. The most acceptable *camotes* and APF combination of crackers were used for the succeeding nutrient content analyses.

Proximate composition analysis was carried out following the standard methods of the Association of Analytical Chemist (AOAC) (1990). Moisture content was determined by oven-drying and fat content was estimated by soxhlet extraction. Protein content was estimated by micro Kjeldahl method and ash content by combustion. Lysine and tryptophan contents were determined based on the colorimetric methods described by Tsai, Hansel & Nelson (1972) and Opienska-Blauth, Charezinski & Berbec (1963), respectively. Zinc concentration was determined using zincon solution (Valdman, Areco & Alfonso, 2007) while iron concentration was measured following AOAC procedure. Enzymatic-gravimetric method (AOAC, 1990) was followed to determine dietary fibre. The antioxidant activity and phytochemical components were determined following the standard method of AOAC (1990). The percentage of antioxidant activity was assessed by 2,2 diphenyl-1-picryl hydrazyl (DPPH) free radical assay. The total phenolic content was determined based on the Folin-Ciocalteu method and total flavonoid was determined using aluminium chloride colorimetric method. All analyses were carried out in triplicates.

Results of the sensory evaluation of the 11 formulations of *camotes* and APF composite crackers were statistically analysed using non-parametric Analysis of Variance (ANOVA). The results obtained from the chemical analyses were analysed using One-Way ANOVA. If statistical differences were significant, Tukey's honestly significant difference (HSD) test was used for further analysed at < 5% level of significance. The results were expressed as mean values ± standard deviation.

RESULTS

It was observed that when more *camotes* flour was substituted for APF, the colour of the cracker became darker. The mean scores ranged from 3.33 to 11.43, which are described as very light brown to very brown (Table 1). Browning could also be attributed to Maillard reaction which occurs between reducing sugars and principally free amino acids and peptides (usually from proteins) when heated e.g. baking (Manley, 2011).

Taste is a key component in sensory evaluation testing. In the study, the intensity of saltiness was evaluated. The scores ranged from 3.65 to 4.12, which are described as bland. No significant difference in saltiness was found among the crackers.

Crispiness, the noise and force with which the sample breaks or fractures, was perceived in all formulations. It is considered as a desirable quality of crackers as it will determine consumer acceptability and represent a critical factor in limiting cracker shelf-life. Crispiness increased with increasing amounts of APF but were only significantly different between the 100% *camotes* cracker and 50 – 0% *camotes*:APF cracker.

Hardness, or the force required to bite through, is another textural attribute evaluated in the study. Too much hardness in a cracker is not desirable as it can cause difficulty in

Table 1. Mean sensory and acceptability scores of 11 cracker formulations

Camotes: APF Cracker	*Colour*	*Taste*	*Crispiness*	*Hardness*	*Grainy texture*	*Cohesiveness*	*Adhesiveness*	*Aftertaste*	*General acceptability*
(100:0)	11.43±2.50^{a}	4.02±1.63^{a}	7.93±2.96^{b}	11.07±1.50^{a}	9.70±3.77^{a}	9.65±4.23^{a}	5.23±3.25^{a}	7.23±3.70^{a}	9.07±1.0^{b}
(90:10)	11.40±2.36^{a}	3.87±2.26^{a}	8.50±2.70^{ab}	10.90±1.65^{a}	9.10±3.27^{ab}	9.53±3.98^{a}	5.73±3.16^{a}	6.83±4.59^{ab}	9.27±0.9^{ab}
(80:20)	9.87±2.49^{ab}	3.98±1.85^{a}	8.75±3.92^{ab}	9.55±3.11^{ab}	9.17±3.73^{ab}	9.00±3.04^{a}	6.03±3.01^{a}	6.95±3.96^{ab}	9.77±0.7^{a}
(70:30)	8.1±2.99^{bcd}	3.93±2.69^{a}	9.47±3.48^{ab}	9.52±2.47^{ab}	8.58±4.55^{ab}	8.47±2.98^{a}	6.70±3.16^{a}	6.00±3.90^{ab}	9.47±0.9^{ab}
(60:40)	8.47±3.22^{bc}	4.12±2.89^{a}	9.77±3.24^{ab}	9.37±3.19^{ab}	8.32±2.54^{ab}	8.37±2.90^{a}	6.80±4.00^{a}	5.63±3.14^{ab}	9.42±1.0^{ab}
(50:50)	7.57±3.19^{c}	4.07±1.66^{a}	9.73±2.87^{ab}	9.23±3.48^{ab}	8.55±3.33^{ab}	8.37±3.17^{a}	6.80±3.28^{a}	5.65±2.98^{ab}	9.40±1.0^{ab}
(40:60)	6.97±2.65^{cd}	3.83±2.28^{a}	10.37±2.89^{a}	8.57±2.99^{b}	8.00±3.33^{ab}	8.33±3.90^{a}	7.05±4.11^{a}	5.27±3.69^{ab}	9.37±1.2^{ab}
(30:70)	6.07±2.46^{d}	4.03±1.51^{a}	10.50±3.78^{a}	8.80±2.75^{b}	7.60±3.05^{ab}	8.30±2.56^{a}	7.47±3.01^{a}	5.10±3.14^{b}	9.33±0.8^{ab}
(20:80)	4.13±2.21^{e}	3.92±2.31^{a}	10.77±2.66^{a}	8.57±3.31^{b}	6.62±3.84^{b}	8.32±3.03^{a}	7.97±3.03^{a}	4.97±3.30^{ab}	9.30±0.5^{ab}
(10:90)	3.33±1.54^{e}	3.77±1.97^{a}	10.80±2.51^{a}	8.43±3.76^{b}	6.65±3.74^{b}	8.13±3.56^{a}	7.90±3.79^{a}	4.28±2.87^{b}	9.20±0.4^{ab}
(0:100)	3.33±2.48^{e}	3.65±2.25^{a}	10.77±3.07^{a}	8.07±2.99^{b}	6.63±2.66^{b}	7.87±3.21^{a}	8.00±3.83^{a}	4.23±3.26^{b}	9.18±1.0^{ab}

*Data is expressed as means with different superscripts in columns indicating statistically significant difference at p≤0.05, (n=30), using Tukey's HSD test.

Legend:

	Colour	**Taste (Saltiness)**	**Crispiness**	**Hardness**	**Grainy texture**	**Cohesiveness**	**Adhesiveness**	**Aftertaste**	**General acceptability**
15	Dark brown	Very salty	Very crispy	Very hard	Very grainy	Very cohesive	Very sticky	Highly perceptible	Extremely acceptable
0	Very light brown	Bland	Not crispy	Soft	None	Loose	Not sticky	Imperceptible	Not acceptable

chewing the product. The study showed that substituting up to 80% *camotes* flour to APF can produce a cracker with similar hardness as that of the APF cracker. Crackers with more than 80% *camotes* flour yielded a harder texture than the APF cracker. Geometrical attribute (grainy texture) is described as the amount of loose and grainy particles resulting from bite. The cracker with the least rating in the study was the APF cracker (100% APF). The rest of the formulations had higher mean scores than the APF cracker especially those that had more than 20% of *camotes* flour. Although there were notable differences in the mean scores, significant difference was only noted between APF cracker and the cracker with 100% *camotes* flour.

Cohesiveness of the crackers or the degree to which mass holds together was observed in all the formulations but were not statistically different. Adhesiveness refers to the degree to which mass sticks to the roof of the mouth or teeth (Lawless & Heymann, 2010). In this test, judges were instructed to place the sample on their tongue and press it on the palate, and rate the force required to remove it from the palate using the tongue. Results showed no significant differences among all formulations. The corn flavour or aftertaste in the crackers was slightly perceived in all the formulations except in 100% *camotes* flour, which was significantly different from that of the APF cracker. Although no previous study has replaced APF with *camotes* flour in the production of crackers, the results are consistent with other studies where findings showed that aftertaste is positively affected when crop substitution in bakery products is increased (Noor Aziah & Komathi, 2009). The 100% *camotes* cracker had the lowest mean score for general acceptability and was the only cracker that was significantly different from the APF cracker, while the cracker made with 80% *camotes* flour had the highest mean score and was the most acceptable. Among all the sensory attributes, significant difference between 80:20 *camotes*:APF cracker and the APF cracker was noted only in colour.

Proximate composition of *camotes* flour and APF

Significant differences between the *camotes* flour and APF were observed in moisture, ash, fibre, protein, and fat while no significant difference was noted in carbohydrates (Table 2).

Moisture content was almost 50% significantly lower in *camotes* flour at 3.69% than in APF at 6.58%. The low moisture contents of the two flours were within the recommended limit of <10.00% for longer storage periods.

The study also revealed that *camotes* flour was 13.0% significantly higher in ash, 38.4% higher in fibre, 30.0% higher in protein, but 57.0% lower in fat than the APF. These results suggest that if *camotes* flour is used as a substitute for

Table 2. Mean proximate composition of *camotes* flour and APF

Components[†]	*Camotes flour*	*APF*
Moisture (%)	3.69 ± 0.12[b]	6.58 ± 0.24[a]
Ash (g/100g)	1.69 ± 0.05[a]	1.47 ± 0.01[b]
Fibre (g/100g)	2.32 ± 0.15[a]	1.43 ± 0.07[b]
Protein (g/100g)	9.71 ± 0.82[a]	6.85 ± 0.41[b]
Fat (g/100g)	1.23 ± 0.09[b]	2.84 ± 0.20[a]
Carbohydrate (g/100g)	81.35 ± 0.81[a]	80.82 ± 0.67[a]

[†]Values are expressed as g/100g dry basis except for moisture content.
[a,b] Means in same row with different superscripts differ significantly ($p<0.05$) using Tukey's HSD test.

APF, the resulting cracker could have higher fibre and protein content.

Nutrient content analysis of *camotes* flour and APF

All foods of vegetable origin contain fiber, but in variable quantities (Sardesai, 2011). The study revealed that total dietary fiber in *camotes* flour was 19.4% significantly higher than in APF (Table 3).

The iron and zinc analyses revealed that *camotes* flour was 34.1% significantly lower in iron content but more than 25.0% significantly higher in zinc content than APF. *Camotes* flour also had almost three times higher lysine content when compared to APF. Tryptophan was found to be 35.0% significantly higher in *camotes* flour than in APF. This implies that substituting *camotes* flour for APF would yield a cracker that has higher lysine and tryptophan contents.

The red pigment of the *camotes* corn could be attributed to anthocyanidins which, according to research, are a unique subgroup of flavonoids responsible for the distinctive colours in plants (De La Rosa, Alvarez-Parilla & Gonzales-Aguilar, 2009). Pigmented corn is a rich source of phytochemicals which are regarded as an important source of antioxidants in cereals (Khampas *et al.,* 2013). Phytochemicals are bioactive non-nutrient plant compounds that have been associated with reduced risk of major chronic diseases. Phenolics are compounds with one or more aromatic rings and one or more hydroxyl groups such as phenolic acids, plant lignans, alkylresorcinols, and flavonoids. In principle, the higher the amount of phenolics and other antioxidant compounds, the greater the potential to affect antioxidant capacity and this has been well documented *in vitro* using a variety of assays (Allen & Prentice, 2012). Phytochemicals such as phenols and flavonoids were significantly higher in *camotes* flour than in APF, which could have contributed to the significantly higher antioxidant activity observed in *camotes* flour.

Proximate composition of 80:20 *camotes*:APF cracker and APF cracker

The trend in the differences observed in the proximate composition of *camotes* flour and APF was also observed when the flours were processed into crackers. Despite the addition of other ingredients, the 80:20 *camotes*:APF cracker remained significantly lower in moisture content by 35.0% and fat by 15.0%, and significantly higher in ash, fibre, protein, and carbohydrates by 4.0%, 33.0%, 23.6%, and 2.6%, respectively, than the APF cracker (Table 4).

Table 3. Nutrient content and phytochemical components analyses of *camotes* flour and APF

Components[†]	*Camotes flour*	*APF*
Total Dietary Fibre (%)	2.73±0.01[a]	2.20±0.0[b]
Iron (mg/100g)	2.7±0.10[b]	4.10±0.10[a]
Zinc (mg/100g)	2.10±0.00[a]	1.50±0.00[b]
Lysine (mg/100g)	427.66±2.27[a]	158.08±2.27[b]
Tryptophan (mg/100g)	50.51±0.72[a]	32.85±0.10[b]
Antioxidant Activity (%)	51.92±0.41[a]	18.3±0.78[b]
Phenols (mg/100g expressed as g/catechin eq/g)	10.00±0.03[a]	2.50±0.20[b]
Flavonoids (mg/100g expressed as mg gallic acid eq/g)	3.73±0.28[a]	1.05±0.18[b]

[†]Values are expressed as mg/100g dry basis

[a,b] Means in same row with different superscripts differ significantly ($p<0.05$) using Tukey's HSD test.

Table 4. Mean proximate composition of 80:20 *camotes:APF* cracker and APF cracker

Components†	*80:20 camotes:APF cracker*	*APF cracker*
Moisture (%)	4.69±0.20[b]	7.23±0.21[a]
Ash (g/100g)	1.44±0.05[a]	1.38±0.00[a]
Fibre (g/100g)	1.58±0.09[a]	1.06±0.01[b]
Protein (g/100g)	7.95±0.06[a]	6.07±0.02[b]
Fat (g/100g)	10.37±0.15[b]	12.23±0.13[a]
Carbohydrate (g/100g)	73.97±0.21[a]	72.02±0.10[b]

†Values are expressed as g/100g dry basis except for moisture content.
[a,b] Means in same row with different superscripts differ significantly ($p<0.05$) using Tukey's HSD test.

Nutrient content and phytochemical components analyses of 80:20 *camotes*:APF cracker and APF cracker

It was observed that the 80:20 *camotes*:APF cracker was 17.1% significantly higher in dietary fibre than the 100% APF cracker (Table 5). According to Brennan & Grandison (2012), the amount of dietary fibre could have an effect in the textural characteristics of food, in that addition of dietary fibre increases the hardness of the products as a result of its effect on cell wall thickness. This is consistent with the sensory evaluation findings in the study where the hardness score of the cracker increased when substitution of *camotes* flour to APF was increased.

The 80:20 *camotes*:APF cracker had 11.6% significantly less iron but 26.0% significantly more zinc than the APF cracker. This was expected since *camotes* flour, which had lower iron but higher zinc contents than APF, was used in higher proportion.

The 80:20 *camotes:*APF cracker had 50.0% significantly more lysine content and 25.0% significantly more tryptophan content than the APF cracker. This proved that supplementation of *camotes* flour to APF could produce a cracker that has a better protein quality because of the increased levels of lysine and tryptophan.

The trend in the phytochemical components and antioxidant activity observed in flours was also observed in the crackers. Phenols and flavonoids were still significantly higher in 80:20 *camotes*:APF cracker than in APF cracker at 75.0% and 65.0%, respectively,

Table 5. Nutrient content and phytochemical components analyses of 80:20 *camotes:*APF cracker and APF cracker

Components†	*80:20 camotes: APF cracker*	*APF cracker*
Total Dietary Fibre (%)	2.11±0.12[a]	1.75±0.12[b]
Iron (mg/100g)	2.91±0.00[b]	3.29±0.10[a]
Zinc (mg/100g)	1.90±0.00[a]	1.40±0.00[b]
Lysine (mg/100g)	400.40±2.27[a]	146.68±3.10[b]
Tryptophan (mg/100g)	45.50±0.57[a]	33.31±0.55[b]
Antioxidant Activity (%)	40.10±0.96[a]	12.90±0.41[b]
Phenols (mg/100g expressed as g/catechin eq/g)	5.30±0.01[a]	1.41±0.02[b]
Flavonoids (mg/100g expressed as mg gallic acid eq/g)	2.38±0.21[a]	0.85±0.13[b]

†Values are expressed as mg/100g dry basis.
[a,b] Means in same row with different superscripts differ significantly ($p<0.05$) using Tukey's HSD test.

despite having other added ingredients. These components have contributed to the antioxidant activity which was 67.8% higher in 80:20 *camotes*:APF cracker than in APF cracker. This showed that using *camotes* flour as a substitute for APF could produce crackers with improved levels of phytochemical components and antioxidant activity.

DISCUSSION

The study revealed that more protein and less fat were obtained from the cracker with 80% *camotes* flour and 20% APF than from the 100% APF, although the 80:20 *camotes*:APF cracker and APF cracker yielded almost the same amount of energy per 100g. The 80:20 *camotes:*APF cracker had better protein quality than the APF cracker, as the former provides almost 50% of both lysine and tryptophan requirements for children. On the other hand, APF cracker provides <20% of lysine and <30% tryptophan requirements for children.

Iron deficiency is considered the most common single-nutrient deficiency disease in the world, particularly affecting women, children, and female adolescents. Meanwhile, zinc deficiency is a serious nutritional problem that negatively affects growth and intellectual and sexual development (Preedy & Watson, 2014). The study showed that 80:20 *camotes*:APF cracker can be a source of iron in children up to 5 years and a source of zinc in children up to 12 years. Inclusion of the 80:20 *camotes:*APF cracker in the daily diet could contribute to the iron and zinc requirements and help prevent inadequacy.

The study also revealed that consuming one serving (20 g) of the 80:20 *camotes*:APF cracker provides 11-14% of the daily requirement for dietary fibre in adults, compared to 9-11% from 20 g of APF cracker. Adequate intake of dietary fibre each day helps to maintain bowel integrity by increasing stool weight and promoting normal laxation (Wilson *et al.*, 2010). It may also protect against heart attack and stroke by lowering blood pressure, improving blood lipids, reducing inflammation, and reducing the risk of type 2 diabetes by slowing glucose absorption which helps to prevent glucose surge and rebound (Whitney *et al.*, 2011).

The study showed that 80:20 *camotes:*APF cracker has significantly higher phytochemical compounds and antioxidant activity than APF cracker. This is beneficial because dietary antioxidant substances contribute to health and well-being by delaying or preventing the negative impact of oxidation that plays a role in many diseases like diabetes, heart disease, cancer, and neurodegenerative disorders (Divya & Pandey, 2014). Phenolic compounds and flavonoids found in the 80:20 *camotes*:APF cracker have the potential to function as important radical-scavenging antioxidants. It has been said that flavonoids exert their antioxidant activity by inhibiting the activities of enzymes including xanthine oxidase, myeloperoxidase, lipoxygenase, and cyclooxygenase, by chelating metal ions, and by interacting with other antioxidants such as ascorbate (Divya & Pandey, 2014).

CONCLUSION

Crackers made from 80:20 *camotes*:APF blend was found to be most acceptable based on sensory evaluation, and nutrient and phytochemical contents. Hence, the *camotes* flour showed potential for use in producing nutrient-dense alternative foods to address food and nutrition insecurity concerns in developing countries.

Acknowledgements
The authors are grateful to the DOST-ASTHRDP NSC for funding this research.

Authors' contributions

ZGS, principal investigator, conceptualised and designed the study, data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript; CBJ advised on the data analysis and reviewed the manuscript; EID advised on the interpretation and reviewed the manuscript, WAH advised on the data analysis and interpretation and reviewed the manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Mal J Nutr 24(4): 627-635, 2018

SHORT COMMUNICATION

Clinical audit on adherence to using Malnutrition Screening Tool and dietitian referral in the Oncology Outpatient Clinic, National Cancer Institute, Malaysia

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ABSTRACT

Introduction: Malnutrition is a frequent complication in cancer patients and can negatively affect treatment outcome. Preliminary audit conducted at the Oncology Clinic, National Cancer Institute (NCI), found that only 5.8% of outpatients underwent nutrition screening using the Malnutrition Screening Tool (MST), and only 2.6% of dietitian referrals were recorded. This audit aims to determine the rate of adherence to nutritional screening, and to implement remedial measures for improved patient care. **Methods**: This was a cross-sectional audit comprising three phases, namely initial audit, remedial measures and re-audit. Criteria audited include screening rate using MST and dietitian referral based on MST scores. Standards were set at 100% for both criteria. Data collected for initial audit were patients' MST scores records and total dietitian referral forms retrieved from Electronic Medical Records. After initial audit, self-administered questionnaires for nurses and physicians were developed to identify barriers. Measures implemented for change included patient-administered MST to shorten screening time, and procedure flowchart to facilitate referral. After 6 months, a re-audit was conducted. **Results**: Total subjects for initial audit and re-audit were 349 and 390, respectively. Initial audit and re-audit showed screening rate using MST increased significantly from 6.3% to 79.5%, but there was no significant change for the dietitian referral rate. **Conclusion**: This clinical audit has led to a change in the policy in NCI outpatient clinics whereby nurses directly schedule dietitian referrals without going through physicians for patients with MST scores ≥2. Continuous audit and monitoring are necessary to facilitate improvement in MST implementation for better outpatient care.

Keywords: Malnutrition Screening Tool (MST), cancer patients, clinical audit, dietitian referral

INTRODUCTION

Malnutrition is a frequent complication in patients with cancer and can negatively affect the outcome of treatment besides being associated with higher rates of morbidity and mortality (Santarpia, Contaldo & Pasanisi, 2011). The prevalence of malnutrition among cancer patients has been estimated between 15% and 80% with weight loss and lethargy as the main symptoms being reported (Haehling & Anker,

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2010). In a study conducted in the East Coast of Peninsular Malaysia, more than one third of newly-diagnosed cancer patients were found to be underweight and undernourished (Menon *et al.*, 2014). Almost 20% of cancer patients have been reported to die from the effect of malnutrition, or its complications rather than the cancer diagnosed (Wu *et al.*, 2009). A significant number of cancer patients at risk of malnutrition remained undetected due to lack of nutrition screening during diagnosis and absence of nutritional evaluation as part of routine practice in the clinical setting (Koom, Ahn & Song, 2012). Therefore, there is increased probability of missed early intervention despite its importance towards nutritional benefit of cancer patients. It has been reported that only 30% to 60% of cancer patients who were at risk for malnutrition received nutritional treatment, and in many instances, even patients diagnosed with severe malnutrition failed to receive an appropriate nutritional intervention (Attar *et al.*, 2012; Segura *et al.*, 2005).

Nutrition screening is the process of identifying specific patients from the broader cancer population who require nutritional assessment followed by intervention if needed, because they may be at risk of malnutrition (Biggs, 2012). This process aims to increase awareness and enable early recognition and treatment needed in cancer care. Nutritional screening process is necessary even when nutritional risk is not obviously present since the impact of early nutritional intervention on the quality of life of cancer patients is well recognised. Moreover, it is essential to evaluate thoroughly the nutritional status of patients during treatment, particularly those undergoing radiotherapy or concomitant radio chemotherapy (Arends *et al.*, 2017). Therefore, it is important to implement an appropriate nutrition screening tool to identify cancer patients at risk of malnutrition early in order to plan the best possible intervention and follow-up during cancer treatment and progression (Santarpia *et al.*, 2011; Bauer & Capra, 2003).

Several guidelines have been published recommending that the implementation of nutrition screening must be completed at the time of diagnosis of cancer patients to detect nutritional disturbance at an early stage (Arends *et al.*, 2017; Thompson *et al.*, 2017; August *et al.*, 2009). Lack of screening and referral can result in late detection and intervention of malnutrition leading to reduced treatment outcome. In order to be efficient, screening should be brief, inexpensive, and highly sensitive and have good specificity. Validated nutrition screening tools, such as Nutrition Risk Screening 2002 (NRS-2002), Malnutrition Universal Screening Tool (MUST) and Malnutrition Screening Tool (MST), Mini Nutritional Assessment Short Form Revised are recommended (Isenring & Elia, 2015).

In view of the usefulness of nutritional screening in identifying cancer patients at risk of malnutrition for early nutrition intervention, the Dietetic Unit in National Cancer Institute (NCI) Putrajaya, established a departmental policy requiring every outpatient of the Oncology Clinic and other specialist clinics, either as new or follow-up visit, to be screened using MST by nurses. The MST was designed for use by non-nutrition-trained staff so as to provide a basis for dietetic referrals and interventions (Barker, Gout & Crowe, 2011; Ferguson *et al.*, 1999). The MST is reported to have a sensitivity of 66% and a specificity of 83% for oncology patients (Shaw *et al.*, 2015). It has also been shown to be quick, valid and a reliable tool to identify chemotherapy and radiation oncology outpatients at risk of malnutrition (Isenring *et al.*, 2006; Academy of

Nutrition and Dietetics, 2017). It is a simple, three questions screening tool assessing recent unintentional weight loss and appetite changes (Anthony, 2008). The sum of these two parameters is obtained to give a score between zero and five. Patients receiving a score of two or more are then considered to be at risk of malnutrition (Ferguson *et al.*, 1999) requiring dietitian referral.

A pre-initial audit undertaken in the Oncology Clinic, NCI in September 2016, found a low screening rate using the MST (5.8%) and only 2.6% of the dietitian referrals were recorded. Therefore, this audit aims to determine the rate of adherence to nutritional screening and to implement remedial measures so as to provide improved patient care.

MATERIALS AND METHODS

The National Cancer Institute (NCI), Putrajaya is a tertiary hospital in Malaysia, which specialises in oncology. Besides inpatient service, the NCI provides a range of outpatient specialist clinics including Radiotherapy Oncology Clinic, Traditional and Complementary Medicine Clinic, Nuclear Medicine Clinic and Multidisciplinary Clinic. Out of the total outpatient visits to NCI in 2015, an average of 61.5% were visits to the Oncology Clinic. Thus, the Oncology Clinic was selected for this study in view of its highest number of visits, presenting a greater chance for early detection of patients at risk of malnutrition. Permission to conduct the study was obtained from the Director of NCI. This audit was registered with the National Medical Research Registration (NMRR) (ID #NMRR-17-18-34027). Ethical approval was not required from NCI because nutrition screening using the MST is part of the compulsory routine care for every outpatient seeking treatment in NCI specialist clinics. This is stated in the Dietetic Unit Policy approved by the NCI Hospital Director.

Data collection

This was a cross-sectional audit conducted for a period of six months between November 2016 to May 2017 in three different phases, namely initial audit, implementation of remedial measures and re-audit. A five working days data collection was conducted for each initial audit and re-audit phase. Every outpatient registered at the Oncology Clinic within the data collection period was included as subjects. The exclusion criterion for this audit was patients who came for blood taking procedure only.

Initial audit phase

Initial audit took place for five consecutive working days during the second week of November 2016. Data collected were patients' MST scores records and total dietitian referral forms, both of which were retrieved from Electronic Medical Records (EMR) and recorded into an Excel spreadsheet.

The process of nutrition screening began with outpatients who came to the clinic being assigned to the Screening Room for assessment by a nurse. The nurse weighed the patient, and carried out a face-to-face interview for weight history and appetite changes, followed by filling up the electronic MST questionnaire. (The version of MST questionnaire provided in the EMR was only available in English language. For non-English-speaking patients, the questions were translated verbally into the Malay language by the nurse. A MST score was computed for each patient (Table 1).

After MST screening was completed, the nurse informed the attending physician to complete the electronic dietitian referral form in EMR. This was followed by confirmation of the Dietetic Clinic for having received patients with MST score ≥ 2. As dietitian referrals

Table 1. MST questionnaire and scoring

1. Have you lost weight recently (within the last 6 months) without trying?	
No	0
Unsure	2
Yes	See question 2
2. If YES, how much weight have you lost?	
1-5kg	1
6-10kg	2
11-15kg	3
>15kg	4
Unsure	2
3. Have you been eating poorly because of decreased appetite?	
No	0
Yes	1
Total score	

could be made either based on MST scores, or solely on the physician's clinical judgement, all dietitian referral forms received were checked to ensure that patients with MST scores <2 were excluded.

Remedial measures phase

Improvement measurements were implemented between initial audit and re-audit phase from December 2016 to April 2017. In this phase, self-administered questionnaires were developed for nurses and physicians to identify barriers in using the MST for screening and dietitian referral.

Nurses were asked several questions including awareness on the existence of the MST form in EMR and whether it was compulsory to perform the screening for every outpatient, compliance and frequency of performing MST, reasons for non-compliance in using MST and whether they proceed to inform physicians for dietitian referral based on MST scores. All nurses on duty at Oncology Clinic during the audit period (n=18) completed the questionnaire. As for physicians, they were asked on their awareness about the existence of the MST form in EMR, awareness on the policy that every outpatient has to be screened using MST by nurses, minimum scores of MST that required dietitian referral and how to refer an outpatient to a dietitian using EMR. All physicians on duty at Oncology Clinic during audit period (n=15) completed the questionnaire.

Remedial measures were planned accordingly to the identified factors contributing towards poor adherence to conducting the nutritional screening. Among the remedial measures taken to improve adherence to nutritional screening implementation were re-emphasis of the nutritional screening policy in specialist clinics by written memo and circulation of standard operating procedure for nutrition screening process, implementation of the MST form in Malay and English, circulation of dietitian's referral procedure for nurses and physician reference, weekly scheduled dietitian clinic hour at the Oncology Clinic and minor policy changes in the dietitian's referral procedures.

Re-audit phase

Re-audit took place for five consecutive working days during the second week of May 2017. Data collected were patients' MST scores records and total dietitian

Table 2. Screening rate and dietitian referral based on MST

Variables	*Initial Audit*		*Re-audit*		χ^2 *(df)*	*p-value*
	n	*%*	*n*	*%*		
Total outpatients visited Oncology Clinic	349		390			
Screening rate using MST	22	6.3	310	79.5	398.7 (1)	<0.001†
Outpatients at risk of malnutrition (MST score ≥ 2)	7	31.8	44	14.2		
Dietitian referral received	1	14.3	7	15.9		1.00‡

†Chi-square test
‡Fisher's exact test

referral forms, both of which were retrieved from EMR, and recorded into Excel spreadsheet.

Data analysis

All data collected were recorded in Excel spreadsheet while analysis was carried out using simple descriptive analysis and IBM SPSS statistics software version 22. Two audit criteria implemented were (1) All outpatients in the Oncology Clinic should be screened using MST, and (2) Patients with MST score ≥ 2 need to be referred to a dietitian for intervention. A rate of 100% was set for both criteria based on the Standard of Procedure for Cancer Patient Individual Diet Management (MOH Malaysia, 2013). Calculation on nutrition screening rate and Dietitian referral rate was done using the formulas below:

Screening rate using MST was calculated based on:

$$\frac{\text{Total number of MST conducted on outpatients}}{\text{Total number of outpatients in Oncology Clinic}} \times 100\%$$

Dietitian referral based on MST was calculated based on:

$$\frac{\text{Total number of dietitian referrals for outpatients with MST scores} \geq 2}{\text{Total number of outpatients with MST scores of} \geq 2} \times 100\%$$

A chi-square test was used to determine whether there was any significant difference for screening rate, while Fisher's exact test was used to determine the significant difference for dietitian referrals between initial audit and re-audit phase. A statistical probability of $p<0.05$ was considered as significant.

RESULTS

Initial audit phase

As shown in Table 2, the total number of outpatients involved in the initial audit was 349. Out of this, only 6.3% were screened using MST. Among the subjects screened, seven patients were found to be at risk, out of whom, only one was referred to a dietitian.

Re-audit phase

After implementation of the remedial actions, the re-audit was carried out involving a total number of 390 outpatients. Screening rate using MST was found to have improved significantly from 6.3% to 79.5% between the initial and re-audit phases ($p<0.001$). However, there was no parallel significant improvement with the dietitian referral rate. While 14.2% of the outpatients with MST score of ≤2 were referred to dietitians in the initial phase, the rate was 15.9% during the re-audit phase.

Table 3. Perceptions regarding MST among nurses and physicians

Variables	*Nurses (N=18)*		*Physician (N=15)*	
	n	*%*	*n*	*%*
Aware about existence of MST screening form in EMR				
Yes	18	100.0	2	13.3
No	0	0.0	13	86.7
Aware that every outpatient has to be screened using MST				
Yes	18	100.0	0	0.0
No	0	0.0	15	100.0
Conducted MST screening among outpatient				
Yes	18	100.0	NA	
No	0	0.0	NA	
Frequency of conducting MST screening among outpatient				
Always	4	22.2	NA	
Sometimes	13	72.2	NA	
Rarely	0	0.0	NA	
Never	1	5.6	NA	
Reason of not performing MST screening regularly				
Not enough time	14	77.8	NA	
Forget	4	22.2	NA	
Feels that it is not important	0	0.0	NA	
Others	0	0.0	NA	
Minimum score of MST to refer to dietitian				
1	NA		0	
2	NA		12	80.0
3	NA		0	0.0
4	NA		2	13.3
Not sure	NA		1	6.7
Action taken for patient with MST scores ≥ 2				
Inform physician	18	100.0	NA	
No action taken	0	0.0	NA	
How to refer an outpatient to dietitian				
Fill up referral form	NA		12	80.0
Fill up referral form + direct call to dietetic clinic	NA		2	13.3
No need to refer as outpatient is already auto-referred	NA		1	6.7

NA = not applicable

Remedial measures phase

The main barrier identified among the nurses on why screening using the MST was not always performed was time limitation (77.8%). As for dietitian referrals, although 80.0% of the physicians were aware of the minimum MST scores (2 or more) to refer a patient, only 13.3% knew the complete procedure, which is filling up the referral form, followed by phone call confirmation to the dietetic clinic (Table 3).

DISCUSSION

Majority of oncology patients are already malnourished by the time of hospital admission (61.9%), as shown in a study among oncology inpatients conducted

at NCI (Norshariza *et al.,* 2017). This highlight the importance of routine nutrition screening among outpatients for early detection of malnutrition before patients requires hospital admission. The prevalence of outpatients at risk of malnutrition in this study during re-audit phase was 14.2%, compared to 13.4% reported in Kenya among cancer outpatients using MUST as nutrition screening tool (Kaduka *et al.*, 2017).

Identification of malnutrition risk at an early stage of patient diagnosis provides an opportunity to initiate the best form of nutritional support.

Nutritional screening and its early identification can identify patients who may be overlooked in establishing reliable pathways of care for patients with undernutrition (Rashidian *et al.*, 2005). According to Kelly *et al.* (2013), effective management of malnutrition requires a more holistic and interdisciplinary process, whereby all members of the clinical team must be involved. This includes nurses who perform initial nutrition screening, dietitians who complete nutrition assessment, nutrition diagnosis and develop evidence-based interventions, pharmacists who evaluate drug-nutrient interactions and physicians overseeing the overall care plan and, in this case, to facilitate referral for nutrition care. This audit highlights a lack of nutrition screening being carried out in outpatient setting due to time limitation, as experienced by nurses. Further investigations suggest that time limitation was due to inadequate staff assigned for screening, lengthy screening time due to lack of familiarity and extra time needed during interview for translation to the Malay language for patients who are non English speaking and delay in the electronic system for charting. Nurses are critical resources as they maintain communication between patients and providers; therefore nurse unavailability creates a gap in the care delivery process (Anali *et al.*, 2014).

Remedial measures that focussed on ways to reduce length of screening time and increase manpower availability had been performed. First, the MST form in both Malay and English has been developed to eliminate the need for translation. By using this bilingual MST form, screening procedure has been changed from staff-administered screening (MST carried out by nurses through interviewing patients) to patient-administered screening. The MST form is then collected for charting into EMR for record purposes. Screening results must be documented within the electronic health record to allow for prompt communication between the nursing staff and other health care team members (Kelly *et al.,* 2013). The involvement of patients in MST screening not only speeds up screening process and reduced staff workload; it also empowered patients to be more aware of their own weight changes and nutritional status.

As for dietitian referral, the main barrier identified was lack of awareness on proper referral procedure. Therefore, a flowchart to explain the referral procedure based on MST scores has been distributed to all physicians on duty at Oncology Clinic. A weekly scheduled dietitian clinic hour at Oncology Clinic has also been initiated to facilitate referrals for patients at risk, and to ensure that patients referred were seen and nutrition intervention was carried out. However, although the nutrition screening rate improved, it did not concur with the referral rate to the dietitian. This might be due to lack of nutrition awareness among physicians. Physicians receive limited formal nutrition education during training and often do not prioritise nutrition among the competing priorities within patient care (Kelly *et al.,* 2013). Furthermore, the electronic health record should be configured to trigger a query for appropriate intervention if a positive

nutrition screening result was obtained (Kelly *et al.,* 2013). However, the electronic system of NCI still required nurses to be aware of the nutritional screening result and to alert physicians to make an effort to complete the electronic referral form to the dietitian. In view of this drawback, this clinical audit has led to a change of policy in NCI outpatient clinics. The policy of needing referral from physicians has been changed to auto-referral whereby nurses can directly schedule dietitian appointment in the electronic system without going through the physician for patients at risk once the MST score is found to be 2 or more.

Limitations of study

As the scoring of MST requires self-reported weight change and perceived appetite change, subjective biases owing to memory and feelings may arise. This audit was not blinded to the staffs in the Oncology Clinic. While they were initially unaware about the ongoing audit during initial audit, however, this audit was disclosed to them during the remedial measures phase and this may lead to bias reporting during the re-audit phase.

CONCLUSION

This clinical audit demonstrated that use of the MST with proper remedial measures was able to significantly improve the screening rate among cancer outpatients at Oncology Clinic, NCI to allow early intervention of patients at risk of malnutrition. Based on this audit, several weaknesses in MST implementation in the Oncology Clinic were identified and rectified. Although there was a significant improvement in the screening rate for malnutrition risk, there was no parallel improvement in the dietitian referral rate. Continuous audit, routine reminders and support from multidisciplinary team are necessary to facilitate further improvement in MST implementation in NCI.

Acknowledgement

We would like to thank the Director General of Health Malaysia for his permission to publish this article.

Authors' contributions

NWH, principal investigator, conceptualised and designed the study, led the data collection, prepared the draft of the manuscript and reviewed the manuscript; NJ, conceptualised and designed the study, advised on the data analysis and reviewed the manuscript; ZAR, conceptualised and designed the study and reviewed the manuscript; BSMHL, assisted in data collection and reviewed the manuscript; SNMS, assisted in data collection and reviewed the manuscript.

Conflict of interest

There is no conflict of interest for this audit.

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