Volume 18, Number 3, November 2023

ISSN 1978-1059
EISSN 2407-0920

JURNAL GIZI DAN PANGAN

INDONESIAN JOURNAL OF NUTRITION AND FOOD

Articles

Published by:

Food and Nutrition Society of Indonesia

Department of Community Nutrition, Faculty of Human Ecology, IPB

Jurnal Gizi dan Pangan

Volume 18, Number 3, November 2023

Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2023, 18(1):1-10***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2023.18.1.1-10*
ISSN 1978-1059 EISSN 2407-0920

Minimum Acceptable Diet and its Associated Factors among Children Aged 6–23 Months in Indonesia

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ABSTRACT

This study aimed to assess the fulfillment of the Minimum Dietary Diversity for children (MDD), the Minimum Meal Frequency (MMF), and the Minimum Acceptable Diet (MAD) and analyze the correlation between individual, socioeconomic, and demographic characteristics and the MAD in children between the ages of 6 and 23 months in Indonesia. This study used a cross-sectional study design. The subjects were 798 children between the ages of 6 and 23 months in Indonesia from a National Total Diet Study. The data collection on the MDD, the MMF, and the MAD used twenty-four-hour dietary recall. The chi-square and multiple logistic regression models were applied in this study with a p-value<0.05 considered significant predictors of outcome variables. The MDD, MMF, and MAD were met by 63.9%, 91.3%, and 61.8% of children, respectively. Significant positive relationships of child's age, mother's educational status, and area of residence (p<0.001) to the MAD. The determinants factors of MAD were the child's age (p<0.05; OR=7.06; 95% CI:3.46–14.38) and area of residence (p<0.05; OR=1.61; 95% CI:1.19–2.15). In conclusion, it is still necessary to improve the fulfillment of the MAD (a combined indicator of the MDD and the MMF). The government's role is to provide balanced nutritional child feeding programs based on local food, strengthen behavior change communication to meet nutritionally balanced complementary foods for children, and improve child nutrition services at *posyandu.*

Keywords: children, dietary diversity, meal frequency, minimum acceptable diet

INTRODUCTION

The age of 0–23 months, or a child's first 1,000 days of life, is a crucial time in their development when they must fulfill exclusive breastfeeding and complementary nutritious, adequate, safe feeding (WHO 2020). It is chiefly true with children ranging from 6 until 23 months of age who begin to require more nutrients other than breast milk and need additional complementary foods to meet the energy gap. Providing complementary food to meet nutritional needs indirectly affect changes in child's maturity process. According to the Indonesia Nutritional Status Study (Ministry of Health Republic of Indonesia (MoH RI) 2021), low proportions of children who were exclusively breastfed, began supplementary feeding at 6 months old and consumed a variety of foods (52%, 44.7%, and 52.5%, respectively). Non-fulfillment of complementary feeding will impact children's nutritional status, health status, and cognitive development into adulthood (Hasanah *et al.* 2020; Zhu *et al.* 2020).

Some of the indicators for assessing Infant and Young Children Feeding (IYCF) are simple, valid, and reliable for assessing, collecting, tabulating, and interpreting complementary feeding practices. The indicators are the Minimum Dietary Diversity for children (MDD), the Minimum Meal Frequency (MMF), and the Minimum Acceptable Diet (MAD). These three indicators evaluate complementary feeding techniques as easier and simpler (WHO & UNICEF 2021).

Research in Indonesia based on the Indonesia Demographic and Health Survey of 2012 and 2017 showed that the MDD among provinces was met at around 39.3%, with West Sulawesi (3.67 types of food) and Yogyakarta (5.28 types of food) being the lowest in dietary diversity for children.

Other findings in Aceh showed that approximately 39% of children who received

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(Received 01-07-2022; Revised 25-10-2022; Accepted 11-11-2022; Published 30-03-2023)

exclusive breastfeeding met the MMF at about 74%, the MDD at about 50%, and the MAD at about 40% (of 392 children aged 6 until 23 months) (Ahmad *et al.* 2019). Previously in 2018, only 28.6% of children met the MDD, 33.1% did the MMF, and around 23.4% did the MAD (of 154 children) in Aceh (Ahmad *et al.* 2018). These figures were still relatively low. It has been proven that the MDD, the MMF, and the MAD were still not evenly met. In addition, research on the factors that influence the MDD, the MMF, and the MAD in Indonesian children aged 6 until 23 months is still limited. Research should not be restricted to the rural or urban level. There is a need to conduct research assessing the MDD, the MMF, and the MAD, along with their influencing factors, at the state level.

This research is the first extensive study covering all regions in Indonesia using the Total Diet Study (TDS) and Individual Meal Consumption Survey (IFCS) data with a twenty-four-hour dietary recall. Collection of child consumption data with MDD, MMF, and MAD indicators can be determined using twenty-four-hour dietary recall (WHO 2010). In addition, this study analyzed the factors that could affect the MAD. Based on the description above, this study's objective was to determine the quality of the MAD (a combined indicator of the MDD and the MMF) and analyze the relationship of individual as well as socioeconomic and demographic characteristics to the MAD in children between the ages of 6 and 23 months.

METHODS

Design, location, and time

The research used a cross-sectional study design at one time and secondary data from the Total Diet Study (TDS) of 2014. The study was conducted in Indonesia, including 33 provinces, representing households with children in the ages range of 6 until 23 months. The subjects were 789 children between the ages of 6 and 23 months. This study used the total diet study data related to the individual twenty-four-hour dietary recall. This research had obtained ethical clearance from the Ethics Committee of the Health Research and Development Agency with a letter numbered LB.02.01/5.2/KE.189/2014. The ethical clearance was used to obtain qualification for research involving humans and permission from the Ministry of Health of the Republic of Indonesia.

Sampling

Overall, the subjects totaling 817 children between the ages 6 and 23 months from the total diet study were grouped into two age groups, 6 until 11 months and 12 until 23 months. When performing a calculation of the minimum number of subjects (Lemeshow *et al.* 1997), the presumption was that 37% of children under the age of five previously met the MAD (including the MDD and the MMF) (IDHS 2012), with a confidence coefficient of 99% ($Z=2.58$ for $\alpha=0.05$) and a sampling error of 5% (0.05). Hence, the sample size needed was 354 children plus an estimated dropout of 10%, or 364 children. Meanwhile, this study used the whole subjects selected to meet the criteria, namely, about 798 of 817 children. The inclusion criteria in this study were children between the ages of 6 and 23 months whose food consumption was recorded with an individual twenty-four-hour dietary recall and children whose body weights (for age) were measured. The exclusion criteria in this study were children with incomplete individual twenty-four-hour dietary recall data and children whose biological status was ill during the twenty-four-hour dietary recall. Sampling method, inclusion, and exclusion criteria can be seen in Figure 1.

Data collection

The data collected in this study were individual characteristic variables, including the child's age, gender, birth order, and nutritional status, and socioeconomic and demographic characteristic variables, including the mother's age, the mother's educational status, the mother's

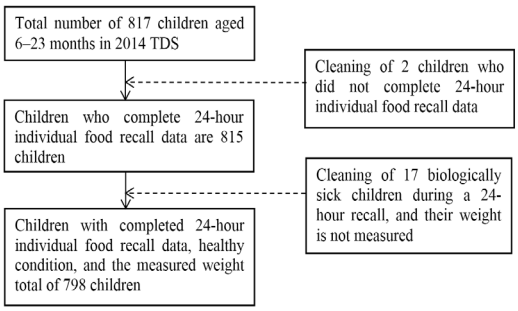

Figure 1. Sampling method, inclusion, and exclusion criteria

employment status, area of residence, and family size. The MDD variable was said to be fulfilled if breastfed children aged 6 until 23 months consumed foods of at least 5 of 8 food groups (with the eight MDD food groups for breastfed children being (1) breast milk; (2) grains and tubers; (3) legumes and beans; (4) dairy products; (5) meat foods (beef, fish, poultry, and liver/ organ); (6) eggs; (7) fruits and vegetables rich in vitamin A; and (8) other fruits and vegetables) and if non-breastfed children consumed foods of at least 4 of 7 food groups (excluding breast milk).

The MMF variable fulfillment was considered in the following way. The MMF was considered fulfilled if the meal frequency was 2 times (scored 1) and unfulfilled if the meal frequency was <2 times (scored 0) in breastfed children the age range of 6 until 8 months who should consume soft, semi-solid, or solid foods. The MMF was considered to be fulfilled if the meal frequency was 3 times (scored 1) and unfulfilled if the meal frequency was <3 times (scored 0) in breastfed children the age range of 9 until 23 months. The MMF was considered to be fulfilled if the consumption frequency was 4 times (scored 1) and unfulfilled if the consumption frequency was <4 times (scored 0) in non-breastfed children the age range of 6 until 23 months. .

The MAD variable was said to be fulfilled if breastfed children met the MDD and MMF or if non-breastfed children had a meal containing foods of at least four MDD food groups, met the MMF, and had a history of consuming breast milk at least 2 times.

Quality control of the data was carried out by checking the completeness of the data, selecting the subjects strictly, ensuring that the data represented the subjects being measured (representativeness), and selecting the data according to appropriate inclusion and exclusion criteria to minimize bias. In addition, we controlled for confounding variables through multivariate analysis.

Data analysis

Data processing included verification, coding, entry, cleaning, analysis, and interpretation. Bivariate analysis was conducted using chi-square for nominal data. Multivariate analysis used multiple logistic regression with SPSS version 22. Multivariate analysis was performed on the relationship between the MAD and individual, socioeconomic, and demographic characteristics. The correlation was considered significant if $p<0.05$. The odds ratio and 95% confidence interval were included.

RESULTS AND DISCUSSION

Individual, socioeconomic, and demographic characteristics. Individual characteristics were observed from the child's age, gender, birth order, and nutritional status, whereas socioeconomic and demographic characteristics were observed from the mother's age, the mother's educational status, the mother's employment status, area of residence, and family size. The distribution of data based on individual, socioeconomic, and demographic characteristics is provided in Table 1.

The data on individual characteristics showed that most children were between the ages of 12 and 23 months (93.7%), and the minority were aged 6–11 months (6.3%). Most of the children were male (54.4%), and some were female (45.6%). In terms of birth order, 36.2% of the children were second-born. Most of the children had a normal nutritional status (76.9%), but some children were found to be with an overweight nutritional status (11.7%) and an underweight nutritional status (8.9%).

The data on socioeconomic and demographic characteristics showed that most of the mothers were 20–30 years old (73.6%). The fact that most of the mothers (55.5%) had low education level means that they did not attend school, had not completed primary school, or completed education until primary and secondary school levels. In addition, most of the mothers were unemployed (61.6%), most of the children resided in urban environments (53.3%), and most of the families (52.8%) were of medium size (consisting of 5–7 people).

Minimum dietary diversity, minimum meal frequency, and minimum acceptable diet in children aged 6–23 months. Giving complementary foods gradually according to age was useful for identifying and evaluating nutrient intake during childhood development. The distribution of subjects according to the MDD, the MMF, and the MAD among breastfed and non-breastfed children is provided in Table 2.

This study found that 63.9% of children met the MDD median score 4. This rate is higher than

Table 1. Individual characteristics, socioeconomic, and demographic characteristics

Individual characteristics, socioeconomic, and demographic characteristics	Frequency (n)	Percentage (%)
Child's age		
6–11 months	50	6.3
12–23 months	748	93.7
Gender		
Male	434	54.4
Female	364	45.6
Birth order		
First	179	22.4
Second	289	36.2
Third	181	22.7
Fourth and so on	149	18.7
Nutritional status (weight for age)		
Severely underweight (<-3 SD)	20	2.5
Moderately underweight (-3 to<-2 SD)	71	8.9
Normal (-2 to+2 SD)	614	76.9
Overweight (≥+2 SD)	93	11.7
Mother's age[1]		
(<20 years old)	14	1.7
(20–35 years old)	587	73.6
(>35 years old)	197	24.7
Mother's educational status		
Low (No school, not completed primary school, or completed primary/secondary school)	443	55.5
High (High school graduate and college)	355	44.5
Mother's employment status		
Doesn't work	490	61.6
Work	308	38.6
Area of residence		
Rural	373	46.7
Urban	425	53.3
Family size		
≤4 people	300	37.6
5–7 people	421	52.8
≥8 people	77	9.6

the rates found in studies conducted in Ethiopia, e.g., 18.8% in Arsi Negele Districts and 59.9% in Addis Ababa City (Beyene *et al.* 2015; Solomon *et al.* 2017). The MDD scores of children aged 6 until 11 months who were breastfed and not breastfed were around 12.5% (median score 3) and 38.8% (median score 3), respectively. Meanwhile, the MDD scores of children aged 12 until 23 months who were breastfed and not breastfed were 65.9% (median score 5) and 66.9% (median score 4), respectively. The scores of the latter were higher than those of the former. Breastfed children consumed a diet consisting of at least five food types on average, while non-breastfed children did a diet consisting of at least four food types. Children between the ages of 12 and 23 months were five times greater chance to fulfilling the MDD than children between the ages of 6 and 11 months (Sekartaji *et al.* 2021). The minimum dietary diversity fulfillment increased as children grew older and shifted to family diets (Keno *et al.* 2021). Non-breastfed children had higher MDD fulfillment than breastfed children. Non-breastfed children tended to be introduced

Table 2. MDD, MMF, and MAD in breastfed children and not breastfed children (n=798)

Variable	Breastfed children				Not breastfed Children				Total	
	6–11		12–23		6–11		12–23			
	n	%	n	%	n	%	n	%	n	%
MDD										
Unmeet	28	87.5	64	34.1	11	61	185	33	288	36.1
Meet	4	12.5	124	65.9	7	38.8	375	66.9	510	63.9
MMF										
Unmeet	3	9.4	4	2.1	4	22.2	58	10.4	69	8.6
Meet	29	90.6	184	97.8	14	77.8	502	89.6	729	91.3
MAD										
Unmeet	28	87.5	63	33.5	12	66.6	202	36.1	305	38.2
Meet	4	12.5	125	66.5	6	33.3	358	63.9	493	61.8

MDD: Minimum Dietary Diversity; MMF: Minimum Meal Frequency; MAD: Minimum Acceptable Diet

to complementary and more varied foods to meet their nutritional needs. More varied feeding of children, earlier introduction to complementary foods and addition of formula milk could improve children's nutrition. Children in the ages range of 6 until 23 months who achieved the MDD were three or four times greater chance to adhere to the MMF than children who did not achieve the MDD (Mulaw *et al.* 2020).

Breastfed and non-breastfed children met the minimum meal frequency collectively at 91.3% (median score 6). This result is higher than the rates obtained by studies conducted in Ethiopia (11.9%) and Gambia (15%) (DHS 2021). Children in the ages range of 6 until 11 months who were breastfed and not breastfed met the MMF at 90.6% (median score 4) and 77.8% (median score 5), respectively, while children in the ages range of 12 until 23 months who were breastfed and not breastfed did at 97.8% (median score 6) and 89.6% (median score 7), respectively. This means that the latter outscored the former. On average, breastfed children had a meal frequency of 5 times, while non-breastfed children did 6 times. Children aged 12 up to 23 months were offered a wider food variety at an increased meal frequency in proportion to their nutritional needs based on their age of continued development. Children who consumed breast milk were seven or eight times greater chance to fulfill the MMF than those who did not (Wagris *et al.* 2019).

Meanwhile, the collective MAD fulfillment of breastfed and non-breastfed children was at 61.8%. This finding is higher than the rates obtained in Africa, e.g., 10.70% in Zimbabwe and 12.30% in Zambia (Gizaw & Tesfaye 2019; WHO 2020). The MAD adherence rate in breastfed children between the ages of 6 and 11 months was 12.5%, and it was 63% in non-breastfeeding children. Meanwhile, the MAD in children between the ages of 12 and 23 months who were breastfed and not breastfed was achieved at around 66.5% and 63.9%, respectively. On average, both breastfed and non-breastfed children met the MAD, with the MDD and MMF scores complying with recommendations. Non-breastfed children had higher MAD fulfillment than breastfed children. According to WHO recommendations, the difference was that non-breastfed children should consume milk (other than breast milk) at least twice a day. As a result, compliance with MAD scores had become more stringent (Khor *et al.* 2016). In this study, it is still considered necessary to improve the fulfillment of the MAD (a combined indicator of the MDD and the MMF). Although the child's meal frequency was relatively high, the minimum dietary diversity achievement was still low.

Correlation of individual, socioeconomic, and demographic characteristics with the MAD in children aged 6–23 months. In developing countries, the MAD has been used as one of the main indicators to evaluate the quality and adequacy of complementary feeding practices. The association between the MAD and individual, socioeconomic, and demographic characteristics is provided in Table 3.

Results demonstrated a significant association between the child's age (p=0.000) and the MAD, meaning that the greater the child's age, the greater the fulfillment of the MAD. Children

Table 3. The association of individual characteristics, socioeconomic, and demographic characteristics with MAD

Individual characteristics, socioeconomic, and demographic characteristics	MAD						*p*
	Unmeet		Meet		Total		
	n	%	n	%	n	%	
Child's age							
6–11 months	40	80	10	20	50	6.3	0.000*
12–23 months	265	35.4	483	64.6	748	93.7	
Gender							
Male	163	37.6	271	62.4	434	54.4	0.674
Female	142	39	222	61	364	45.6	
Birth order							
First	66	36.9	113	63.1	179	22.4	0.105
Second	102	35.3	187	64.7	289	36.2	
Third	67	37	114	63	181	22.7	
Fourth and so on	70	47	79	53	149	18.7	
Nutritional status (weight for age)							
Severely underweight (<-3 SD)	8	40	12	60	20	2.5	0.801
Moderately underweight (-3 to<-2 SD)	29	40.8	42	59.2	71	8.9	
Normal (-2 to+1 SD)	229	37.3	385	62.7	614	76.9	
Overweight (≥+1 SD)	39	41.9	54	58.1	93	11.7	
Mother's age							
(<20 years old)	5	35.7	9	64.3	14	1.8	0.904
(20–35 years old)	227	38.7	360	61.3	587	73.6	
(>35 years old)	73	37.1	124	62.9	197	24.7	
Mother's educational status							
Low	186	42	257	58	443	55.5	0.014*
High	119	33.5	236	66.5	355	44.5	
Mother's employment status							
Doesn't work	178	36,3	312	63.7	490	61.4	0.165
Work	127	41.2	181	58.8	308	38.6	
Area of residence							
Rural	166	44.5	207	55.5	373	46.7	0.000*
Urban	139	32.7	286	67.3	425	53.3	
Family size							
≤4 people	110	36.7	190	63.3	300	37.6	0.241
5–7 people	159	37.8	262	62.2	421	52.8	
≥8 people	36	46.8	41	53.2	77	9.6	

MAD: Minimum Acceptable Diet; SD: Standard Deviation

*p<0.05 is consider significantly associated (chi-square test)

between the ages of 12 and 17 months had twice as high a chance, and those between the ages of 18 and 23 months had thrice, of achieving the MAD (Acharya *et al.* 2021). The mother's educational status and the MAD were positively associated in this study (p=0.014), meaning that the higher the mother's educational status, the higher the minimum diet acceptable to the child. Children whose parents had formal high school and college education were more probably to have the MAD fulfilled than children with parents without formal education (Mulat *et al.* 2019).

It is also indicated that there was a highly significant link between area of residence (p=0.000) and the MAD. Children living in urban areas (67.3%) were higher in MAD fulfillment than those living in rural areas (55.5%). In other words, compared to children living in urban environments, children in rural environments were more susceptible to MAD non-fulfillment. Easier access to foods in urban areas and the availability of more diverse types of foods were attributable to such MAD fulfillment rate in urban areas (Ng *et al.* 2012).

On the other hand, birth order and the MAD had no significant correlation (p=0.105). Acharya *et al.* (2021) and Ali *et al.* (2021) revealed that children born in the second order had a higher probability of having the MAD fulfilled than children born in the first order. Nutritional status (p=0.801) and the MAD had an insignificant negative correlation. The food index may not be sensitive to chronic malnutrition because it is judged from twenty-four-hour dietary recall, which may not give a picture of a child's typical food intake, which could partly explain why the association is so low (Saaka *et al.* 2016). Family size and the MAD had an insignificant negative correlation too (p=0.241). Children with more than five family members had a lower chance of meeting the MAD (Guirindola *et al.* 2018).

There were non-significant correlations between gender (p=0.674) and the mother's employment status (p=0.165) and the MAD. Children with mothers who did not work (63.7%) were higher in MAD fulfillment than children with mothers who worked (58.8%), meaning that the proportion of minimum diet for children with working mothers was lower than children with mothers who did not work. Children whose mothers did not have a workload had a higher likelihood of receiving proper complementary eating, and thus better MAD fulfillment, than children whose mothers did (Yisak *et al.* 2020).

Determinant factors associated with the MAD. Factors that affected the MAD were encountered in children ranging from 6 until 23 months. Multiple logistic regression analysis is an approach for modeling the relationship between more than one independent variable and one dependent variable. If the result of multiple logistic regression analysis between the dependent and independent variables is lower than 0.05 ($p<0.05$), then the independent variable is significantly related to the dependent variable. Usually, a variable is selected for multivariate analysis if it has $p<0.25$. Thus, in this case, gender, nutritional status, and maternal age should not be selected. The selected independent variables were the child's age, the child's birth order, the mother's educational status, the mother's employment status, area of residence, and family size. Based on the final results of multiple logistic regression, the child's age and area of residence ($p<0.05$) were found to be the determinant of MAD.

Table 4 shows that one determinant factor for MAD was the child's age ($p<0.05$; OR=7.06; 95% CI=3.46–14.38). Children between the ages of 12 and 23 months were seven times greater chance than younger children to meet the MAD (6 to 11 months). As children grew older, the fulfillment of the MAD also increased. Toddler mothers' perceptions of the digestive condition of children who aren't ready to be given complementary foods with a solid or semi-solid textured are the cause of sub-optimal MAD fulfillment in younger children (Molla *et al.* 2021). This finding is consistent with that of Tassew *et al.* (2019), who revealed that children

Table 4. Determinant factors associated with minimum acceptable diet

Variable	Minimum acceptable diet		OR (95% CI)	*p*
	Unmeet	Meet		
Child's age (months)				
6–11	40 (80)	10 (20)	Ref.	
12–23	265 (35.4)	483 (64.6)	7.06 (3.46–14.38)	0.000*
Area of residence				
Rural	166 (44.5)	207 (55.5)	Ref.	
Urban	139 (32.7)	286 (67.3)	1.61 (1.19–2.15)	0.002*

*$p<0.05$ as analyzed with multiple logistic regression; Ref: Reference OR: Odds Ratio; CI: Confidence Interval

between the ages of 18 and 23 months were 4 times higher likely to meet the MAD than younger children (6–8 months).

Another factor determining fulfillment of the MAD was area of residence ($p<0.05$; OR=1.61; 95% CI=1.19–2.15). Children living in urban areas had a one- or two-fold chance of meeting the MAD than children living in rural areas. Mothers living in urban areas found it easier to obtain information, available healthcare facilities, internet, television, newspapers, and other media exposures to increase their knowledge to practice good eating patterns (Teshome & Tadele 2022). Living in urban areas made it easier for mothers to access various foodstuffs in places such as markets, vegetable shops, and malls or find ready-to-eat foods in places such as restaurants, cafes, food stalls, bakeries, and urban food courts (Woldegebriel *et al.* 2020). This finding is consistent with those of Worku *et al.* (2022) and Birie *et al.* (2021), who stated that compared to children whose mothers resided in rural environments, children whose mothers resided in urban environments were five times higher likelihood of having the MAD fulfilled. Another finding is that rural children were more prone to experiencing low food diversity scores. Better equitable access is needed to reach food producers, utilize various types of locally available foods, use vegetable gardens, and increase mothers' knowledge in managing foods (dela Luna *et al.* 2020).

Strengths and limitations. This study has several limitations, one of which is twenty-four-hour recall data collection being only carried out for one meal. A twenty-four-hour recall based on the mother's memory had the potential for response bias, such as errors in remembering. The twenty-four-hour recall data collection time should be extended to two days or more to allow for accurate, precise description of the amount of food consumed by children. The use of secondary data was limited, leading to the use of what variables available. However, this study has an advantage in the use of large-scale data of representative subjects throughout Indonesia. This study provides an overview of information to the public, especially parents with children between the ages of 6 and 23 months, to increase awareness about giving complementary foods in greater diversity, frequency, and minimum adequacy of diet for children.

CONCLUSION

The research subjects were mainly children aged 12–23 months, male, second-born, and of average weight nutritional status. The majority of mothers were in the age range between 20 and 35 years, of low educational status, unemployed, resided in urban environments, and with medium family size (5–7 people). The percentages of children that met the MDD, the MMF, and the MAD were 63.9%, 91.3%, and about 61.8%, respectively. Several factors of individual and socioeconomic characteristics could affect the fulfillment of the minimum acceptable diet, namely, the child's age and area of residence ($p<0.05$). It is suggested for further research to use a more balanced proportion of sample units between children in the age range of 6 until 11 months and 12 until 23 months to evaluate the fulfillment of the MAD. The government's role is to provide balanced nutritional child feeding programs based on local food, strengthen behavior change communication to meet nutritionally balanced complementary foods for children, and improve child nutrition services at *posyandu.*

ACKNOWLEDGEMENT

The authors express gratitude to the Health Research and Development Agency, Ministry of Health of the Republic of Indonesia, for allowing an analysis of the research on a large scale based on the 2014 total diet study in children between the ages of 6 and 23 months.

DECLARATION OF INTERESTS

The authors assert to have no conflicts of interest.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2023, 18(1):11-20***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2023.18.1.11-20*
ISSN 1978-1059 EISSN 2407-0920

Supplementary Biscuits for the Recovery of Malnourished Children in Indonesia

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ABSTRACT

The objective of this research was to study the nutritional status of children before receiving biscuits, the biscuit supplementation regularity, the adequacy of the biscuits received and consumed by the children according to recommendations, and the relationship between supplementary biscuits consumption and nutritional status. Using a cross-sectional study design, the study was conducted in 2017 involving 586 children in three regions (11 provinces) in Indonesia. A chi-square test was performed to see the relationship between supplementary biscuits consumption and nutritional status. The most common nutritional problems were stunting-wasting (34.1%), stunting (32.6%), and wasting (23.9%). The nutritional problems occurred most often in the eastern region (96.4%), followed by the central region (95.5%) and the western (87.1%). Within three months, 66–78% of respondents received supplementary biscuits regularly, but only 10–29% received the supplementary biscuits as recommended in the same period. Within the three months' period, from respondents that received the supplementary biscuits as recommended, the region in which children consumed the biscuits according to the recommendations the most was the eastern region (45.2%), followed by the western (23.3%) and central regions (5.3%). Children who consumed biscuits as recommended in the three months' period had a 2.9 times possibility of having a normal nutritional status (categorized based on the weight-for-age index) (p-value=0.049).

Keywords: malnourish children, nutritional status, region, supplementary biscuits

INTRODUCTION

Children under five years old are the most vulnerable group of children to suffer from undernutrition, given that they require high levels of nutrients for growth. Malnutrition has serious, lasting impacts on individuals, their families, and the society, and it may cost countries medically, socially, and economically (World Health Organization (WHO) 2021).

The largest portions of malnourished under-fives in Asia suffer from stunting (54%) and wasting (69%) (UNICEF/WHO/World Bank 2020). In Indonesia, the stunted status in 2013 and 2018 was high (37.2% and 30.8%, respectively), and so was the wasted status (12.1% and 10.2%, respectively) (Ministry of Health Republic of Indonesia (MoH RI) 2013; 2019). Stunting has negative impacts related to low cognitive abilities, leading to low completed education levels and potentially to low income and welfare levels in the future (Schneider 2017). Meanwhile, the negative impacts of wasting are low body immunity, susceptibility to diseases, developmental delays, and the risk of death (UNICEF/WHO/World Bank 2020), thereby necessitating feeding, medication, and care for individuals to stay alive.

One of the efforts to reduce nutritional problems and to increase nutritional adequacy is providing biscuit supplementation for the recovery of malnourished children under five years (6–59 months). In addition to nutritional adequacy, the quality of nutritional sources needs to be considered (Randani *et al.* 2022). Therefore, the supplementary biscuits should be made with a special formula containing energy, protein, and fat and enriched with vitamins and minerals (MoH RI 2017).

Some literature provides information about the effect of giving supplementary biscuits in changing, or improving, the nutritional statuses

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(Received 09-09-2022; Revised 08-11-2022; Accepted 17-11-2022; Published 30-03-2023)

of children. Dina's study showed that there was an improvement and difference in the Weight-for-Age Index (WAZ) after biscuit supplementation but no significant difference in the Weight-for-Height Index (WHZ) (Rini *et al.* 2017). Another work reported no significant difference in the nutritional statuses of children under five years old based on the WHZ after supplementary biscuits consumption (p=0.585) (Putri & Mahmudiono 2020).

The objective of this study was to determine nutritional problems in children prior to biscuit supplementation, the biscuit supplementation regularity, the adequacy of the biscuits received and consumed by the children according to recommendations, and the relationship between the adequacy of the three months' biscuit supplementation and nutritional status.

METHODS

Design, location, and time

This research is part of the research on "the evaluation of biscuit supplementation for the recovery of malnourished children and pregnant women with chronic energy deficiency". This study is an analytical descriptive observational study with a cross-sectional study design. The research was conducted in 2017 across 11 provinces in three (3) regions: Western region (Banten, East Java, North Sumatra, and Riau); Central region (West Kalimantan and South Kalimantan), and Eastern region (East Nusa Tenggara, North Sulawesi, South Sulawesi, Maluku, and North Maluku).

An ethical approval was obtained from the Ethics Committee of the Indonesian Health Research and Development Agency through a letter numbered LB.02.01/2/KE.112/2017 set in Jakarta on March 31, 2017.

Sampling

Provinces were selected based on the highest prevalence of wasting in children in each region, and districts were selected based on the highest prevalence of wasting in children in the selected provinces. In each district, two locations with the highest wasting prevalence rates were selected. The inclusion criterion was children living in the selected study sites who received biscuit supplementation in 2016, whereas the exclusion criterion was children receiving biscuit supplementation after July 2017 or children having no anthropometric (weight and height) data.

All sampling units (586 children) were analyzed for nutritional problems before the biscuit supplementation, biscuit supplementation regularity (1–3 months), and the amount of biscuit supplement received in three months according to recommendations. On sampling units who received biscuit supplement in the recommended amount in three months (110 children) an analysis was conducted regarding how they consumed the biscuits. All sampling units (586 children) were involved in the analysis of the relationship between the adequacy of the biscuits consumed in three months and nutritional status.

Data collection

The nutritional problems as illustrated by the nutritional statuses of children before receiving supplementary biscuits were assessed based on anthropometric (height and weight) measurements by nutritionists at local health centers (TPG) or cadres at integrated services posts (*Posyandu*).

Supplementary biscuits contain calories, protein, and fat. They are enriched with ten kinds of vitamins (A, D, E, K, B1, B2, B3, B6, B12, and folic acid) and seven kinds of minerals (iron, iodine, zinc, calcium, sodium, selenium, and phosphorus). Supplementary biscuits are usually given by nutritionists at local health centers (TPG) or cadres at integrated services posts (*Posyandu*).

Data on the regularity of biscuit supplementation and the amount of biscuit supplement received and consumed according to recommendations in three months were obtained from a structured questionnaire. Interviews were conducted based on the qualitative questionnaire to support the quantitative data collected.

Data analysis

The nutritional statuses of children were determined based on the Height-for-Age Index (HAZ) and Weight-for-Height Index (WHZ) according to a Regulation of the Minister of Health (Ministry of Health of Republic of Indonesia (MoH RI) 2020). A child would be classified as stunted if the the Height-for-Age Index (HAZ) was less than -2 SD and wasted if the Weight-for-Height Index (WHZ) was less than -2 SD. The combined nutritional status (based on the HAZ

and WHZ) determined whether the nutritional problems were chronic, acute, or a combination of both. The combined nutritional statuses used in this study were stunted, wasted, stunted-wasted, and not stunted-wasted (underweight, normal, and overweight).

The data on the amount of biscuit supplement received and consumed were processed by comparing them against adequacy recommendations. For daily consumption of supplementary biscuits, it was recommended for a child aged 6–11 months to take eight pieces and for a child aged 12–59 to take 12 pieces (MoH RI 2017).

A univariate analysis was carried out to obtain an overview of children who received supplementary biscuits. Additionally, a bivariate analysis with a chi-square test was carried out to see the association between the adequacy of biscuits consumed in three months and nutritional status (based on the HAZ, WAZ, and WHZ). In addition, an analysis of differences in nutritional problems and regularity of biscuit supplementation was carried out across regions using a difference test between proportions (chi-square test).

RESULTS AND DISCUSSION

Nutritional problems of children before biscuit supplementation

Nutritional problems are reflected in the nutritional status. The nutritional statuses of children under five years prior to biscuit supplementation are presented in Figure 1.

A combination of two anthropometric indexes (HAZ and WHZ) provides a clearer picture of the nutritional problems that occurred in children before biscuit supplementation. Double nutritional problem of stunting and wasting had the highest prevalence rate (34.1%), followed by stunting-only (32.6%) and wasting-only (23.9%). Non-stunting-wasting was found in 9.4% of children, 2.4% of whom were underweight, 5.8% were normal, and 1.2% were overweight. Before receiving supplementary biscuits, most of the sample experienced undernutrition problems, either singly or in combination (93%). Another study reported a nearly identical finding to this finding (90.7%) (Sarimah & Hutagalung 2013).

Before received biscuit supplementation, 68% of children suffered from wasting. Based on the technical guidelines to which this study referred, the main targets of the biscuit supplementation are children aged 6–59 months who are suffering from wasting (WHZ<-2 SD) and are neither hospitalized nor receiving outpatient care (MoH RI 2017). Other targets are children who are in a recovery period after receiving a malnutrition treatment at a TFC/ hospital/community health center (*Puskesmas*), underweight, not gaining weight twice in a row (2T), and on the red growth line trajectory (supplementation for recovery program technical guidelines 2011). Supplementary biscuits are rich in nutritional content and thus unfit to be given to overweight children.

The nutritional problems in children who were selected to receive supplementary biscuits (before biscuit supplementation) in each region are detailed in Table 1.

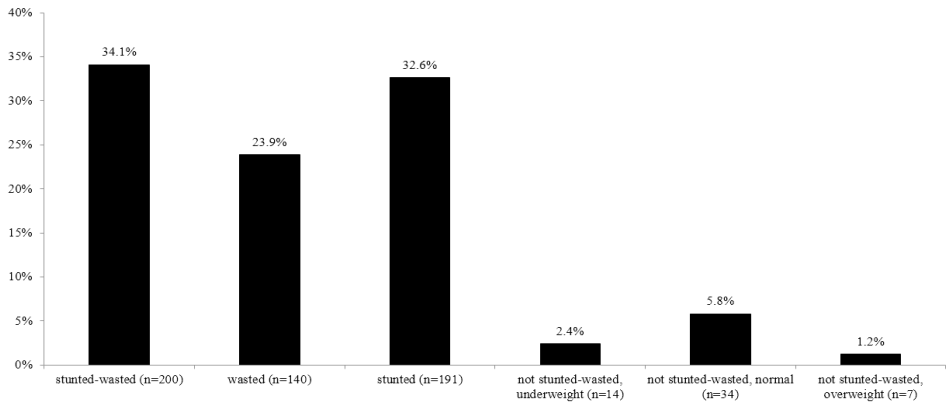

Figure 1. Type of malnutrition problem on children under five before receiving supplementary biscuits

The overall nutritional problems (stunting-wasting, stunting, wasting, and underweight) were the highest in the eastern region (96.4%) and lower in the central region (95.5%) and western region (87.1%). The nutritional problems between regions showed statistically significant differences (p-value=0.000). There were different patterns of nutritional problems in each region. In the eastern region, the main problem was stunting-wasting (41.9%), followed by stunting (32.3%) and wasting (20.6%). The central region had stunting as the most problematic (42.4%), followed by stunting-wasting (30.3%) and wasting (22.7%). In the western region, the problems in the order from the most common were stunting (30%), wasting (29%), and stunting-wasting (23.8%). This shows a disparity in patterns of nutritional problems between regions. The literature states that nutritional problems persist in high percentages in some provinces in the eastern region (Hastoety *et al.* 2018).

In terms of severity, stunting-wasting is considered the most severe because it has a higher risk of death. The percentage of stunted-wasted children was high in all regions. The highest percentage of stunting-wasting was found in the eastern region (41.9%), followed by the central (30.3%) and western region (23.8%).

Table 1. Nutritional problems of children under five before receiving supplementary biscuits by region

Region	Stunted-wasted	Wasted	Stunted	Not stunted-wasted		
				Underweight	Normal	Overweight
Western region (n=210)	23.8	29.0	30.0	4.3	11.0	1.9
North Sumatera (n=57)	22.8	40.4	29.8	0.0	5.3	1.8
Riau (n=61)	21.3	36.1	11.5	3.3	24.6	3.3
East Java (n=43)	7.0	18.6	55.8	7.0	11.6	0.0
Banten (n=49)	42.9	16.3	30.6	8.2	0.0	2.0
Central region (n=66)	30.3	22.7	42.4	0.0	4.5	0.0
West Kalimantan (n=28)	46.4	17.9	35.7	0.0	0.0	0.0
South Kalimantan (n=38)	18.4	26.3	47.4	0.0	7.9	0.0
Eastern region (n=310)	41.9	20.6	32.3	1.6	2.6	1.0
NTT (n=93)	63.4	8.6	24.7	2.2	1.1	0.0
North Sulawesi (n=25)	36.0	32.0	20.0	4.0	4.0	4.0
South Sulawesi (n=42)	26.2	47.6	23.8	2.4	0.0	0.0
Maluku (n=62)	25.8	24.2	45.2	0.0	4.8	0.0
North Maluku (n=88)	39.8	14.8	38.6	1.1	3.4	2.3

The different test of nutritional problems of children under five before receiving supplementary biscuits between regions using the chi-square test showed a p-value of 0.000

The province with the highest prevalence of stunting-wasting was East Nusa Tenggara (63.4%), followed by West Kalimantan (46.4%) and Banten (42.9%).

Stunting is an indicator of chronic malnutrition, and wasting is an indicator of acute malnutrition (Barth *et al.* 2020). The high prevalence of stunting-wasting in children shows that children have been experiencing long-standing nutritional problems that begun in the past and still continues until now (when the anthropometric measurement was carried out). The literature states that double-malnourished under-fives are at greater risks of diseases (acute respiratory infection, diarrhea, and measles) than single-malnourished children. Therefore, double malnutrition, underweight, and wasting in children need immediate intervention with supplementary foods, medication, micro-nutrition, education, and family empowerment (Andini *et al.* 2020).

In contrast to stunting-wasting, the highest prevalence of wasting was encountered in the western region (29%), followed by the central region (22.7%) and the eastern (20.6%). The highest prevalence of stunting was found in the central region (42.4%), followed by the eastern region (32.3%) and the western (30%). The WHO (2021) describes wasting as acute malnutrition due to inadequate intake or infectious diseases (especially diarrhea) and stunting as reflecting growth retardation, the cumulative long-term effects of malnutrition, repeated infections, and poor environmental conditions. It is also stated in the literature that the indicators of WHZ and HAZ reflect past nutritional status due to the use of more stable height parameters (Handayani *et al.* 2012).

The nutritional problems of children under five years old are complex in terms of individual and household characteristics, mother's education, sanitation, drinking water sources, child morbidity, access to weighing, income, poverty, and affordability. While natural poverty reflects the limited quality of natural and human resources, which leads to low productivity, cultural poverty originates from living in a poor environment since birth. Income and prices affect food affordability and consumption behavior, which in turn affects nutritional status. These problems are often found in eastern Indonesia (Fauziyah 2016; Ariesthi *et al.* 2015; Ariesthi 2019).

Provinces that are relatively close together (NTT and North Maluku) have almost the same nutritional problems (high prevalence of stunting-wasting). This is in line with the results of Elisanti's study which revealed that there is a tendency for the nutritional status of an area to be influenced by, and affect, the nutritional status of adjacent (intersecting) areas (Elisanti 2017). It is also consistent with the First Law of Geography according to Waldo Tobler that everything is related to everything else, but things that are near are more related than things that are far away (Elisanti 2017). Adjacent areas generally tend to have almost the same geographical conditions, so the problems also tend to be the same.

Biscuit supplementation regularity and amount of biscuit received and consumed

The following table illustrates how regularly the biscuit supplement was distributed by the local government to targeted malnourished children regardless of the amount of the biscuit supplement (Table 2).

By region, the children who received supplementary biscuits regularly every month during the three months' period were mostly in the western region (78.1%), followed by those in the eastern region (72.6%) and the central region (66.7%). The differences between regions were not statistically significant (p-value=0.334). It appeared that the children in the eastern region were regularly given supplementary biscuits in three months, even though the amount given each month was less than the recommended adequacy (provisions).

The amounts in which the children received the supplementary biscuits based on the recommendations in the three months' period are presented in Table 3.

Disparity between regions was observed in the distribution of biscuits according to the recommendations. In three months, the eastern region saw the smallest percentage of children who received supplementary biscuits according to the recommendations (10%), followed by the western region (26.6%) and the central region (28.8%). Children who received supplementary biscuits less than the recommendations in the three months were mostly found in the eastern region (90%).

Regular biscuit supplementation for children in the recommended amount was faced

Table 2. Percentage routinity/time duration of children received supplementary biscuits

Region	n	Routinity/time of receiving supplementary biscuits					
		One month		Two month		Three month	
		n	%	n	%	n	%
Western (Jawa, Sumatera)	210	23	11.0	23	11.0	164	78.1
Central (Kalimantan)	66	9	13.6	13	19.7	44	66.7
Eastern (Sulawesi, NTT, Maluku)	310	38	12.3	47	15.2	225	72.6
Total	586	70	11.9	83	14.2	433	73.9

The different test on the routinity or time duration of children received supplementary biscuits between regions using the chi-square test obtained a p-value of 0.334

with various constraints, one of which was encountered in the distribution process to the targets. The qualitative interviews carried out revealed that the main constraint to distribution was geographical conditions (difficulty to reach the destination, great distance, damaged roads, weather, and uncertain ship schedules). Other constraining factors were inadequate supplementary biscuits available, the lack of manpower, and the absence of a special budget for distribution (Hermina 2017). These constraints can be anticipated through planning in which problems are mapped based on information, data, and facts available to achieve the expected results (Komala & Irwan 2019).

The percentages of children consuming supplementary biscuits according to the recommendations in three months of the sample (110 children) are presented in the following Table 4.

This study found that of the children who received and consumed the supplementary biscuits according to the recommendations in the three months' period (26.4%), mostly were from

Table 3. Percentage of children under five received amount of supplementary biscuits according to the provisions in three months

Region	n	Children received PMT-P biscuits less than the provisions*		Children received PMT-P biscuits according to the provisions	
		n	%	n	%
Western (Jawa, Sumatera)	210	150	71.4	60	26.6
Central (Kalimantan)	66	47	71.2	19	28.8
Eastern (Sulawesi, NTT, Maluku)	310	279	90	31	10
Total	586	476	81.2	110	18.8

*Provisions: The number of biscuits given to children aged 6–11 months is as much as 240 pieces/month and for children aged 12–59 months as much as 360 pieces/month

PMT-P: *Pemberian Makanan Tambahan Pemulihan* (Supplementary Biscuits for Recovery Program)

Table 4. Percentage of children under five consumed amount of supplementary biscuits according to the provisions in 3 months

Region	n	Children consumed biscuits less than the provisions*		Children consumed biscuits according to the provisions	
		n	%	n	%
Western (Jawa, Sumatera)	60	46	76.7	14	23.3
Central (Kalimantan)	19	18	94.7	1	5.3
Eastern (Sulawesi, NTT, Maluku)	31	17	54.8	14	45.2
Total	110	81	73.6	29	26.4

*Provisions: The number of biscuits consumed for aged 6–11 months is as much as 240 pieces/month and for children aged 12–59 months is as much as 360 pieces/month

the eastern region (45.2%), followed by those who were from the western region (23.3%) and central region (5.3%). The eastern region showed the highest percentage of supplementary biscuits consumption probably due to less access to food than in other regions. The consumption of supplementary biscuits that did not comply with the recommendations was because the biscuits were provided in insufficient amounts or because the consumption did not follow the recommendations although the biscuits were received in adequate amounts. A study by the National Development Planning Agency (Bappenas) found that the management of supplementary biscuits for moderately acute malnutrition (wasting) was not conducted comprehensively, only high-energy, high-protein supplementary foods were provided, and counseling activities were not performed adequately to raise public awareness (Ministry of National Development Planning of the Republic of Indonesia (Bappenas) 2019). Therefore, in addition to distributing supplementary biscuits adequately, it is also necessary to provide counseling and assistance for the children's families to increase their awareness of the importance of children consuming supplementary biscuits in the amount recommended.

The relationship between biscuits intake and nutritional status

Significant results ($p<0.005$) were found on the relationship between supplementary biscuits consumption and nutritional status according to the Weight-for-Age Index (WAZ). Children who consumed supplementary biscuits less than the recommendations were at 2.9 times the risk of being underweight (Table 5). In other words, sufficient consumption of supplementary biscuits by children according to the recommendations in three months would increase the possibility of having a normal nutritional status by 2.9 times.

Consuming supplementary biscuits according to the recommendations is expected to help alleviate nutritional problems in children under five years old. Another study supports this finding with evidence that supplementary biscuits have a role in improving nutritional status by increasing the body weights of undernourished children (Herianto 2017). Children who consumed supplementary biscuits had increased body weights by 18.16% (Adibin 2018). Consumption of supplementary biscuits by toddlers for 90 days improved the nutritional statuses of the toddlers based on the WAZ and WHZ (Mahmudah & Ulvie 2018).

The relationship between biscuits consumption and nutritional status according to the HAZ and WHZ was not statistically significant ($p\geq0.005$). This could be because the HAZ and WHZ reflect past nutritional status with more stable height parameters (Handayani *et al.* 2012) and describe nutritional status in the long term, while this study only analyzed the relationship after consumption of supplementary biscuits for only three months.

Table 5. The relationship between biscuits consumed and the nutritional status of children after 3 months

Amount of biscuits consumed	n	Nutritional status		OR (95% CI)	*p*
		Category WHZ			
		Wasted (%)	Normal (%)		
< Provisions	142	38.7	61.3	1.138 (0.362–3.573)	1.000
≥ According to the provisions	14	35.7	64.3		
		Category WAZ			
		Underweight (%)	Normal (%)		
< Provisions	142	74.6	25.4	2.944 (0.987–8.968	0.049*
≥ According to the provisions	14	50.0	50.0		
		Category HAZ			
		Stunted (%)	Normal (%)		
< Provisions	142	66.2	33.8	1.088 (0.345–3.426)	0.885
≥ According to the provisions	14	64.3	35.7		

WHZ: Weight for Height Index
WAZ: Weight for Age Index
HAZ: Height for Age Index
≥ According to the provisions as score 1 in all variable that tested (category WHZ, WAZ, and HAZ)

CONCLUSION

Before receiving supplementary biscuits, children were mostly of the stunted-wasted nutritional status (34.1%), and the rest were of the stunted (32.6%), wasted (23.9%), and not stunted-wasted (9.4%) statuses. The greatest prevalence of undernutrition problems was found in the eastern region (96.4%), followed by the central (95.5%) and western (87.1%) regions.

The percentage of children who received biscuits every month in three months across all regions was in the range 66–78%, but only 10–29% received the supplementary biscuits according to the recommendations in the three months' period. Of the children who received biscuits as recommended within the three months, the highest percentage of children who consumed the biscuits according to the recommendations was found in the eastern region (45.2%), followed by the western (23.3%) and central regions (5.3%).

Children who consumed supplementary biscuits in the recommended amount for three months had 2.9 times the possibility of having a normal nutritional status (based on the WAZ, statistically significant with p=0.049).

ACKNOWLEDGEMENT

Acknowledgments are given to The Center for Research and Development in Public Health Effort and National Institute of Health Research and Development (NIHRD) for funding the research on the evaluation of the implementation of the supplementary biscuits for the recovery of malnourished children and pregnant women with chronic energy deficiency program. Thanks are also expressed to Hermina as the head of research; Nazarina and Noviati Fuada as supervisors of article writing.

DECLARATION OF INTERESTS

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

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2023, 18(1):21-30***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2023.18.1.21-30*
ISSN 1978-1059 EISSN 2407-0920

Hepatoprotective Effect of Parijoto Fruit Extract (*Medinilla speciosa* Blume) on Male Mice Fed with High-Fat Diet

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ABSTRACT

This study aims to determine the effectiveness of parijoto fruit extract (*Medinilla speciosa* Blume) in improving the condition of fatty liver in male balb/c mice (*Mus musculus* L.) fed with a high-fat diet. Thirty male balb/c mice weighing 20 to 30 g were randomly divided into six groups, i.e: 1) Standard feed and Carboxymethyl Cellulose Sodium or CMC-Na 0.5% (K1); 2) High-fat diet and CMC-Na 0.5% (K2); 3) High-fat diet and simvastatin 0.026 mg/day (K3); 4) High-fat diet and parijoto fruit extract 5.6 mg/20 g BW (P1); 5) High-fat diet and parijoto fruit extract 8.4 mg/20 g BW (P2); 6) High-fat diet and parijoto fruit extract 11.2 mg/20 g BW (P3). Standard feed, high-fat diet, simvastatin, and parijoto fruit extract were administered for 56 days. On the 57th day, the total of 30 mice were terminated and the livers were then removed for H&E staining histopathological slides. Data on the degree of fatty liver on histopathological slides were collected and analyzed using the Kruskal-Wallis test and followed by the Mann-Whitney test. The histopathological analysis showed Medinilla speciosa Blume extract at a dose of 5.6 mg/20 g BW in group P1 prevent the steatosis degree compared to high-fat feed mice in group K2 ($p>0.05$). Parijoto fruit extract could act as the potential treatment for fatty liver.

Keywords: fatty liver, histopathological, high-fat diet, medinilla speciosa blume, mice

INTRODUCTION

Non-Alcoholic Fatty Liver Disease (NAFLD) is defined as a liver condition with a fat content exceeding 5% of the total normal liver weight in people who rarely or do not drink alcohol (Kneeman *et al.* 2012). The spectrum of fatty liver disease ranges from simple hepatic steatosis to cirrhosis. Although not all cases of hepatic steatosis will progress, early intervention is important because these patients are at risk of developing liver cirrhosis and associated complications such as hepatocellular carcinoma (Huang *et al.* 2020).

NAFLD is the most common cause of liver disease worldwide. Globally, the prevalence of NAFLD has continued to increase over the last three decades, increasing from 391.2 million in 1990 to 882.1 million in 2017 (Ge *et al.* 2020). In Indonesia, the prevalence of NAFLD reaches around 30% of the total adult population. The incidence of NAFLD is believed to be influenced by high-calorie diets, particularly those heavy in cholesterol, saturated and trans fatty acids, and other fats (Salehi-Sahlabadi *et al.* 2021). NAFLD is most commonly found in hyperlipidemic patients with a prevalence of 90%, followed by 80–90% in obese adults and 30–50% in diabetic patients (Milić & Štimac 2012). The high prevalence of NAFLD due to hyperlipidemia indicates that effective therapy is needed in this disease to prevent disease progression leading to cirrhosis and liver failure (Kneeman *et al.* 2012). Statins are a class of drugs that are often used to treat hyperlipidemia. This drug can lower Low-Density Lipoprotein (LDL) cholesterol, triglycerides, and increase High-Density Lipoprotein (HDL) through the mechanism of HMG-CoA Reductase inhibition. However, long term ingestion of statins causes adverse health effects such as liver injury and muscle toxicity. Other side effects include myopathy,

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(Received 08-09-2022; Revised 28-11-2022; Accepted 22-12-2022; Published 30-03-2023)

rhabdomyolysis, and acute renal failure. Thus, attention is now directed to natural products of plant origin (Salvamani *et al.* 2014).

So far, only a few studies have been conducted on the effect of parijoto fruit on the treatment of NAFLD. Research results from Elfrida (2015) showed that parijoto fruit extract at a dose of 0.9 mg/200 g BW/day had a significant effect on fatty liver tissue of the rats. Apart from the small number of studies, this study also modified the duration of research time and selected more suitable test animals. Balb/c strain mice were used as test animals because a high-fat diet in balb/c mice was able to induce hyperlipidemia more effectively than mice and C57BL/J6 strain mice, which are atherogenic strains of mice that have been genetically modified (Madariaga *et al.* 2015), so it is hoped that a clear fatty liver can be formed to be assessed. As a result, the purpose of this study was to assess the effect of parijoto fruit extract on improving histopathological features of fatty liver in male mice balb/c strain. Parijoto is a typical plant of the slopes of Mount Muria in Kudus Regency, Central Java which is used by local people as traditional medicine to treat various diseases (Wibowo *et al.* 2012). The benefits of parijoto are believed to be uterine fertilizers, diarrhea medicine, thrush, anti-inflammatory, anticancer, antibacterial, and lowering lipid profiles (Hanum *et al.* 2017). Based on research conducted by Wachidah (2013), the fruit of the parijoto plant contains tannins, flavonoids, and saponins which have the potential as lipid profile-lowering drugs because they have antioxidant and antihyperlipidemic activity. Study of 70% ethanol extract of parijoto fruit (*Medinilla speciosa* Blume) on the liver histopathology of male white rats induced by hyperlipidemia that has been done previously gave effective results at a dose of 90 mg/200 g BW in liver histopathology improvement carried out for 42 days (Elfrida 2015). The researchers expected that this study could show the potentials of parijoto fruit extract in improving histopathological feature of fatty liver with modified doses.

METHODS

Design, location, and time

This is true-experimental research, using a randomized post-test only control group design. The material origin located in the slopes of the Muria Mountains in Kudus, Central Java, Indonesia. Maintenance and treatment on experimental animal was conducted at the Pharmacology and Therapeutic Laboratory, Faculty of Medicine, University of Padjadjaran, Bandung, West Java, Indonesia. The research was carried out from November 2021 to June 2022. This study had obtained approval by The Research Ethics Committee of Medicine Faculty, Universitas Pembangunan Nasional Veteran Jakarta, Jakarta, Indonesia (2/1/2022/KEPK).

Materials and tools

Medinilla speciosa Blume for the treatment were extracted with 70% ethanol as solvent. Modeling of fatty liver was done by inducing high-fat diet for 56 days. Mice liver tissue stained with hematoxylin and eosin, were observed under a light microscope from the entire field of view at 40 times magnification to determine area and 400 times magnification to confirm the fat accumulation.

The tools used in this study were rotary evaporator, water bath, O'Hauss scale, gastric probe for oral administration, disposable syringe, surgical instruments, alcohol swabs, and blanks.

Procedures

Parijoto fruit extraction. A sample of 3,000 g of parijoto fruit with a purplish pink color was separated from foreign matter and twigs that were carried away, then washed, drained, and dried at a temperature of 70˚C. Parijoto fruit was mashed and then weighed as much as 800 g for maceration. Parijoto fruit extract (*Medinilla speciosa* Blume) was prepared by maceration method using 70% ethanol solvent for 24 hours then filtered using filter paper. The extract was evaporated in a rotatory evaporator at 70˚C to separate 70% ethanol solvent from the extract (Kurniawati 2015; Legawati *et al.* 2020) The remaining filtrate was followed by evaporation using a waterbath at a temperature of 70˚C. The weight of parijoto fruit extract obtained was 12 g and the yield was 1.5% of the total extracted sample.

Phytochemical screening. Phytochemical screening was carried out to ensure the presence of active secondary metabolites that have biological activity from parijoto fruit simplicia used in this study. Tests were qualitatively carried out on saponin, tannin, flavonoid, alkaloid, steroid, terpenoid by by color reaction or precipitation.

Parijoto fruit extract suspension preparation. Parijoto fruit extract suspension was made every week. Parijoto fruit extracts were weighed at 0.294 g (280 mg/kg BW), 0.441 g (420 mg/kg BW), and 0.588 g (560 mg/kg BW) respectively. Doses were modified from prior research on parijoto fruit on liver rats with a dose modification close to 90 mg/200 g BW. Each extract was dissolved in 0.5% CMC-Na gradually until a suspension was formed and the volume was made up to 35 ml (Kurniawati 2015; Legawati *et al.* 2020).The extract was administered orally to mice using oral gavage, 1 ml each mice, expressed as mg/20 g of body weight for 56 days.

Simvastatin suspension preparation. The dose of simvastatin in mice was 0.026 mg/20 g BW then dissolved in a 0.5% CMC-Na (Elfrida 2015). The suspension was administered orally to mice using oral gavage, expressed as mg/20 g BW for 56 days along with high-fat feed induction period.

High-fat feed induction. The high fat feed given to mice was prepared by mixing and stirring until evenly distributed 8,000 g of standard pellets, 2,500 g of flour, 750 g of palm oil. Sixteen duck egg yolks are mixed with 1,000 g of goat fat until soft and runny while adding hot water as needed. The two mixtures then mixed together until smooth (Kodariah & Wahid 2020).

Animals study. Total number of samples in this study was thirty male balb/c mice (*Mus musculus* L.). The mice were 8 weeks old and weighed 20–30 g. The mice were acclimiatized for seven days according to standard animal housing conditions (with the temperatur was kept at 25±2˚C and maintained with 12 hour light-dark cycles), fed standard CP511 pellet and water ad libitum.

Experimental procedure. The randomization was done where the mice were divided into 6 groups (n=5); each group was given different treatments and treated for a period of 56 days: standard feed and CMC-Na 0.5% for group I (K1), high-fat diet and CMC-Na 0.5% for group II (K2), high-fat diet and simvastatin 0.026 mg/day for group III (K3), high-fat diet and parijoto fruit extract for group IV–VI (P1–P3). The dose of parijoto fruit extract for P1–P3 was 5.6, 8.4 and 11.2 mg/20 g BW, respectively. The doses in this study were base on modification of previous studies by Kurniawati (2015) and Elfrida (2015). The diet was administered ad libitum and the treatment was administered using oral gavage for 56 days. The animal handling during treatment was the same as during the acclimatization phase.

Microscopic study. At day 57, part of liver tissue was drawn from the terminated mice, fixed in 10% formalin, embedded in paraffin, and finally cut into 5 mm sections. The slides were stained with hematoxylin-eosin. The histopathological fatty changes of the liver were observed using Olympus CX23 Microscope from the entire field of view at 40 times magnification to determine area and 400 times magnification to confirm the fat accumulation.

The percentage of distribution of fat deposits was assessed semi-quantitatively using the Brunt method histological criteria that degreed from 0 to 3, as follows: 1) Degree 1: Fatty liver was found in 33% hepatocytes; 2) Degree 2: Fatty liver was found in 34%–66% hepatocytes; 3) Degree 3: Fatty liver was found in >66% hepatocytes (Brunt 2016; Aufazhafarin *et al.* 2021). Fat deposits distribution was firstly by estimating the liver lobes area which contained fat accumulation in hepatocytes (40 times), confirmating the steatosis (400 times), and then categorized into three degree.

Data analysis

All data obtained were presented as mean. Analysis was done with Kruskall-Wallis statistical test, followed by post-hoc Mann Whitney test with a significant value of $p<0.05$.

RESULTS AND DISCUSSION

Phytochemical screening was carried out to ensure the content of active compounds contained in the extract of parijoto fruit used in this study. The results of the phytochemical tests carried out as shown in Table 1 show that extract of the parijoto fruit used contained compounds in the form of saponin, tannin, flavonoid, alkaloid, and steroid.

The degree of steatosis

The results of this study based on the degree of steatosis indicate that there is impact of treatment of parijoto fruit extract in preventing fatty liver.

The degrees based on the microscopic observations represent the percentage of the fatty

Table 1. The secondary metabolites of parijoto fruit

Secondary metabolite	Conclusion	Explanation
Saponin	c	Foam is formed for more than one second
Tannin	+/Galat	Formed in black
Flavonoid	+	A clear red layer forms on top of the amyl alcohol layer
Alkaloid	+	Formation of orange color
Steroid	+	Formed in green and red
Terpenoid	-	No color formed

liver found in hepatocytes (Table 2). Based on the degree of steatosis of the liver of mice (Table 2), the highest percentage of steatosis occurred in the negative control group (K2). Eighty percent of mice in group K2 had a severe degree of steatosis (>66%) and as many as 20% had a moderate degree of steatosis (34–66%).

Second, the administration of simvastatin 0.026 mg/20 g BW in mice or a dose of 10 mg/70 g BW (K3) was able to reduce the incidence of steatosis by more than 66%, as much as 60% is in accordance with the baseline (≤33%) and 20% at a moderate degree.

Third, the administration of parijoto fruit extract at a dose of 5.6 mg/20 g BW/day (P1) can prevent the degree of steatosis to not exceed 66%, and only as much as 60% is in accordance with the baseline, which is a value that is a normal benchmark range, found in the normal group without a high-fat diet (K1).

Fouth, administration of parijoto fruit extract with increasing doses of 8.4 and 11.2 mg/20 g BB (P2 and P3) did not show the prevention in the degree of steatosis. Sixty percent of mice in each group P2 and P3 had a severe degree of steatosis (>66%) and as many as 40% had a moderate degree of steatosis (34–66%).

Statistical analysis based on degree of steatosis from histopathological observation

Data on the degree of steatosis were analyzed using the Kruskal Wallis test. It shows a significant difference between the six treatment

Table 2. The effect of parijoto fruit extract on the degrees of steatosis

Group	Degree of steatosis			n
	Degree 1	Degree 2	Degree 3	
K1	4	1	0	5
K2	0	1	4	5
K3	3	1	1	5
P1	3	0	2	5
P2	0	2	3	5
P3	0	2	3	5

K1:Standard feed and carboxymethyl cellulose sodium or CMC-Na 0.5%
K2:High-fat diet and CMC-Na 0.5%
K3:High-fat diet and simvastatin 0.026 mg/day
P1:High-fat diet and parijoto fruit extract 5.6 mg/20 g BW
P2:High-fat diet and parijoto fruit extract 8.4 mg/20 g BW
P3:High-fat diet and parijoto fruit extract 8.4 mg/20 g BW

Table 3. The results of the difference in the significance of steatosis between groups

Groups	K1	K2	K3	P1	P2	P3
K1	x	0.007*	0.439	0.366	0.011*	0.011*
K2	0.007*	x	0.041*	0.120	0.513	0.513
K3	0.439	0.041*	x	0.811	0.077	0.077
P1	0.366	0.120	0.811	x	0.212	0.212
P2	0.011*	0.513	0.077	0.212	x	1
P3	0.011*	0.513	0.077	0.212	1	x

*Significant difference ($p<0.05$) as analysed by Mann-Whitney test
K1:Standard feed and carboxymethyl cellulose sodium or CMC-Na 0.5%
K2:High-fat diet and CMC-Na 0.5%
K3:High-fat diet and simvastatin 0.026 mg/day
P1:High-fat diet and parijoto fruit extract 5.6 mg/20 g BW
P2:High-fat diet and parijoto fruit extract 8.4 mg/20 g BW
P3:High-fat diet and parijoto fruit extract 8.4 mg/20 g BW

groups with a result of 0.021 ($p<0.05$). Then, followed by Mann-Whitney Test (Table 3).

The Mann-Whitney result showed that the negative control group (K2) fed a high-fat diet for 56 days had a significant difference with the normal control group (K0) with p=0.007. These results indicate that the administration of HFD in eight weeks succeeded in forming fatty liver in the presence of steatotic cells in hepatocytes.

The K3 group that was given simvastatin had a significant difference with the K2 group that was fed a diet high in fat and cholesterol (p=0.041). These results showed that the administration of simvastatin at a dose of 0.026 mg/20 g BW showed the prevention in the degree of steatosis which was better than the treatment groups P1, P2, and P3.

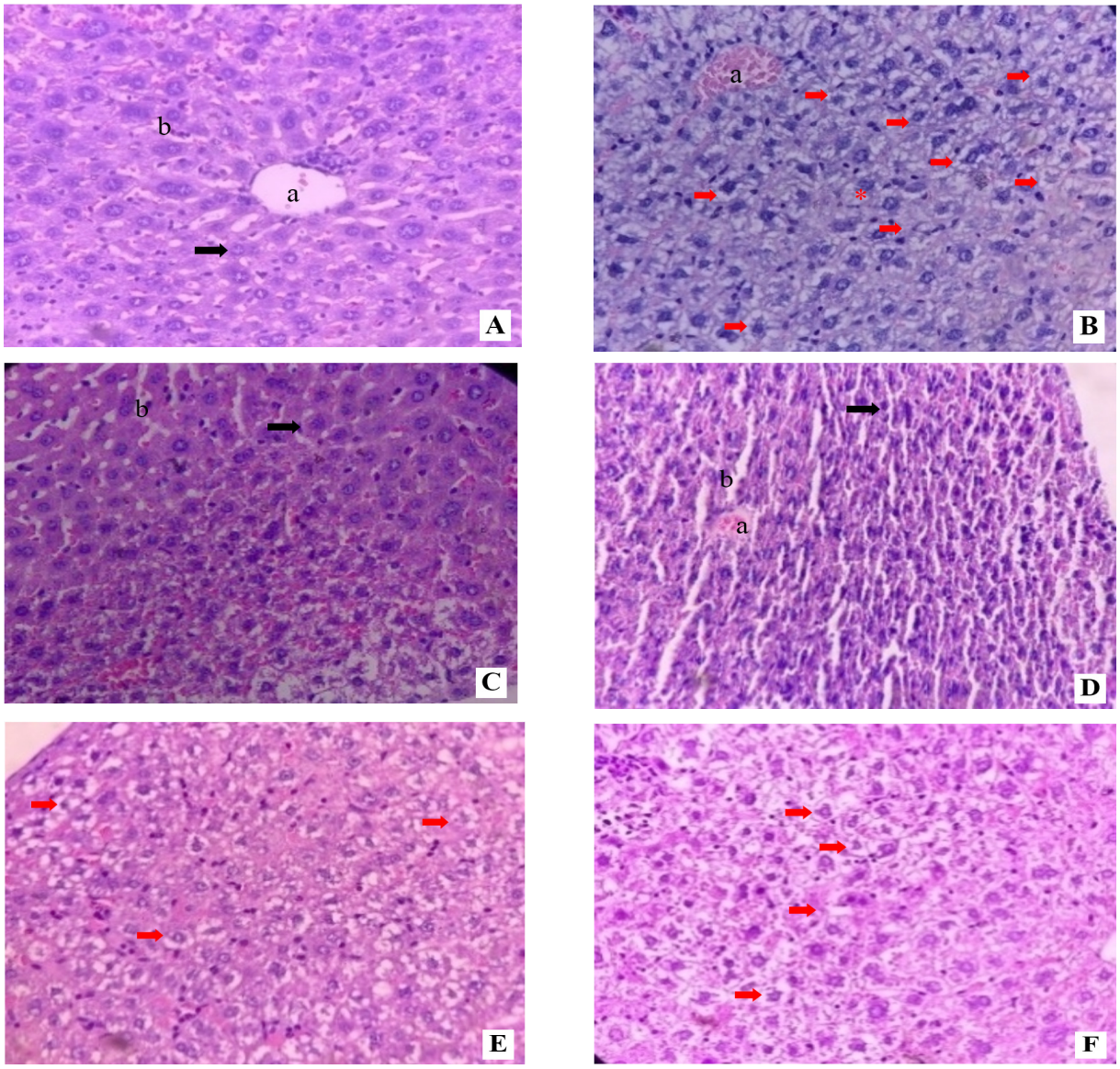
Based on the Mann-Whitney statistical result, Group P1 did not find a significant difference with K2 (p=0.120). These results showed that the administration of P1 parijoto fruit extract was not statistically significant. Although it was not statistically significant, but the histopathological picture in this study showed the administration of P1 parijoto fruit extract was able to improve the histopathological picture of fatty liver (Figure 1 (D)). This prevention is thought to be related to the proven active compound content of saponins, tannins, and flavonoids in the parijoto fruit extract in the phytochemical analysis that was also carried out in this study.

The results of Mann-Whitney statistical test, Group P2 and P3 did not find significant differences with all groups K2 (p=0.513, 0.513). In addition, Group K1 given standard feed had a significant difference with groups P2 and P3 (p=0.011 and 0.011). These results showed that the doses of parijoto fruit extract at 8.4 and 11.2 mg/20 g BW (P2 and P3) for 8 weeks were not optimal in preventing fatty liver.

Histopathology observation

The effects of parijoto fruit extract on liver histopathology of non-alcoholic fatty liver induced mice are offered in Figure 1. The histopathological results of HE staining are shown in Figure 1. Images were obtained at 400 times magnification. Group K1 is presented in Figure 1 (A). Liver tissue shows normal morphological structures in lobular architecture. The cell boundaries in the sinusoids are clear and regular. Hepatocytes are evenly distributed and tightly packed to form a plate arranged radially around the central vein, hepatocytes within normal limits, the cell nucleus in the middle, and there are no fat vacuoles in the cytoplasm.

In the K2 group, it was shown that high-fat diet can cause fatty liver with the presence of



(A) K1: Standard feed
(B) K1: High-fat diet
(C) K3: High-fat diet + Simvastatin
(D) P1: High-fat diet + Parijoto fruit extract (5.6 mg/20 g BB)
(E) P2: High-fat diet + Parijoto fruit extract (8.4 mg/20 g BB)
(F) P3: High-fat diet + Parijoto fruit extract (11.2 mg/20 g BB)
a:central vein; b:sinusoids; ➡:Normal hepatocytes; ➡:Steatosis

Figure 1. The effect of parijoto fruit extract on histopathological fatty changes of H&E staining in the liver of mice

steatotic cells in hepatocytes (Zhang *et al.* 2021). A greater amount of steatosis was seen in group K2 (Figure 1 (B)) compared to control groups K1 and K3 (Figure 1 (A and C)). In group K2, there was a change in the structure of the liver cells which became irregular, the cell boundaries were not clear, the arrangement of hepatocytes was wide and the sinusoids became irregular. Liver cells showed marked fat degeneration with clear fat vacuoles with cell nuclei pushed to the

periphery. Administration of high-fat diet will cause FFA in the liver to undergo β-oxidation in the mitochondria of hepatocytes or accumulate in the form of triglycerides. The accumulation of fat in the form of triglycerides in the liver can trigger an increase in liver lipotoxicity from an increase in free fatty acids, free cholesterol and other lipid metabolites. As a result, mitochondrial dysfunction can occur, causing a decrease in the results of burning fatty acids into energy from the β -oxidation process of triglycerides (Buzzetti *et al.* 2016). Triglycerides from the liver that are not transported on time can damage the lipid metabolism pathway that causes fatty liver (Fan *et al.* 2017).

The prevention of the fatty liver histopathological appearance occurred in group P1 which is presented in Figure 1 (D). In the P1 group, the liver parenchyma began to approach the normal hepatocyte picture, the parenchymal cell boundaries were clearer and the lipid degeneration was reduced with smaller vacuoles when compared to the K1 group, although the degree of steatosis in the P1 group was still higher than in the K3 and K1 groups. The improvement in the P1 group is thought to be related to the proven active compound content of saponins, tannins, and flavonoids in the extract of parijoto fruit in the phytochemical analysis which was also carried out in this study. Thus, parijoto fruit extract has the potential to improve the histopathology of fatty liver.

The mechanism of saponin content in improving the appearance of steatosis is by inhibiting the absorption of cholesterol and triglycerides and the reabsorption of bile acids in the intestine. Saponin can inhibit this absorption through inhibiting pancreatic lipase activity (Marrelli *et al.* 2016) and due to its heavy molecular weight, it is able to displace cholesterol from the food-mixed micelles and form cholesterol deposits that are difficult to pass through the intestinal mucus barrier, thereby causing a decrease in serum cholesterol concentrations (Luo *et al.* 2020). Tannin have the ability to increase the fecal excretion of cholesterol and bile acids through inhibition in the intestine, and also inhibit the occurrence of fatty liver through the activation of Adenosine Monophospate Activated Protein Kinase (AMPK) which is involved in the regulation of lipid metabolism. Activated AMPK inhibits the activity of enzymes and factors that regulate lipogenic processes in triglyceride synthesis. AMPK activation is also known to increase the expression of genes involved in fatty acid oxidation resulting in an increase in β-oxidation (Zou *et al.* 2014). Flavonoid act by reducing cholesterol formation in the liver through inhibition of HMG-CoA reductase (Zeka *et al.* 2017). Flavonoid also increase the activity of Lecithin Acyl Transferase (LCAT) which can reduce free cholesterol levels in the blood, increase the release of cholesterol from macrophages and increase the expression of ATP-binding Cassette (ABC) A1 and apolipoprotein A1 which is the basic material for the formation of HDL (Puspasari *et al.* 2016). The content of alkaloid also has antioxidant activity through the donation of hydrogen ions to free radicals and can act as pancreatic lipase inhibitors that can increase fat secretion in the faeces by reducing the breakdown of triglycerides into free fatty acids and glycerol (Artha *et al.* 2017).

Figures of hepatocytes with steatosis in the histopathology of groups P2 and P3 are presented in Figures 1 (E and F). In the histopathological picture of the group treated with parijoto fruit extract groups P2 and P3, fatty liver did not appear to have improved, macro and microvesicular steatosis was found where hepatocytes filled with fat granules with enlarged sizes. Cell boundaries and sinusoids appear indistinct and irregular. Hepatocytes with micro and macrovesicular steatosis were seen in groups P2 and P3 (Figure 1 (E and F)). In macrovesicular steatosis, there are lipid droplets in the hepatocyte cytoplasm that push the nucleus to the cell periphery. In microvesicular steatosis, there are small lipid droplets in the hepatocyte cytoplasm and the nucleus remains in the center of the cell (Takahashi & Fukusato 2014). These results showed that the doses of parijoto fruit extract at 8.4 and 11.2 mg/20 g BW (P2 and P3) for 8 weeks did not show any improvement in the histopathological picture of fatty liver compared to 5.6 mg/20 g BW (P1). The lack of improvement in the histopathology of fatty liver of mice in the treatment group of parijoto fruit extract doses 2 and 3 may be a condition that often occurs when testing new drug candidates which is a dose optimization phenomenon, where the dose will provide a maximum pharmacological response at a certain dose with minimum likelihood of undesirable symptoms. Thus, these

results indicate that the doses of 8.4 and 11.2 mg/20 g BW are not optimal in improving the histopathology of fatty liver.

Improvement of the histopathological picture of fatty liver occurred in the K3 group which is presented in Figure 1 (C). The degree of steatosis in the K3 group given simvastatin showed a better histopathological improvement than the P1, P2, and P3 treatment groups. The liver parenchyma began to approach the normal hepatocyte picture, was more clearly demarcated, and reduced lipid degeneration compared to the K1 group. This improvement in the degree of steatosis is due to simvastatin, a pharmacological agent that is commonly used in antihyperlipidemic therapy that works by inhibiting the HMG-CoA reductase enzyme during the cholesterol synthesis process (Indonesian Society for Endocrinology (PERKENI) 2019). The degree of steatosis in the K3 group given simvastatin at a dose of 0.026 mg/20 g BW showed better histopathological improvement compared to the P1, P2, and P3 treatment groups. Thus, simvastatin is able to improve the histopathological picture of fatty liver, and its effectiveness is still better than the administration of parijoto fruit extract therapy.

The current standard for the management of non-alcoholic fatty liver disease in humans, which has been evaluated histologically and has been included in the American Association for the Study of Liver Diseases is given Vitamin E (Perumpail *et al.* 2018). Vitamin E (rrr a-tocopherol) given at a daily dose of 800 IU/day can improve the improvement of liver steatosis in adults, especially non-diabetics. With the findings of this study, it is hoped that parijoto fruit extract can be used as a supplementation modality or can be used daily, especially by the local community which can be given as a non-alcoholic fatty liver disease prevention therapy in humans.

CONCLUSION

The administration of parijoto fruit extract 5.6 mg/20 g BB/day was able to improve the degree of steatosis which was more than 66%, but only 60% was in accordance with the baseline. Increasing the dose of parijoto fruit extract (*Medinilla speciosa* Blume) at doses of 8.4 and 11.2 mg/20 g BW did not increase the response to improvement in the degree of steatosis in the histopathological picture of fatty liver of male mice (*Mus musculus* L) balb/c strain. It can be concluded that parijoto fruit extract dose of 5.6 mg/20 g BW/day could act as the potential treatment for fatty liver. Thus, further research is needed on toxicity tests for knowing the side effects that can be caused by parijoto fruit extract before it can be implemented in human.

ACKNOWLEDGEMENT

The authors would like to express our gratitude to parties who have helped us in conducting the research.

DECLARATION OF INTERESTS

The authors have no conflict of interest.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2023, 18(1):31-40***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2023.18.1.31-40*
ISSN 1978-1059 EISSN 2407-0920

Breastfeeding Trends and it's Related Factors in Indonesia: A National Survey

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ABSTRACT

This cross-sectional study aimed to examine breastfeeding trends and factors in Indonesia using Demographic Health Survey (DHS) data from 2007 to 2017. The research data were obtained from three Indonesia Demographic Health Surveys. The data covered households and women aged 15–49 years old, including 40,701 households and 32,895 women in 2007; 43,852 households and 45,607 women in 2012; and 47,963 households and 49,627 women in 2017. Descriptive statistics was deployed to analyze the sociodemographic factors of the respondents. A questionnaire was employed to obtain data on the mothers' age, residence, education, economic status, mother working, marital status, literacy, place of delivery, first Antenatal Care (ANC) place, child size at birth, and gender of the child. Multinomial logistic regression analysis was used to analyze factors related to breastfeeding and how big the impact is. The findings indicate that the trend of breastfeeding in Indonesia significantly decreased based on the characteristics of mothers and children. The rates of breastfeeding (exclusive breastfeeding infants aged 0–5 months who received only breast milk) among mothers living in urban areas decreased significantly from 41.6% in 2012 to 38.4% in 2017. In 2017, children with normal birth weight (OR=0.87, 95% CI:0.53–1.45), boys (OR=1.01, 95% CI:0.92–1.10), and non-illiterate mothers (OR=0.50, 95% CI:0.46–0.55) had higher odds of breastfeeding compared to children with small birth weight, girls, and illiterate mothers. Factors associated with breastfeeding also change every year. In 2012, breastfeeding was related to marital status and delivery, but in 2017 it was not associated with those factors. Factors related to breastfeeding in Indonesia are age, residence, education, weight index, size of child at birth, mother's occupation, marital status, literacy, place of delivery, and first ANC place. These results are important for developing policies to improve maternal and child health in Indonesia by increasing education and mother training for early initiation of breastfeeding.

Keywords: breastfeeding, demographic health survey, Indonesia, trends, under five children

INTRODUCTION

Breastfeeding is one of the foundations of child health (World Health Organization (WHO) 2018). Breastfeeding is essential for a child's survival, nutrition, and development, as well as maternal health (WHO 2017). It is also the best way to build a bonding between mother and baby (Piro & Ahmed 2020). Around 800,000 neonatal deaths are generally associated with delayed breastfeeding initiation and a lack of exclusive breastfeeding. Breastfeeding should begin immediately after birth to reduce the risk of neonatal death in the first week of life by 22% (Woldeamanuel 2020). In 2017, the infant mortality rate in Indonesia was 21.4 per 1,000 live births, higher than in other Southeast Asian Low and Middle-Income Countries (LMIC), including Vietnam (17 per 1,000 live births), Thailand (8 per 1,000 live births), and Malaysia (7 per 1,000 live births) (Saputri *et al.* 2020). LMICs are facing a lack of access to clean water, low levels of adequate sanitation, low levels of adequate sanitation, and limited essential health and social services resulting in a lack of breastfeeding (Woldeamanuel 2020).

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(Received 12-10-2022; Revised 28-11-2022; Accepted 18-01-2023; Published 30-03-2023)

Only about 41% of infants aged less than six months were exclusively breastfed in 2017, with 45% continuing breastfeeding until two years (WHO 2018). Meanwhile, in Indonesia in 2018, the percentage of early initiation breastfeeding was 71.17%, which exceeded the national target (45%). However, there are provinces in Indonesia that have yet to reach the target: Maluku (23.18%), Central Sulawesi (30.37%), and North Sulawesi (37.70%). Meanwhile, limited data was found in the West Papua province (Ministry of Health Republic of Indonesia (MoH RI) 2019). The breastfeeding trend in Asian countries tends to decrease drastically yearly due to the promotion of the formula milk industry. Millions of babies, two-thirds of the worldwide population now consume formula milk, with breastfeeding falling in Asia (Smith 2019).

The Indonesian government's policy regarding breastfeeding is stated in the decree of the Minister of Health of the Republic of Indonesia No. 450/2004, regarding the provision of exclusive breast milk to babies in Indonesia with ten steps towards successful breastfeeding, one of which is helping mothers breastfeed correctly (MoH RI 2004). The Indonesian government's policy regarding the restriction of formula milk is contained in the Regulation of the Minister of Health of the Republic of Indonesia Number 14 of 2014 concerning the imposition of sanctions on health officers and institutions, as well as manufacturers of formula milk, which can hinder the success of the exclusive breastfeeding program. The sanctions may be in the form of a verbal or written warning and revocation of business licenses (MoH RI 2014). The decreasing trend in breastfeeding in Indonesia and the lack of evidence about changes over time in factors associated with breastfeeding require further investigation using national data. This study examines trends and characteristics associated with breastfeeding in Indonesia from 2007 to 2017.

METHODS

Design, location, and time

The study employed cross-sectional research design with a national dataset of children from Indonesia Demographic and Health Surveys (IDHS) in 2007, 2012, and 2017. The Ministry of Health Republic of Indonesia provided ethical clearance for this study, and permission to utilize the data was obtained from Inner City Fund (ICF) International.

Sampling

The surveys employed a two-stage stratified cluster design based on administrative regions and locations. The first step was to identify primary sampling units, and the second was to select households.

Data collection

Data were collected from the three national surveys using the Woman's Questionnaire. The validity and reliability are very satisfactory, as evidenced by the questionnaire from Demographic Health Survey (DHS) that has been used globally and has officially obtained permission from the state. Questionnaires have also been modified using local languages to ensure validity and reliability (Mohammadi *et al.* 2020; Prasetyo *et al.* 2022). The woman's questionnaire contains information on the following topics: mother's age, residence, education, economic status, mother's occupation status, marital status, literacy, place of delivery, first ANC place, child size at birth, and sex of the child. This study used data of households and women aged 15–49 from three surveys: 40,701 households and 32,895 women in 2007; 43,852 households and 45,607 women in 2012; and 47,963 households and 49,627 women in 2017.

The dependent variable in this research is the provision of breastfeeding. The provision of breastfeeding was defined when a through a survet on whether was carried out on whether the mother was breastfeeding. The questionnaire assesses breastfeeding by asking respondents, "Are you currently giving breastfeeding children?". The responses from respondents were categorized into two categories: yes and no.

The independent variable in this study is eleven factor that affects the provision of breastfeeding, including age (15–19 years, 20–34 years, and 35–49 years), residence (urban and rural), education (no education, primary, secondary, higher education), wealth quintiles (poorest, poorer, average, richer, richest), baby birth size (very large, large than average, average, smaller than average, very small, do not known), working mother (no, yes), sex of child (boys, girls), marital status (never married, married/ cohabiting, divorced/separated / widow), literacy

(no, yes), place of delivery (health facility, home/other), and place of first ANC (health facility, home/other).

Data analysis

Data analysis used Statistical Package for Social Science version 21 (IBM USA). We used frequencies and percentages to report sample characteristics and breastfeeding trends and a chi-square test to examine the relationship between the independent variable and breastfeeding in each survey (the Fisher's Exact test is an alternative test for the Chi-Square test which does not meet the requirements for use). Multinomial logistic regression was used to identify odds ratios and 95% Confidence Intervals (CI) of the factors influencing the breastfeeding description. P-value<0.05 means a significant influence exists between the provision of breastfeeding and the independent variable. Ethical clearance was obtained from ICF International, headquartered in Rockville, Maryland, United States, with Authletter number 142047.

RESULTS AND DISCUSSION

Respondents' characteristics

Table 1 shows the characteristics of the mothers and children. Data from 2007–2017 shows that the number of mothers aged 20–34 years old has decreased, while the number of mothers aged 35–49 years old has increased by 4%. Respondents in rural areas have decreased, while those in urban areas have increased by around 11%. The proportion of mothers' education has increased by up to 10% at the higher education level.

Trends and factors associated with breastfeeding administration in Indonesia

Table 2 shows breastfeeding rates by mothers' and children's characteristics. Overall, breastfeeding trends in Indonesia from the three surveys show an undesirable decrease based on the factors of mothers and children. The breastfeeding rates among mothers in urban areas decreased significantly from 41.6% in 2012 to 38.4% in 2017. Similarly, breastfeeding among mothers without education decreased from 58.6% in 2012 to 46.7% in 2017. It reduced breastfeeding in boys from 44.9% in 2012 to 41.4% in 2017. Breastfeeding also decreased significantly in mothers who used health facilities for the first time of ANC, from 40.9% in 2012 to 39.8% in 2017.

Factors related to breastfeeding in Indonesia are age ($p<0.001$), residence ($p<0.001$), education ($p<0.001$), weight index ($p<0.001$), size of child at birth ($p<0.05$), mother occupation ($p<0.001$), marital status (2007 and 2012) ($p<0.05$), literacy ($p<0.05$), place of delivery (2007 and 2012) ($p<0.05$), and first ANC place (2007 and 2017) ($p<0.001$). Breastfeeding in Indonesia is not related to the factors of sex of children, marital status (2017), place of delivery (2017), and first ANC place (2012) ($p>0.05$).

The multivariate analysis in Table 3 showed that in 2007, the odds of breastfeeding were 27% higher among teenage mothers than older mothers (OR=0.27, 95% CI:0.22–0.33). Mothers living in urban areas have a higher odds ratio for practicing breastfeeding compared to mothers living in rural areas (OR=1.15, 95% CI:1.04–1.26 in 2007; OR=1.09, 95% CI:0.98–1.22 in 2012; OR=1.15, 95% CI:1.03–1.27 in 2017). In 2007, the odds of breastfeeding were 79% higher for mothers with no education compared to mothers with higher education, 71% for the poor compared to wealthy families, 61% for mothers who were not working compared to working mothers, and 81% of mothers who were married compared to unmarried/divorced mothers. In 2017, children with normal birth weight (OR=0.87, 95% CI:0.53–1.45), boys (OR=1.01, 95% CI:0.92–1.10), and non-illiterate mothers (OR=0.50, 95% CI:0.46–0.55) had higher odds of breastfeeding compared with children at birth, girls, and illiterate mothers. In 2012, mothers who gave birth in a health facility were 73% more likely to perform breastfeeding compared to mothers who gave birth at home (OR=0.73, 95% CI:0.63–0.85), and mothers who served ANC at a health facility had higher odds to provide breastfeeding compared to mothers who did ANC at home (OR=1.03, 95% CI:0.89–1.19) (Table 3).

The conditions in Indonesia regarding early breastfeeding have reached the national target. However, provinces in eastern Indonesia need attention in this regard. Several provinces have not yet achieved the national target, such as Maluku, Central Sulawesi, North Sulawesi, and Papua (Sugawara & Nikaido 2014). This trend is similar to that of middle-income countries such as Vietnam and Haiti (Kavle *et al.* 2019; Ndirangu

Table 1. Respondents' characteristic

Characteristic	2007 (n=18,645)		2012 (n=18,021)		2017 (n=17,848)	
	n	%	n	%	n	%
Age, years old						
15–19	565	3.0	586	3.3	440	2.5
20–34	13,588	72.9	12,901	71.6	12,098	67.8
35–49	4,492	24.1	4,534	25.2	5,310	29.8
Residence						
Urban	7,013	37.6	8,170	45.3	8,760	49.1
Rural	11,632	62.4	9,851	54.7	9,088	50.9
Education						
No education	797	4.3	584	3.1	270	1.5
Primary	7,361	39.5	5,550	29.8	4,455	25.0
Secondary	9,046	48.5	9,489	50.9	9,920	55.6
Higher	1,439	7.7	2,398	12.9	3,203	17.9
Wealth index						
Poorest	5,747	30.8	5,477	30.4	4,963	27.8
Poorer	3,722	20.0	3,591	19.9	3,483	19.5
Middle	3,229	17.3	3,249	18.0	3,257	18.2
Richer	3,033	16.3	3,010	16.7	3,138	17.6
Richest	2,914	15.6	2,694	14.9	3,007	16.8
Size child at birth						
Very large	1,156	6.3	744	4.2	892	5.0
Larger than average	4,406	23.9	4,707	26.4	4,795	27.0
Average	9,078	49.3	9,404	52.6	9,383	52.9
Smaller than average	2,312	12.6	2,101	11.8	1,993	11.2
Very small	542	2.9	337	1.9	380	2.1
Don't know	906	4.9	570	3.2	298	1.7
Working mother						
No	10,007	53.9	9,229	51.2	9,511	53.3
Yes	8,573	46.1	8,783	48.8	8,324	46.7
Sex of child						
Boys	9,834	52.7	9,358	51.9	9,202	51.6
Girls	8,811	47.3	8,663	48.1	8,646	48.4
Marital status						
Never married	30	0.2	13	0.1	0	0.0
Married	17,287	96.9	17,542	97.3	18,183	97.5
Divorced	531	3.0	466	2.6	462	2.5
Literacy						
No	1,508	8.1	1,099	6.1	645	3.6
Yes	17,007	91.9	16,781	93.9	17,158	96.4
Place of delivery						
Health facility	6,844	38.3	9,225	53.5	13,104	73.7
Home/Other	11,031	61.7	8,030	46.5	4,665	26.3
First ANC, place						
Health facility	3,973	33.4	4,353	57.5	4,531	51.1
Home/Other	7,921	66.6	3,218	42.5	4,338	48.9

ANC:Antenatal Care

Table 2. Rates of breastfeeding in Indonesia (2007, 2012, 2017) by demographic and socioeconomic characteristics

Characteristic	Breastfeeding (2007)			Breastfeeding (2012)			Breastfeeding (2017)		
	No (n/%)	Yes (n/%)	*P*	No (n/%)	Yes (n/%)	*P*	No (n/%)	Yes (n/%)	*P*
Age									
15–19	163/28.8	402/71.2	0.000	182/31.1	404/68.9	0.000	162/36.8	278/63.2	0.000
20–34	7,009/51.6	6,579/48.4		6,976/54.1	5,925/45.9		6,761/55.9	53,378/44.1	
35–49	2,671/59.5	1,821/40.5		2,751/60.7	1,783/39.3		3,469/65.3	1,841/34.7	
Residence									
Urban	4,125/58.8	2,888/41.2	0.000	4,769/58.4	3,401/41.6	0.000*	5,397/61.6	3,363/38.4	0.000*
Rural	5,718/49.2	5,914/50.8		5,140/52.2	4,711/47.8		4,995/55	4,098/45	
Education									
No education	397/49.8	400/50.2	0.000	242/41.4	342/58.6	0.000	144/53.3	126/46.7	0.020
Primary	3,628/49.3	3,733/50.7		2,939/53	2,611/47		2,535/56.9	1,920/43.1	
Secondary	4,947/54.7	4,099/45.3		5,323/56.1	4166/43.9		5,793/58.4	4,127/41.6	
Higher	871/60.5	568/39.5		1,405/58.6	993/41.4		1,920/59.9	1,283/40.1	
Wealth index									
Poorest	2,597/45.2	3,150/54.8	0.000	2,674/48.8	2,803/51.2	0.000	2,586/52.1	2,377/47.9	0.000
Poorer	1,944/52.2	1,778/47.8		1,871/52.1	1,720/47.9		1,995/57.3	1488/42.7	
Middle	1,706/52.8	1,523/47.2		1,896/58.4	1,353/41.6		1,943/59.7	1,314/40.3	
Richer	1,766/58.2	1,267/41.8		1,794/59.6	1,216/40.4		1,928/61.4	1,210/38.6	
Richest	1,830/62.8	1,084/37.2		1,674/62.1	1,020/37.9		1,940/64.5	1,067/35.5	
Size child at birth									
Very large	588/50.9	568/49.1	0.000	419/56.3	325/43.7	0.000	534/59.9	358/40.1	0.014
Larger than average	2,318/52.6	2,088/47.4		2,632/55.9	2,075/44.1		2,769/57.7	2,028/42.3	
Average	4,911/54.1	4,167/45.9		5,120/54.4	4,284/45.6		5,410/57.7	3,973/42.3	
Smaller than average	1,170/50.6	1,142/49.4		1,161/55.3	940/44.7		1,190/59.7	803/40.3	
Very small	301/55.5	241/44.5		211/62.6	126/37.4		249/65.6	131/34.5	
Don't know	408/45.0	498/55.0		266/46.7	304/53.3		163/54.7	135/45.3	
Working mother									
No	4,715/47.1	5,292/52.9	0.000*	4,348/47.1	4,881/52.9	0.000*	4,829/50.8	4,682/49.2	0.000*
Yes	5,098/59.5	3,475/40.5		5,554/63.2	3,229/36.8		5,555/56.7	2,769/33.3	
Sex of child									
Boys	5,184/52.7	4,650/47.3	0.418*	5,160/55.1	4,198/44.9	0.338*	5,395/58.6	3,807/41.4	0.133*
Girls	4,659/52.9	4,152/47.1		4,749/54.8	3,914/45.2		4,997/57.8	3,649/42.2	

Continue from Table 2

Characteristic	Breastfeeding (2007)			Breastfeeding (2012)			Breastfeeding (2017)		
	No (n/%)	Yes (n/%)	*P*	No (n/%)	Yes (n/%)	*P*	No (n/%)	Yes (n/%)	*P*
Marital status									
Never married	11/36.7	19/63.3	0.020	5/38.5	8/61.5	0.000	-	-	0.259*
Married	9,111/52.7	8,176/47.3		9,571/54.6	7,971/45.4		10,124/58.2	7,276/41.8	
Divorced	305/57.4	226/42.6		333/71.5	133/28.5		268/59.8	180/40.2	
Literacy									
No	726/48.1	782/51.9	0.000*	507/46.1	592/53.9	0.000*	346/53.6	299/46.4	0.009*
Yes	9,048/53.2	7,959/46.8		9,325/55.6	7,456/44.4		10,018/58.4	7,140/41.6	
Place of delivery									
Health facility	3,956/57.8	2,888/42.2	0.000*	5,169/56	4,056/44	0.002*	7,638/58.3	5,466/41.7	0.275*
Home/Other	5,482/49.7	5,549/50.3		4,322/53.8	3,708/46.2		2,695/57.8	1,970/42.2	
First ANC, place									
Health facility	2,291/57.7	1,682/42.3	0.000*	2,574/59.1	1,779/40.9	0.329*	2,908/64.2	1,623/35.8	0.000*
Home/Other	3,935/49.7	3,986/50.3		1,920/59.7	1,298/40.3		2,616/60.3	1,722/39.7	

ANC: Antenatal Care
Chi-square test

et al. 2018). This condition is due to structural, individual, and environmental factors such as lack of access to health services, insufficient knowledge, socioeconomic conditions, and family support (Blackwell & Morrell 2021; Hauck *et al.* 2020; Horwood *et al*. 2020).

The results showed that mothers living in urban areas have a higher odds ratio of practicing breastfeeding than those living in rural areas. This also relates to working mothers. In urban areas, some mothers who provide breastfeeding are working mothers. The proportion of children aged 0–23 months who have been breastfed among homemakers is lower than in mothers who work. *Riset Kesehatan Dasar*/ RISKESDAS (Basic Health Research) results in 2013 showed that 90.8% of mothers who did not work breastfeed compared to 93.2% of working mothers who breastfeed. Mothers, who work extra hours or mothers who work, practice breastfeeding better than those who do not work (MoH RI 2019). Mothers occupation will have access to information, economic capabilities and knowledge that is more supportive of breastfeeding than mothers who were housewives (Horwood *et al.* 2020; Rujumba *et al*. 2020).

The results showed that 81% of married mothers breastfed more than unmarried/ divorced mothers. Family support is an essential factor for married mothers. Family support can provide knowledge, motivation, and experience to continue breastfeeding (Anstey *et al.* 2018; Gharaei *et al.* 2020). Support from family can also increase mothers' self-efficacy in facing difficulties while breastfeeding (Gharaei *et al.* 2020; Piro & Ahmed 2020). A variety of factors contribute to the creation of a suitable environment for breastfeeding. At the national level, policies guaranteeing maternity leave and the right to breastfeed in the workplace are critical, as are restrictions on sales of infant formula. Mothers need information and support in health facilities to breastfeed immediately after birth. Breastfeeding mothers are motivated by positive social norms that support and encourage breastfeeding, including in public places (Pangestuti 2018). Support from trained counselors and peers, such as other mothers and

Table 3. Multivariate logistic regression analyzes factors associated with breastfeeding

Characteristic	2007 (n=18,645)	2012 (n=18,021)	2017 (n=17,848)
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Age, years old			
15–19	0.27 (0.22–0.33)	0.29 (0.24–0.35)	0.30 (0.25–0.37)
20–34	0.72(0.67–0.77)	0.76 (0.71–0.81)	0.67 (0.62–0.71)
35–49	Ref	Ref	Ref
Residence			
Urban	1.15 (1.04–1.26)	1.09 (0.98–1.22)	1.15(1.03–1.27)
Rural	Ref	Ref	Ref
Education			
No education	0.79 (0.55–1.02)	1.12 (0.68–1.86)	1.08 (0.61–1.91)
Primary	0.96 (0.82–1.13)	1.16 (0.98–1.39)	1.17 (1.00–1.37)
Secondary	1.04 (0.89–1.21)	1.28 (1.10–1.48)	1.26 (1.11–1.44)
Higher	Ref	Ref	Ref
Wealth index			
Poorest	0.71 (0.60–0.83)	0.63 (0.53–0.76)	0.65 (0.55–0.78)
Poorer	0.87 (0.74–1.01)	0.68 (0.57–0.81)	0.78 (0.66–0.92)
Middle	0.80 (0.69–0.93)	0.85 (0.71–1.02)	0.85 (0.72–0.99)
Richer	0.95 (0.82–1.09)	0.82 (0.69–0.98)	0.87 (0.75–1.01)
Richest	Ref	Ref	Ref
Size child at birth			
Very large	1.33 (1.03–1.71)	1.07 (0.70–1.63)	0.94 (0.54–1.61)
Larger than average	1.41 (1.14–1.75)	1.06 (0.74–1.54)	0.84 (0.51–1.40)
Average	1.57 (1.28–1.93)	1.02 (0.71–1.47)	0.87 (0.53–1.45)
Smaller than average	1.40 (1.12–1.75)	1.10 (0.75–1.61)	0.98 (0.58–1.64]
Very small	2.14 (1.59–2.87)	1.51 (0.90–2.52)	1.46 (0.79–2.70)
Don't know	Ref	Ref	Ref
Working mother			
No	0.61 (0.56–0.68)	0.54 (0.49–0.59)	0.50 (0.46–0.55)
Yes	Ref	Ref	Ref
Sex of child			
Boys	1.00 (0.93–1.08)	1.00 (0.90–1.10]	1.01 (0.92–1.10)
Girls	Ref	Ref	Ref
Marital status			
Never married	0.29 (0.10–0.78)	0.46 (0.10–2.00)	0.48 (0.48–0.49)
Married	0.81 (0.65–1.02)	0.49 (0.35–0.68)	0.45 (0.45–0.46)
Divorced	Ref	Ref	Ref
Literacy			
No	1.04 (0.86–1.25)	0.76 (0.56–1.02)	0.81 (0.58–1.13)
Yes	Ref	Ref	Ref
Place of delivery			
Health facility	0.85 (0.70–1.02)	0.73 (0.63–0.85)	0.69 (0.61–0.78)
Home/Other	Ref	Ref	Ref
First ANC, place			
Health facility	1.27 (1.06–1.53)	1.03 (0.89–1.19)	1.13 (1.02–1.25)
Home/Other	Ref	Ref	Ref

ANC: Antenatal Care
OR: Odds Ratio; CI: Confidence Interval
Regression logistic multinominal test

family members, is vital in communities. Support from husbands and partners can also motivate mothers to breastfeed their children (UNICEF 2018).

The study found that mothers who gave birth in a health facility were 73% more likely to breastfeed than mothers who gave birth at home (OR=0.73, 95% CI:0.63–0.85) and mothers who served ANC at a health facility had higher odds to give breastfeeding compared to mothers who did ANC at home/other (OR=1.03, 95% CI:0.89–1.19). This is because mothers who get services in health service facilities will gain knowledge from health workers and therefore have better self-efficacy, knowledge, and attitudes toward breastfeeding (Piro & Ahmed 2020). Good antenatal care increases the mothers' ability to benefit from breastfeeding for the growth and development of children. Breastfeeding is the best food for children and is more economical than formula milk (McNellan *et al.* 2019; Piro & Ahmed 2020). As the advantages of exclusive breastfeeding, the baby will be sick less often and the expenses for going to the doctor or hospital will also be reduced (Horwood *et al.* 2020; Mallick *et al.* 2020; Woollard 2019)

CONCLUSION

Factors related to breastfeeding in Indonesia are age, residence, education, weight index, size of child at birth, mother working, marital status, literacy, place of delivery, and first ANC place. The findings of this research are important for developing policies to improve maternal and child health in Indonesia by increasing health promotion, education, and trainings for mothers. Further research can analyze the obstacles to giving breastfeeding to working mothers or developing learning methods about giving breastfeeding to children for mothers who experience problems with access to education and health services

ACKNOWLEDGEMENT

First, we would like to thank the Measure DHS program for providing access to the datasets. Second, we would also like to thank all mothers who agreed to be interviewed and gave informed consent to participate in this study.

DECLARATION OF INTERESTS

The authors have no conflict of interest.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2023, 18(1):41-50***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2023.18.1.41-50*
ISSN 1978-1059 EISSN 2407-0920

Protective Effects of the Polyphenolic-Rich Fraction of Cornsilk against Oxidative Stress in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

The current study investigated the anti-hyperglycemic and antioxidative properties of the Phenolic-Rich Fraction of Cornsilk (PRF-CS) in Streptozotocin (STZ)-induced diabetic rats. Five groups of 30 male Sprague Dawley rats were employed in this study. A sample size of six rats each is placed in five groups: Normal-Control (NC), Diabetic-Control (DC), Diabetic-PRF-CS treated 100 mg/kg (DPRF100) and 200 mg/kg (DPRF200), and Diabetic-Metformin Treated (Dmet) groups. The PRF-CS was administered at 100 and 200 mg/kg doses for 28 consecutive days to the diabetic rats. Treatment with both doses of PRF-CS (DPRF100 and DPRF200) significantly decreased the blood glucose levels of the rats ($p<0.05$). Additionally, the PRF-treated rats demonstrated significantly decreased ($p<0.05$) lipid peroxidation (3.60±0.23 and 3.31±0.56 µmol/g, respectively). The hepatic antioxidant enzyme activities of Superoxide Dismutase (SOD) (169.35±4.75 and 175.30±3.69 U/mg, respectively), Catalase (CAT) (1457.51±152.74 and 2011.99±396.96 U/mg), and Glutathione Peroxidase (GSH-Px) (63.43±2.99 and 78.47±4.51 U/mg) were also elevated in contrast to the DC group. Furthermore, the PRF-CS administration improved the histological alterations in the liver tissues of the DPRF100 and DPRF200 rats. In conclusion, PRF-CS treatment exhibited protective effects in the diabetic rat model by decreasing oxidative stress and preserving liver integrity.

Keywords: cornsilk, diabetes, oxidative stress, phenolic-rich fraction, STZ-induced diabetic rats

INTRODUCTION

Hyperglycemia is when blood sugar or glucose levels are higher than normal. The condition can be caused by several factors, including pancreatic cancer, cystic fibrosis, chronic pancreatitis, pheochromocytoma, acromegaly, and Cushing syndrome, but Diabetes Mellitus (DM) is the most common cause of hyperglycemia (Winter *et al.* 2021). Diabetes has a strong connection with oxidative stress as hyperglycemia stimulates the production of free radicals, leading to oxidative stress (Asmat *et al.* 2016). The imbalance between antioxidants and free radical production is known as oxidative stress. The phenomenon is caused by the inability of the body's defense system to counteract the increased radical generation, leading to high levels of free radicals and weaker antioxidant defense mechanisms (Golbidi *et al.* 2011).

Prolonged hyperglycemia induces liver disease because the escalated generation of Reactive Oxygen Species (ROS) during oxidative stress damages liver tissues (Ramu *et al.* 2016). Several studies suggested that diabetes patients suffer a higher standardized mortality rate from terminal liver disease than cardiovascular disease (Zhang *et al.* 2012). Therefore, effective antioxidant therapy is necessary in order to counteract the negative effects of ROS as antioxidants have been proven to be effective in scavenging ROS and reducing diabetic complications (Bajaj & Khan 2012). Consuming plenty of vegetables, fruits, and other plants on a consistent basis, which are all high in different kinds of natural antioxidants, has been linked to lowering the risk of developing diabetes (Zhang *et al.* 2015). In addition, it has been found that agricultural by-products which some are commonly recognized as waste have

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(Received 12-12-2022; Revised 15-01-2023; Accepted 07-02-2023; Published 30-03-2023)

substantial antioxidative activity. Hence, there is a growing interest in using agricultural by-products as food additives or supplements due to their specific nutritional and pharmacological properties (Faustino *et al.* 2019).

The agricultural by-products such as peels, pomace, seeds, stalks, and pulp were proved to contain various functional compounds, where some of these by-products contained the same amount or more bioactive compounds than the finished product (Silva *et al.* 2014). Bioactive compounds such as vitamins, carotenoids, phenolics, and other essential nutrients are abundant in agricultural by-products. Among these bioactive compounds, phenolics are recognized for their anti-diabetic and antioxidant activities (Lin *et al.* 2016). Recently, plant-derived phenolic compounds have gained considerable interest due to a wide range of pharmacological properties, including anti-microbial, hepatoprotective, antioxidant, anti-diabetes, and anti-inflammatory activities. In this context, the Phenolic-Rich Fraction (PRF) is beneficial due to its powerful free radicals scavenging and antioxidant actions (Dragan *et al.* 2015).

Cornsilk (CS) is a by-product or agricultural waste of maize farming since it is not edible. Each year, approximately 50,000 tonnes of CS are disposed of in Malaysia alone (Nurhanan *et al.* 2012). Cornsilk is a bundle of silky, long, and yellowish strands on top of a maize fruit that could grow to 10 to 20 cm as the fruit develops (Rahman & Wan Rosli 2014). Apart from functioning as a stigma for the female flower, CS is also rich in phenolic compounds, such as flavonoids (Liu *et al.* 2011). A few studies demonstrated that CS from baby corn (young maize) contained higher antioxidant activities than CS from mature maize fruits (Rahman & Wan Rosli 2014; Sarepoua *et al.* 2013). Hence, the PRF of CS from baby corn could potentially be the source of natural antioxidants to hinder oxidative stress. The current investigation examined the protective effects of PRF of CS from baby corn (PRF-CS) on lipid peroxidation and antioxidative actions in diabetic rats induced with Streptozotocin (STZ). The effects of PRF-CS treatment on the liver damage generated by STZ were also highlighted in this study. This present study will foster the use of agro-residue from corn as a new functional food ingredient that can assist our health-conscious population, which places an emphasis on well-being and wellness.

METHODS

Design, location, and time

The study was a randomized experimental study. The study was carried out in the Animal Research and Service Centre (ARASC), Health Campus, Universiti Sains Malaysia (USM), Kelantan, Malaysia. The study was carried out from January to April 2019 and the procedures related to the animals' section of the current study use were outlined and carried out based on the approval of the Animal Ethics Committee, USM (USM/IACUC/2017/(832)).

Materials and tools

The main material for this study was cornsilk from fresh baby corn (*Zea mays* L.). Fresh baby corn harvested 45–55 days post-planting was bought from a farm in Kampung Tendong, Pasir Mas, Kelantan, Malaysia. Two to three months old male Sprague Dawley rats weighing 250 to 300 g were employed for this study. The ARASC, Health Campus, USM supplied all 30 rats for this experiment. Chemicals and reagents used in this study were: 10% neutral buffered formalin, 1,1,3,3-tetraethoxypropane,Thiobarbituric acid (TBA), and Tris-Hydrochloric Acid (Tris-HCl) buffers were obtained from Sigma (Saint Louis, Missouri (MO), United States of America (USA)), acetic acid, ethyl acetate, hexane, and ethanol were from HmbG (Hamburg, Germany), and Albumin (BSA) were by Amresco (Solon, USA). The CAT, SOD, and GSH-Px assay kits were produced by Elabscience (Texas, USA). The phosphate buffer solution was obtained from the 1st Base (Singapore Science Park II, Singapore), n-butanol was from Fisher (Waltham, United Kingdom (UK)), metformin from Metcheck 850 (India), sodium citrate buffer solution from R & M (Essex, UK), STZ was bought from Merck (Darmstadt, Germany), and trichloroacetic acid (TCA) was obtained from Fisher (Waltham, UK).

Procedures

Preparation of the PRF-CS. The PRF from the CS of the baby corn was prepared based on the methodology reported in the study by Nurraihana *et al.* (2018).

Animal study. Subsequently, 30 diabetic rats were arbitrarily grouped into five clusters of six rats. Group I housed Non-Diabetic rats (NC), group II contained Diabetic Untreated rats (DC), groups III, IV, and V comprised diabetic

rats treated with PRF-CS at 100 (DPRF100), PRF at 200 (DPRF200) and metformin at 150 (Dmet) mg/kg/day, respectively. The treatments were administered once a day for four weeks (day 28) through oral gavage.

Induction of experimental diabetes. On the first day of administration (Day 0), STZ was dissolved in 0.1 M sodium citrate buffer (pH 4.5) and administered intraperitoneally (i.p.) to the rats at a dose of 55 mg/kg. Food and water intake were closely monitored, and diabetes was validated by checking the fasting blood glucose (FBG) level using a glucose strip on the third and seventh-day post-STZ injection. Rats with FBG levels of ≥13 mmol/l were regarded as diabetic (Rahman 2016).

Determination of body weight and FBG. The body weight of each rat was recorded weekly for four weeks. The rats fasted overnight prior to the FBG test. Blood samples from the rats were collected from the veins at the tip of their tails by employing the Accu-Chek glucometer (Roche, Germany) on the dosing days and every subsequent week for FBG estimation.

Determination of lipid peroxidation and antioxidant enzymes activities. The rats were terminated after four weeks while under anesthesia. The livers of the rats were swiftly removed, weighed, and washed twice with a cold phosphate buffer solution. Subsequently, the liver tissues were split into two parts. One part was subjected to histopathological studies, while the other was used for enzymatic studies. The rat liver tissues were minced, weighed, and homogenized at a weight-to-volume ratio of 1:9 in Tris-HCl buffer (pH 7.4). The temperature was kept as low as possible by placing the homogenate on ice. The tissues homogenate was then centrifuged (1,041 g, 10 min) (Hettich, Germany) and the supernatant was collected and preserved at a −80 ^{O}C deep freezer (ilShin, South Korea) for the total protein and enzymatic tests, including SOD, CAT, GSH-Px, and lipid peroxidation activities. Protein concentrations were established according to the Bradford method. The amount of Malondialdehyde (MDA) that reacted with TBA in the homogenates was determined based on the technique reported by Angirekula *et al.* (2018). The SOD, CAT, and GSH-Px activities were assessed by employing a commercial assay kit from Elabscience (Texas, USA) following the guidelines provided by the manufacturer.

Histopathological study. The liver samples were fixed in 10 % formalin for two weeks before being processed into wax blocks and sectioned to a thickness of 4 µm. Tissue sections were placed on silane-coated slides and stained with hematoxylin and eosin. The stained tissues from every group were evaluated for histopathological alterations through the light microscope (Leica, Germany) at 10× and 40× magnifications, and an image analyzer captured the photomicrographs. Additionally, the tissues were assessed by a blind histologist. Any morphological alterations were observed, and the severities of each histopathological change were denoted as not (−), mildly (+), moderately (++), or severely (+++) damaged.

Data analysis

The results were evaluated for statistical significance using the IBM Statistical Package of Social Sciences (SPSS) Statistics Data Editor Version 24. The results were conveyed as means±standard error of the means (S.E.M). A p-value of less than 0.05 was deemed significant. The differences between the means were analyzed for significance using a one-way analysis of variance (ANOVA) test by employing the Post-hoc Tukey test.

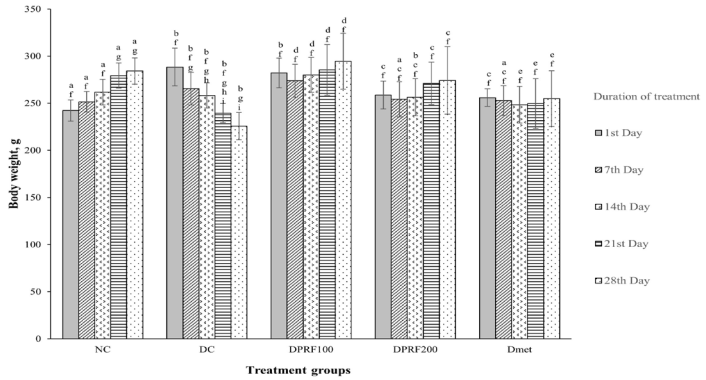
RESULTS AND DISCUSSION

The effects of PRF-CS on the rat body weight

The changes in body weights observed in NC and DC groups are displayed in Figure 1. The STZ-induced rats exhibited a considerable weight loss ($p<0.05$) compared to Normal rats (NC group). The DC group lost weight throughout the study, while the DPRF100, DPRF200, and Dmet groups exhibited significant improvements in body weight compared to the DC group.

The DPRF100 and DPRF200 groups demonstrated a significant body weight elevation compared to the NC and DC groups from the second to the fourth week of treatment. The Dmet group exhibited a significantly increased weight starting from the third week of treatment. After 28 days, the weight of the NC group significantly increased by 17.34% compared to the baseline. Conversely, the weight of the rats in the DC group was significantly reduced at 21.73% compared to the baseline. PRF-CS-treated groups showed no notable difference in the percentage of body weight increment as compared to the baseline.

A loss in body weight characterized the STZ-induced diabetic rat. The weight loss was



The values indicated by the different letters are statistically significant ($p<0.05$). Letters a–e each represents pairwise comparisons among different groups based on time, while f–i are pairwise comparisons within each group based on time
NC: Normal-Control; DC: Diabetic-Control; DPRF100: Diabetic-PRF-CS Treated (100 mg/kg); DPRF200: Diabetic-PRF-CS Treated 200 mg/kg; Dmet: Diabetic-Metformin Treated (150 mg/kg)

Figure 1. The body weights of phenolic-rich fraction of cornsilk- and metformin-treated streptozotocin-induced diabetic rats

due to the extreme breakdown of tissue proteins and fatty acids. Diabetic cells are unable to properly utilize glucose to produce energy due to inadequate insulin (Codella *et al.* 2017), causing the body to break down protein (proteolysis) and lipid (lipolysis) for energy (Syamsudin *et al.* 2010). Other than β-cells functions and insulin resistance defects, type II diabetes is also correlated to α-cells disabilities which are linked to relative glucagon hypersecretion (Lund *et al.* 2011). Glucagon resists the effects of insulin, elevating the glucose concentration in the bloodstream, inhibiting the storage of metabolic fuels, and activating gluconeogenesis (Coelho *et al.* 2013). Consequently, the condition might prevent fat and protein storage, inducing weight loss in diabetic rats (Martínez *et al.* 2014).

Studies indicated that untreated STZ-induced diabetic rats exhibited a markedly reduced body weight (Rahman 2016), which is consistent with the findings of the present study. Nevertheless, the PRF-CS treatments increased the body weight of diabetic rats. The increment might be related to increased muscle glucose uptake, preventing tissue loss. The treatments enable body tissues to access glucose to obtain energy and build necessary tissue materials for growth.

The effects of PRF-CS on the FBG levels

The effects of PRF-CS treatment on the FBG level of STZ-induced diabetic rats are displayed in Table 1. The diabetic rats demonstrated a significant increase in FBG levels than normal rats.

After four weeks, the diabetic rats administered with 100 and 200 mg/kg of PRF-CS experienced significantly diminished blood glucose levels, which were reduced by 67.45% and 66.85%, respectively, compared with the DC rats. The Dmet group also demonstrated a significant decrease of 66.76 % in blood glucose level compared with the DC group.

The diabetic rats demonstrated a high level of FBG, which is a crucial indicator of the disease. After the STZ injection, FBG levels in the DC group were notably elevated in contrast to those in the NC group, indicating that a DM model was successfully induced via STZ injection. Furthermore, the administration of PRF-CS significantly lowered the FBG level in diabetic rats, which is consistent with the studies by Ghada *et al.* (2013) that demonstrated that FBG levels decreased significantly with a crude extract of CS from mature corn fruits.

The phytochemical evaluation of the PRF-CS demonstrated the presence of phenolic compounds (Nurraihana *et al.* 2018). Phenolic compounds display various biological characteristics, where some are potent antioxidants, and several display anti-diabetic properties (Sarian *et al.* 2017). Jassim *et al.* (2016) reported that phenolic substances from Solanum melongena peel demonstrated remarkable

Table 1. The effects of phenolic-rich fraction of cornsilk (PHRF-CS) on fasting blood glucose (FBG) levels

Groups	Blood glucose level				
	0 day	7 day	14 day	21 day	28 day
NC	5.5±0.19[a]	5.4±0.14[a]	5.53±0.35[a]	5.5±0.1[a]	5.17±0.45[a]
DC	22.38±4.13[b]	20.3±2.21[b]	27.9±3.18[b]	31.85±1.45[b]	33.33±1.08[b]
DPRF100	19.85±2.13[b]	10.23±3.36[a,b]	14.73±6.26[a,b]	11.53±3.55[a,c]	10.85±3.31[a,c]
DPRF200	17.25±2.33[b]	10.7±3.05[a,b]	15.95±5.12[a,b]	12.23±4.41[a,c]	11.05±4.53[a,c]
Dmet	18.13±3.73[b]	10.8±1.79[a,b]	13.45±5.52[a,b]	12.5±3.26[a,c]	11.08±3.79[a,c]

The values indicated by the different letters are statistically significant ($p<0.05$) by one-way ANOVA
NC: Normal-Control; DC: Diabetic-Control; DPRF100: Diabetic-PRF-CS treated (100 mg/kg); DPRF200: Diabetic-PRF-CS treated 200 mg/kg; Dmet: Diabetic-Metformin treated (150 mg/kg)

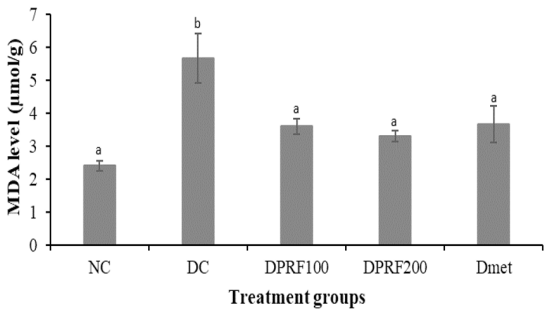
hypoglycemic and hypolipidemic properties in rats that are alloxan-induced diabetics. Therefore, phenolics, especially flavonoids in PRF-CS, might induce hypoglycemic actions. Nonetheless, the potential of phenolic compounds as a Reactive Oxygen Species (ROS) scavenger could excise the toxic effects of STZ on β-cells and lower the blood glucose level in rats treated with PRF-CS (El Hawary *et al.* 2016).

Effect of PRF-CS on oxidative stress markers

The MDA level in the DC group was significantly elevated compared to the NC group (Figure 2). Nonetheless, the DPRF100, DPRF200, and Dmet administrations exhibited significantly reduced MDA levels than the DC group.

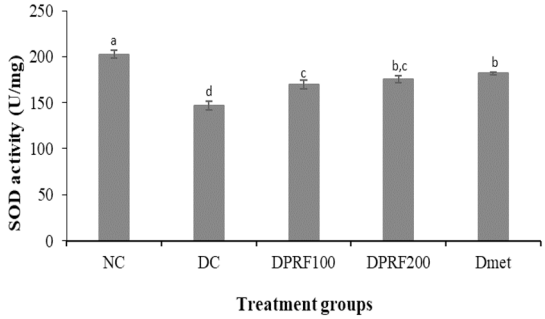
Figure 3 displays the SOD activities in the liver of negative control, treated, and untreated diabetic rats. The SOD activity of the DC rats was significantly diminished than in the NC rats. Furthermore, the rats treated with PRF-CS and metformin demonstrated significantly enhanced SOD activities when compared to the DC rats. Nevertheless, the SOD level of activities of the DPRF100, DPRF200, and Dmet groups was significantly less in contrast to the NC group.

Based on Figure 4, significantly reduced CAT activity levels in the DC, DPRF200,



The data are expressed as mean±S.E.M. (n=6). The values indicated by the different letters are statistically significant ($p<0.05$)
NC: Normal-Control; DC: Diabetic-Control; DPRF100: Diabetic-PRF-CS Treated (100 mg/kg); DPRF200: Diabetic-PRF-CS Treated 200 mg/kg; Dmet: Diabetic-Metformin Treated (150 mg/kg)

Figure 2. The malondialdehyde levels (umol/g) of each group



The data are expressed as mean±S.E.M. (n=6). The values indicated by the different letters are statistically significant ($p<0.05$)
NC: Normal-Control; DC: Diabetic-Control; DPRF100: Diabetic-PRF-CS Treated (100 mg/kg); DPRF200: Diabetic-PRF-CS Treated 200 mg/kg; Dmet: Diabetic-Metformin Treated (150 mg/kg)

Figure 3. The superoxide dismutase activity levels (U/mg) of each group

DPRF100, and Dmet groups were observed when juxtaposed with the NC group. But, there was no significant elevation of the activity levels in the DPRF100, DPRF200, and Dmet groups compared to the DC group.

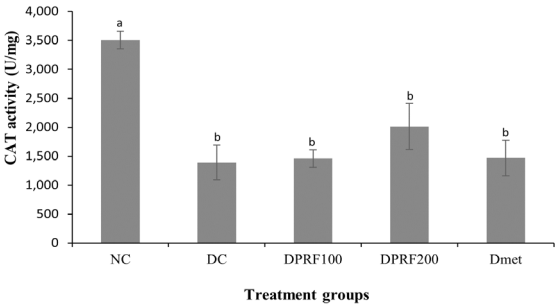
Figure 5 demonstrates the GSH-Px activities of the rats in each group. Compared to the GSH-Px activity level of the NC group, the DC and the DPRF100 groups exhibited significantly reduced activity levels. Nonetheless, the GSH-Px activities of the rats treated with DPRF200 and Dmet were significantly diminished compared to the DC group. Moreover, the diabetic group administered with 200 mg/kg of PRF-CS had a considerable elevation in GSH-Px activity in contrast to those administered with the lower dose.

The liver is the main organ responsible for the free radical reactions and oxidation and detoxification processes. In various diseases, the liver displays increased levels of oxidative stress indicators at the beginning stages (Sanchez-Valle *et al.* 2012). Since the liver was susceptible to ROS-imparted injuries, the present study investigated the effects of PRF-CS on antioxidant enzyme activities and lipid peroxidation in the liver of diabetic rats to determine if the substance could alleviate oxidative stress.

In DM, hyperglycemia induces excessive ROS production. The ROS reacts with unsaturated lipids present in cell membranes, resulting in lipid peroxidation. In lipid peroxidation, increased MDA level damages cell membranes from the inactivation of numerous cellular proteins and receptors (Birben *et al.* 2012). Lipid peroxidation is commonly measured in terms of MDA. A significant increment in MDA concentration in the STZ-induced diabetic rats reflected elevated lipid peroxidation, which led to tissue injury. The observation also suggested that the endogenous antioxidant defense mechanisms were incapable of preventing free radical overproduction. The PRF-CS treatment impeded hepatic lipid peroxidation in diabetic rats, as demonstrated by the reduction of the MDA concentration to the normal level. The findings suggested that the PRF-CS could protect tissues from lipid oxidation-induced damage.

DM is linked with escalated oxidative stress (Asmat *et al.* 2016). The increment resulted from an impaired body antioxidant system. Jayaraman *et al.* (2018) indicated that diabetic rats had lower levels of antioxidant enzymes. Similar findings were obtained in the DC group in the present study.

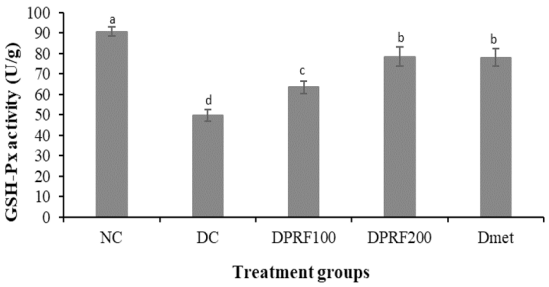
The SOD is the frontline enzyme that converted superoxide radicals into less reactive substances, such as hydrogen peroxide (H_2O_2). Enzymes called CAT and GSH-Px, which are part of the antioxidant system's second line of defense, are responsible for the transformation of H_2O_2 into molecules of oxygen and water (Alatawi *et*



The data are expressed as mean±S.E.M. (n=6). The values indicated by the different letters are statistically significant ($p<0.05$)

NC: Normal-Control; DC: Diabetic-Control; DPRF100: Diabetic-PRF-CS Treated (100 mg/kg); DPRF200: Diabetic-PRF-CS Treated 200 mg/kg; Dmet: Diabetic-Metformin Treated (150 mg/kg)

Figure 4. The catalase activity levels (U/mg) of each group



The data are expressed as mean±S.E.M. (n=6). The values indicated by the different letters are statistically significant ($p<0.05$)

NC: Normal-Control; DC: Diabetic-Control; DPRF100: Diabetic-PRF-CS Treated (100 mg/kg); DPRF200: Diabetic-PRF-CS Treated 200 mg/kg; Dmet: Diabetic-Metformin Treated (150 mg/kg)

Figure 5. The Glutathione Peroxidase activity levels (U/g) of each group

al. 2018). Therefore, a decrease in SOD activity may result in an accumulation of free radicals, which may in turn induce oxidative stress, tissue damage, and metabolic abnormalities. GSH-Px is the primary enzyme that protects cells from the damage that ROS can cause when oxidative stress is low (Tiwari *et al.* 2013). The PRF-CS-treated diabetic groups in the current investigation demonstrated marked increased hepatic SOD and GSH-Px actions when juxtaposed to the DC groups. Parallel effects were observed in metformin-treated diabetic rats.

The improved antioxidant enzyme activities demonstrated by the PRF-CS indicated that this fraction might prevent enzymes from glycating or decrease reactive oxygen free radicals and enhance the antioxidant enzyme's activities. The observation demonstrated the free radicals scavenging activity abilities of the PRF-CS, exerting an advantageous action through protection against pathogenic changes generated by superoxide radicals and H2O2 radicals (Andrestian *et al.* 2019). The PRF-CS activities observed might be due to the phenolic compounds. Therefore, the PRF-CS was a significantly successful treatment in STZ-induced diabetic rats, potentially by enhancing the endogenous antioxidative actions.

The effects of PRF-CS on histological damage of the rat livers

The H & E stain was employed to analyze the pathological observations of the liver, which were assessed through a light microscope. The histopathological liver alterations in the experimental groups were scored against the control, as shown in Table 2.

Resultantly, the liver sections from the NC group exhibited normal hepatocytes with well-spaced sinusoids and the central vein (Figure 6A). The liver morphology of the rats in the DC group demonstrated moderate histopathological changes, denoted by the degeneration of hepatocytes. The degenerative modifications included the formation of fatty vacuoles, revealing fatty alterations (Figure 6B).

Diabetic rats from the DPRF100 group exhibited no significant variations, and no improvements were detected in contrast to the DC group (Figure 6C). Nonetheless, the rates from DPRF200 and Dmet groups exhibited mild histopathological improvements (Figure 6D and Figure 6E, respectively). The hepatocytes and blood sinusoidal space in both groups' livers gradually returned to near-normal morphology.

The initiation of oxidative stress caused by the exhaustion of the antioxidant scavenger system was among the diabetogenic characteristic of STZ, mediated via the destruction of pancreatic β-cell. Enhanced oxidative stress indicated an elevated generation of free radicals. The phenomenon could cause tissue damage and histopathological alterations in the organs. Since the liver plays a prominent and crucial role in controlling carbohydrate metabolism, several structural and functional abnormalities that influence glycogen and lipid metabolism can occur in the liver due to diabetes. Degeneration of hepatocytes, dilated sinusoids, and steatosis were the histological findings that were identified in the STZ-induced diabetes study (Al-Ani *et al.* 2017). Similar findings were obtained in the DC group in this investigation.

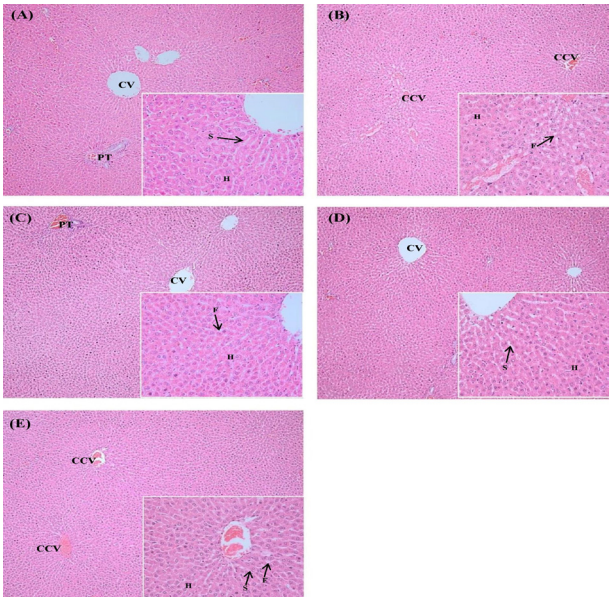
This study demonstrated that both the PRF-CS (200 mg/kg) and metformin treatments improved the morphological changes in the liver, where a marked restoration of hepatocytes and blood sinusoids was observed. The results

Table 2. The grading of the morphological changes in the liver of the rats

Histopathological changes	Groups				
	NC	DC	DPRF100	DPRF200	Dmet
Sinusoidal space	-	++	++	+	+
The density of fatty vacuoles formed	-	++	++	+	+
The presence of degeneration changes in hepatocytes	-	++	++	+	+

(–): Represent none; (+): Mild; (++): Moderate; (+++): Severe damage

NC: Normal-Control; DC: Diabetic-Control; DPRF100: Diabetic-PRF-CS Treated (100 mg/kg); DPRF200: Diabetic-PRF-CS Treated 200 mg/kg; Dmet: Diabetic-Metformin Treated (150 mg/kg)



(A): Normal-Control (NC); (B): Diabetic-Control (DC); (C): Diabetic-PRF-CS Treated (100 mg/kg) (DPRF100); (D): Diabetic-PRF-CS treated 200 mg/kg (DPRF200); (E): Diabetic-Metformin Treated (150 mg/kg) (Dmet)
All figures are in 10× magnification with 40× inserts; CV: Represents the central vein; CCV: Congestion of the Central Vein; PT: The Portal Triad; S: The Sinusoid; H: The Hepatocyte; F: The Fatty Vacuole

Figure 6. The H & E-stained liver images

indicated that the treatments had protective effects against the hepatic alterations correlated with diabetes. The hepatoprotective effects of the PRF-CS were associated with its antioxidant effects as it was rich in phenolic compounds, particularly flavonoids. Flavonoids, an example of phytoconstituents, are known for their hepatoprotective actions (Kumar & Pandey 2013).

CONCLUSION

Anti-hyperglycemic and anti-hyperlipidemic activities were demonstrated by PRF extracted from CS. Additionally, the PRF-CS exhibited possible antioxidant properties against free radical damage. The favorable effects of PRF-CS in diminishing lipid peroxidation and oxidative stress were observed from the reduced MDA quantities and improved antioxidant enzyme activities, such as SOD, CAT, and GSH-Px. Furthermore, biochemical, and histopathological analyses suggested that PRF-CS impeded liver damage due to antioxidant activity. The current investigation proposed that DM-induced oxidative stress might be impeded or terminated by consuming the PRF-CS.

ACKNOWLEDGEMENT

We would like to express our gratitude to Universiti Sains Malaysia (USM), the Ministry of Higher Education, Malaysia (FRGS/1/2015/SKK06/USM/02/1), Nutrition Laboratory, School of Health Sciences (USM), the Central Research Laboratory (School of Medical Sciences, USM) and Animal Research and Service Centre (USM Health Campus), for granting us permission to use all required equipment and the necessary materials during the study period.

DECLARATION OF INTERESTS

The authors have no conflict of interest.

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Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2023.18.1.51-60*
ISSN 1978-1059 EISSN 2407-0920

Plant-Based Diet during the Prepubertal Period and Age at Menarche: A Systematic Review

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ABSTRACT

This systematic review aimed to analyze the effect of a prepubertal plant-based diet, through dietary patterns and its nutritional components, on the age at menarche. Systematic searches through Medline (EBSCO), Embase, Health & Medical Collection (Proquest), Scopus, and Google Scholar for articles published between January 2000 and September 2022 in English using the keywords of plant-based diet, age at menarche, and their synonyms identified 673 articles. Articles were screened through titles and abstracts, as well as full-text reviews based on inclusion/exclusion criteria and assessment of research quality using the JBI Critical Appraisal Tools with a JBI cut-off final score of >50%, leaving 12 articles in this systematic review presented in the PRISMA 2020 flowchart. Data analysis was conducted descriptively by comparing findings between articles. Age at menarche/puberty was reported as an outcome of food intake through a vegetarian diet, Mediterranean diet, vegetable protein, phytoestrogens and isoflavones, and dietary fiber. Based on the analysis of each subgroup, the plant-based diet did not consistently influence the incidence of menarche. In conclusion, a plant-based diet does not significantly affect age at menarche when it is accompanied by a balanced intake of energy and macro-micronutrients. The principles of balanced nutrition and the assistance of nutritionists are needed in practicing a plant-based lifestyle, especially for children and adolescents who are still in the developmental stage.

Keywords: food fiber, menarche, phytoestrogens and isoflavones, plant-based diet, vegetable protein

INTRODUCTION

Menarche or first menstruation is a marker of puberty that is easy to remember. A menarche is an important event for a woman because it symbolizes physical and sexual maturity related to the ability to reproduce (Lacroix *et al.* 2022). In many countries, the average age at menarche is 11–13 years, for example, 11.71 years in Brazil (Barros *et al.* 2019), 12.3 years in Iran (Hozoori *et al.* 2017), 12.7 years in China (Duan *et al.* 2020), and 12.96 years in Indonesia (Sudikno & Sandjaja 2019).

Early menarche (menarche at the age of ≤10 years) or late menarche (menarche at the age of >15 years) is known to have health impact both physically and psychologically (Lacroix *et al.* 2022). Women who experience early menarche are at risk of developing impaired adult asthma (Minelli *et al.* 2018), obesity, diabetes, breast cancer, cardiovascular disease (Yoo 2016), hypercholesterolemia (Petersohn *et al.* 2019), and short adult stature (Kang *et al.* 2019). Psychologically, early menarche can cause mental health problems and risky sexual behavior for unwanted pregnancies, as well as cause anxiety and stress (Trépanier *et al.* 2013; Yoo 2016). Conversely, late menarche also increases the risk of cardiovascular disease, cervical cancer, musculoskeletal disorders, the possibility of experiencing early menopause (Day *et al.* 2015), miscarriage, and the risk of fertility problems (Canelón & Boland 2020). Adolescent girls with delayed menarche, compared to their peers, will experience anxiety, depression and decreased intelligence (Day *et al.* 2015).

The occurrence of menarche is influenced by several factors, including nutrition (Nasiri *et al.* 2022) as a modifiable factor. Several studies (Hozoori *et al.* 2017; Barros *et al.* 2019) have shown that there is a significant relationship between the age at menarche and nutritional

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(Received 23-12-2022; Revised 01-02-2023; Accepted 27-02-2023; Published 30-03-2023)

status, which is strongly influenced by food intake during the prepubertal period. Higher total fat intake and animal-based foods are associated with earlier age at menarche. Meanwhile, intake of vegetable protein, higher fiber, and dietary isoflavones are associated with delayed puberty (Vilamor & Jansen 2016; Duan *et al.* 2020).

A plant-based diet is widely recommended as a dietary pattern that is beneficial for health. A plant-based diet means choosing food intake that mostly comes from plants, but can also include consuming small amounts of animal-based foods such as meat, fish, milk, and eggs (Figueroa *et al.* 2021). People who tend to choose plant-based diets are potentially lower in energy, protein, fat, and some micronutrients than omnivores (Nugroho *et al.* 2015; Calcaterra *et al.* 2021). This condition can affect the time of menarche and have an impact on subsequent health. Sunuwar *et al.* (2010) in Nepal found that vegetarian diet could delay the onset of menarche. The average age at menarche in the vegetarian group (12.82±0.81 years) was older than non-vegetarians (12.68±0.95 years) (Sunuwar *et al.* 2010). Meanwhile, a literature review by Šetinc (2022) on the effects of vegetarian's macro and micronutrients intake on health concluded that there was no difference in the age at menarche between vegetarian and omnivorous women (Šetinc 2022). This systematic literature review aimed to obtain further learning related to the age at menarche and plant-based diet through diet patterns and its nutritional components such as vegetable protein, phytoestrogens/isoflavones, and dietary fiber.

METHODS

Design, location, and time

This study was a systematic review from systematic article search results on Medline (EBSCO), Embase, Health & Medical Collection (ProQuest), Scopus, and Google Scholar. The inclusion criteria were articles regarding plant-based diets or vegetarianism and age at menarche/puberty from academic journals published in English between January 2000 and September 2022. Meanwhile, the exclusion criteria were publications that had no relevance to the research questions, such as articles on dysmenorrhea, breast cancer, menopause, etc. Article searches were limited to primary research articles and did not include review articles. Paid articles were also not included in this systematic review.

Data collection

The question of this research was how prepubertal plant-based diet influences the age at menarche. It was rendered into the concept of data search with the PICO framework consisting of P/Population, which was children or adolescents; I/Intervention/Exposure, which was the intake of plant-based diets; C/Comparison, which was animal-sourced/omnivorous food; and O/Outcome, which was the age at menarche/puberty. Articles identification was using the Boolean Operator OR/AND with the main keywords of plant-based diet or vegetarian and the age at menarche with their synonyms, which could be found with the help of the controlled vocabulary feature on EBSCO, Embase, and ProQuest.

Data analysis

Research data were presented in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flowchart (PRISMA 2020). Article search results were imported into the reference manager application and duplication checks were carried out. Next, the articles with title and abstract that did not meet the PICO criteria through the team's assessment were excluded from the study. By reading the full texts, articles were filtered according to the inclusion and exclusion criteria. The remaining articles were assessed for the quality of their studies using the Joanna Briggs Institute (JBI) Critical Appraisal Tools (JBI 2020) according to the research methodology used in the article with a JBI cut-off final score of >50%. Then the selected articles were extracted into the table to facilitate data synthesis. The analysis was done descriptively and result synthesis was performed by comparing the findings between the articles. To facilitate analysis, articles containing similar intervention/exposure to plant-based diet components were grouped. The effects of a plant-based diet on menarcheal age were classified as accelerating/delaying menarcheal age or no relationship between a plant-based diet and menarcheal age.

RESULTS AND DISCUSSION

The process of collecting data that met the inclusion criteria in 5 electronic databases

resulted in 673 articles. After deleting duplicate articles, 615 titles and abstract were screened according to the PICO criteria, leaving 27 articles to be assessed for eligibility. Based on the full texts that had been reviewed, 15 articles were excluded because the articles did not include the age at menarche/puberty as an outcome (n=6), the articles were not primary research articles (n=6), and the full text of the articles could not be accessed (n=3). Of the remaining 12 articles, it was found that there were no articles that had a final JBI score of less than 50%, so the 12 articles were included in this systematic review (Figure 1).

This study included all types of primary research articles published in academic journals. Of the 12 articles that had been studied, there were 9 cohort studies, including nested case-controls studies, and 3 cross-sectional studies published between 2002–2022. Most of the studies were conducted in the United Kingdom (4 studies), Germany (2 studies), and the United States of America (2 studies), with the rest conducted in Canada, China, India, and Israel (1 study each). Based on the interventions/exposures presented in the article, the researchers highlighted the age at menarche as an outcome of plant-based

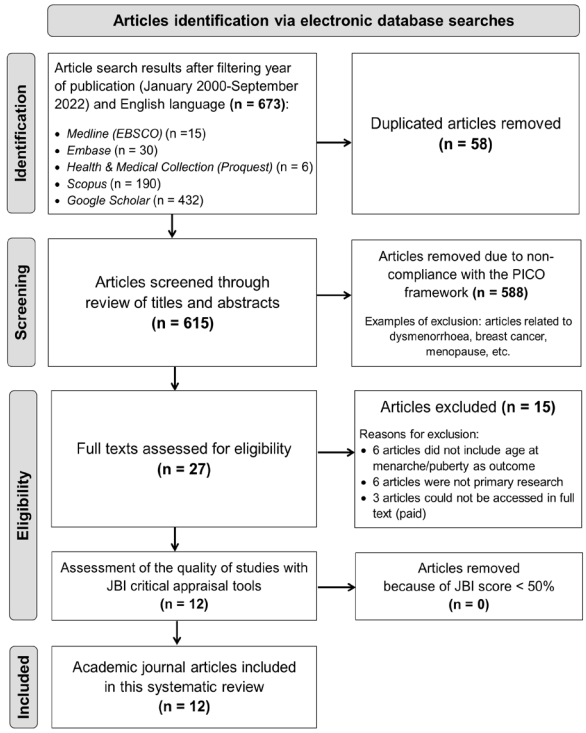

Figure 1. Flowchart of preferred reporting items for systematic reviews (PRISMA 2020)

diet through a prepubertal vegetarian diet and Mediterranean diet, intake of phytoestrogens and isoflavones, vegetable protein, and dietary fiber (Table 1).

The results of this systematic review showed variations in the data, as shown in Table 1. There were 4 out of 12 articles that provided more than one conclusion regarding the effect of a prepubertal plant-based diet on the age at menarche/puberty. Most of the articles (9 articles) stated that there was no effect of a plant-based diet on the age at menarche. However, 4 articles reported delayed puberty related to a plant-based diet and 3 articles stated otherwise. Variations in the results of these studies may be due to differences in research methods, sample size, and the presence of confounding factors that influenced the onset of menarche, such as nutritional status, energy intake, intake of other macronutrients and micronutrients, as well as other factors other than nutrition. In the next paragraphs, the findings will be discussed in groups of several articles that have similar interventions/exposures.

Vegetarian diet and age at menarche

Vegetarian diet is a type of plant-based diet that limits animal-based product intake. Vegetarian diet patterns may vary, but the most commonly used definitions include vegan diet, which is a pure, plant-based diet that completely avoids all types of animal products, and vegetarian diet, which still includes the consumption of eggs (ovo-vegetarian diet) or dairy products (lacto-vegetarian diet) or eggs and milk (lacto-ovo vegetarian diet) (Calcaterra *et al.* 2021).

This systematic search found two articles that studied the effect of a vegetarian diet on the age at menarche. Choudhary *et al.* (2015) conducted a cross-sectional study in India on 100 female students (45 vegetarians, 25 ovo-vegetarians, and 30 non-vegetarians) aged 19–25 years to discover the role of different dietary habits on age at menarche. This study found that there was no significant relationship between vegetarian and ovo-vegetarian dietary habits and the age at menarche, while the non-vegetarian group tended to have early menarche. However, this study had limitations because the subjects were only in a small group. Studies in larger populations are needed to confirm these findings (Choudhary *et al.* 2015).

Table 1. Research data extraction

Author and year of publication	Country	Methods	Sample size[a]	Intervention/ Exposure	Outcome	Results		
						Accelerate the age of menarche/ puberty	Delaying the age of menarche/ puberty	No relationship between exposure and outcome
(Choudhary *et al.* 2015)	India	Cross-sectional study	n=100 (female students aged 19–25 years)	Eating habits (vegetarian, eggetarian, and non-vegetarian)	Age of menarche	p≤0.05 in non-vegetarian diet (compared with vegetarian and eggetarian)	-	p>0.05 in vegetarian and ovo-vegetarian diet
(Rosell *et al.* 2005)	UK	Cross-sectional study	n=35,720 (women aged ≥20 years of the general population, including 12,705 vegetarians)	Vegetarian diet (time when starting a vegetarian diet)	Age of menarche	p≤0.01 in women who became vegetarian at age 10–14 years (compared with age ≥20 years)	-	p>0.01 (no difference between life-long vegetarian women and women who became vegetarian at age ≥20 years)
(Cheng *et al.* 2021)	UK	Longitudinal cohort study (followed from birth to age 17 years)	n=3,919 (girls from general population)	Food intake (type of vegetable/ animal protein)	Onset of female puberty	-	-	p>0.05 (no variation according to the type of protein intake)
(Günther *et al.* 2010)	Germanny	Longitudinal cohort study (followed from birth to age 13 years)	n=112 (children from general population, including 57 girls)	Vegetable protein intake	Onset of puberty	-	p<0.05 in high intake of vegetable protein at the age of 3–4 years (p=0.02) and 5–6 years (p=0.04)	-
(Rogers *et al.* 2010)	UK	Cohort study (followed from birth to age 12 years and 8 months)	n=3,298 (girls from general population)	Food intake	Age of menarche	p≤0.01 in high vegetable consumption at age 3 years	-	p>0.01 in vegetable protein intake
(Sinai *et al.* 2019)	Israel	Nested case-control study (followed from birth to age 3 years for exposure and monitored again at age 8–12 years for outcome)	n case=29 (children with soy-based formula), n control=60 (children from general population)	Soy intake (soy-based formula)	Onset of puberty	-	-	p=0.95 (no difference in the age of puberty in the case group and the control group)

Continue from Table 1

Author and year of publication	Country	Methods	Sample size[a]	Intervention/ Exposure	Outcome	Results		
						Accelerate the age of menarche/ puberty	Delaying the age of menarche/ puberty	No relationship between exposure and outcome
(Segovia-Siapco *et al.* 2014)	USA	Cross-sectional study	n=327 (female students aged 12–18 years)	Soy intake	Age of menarche	-	-	p=0.77 (no difference between the lowest and the highest quartile level of total soybean consumption)
(Adgent *et al.* 2012)	UK	Longitudinal cohort study (followed from birth to age 14.5 years)	n=2,920 (girls from general population)	Soy based baby feeding	Age of menarche	p<0.05 (compared to breast milk or non-soy formula)	-	-
(Xiong *et al.* 2022)	China	Prospective cohort study (followed up for 5 years)	n=2,152 (female students aged 6–8 years)	Soy and fiber intake	Onset of female puberty	-	p=0.03 (high intake of soy)	p=0.06 (total fiber intake)
(Cheng *et al.* 2010)	Germany	Subcohort (followed from birth to age 14 years)	n=119 (girls from general population)	Intake of isoflavones and dietary fiber	Onset of female puberty	-	p=0.04 (isoflavone intake on breast development)	p=0.2 (isoflavone intake on age of menarche) p=0.6 (fiber intake on puberty)
(Koo *et al.* 2002)	Canada	Prospective cohort study (followed up for 3 years)	n=589 (girls from general population aged 6–14 years)	Dietary fiber (and cellulose) intake	Age of menarche	-	p=0.0269 (the highest fiber intake group compared to the lowest fiber intake group)	-
(Szamreta *et al.* 2020)	USA	Longitudinal cohort study (followed up for 2.5 years)	n=202 (girls from general population aged 9–10 years old)	Mediterranean diet (high in fiber-rich plant foods and fish, but low in red meat)	Age of menarche	-	p<0.01 (high adherence compared to low adherence to a Mediterranean-like diet)	-

[a]n: The number of samples that produce research outcomes (age of menarche/female puberty)

Another study was conducted by Rosell *et al.* (2005) in the United Kingdom that compared the age at menarche in a group of vegetarians who started a vegetarian diet at different ages and a group of non-vegetarians. The study, using data from the EPIC-Oxford cohort involving 35,720 British women aged over 20 years, proved that there was no significant difference between the ages at menarche in the vegetarian group for life (since before the age of 1 year) and in the group of vegetarian after age 20 with assuming this group had experienced menarche before becoming

vegetarian. However, the age at menarche was found to be 0.2 years lower in women who became vegetarians around the onset of puberty (10–14 years) than in women who became vegetarian after age 20 ($p<0.01$), but the authors did not explain the cause of this difference (Rosell *et al.* 2005).

Both studies concluded that there was no relationship between vegetarian dietary habits and menarcheal age. The vegetarian diet is determined by the components and variety of food consumed. If there is a balance of energy and macro- and micronutrients, a vegetarian diet may not affect the age at menarche (Calcaterra *et al.* 2021).

Mediterranean diet and age at menarche

Similar to the vegetarian diet, the Mediterranean diet is high in plant-based foods such as fiber-rich whole grains, vegetables, fruit, nuts, and fish, but limits red meat and processed foods. Szamreta *et al.* (2020) studied the relationship between Mediterranean diet and age at menarche in the United States of America through the Jersey Girl Study, a longitudinal cohort study of 202 girls aged 9–10 years. Dietary intake was assessed using the 24-hour Food Recall method for at least 3 different days and grouped them into high, medium, and low Mediterranean Diet Scores. Data on the age at menarche were asked annually and monitored for an average of 2.5 years. Higher adherence to the Mediterranean diet was associated with older age at menarche after controlling for body mass index, body composition measures, and fat distribution. This association might be influenced by higher consumption of vegetables and non-fat or low-fat dairy products. Szamreta *et al.* also highlighted the role of higher sex hormone-bound globulin and lower levels of endogenous estrogen in Mediterranean diet that might result in delayed onset of puberty (Szamreta *et al.* 2020).

A literature review on the role of prepubertal nutrition on the timing of puberty provided reasons for possible causes of menarche delay in followers of a plant-based diet. There is a risk of malnutrition if micronutrient needs are not considered. It can cause developmental disorders in children, including disorders of puberty (Calcaterra *et al.* 2021)

Vegetable protein intake and age at menarche

A longitudinal cohort study in Germany (Günther *et al.* 2010) reported that a higher intake of vegetable protein in childhood was associated with delayed puberty. Günther *et al.* (2010) studied 112 children using data from the Dortmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD Study) which assessed food intake with a 3-day weighted food diary at the age of 12 months, 18–24 months, 3–4 years, and 5–6 years. Puberty-related data were collected from 5 to 13 years of age in girls (n=57). Higher vegetable protein intake at the age of 3–4 years was associated with delayed puberty which was described as a pubertal growth spurt ($p=0.02$). Although the data were not presented in the article, age at menarche was also investigated as another marker of puberty and showed similar results (Günther *et al.* 2010).

A different opinion was conveyed in two studies in United Kingdom (Rogers *et al.* 2010; Cheng *et al.* 2021) that used population-based cohort data from The Avon Longitudinal Study of Parents and Children (ALSPAC). Rogers *et al.* collected FFQ data when the children were 3 and 7 years old, followed by a 3-day food diary when the children were 10–11 years old. Data related to the age at menarche (n=3,298) were collected once when the children were on average 12 years and 8 months old as a limit to whether the child had menarche or not (Rogers *et al.* 2010). Meanwhile, Cheng *et al.* used the FFQ on children aged 3, 4, 7 years, and a 3-day food diary when the children were 7.5 years old. Data on the pubertal development of children were collected using repeated questionnaires every year throughout the children's 8–17 years of age. Menarche in girls (n=3,919) was reported as exact date and/or age in years at which menarche has occured (Cheng *et al.* 2021). The results of both studies showed that there was no significant relationship between vegetable protein intake and menarcheal age (Rogers *et al.* 2010; Cheng *et al.* 2021). The type of protein intake, whether animal or vegetable protein intake, does not cause pubertal age variations if total energy intake and intake of other macronutrients are also considered (Cheng *et al*. 2021).

Phytoestrogen/isoflavone intake and age at menarche

Vegetarian groups often include soy foods as a source of high-quality protein in their daily diet to replace meat. Soybeans contain high concentrations of isoflavones, one of the

main groups of phytoestrogens. Phytoestrogens are plant compounds that are structurally and functionally similar to endogenous estrogens so that they can also influence the sexual maturity of children (Jefferson *et al.* 2012; Segovia-Siapco *et al.* 2014; Xiong *et al.* 2022).

Adgent *et al.* (2012) studied the effect of soy-based infant feeding on the age at menarche. Using ALSPAC data, 2,920 girls were studied longitudinally. About 2% of mothers reported that they introduced soy products into their baby's diet as early as at or before 4 months of age. Compared with girls who were given breast milk or non-soy-based milk, girls who were given soy milk early were 25% more likely to experience menarche during the follow-up period (HR 1.25; 95% CI; 0.92–1.71) (Adgent *et al.* 2012).

However, different results were found by Sinai *et al.* (2019) in Israel who also examined the consumption of soy-based formulas. A nested case-control study of a prospectively followed cohort studied 29 children fed a soy-based formula and 60 children randomly selected as controls. Eating habits were followed from birth until the children were 3 years old, then when the children were 7.8 and 10.5 years old. Nutritional intake was re-evaluated using a food diary for 3 days and a physical examination related to signs of puberty was conducted. The results of the study found that there was no relationship between the consumption of soy-based formulas and the onset of puberty (Sinai *et al.* 2019).

Segovia-Siapco *et al.* (2014), through a cross-sectional study of 339 girls aged 12–18 years in the United States of America, also found no relationship between soybean consumption and the age at menarche. Dietary intake was assessed using a web-based FFQ that grouped 36 types of soy foods from a total of 151 foods. The data analysis showed that there was no significant difference in median age at menarche between those in the lowest quartile and the highest quartile of total soy consumption levels (12.67 years and 12.58 years, respectively) after adjusting for energy levels (Segovia-Siapco *et al.* 2014).

Contrary to previous findings, cohort studies in China (Xiong *et al.* 2022) and Germany (Cheng *et al.* 2010) found that girls with high intakes of isoflavones in prepubertal age actually entered puberty at an older age. Research by Xiong *et al.* (2022) with 2,152 subjects resulted in data on an increase in the age of menarche in girls who consumed 57.2 g of soybeans per day compared to in girls who only consumed 1.6 g of soybeans per day (13.1 years vs. 12.5 years; p=0.03). Research by Cheng *et al.* (2010) on 119 girls also found that girls on a high total isoflavone diet experienced about 8 months slower breast development compared to girls on a low total isoflavone diet (p=0.04). However, data on the age at menarche did not show an association with total dietary isoflavones (p=0.2) although there was actually an increase in age at menarche to 13 years in the group with a high total isoflavone diet, while in the group with a low total isoflavone diet, age at menarche was 12.6 years (Cheng *et al.* 2010). The inconsistency of the findings of the two menarcheal age studies was probably due to differences in samples size and differences in isoflavone intake level, which was higher in the study in China. A higher concentration of isoflavones may be needed to influence the incidence of menarche (Cheng *et al.* 2010; Xiong *et al.* 2022). Meanwhile, the explanation given regarding the delay in puberty is not fully known. It was suspected that the isoflavone can bind directly to the estrogen receptor and cause an antiestrogenic effect that can inhibit the enzyme for the formation of the hormone estrogen (Cheng *et al.* 2010; Xiong *et al.* 2022).

A literature review on the relationship between phytoestrogen intake and the timing of puberty stated that the timing of exposure to phytoestrogens was related to different body developments. Exposure to phytoestrogens since early prepuberty when endogenous estradiol levels were low might influence the incidence of early puberty, whereas exposure to phytoestrogens in later periods might interfere with endogenous estrogen-mediated puberty because of weak estrogen receptor agonist activity (Jefferson *et al.* 2012).

In general, intake of phytoestrogens/ isoflavones did not show a relationship with menarcheal age. Its influence on the age at menarche depends on how much the concentration level and timing of exposure in the prepubertal period. Intake of high concentrations of phytoestrogens or isoflavones at the end of the prepubertal period can delay menarche.

Dietary fiber intake and age at menarche

Dietary fiber is naturally provided by various foods such as grain products, vegetables,

and fruit, which are the main foods for vegetarians. A prospective cohort study conducted in China (Xiong *et al.* 2022) on 2,152 girls aged 6–8 years found that fiber intake was not significantly associated with the timing of puberty. Similar findings were reported in a subcohort study of 119 girls in Germany (Cheng *et al.* 2010). However, a previous study in Canada which examined 637 girls aged 6–14 years prospectively found that intake of dietary fiber and cellulose in the prepubertal period was associated with later age at menarche (Koo *et al.* 2002). Food intake was assessed using a semi-annual FFQ questionnaire for 3 years and the age at menarche was also asked during that period. The results showed that the risk of experiencing menarche in the group with the highest fiber intake (>25.5 g per day) was only about half (HR=0.54; 95% CI:0.31–0.94) compared to the group with the lowest fiber intake (<18.2 g per day). An increase in dietary fiber intake was thought to reduce the availability of circulating estrogen in the body caused by increased estrogen removal through feces along with fiber. It would affect the development of puberty, including menarche (Koo *et al.* 2002).

An interesting point was reported by Rogers *et al.* (2010) in a cohort study in UK. They found weak evidence that higher consumption of vegetables at 3 years of age was positively related to the incidence of menarche. Girls who consumed 14 or more servings of vegetables per week at 3 years of age were 37% more likely (OR=1.37; 95% CI:0.96–1.94) to have experienced menarche before 12.8 years of age than girls who consume less than 3 servings of vegetables per week. Explanations for these findings were not clearly stated, but similar things were not found in association with food intake at an older age, so it could be assumed that dietary intake during early to mid-childhood might be more strongly associated with the incidence of menarche than dietary intake during late childhood before menarche (Rogers *et al.* 2010).

Dietary fiber intake also does not show a definite relationship with menarcheal age. Fiber intake in the early prepubertal period will accelerate menarche, but high dietary fiber intake can delay the onset of menarche.

This systematic review was limited to searching for articles in English, so it may not include similar research from other countries. In this study, there was a possibility of bias in concluding due to the many confounding factors in the relationship between a plant-based diet and menarcheal age. However, this paper has the strength of reviewing the effects of a plant-based diet through its various nutritional components. Currently, the plant-based diet is increasingly in demand, so further research is needed to provide evidence-based on the pattern of relationship between plant-based diets during the prepubertal period and the age at menarche.

CONCLUSION

The results of this systematic review of 12 articles collected from 5 electronic databases indicated variations in the data. The components and types of food consumed, how much the concentration level, and the timing of exposure to plant-based diets in the prepubertal period also affect the time of menarche/puberty. In general, it can be concluded that a plant-based diet did not show a significant relationship with the age at menarche when accompanied by a balanced intake of energy and macro- or micronutrients. The principle of balanced nutrition by consuming a variety of foods must still be considered and the assistance of a nutritionist is needed in practicing a plant-based lifestyle, especially for children and adolescents who are still in the developmental stage.

DECLARATION OF INTERESTS

The authors have no conflict of interest.

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Available online: http://journal.ipb.ac.id/index.php/jgizipangan ***J. Gizi Pangan, March 2023, 18(1):61-70***
Accredited based on DGHE, Republic of Indonesia No. 28/E/KPT/2019 *DOI: https://doi.org/10.25182/jgp.2023.18.1.61-70*
ISSN 1978-1059 EISSN 2407-0920

Awareness, Knowledge, and Practices towards Reading Snack Food Labels among Malaysian Adolescents

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ABSTRACT

This study aimed to determine the awareness, knowledge, and practices regarding food labels and factors that influence their use; a cross-sectional study was conducted among 200 Malaysian adolescents aged 10 to 19. Participants completed an online Food Label Use Questionnaire (FLUQ) that included demographic, nutrition knowledge, label use, and factors affecting label use. SPSS version 28.0 was used for data analysis. The results showed that 93% of adolescents correctly identified the consequences of over-consuming calories. Still, only 84.5% performed well on the nutrition use task, struggling to interpret calorie and carbohydrate amounts in certain foods. The main reasons for not using food labels were time constraints (45.5%), unattractive or confusing labels (36%), absence of labels on certain foods (33%), lack of health concerns (32%), and insufficient knowledge (27%). However, 90.5% of adolescents were concerned about product expiration dates and often read this information. Taste and price were the main reasons for referring to food labels, while time constraints were the main barrier to their use. In conclusion, practicing reading food labels can guide adolescents in making informed decisions about their food choices and portion intake. Awareness campaigns can aid in promoting healthy habits and empowering adolescents to make the right choices for their health.

Keywords: adolescents, food labels, nutrition facts, nutrition information panel

INTRODUCTION

The label means any tag, brand, mark, pictorial or other descriptive matter, written, printed, stencilled, marked, embossed or impressed on, or attached to a container of food (Codex 1985). Food labelling aims to promote healthier food choices by informing customers about nutrient content and making the food selection environment more favorable (Kattelmann *et al.* 2014). Food merchants and policymakers have worked to improve food labelling through design changes and food label laws to assist consumers in making easier and better decisions about various food products (Shen *et al.* 2018). In Malaysia, the mandatory Nutrition Information Panel (NIP) on packaged foods and beverages displays information on energy, protein, carbohydrate, and fat content to ensure consumers are well-informed and more health-conscious (Jefrydin *et al.* 2019).

Urbanisation, tobacco use, harmful alcohol consumption, unhealthy diet and physical inactivity are significant contributors to Non-Communicable Disease (NCD) risk due to the increased availability of unhealthy foods (Talagala & Arambepola 2016; Uwimana-Nicol *et al.* 2021). According to Jefrydin *et al.* (2019), adolescents are at a high risk of obesity due to their frequent consumption of processed foods like bread, biscuits, soft drinks, ice cream, canned sausage, and cheese. The energy intake of adolescents has increased in recent years due to increased consumption of animal products,

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(Received 15-11-2022; Revised 31-01-2023; Accepted 06-03-2023; Published 30-03-2023)

soft drinks, fast foods, and salty snacks (Rezali *et al.* 2012). Kansal *et al.* (2023) found that Indian adolescents face difficulties in comprehending information on food labels, including nutrition information, and are unaware of the adverse effects of consuming unhealthy packaged foods. Most respondents expressed the need for food labels to be available in their local language for better understanding.

The study's objective was to investigate the awareness, understanding, and utilisation of food labels among adolescents and the factors that affect their usage when making food purchases. The study hypothesised that adolescents possess knowledge, understanding, and practice of reading food labels. The factors that impact adolescents' use of food labels during food purchases are linked to their understanding of the information presented on the food label.

The present study focuses on Malaysian adolescents, a group that has received limited attention in previous research on food labels' knowledge, attitude, and practices, which primarily focused on university students and educated young adults (Norazmir *et al.* 2012). Adolescence, defined by the World Health Organization (WHO) as the period between childhood and adulthood (ages 10 to 19), is a crucial stage in life that requires attention to health as young people are particularly susceptible to long-term impacts (Tan *et al.* 2019). Given the critical role of consumers' awareness, knowledge, and practices about food labels in making healthy food choices, and the limited research in this area among Malaysian adolescents, The main objective of this research is to examine the awareness, knowledge, and practices of food label reading among adolescents, and to identify the factors that affect the utilisation of food labels during food purchasing among this demographic.

METHODS

Design, location, and time

A cross-sectional study was conducted among 10 to 19-year-old adolescents in all states of Malaysia to avoid bias in socio-demographic representation. Approval was obtained from UiTM Research Ethics Committee [REC/06/2021 (MR/396)], and online consent was obtained from adolescents who agreed to complete the online questionnaire.

Sampling

Convenience sampling was used in the study. Inclusion criteria: citizen aged 10 to 19 years old and; able to read in Malay or English. Exclusion criteria: non-citizen, permanent residents, can't read in Malay or English. A sample size calculation using Krejcie and Morgan (1970) formula. A precision of 0.05 at a 95% confidence interval. A total of 200 subjects participated in this study.

Data collection

The study took place from February to April 2022, and adolescents took an online survey (Google Forms) promoted on social networks (Facebook, Instagram, WhatsApp). Before analyzing the data, cleaning was done to remove duplicate submissions.

Instrumentation. A self-administered Food Label Use Questionnaire (FLUQ) (Nurliyana *et al.* 2011) was distributed to adolescents. FLUQ are well-established questionnaires; related to the scope of the study. The questionnaire consists of 26 questions and includes 4 main sections to measure the following aspects: (a) socio-demographic; (b) nutrition knowledge; (c) the use of food and nutrition labels; (d) factors affecting the use of food labelling.

Demographic data. In this section, there were questions about adolescents' demographic data, such as age (10–19 years), race (Malay, Chinese, Indian, or other), level of study (primary, secondary, or pre-university), height, and weight. Adolescents only fill up the body weight & height. Body Mass Index (BMI) was categorised as underweight (<18.5); normal (18.5–24.9); overweight (25–29.0); obese (>30), family income per month (≤RM 1,500 (USD330), RM 1,500–RM 2,500 (USD330–USD550), RM 2,500–RM 4,000 (USD550–USD880), or ≥RM 4,000 (USD880), and marital status (single, married, divorced, or widowed).

Nutrition knowledge. Consists of eight questions of nutrition label knowledge. The adolescents had to choose one answer only for the multiple-choice questions.

The use of food and nutrition labels. In this section, respondents were asked how to read the total calories of the whole packaging of bread and a packet of biscuits. They were also assessed to read the carbohydrate amount in one serving of bread and dietary fiber in one serving

of biscuit. Consists of a 4-part Likert-type answer set ranging from 'often' to 'never' to determine the frequency of using food labels among adolescents. Subsequently, several inquiries were made regarding the usefulness of the food label or nutrition label in making decisions about food purchases, with response options including "yes," "sometimes," and "no." Then, a 4-part Likert-type answer for the question of attitude regarding the most important aspects ranging from 'most important to 'not important in using food labels during food purchasing. (i.e., price, taste, nutrient content, ingredient, packaging materials, and expiry date). Finally, a 4-point Likert-type scale was used to assess the frequency of reading and reviewing specific food label items, including the list of ingredients, serving size, calories, total fat, trans fat, cholesterol, sodium, carbohydrates, protein, dietary fiber, sugars, vitamins, and minerals. Response options ranged from "often" to "sometimes," "rarely," and "never."

Factors affecting the use of food labelling. The adolescents can choose more than one answer on the factors they refer to, not the food label.

Data analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 17.0. (SPSS Inc, USA).

RESULTS AND DISCUSSION

Demographic data

A total of 200 adolescents were included in the cross-sectional study. Demographic data are presented in Table 1. The majority of adolescents were from secondary school (57.0%), followed by primary school (15.0%) and pre-university/ university (14.0%). A normal BMI was observed in 44.0% of the adolescents. Of the adolescents surveyed, 36.5% had a household income lower than the minimum wage in Malaysia, which is Ringgit Malaysia (RM) 1,500.

Level of awareness

The score for each question in nutrition knowledge and understanding. From the analysis, most adolescents could answer correctly except for Question 5, which asked about knowledge regarding the nutrient that provides the most energy. More than half (77%) answered wrongly, with (21%) correct and (2%) not sure. On the other hand, 93% of adolescents correctly answered the question about the consequence of the over-consumption of calories.

Table 1. Demographic of the study population (n=200)

Characteristic	Male (n)	Female (n)	%
Ethnicity			
Malay	70	183	91.5
Chinese	0	2	1
Indian	4	12	6
Others	1	3	1.5
Level of study			
Primary	12	30	15
Secondary	41	114	57
Pre-university	11	28	14
University	11	20	14
BMI (kg/m^2)			
Underweight (<18.5)	22	57	28.5
Normal (18.5–24.9)	33	88	44
Overweight (25–29.9)	15	30	15
Obese (>30)	5	25	12.5
Family income/month			
≤RM 1,500	24	73	36.5
RM 1,500–RM 2,500	16	41	20.5
RM 2,500–RM 4,000	9	36	18
≥RM 4,000	26	50	25
Marital status			
Single	74	197	98.5
Married	1	3	1.5

BMI: Body Mass Index
RM: Ringgit Malaysia

Level of knowledge

Based on the responses to the questions above, the level of nutrition knowledge among adolescents was categorized into high, medium, and low. The scoring range for each level was determined as follows: High (6–8), Medium (4–5), and Low (0–3). The finding that the majority of adolescents had only medium knowledge about nutrition suggests that there is a need for increased nutrition education for this age group. Adolescents are at a stage in their lives where they are more likely to make independent

food choices, and therefore, they need to have a good understanding of nutrition to make healthy choices. Nutrition education programs can help adolescents learn about the nutrients required for their growth and development, the importance of a balanced diet, and how to make healthy food choices.

Table 2 shows the percentage of the nutrition label task score. Surprisingly, this study reported that the majority of the respondents were performing poorly (84.5%) in the nutrition use task as they were unable to interpret the total calories in the whole packaging of bread (98%) and biscuits (78%). Moreover, most of them misinterpret carbohydrates amount in one serving of bread (84%) and the gram of dietary fibre in one biscuit (71%).

According to a study by Jung *et al.* (2019), food label education is essential to promote healthy eating habits and prevent chronic diseases. The study found that consumers knowledgeable about food labels were more likely to make healthier food choices. However, many people find food labels confusing and difficult to understand. To address this issue, a systematic review by Jovicic *et al.* (2021) suggests that simplifying food labels or developing apps to help interpret the information could be effective solutions. The review found that simplified food labels, such as the traffic light system, were easier for consumers to understand and make informed choices. Additionally, apps that use technology, such as augmented reality, can help consumers easily access and interpret the information on food labels.

Table 2. Nutrition label task score

Question number	Context of question	Answer			
		Correct		Wrong	
		n	%	n	%
1a	Total calories in the whole packaging of bread (kcal)	4	2	196	98
1b	Carbohydrate amount in 1 serving of bread (g)	32	16	168	84
2a	Total calories in a packet of biscuits	44	22	156	78
2b	Dietary fibre in 1 serving of biscuit (g)	58	29	142	71
2c	Classification of ability to interpret the label	31	15.5	169	84.5

Level of practices

The study showed that 35% of adolescents "sometimes" use food labels when purchasing food, followed by 31% who "rarely" use them, 19.5% who "often" use them, and 14.5% who "never" use them.

The fact that only 19.5% of adolescents reported often using food labels when purchasing food is concerning, as it suggests that many adolescents may need to be made aware of the importance of using food labels. Evidence suggests that the use of food labels is associated with healthier food choices and better dietary quality, particularly among adolescents. Therefore, promoting food labels among adolescents could be an effective strategy to improve their dietary quality and promote healthier eating habits.

Several strategies can be implemented to promote food labels among adolescents. For example, nutrition education programs can include information on reading and interpreting food labels and the importance of using food labels when making food choices. Schools can also provide opportunities for students to practice using food labels, for example, by including activities that involve analysing food labels as part of the curriculum.

Reasons for purchasing snacks

Table 3 showed that 90.5% of adolescents consider the expired date to be the "most important" aspect when purchasing food, but only 19.9% "often" use it during food purchases. As 50.5% and 51.5% of adolescents consider taste and ingredients as the "most important" aspects in making decisions, respectively. Less than half of the adolescents listed price (48%), nutrient content (40%), and packaging (35.5%) as the "most important" factors when buying food.

Table 3. Influence factors on the food label use

Influence	Use of food labels			
	Often (%)	Sometimes (%)	Rarely (%)	Never (%)
Price				
Most important	17 (17.7)	31 (32.3)	28 (29.2)	20 (20.8)
Important	17 (20.5)	32 (38.6)	25 (30.1)	9 (10.8)
Least important	5 (26.3)	6 (31.6)	8 (42.1)	0 (0)
Not Important	0 (0)	1 (50)	1 (50)	0 (0)
Taste				
Most important	25 (24.8)	30 (29.7)	33 (32.7)	13 (12.9)
Important	11 (13.1)	35 (41.7)	25 (29.8)	13 (15.5)
Least important	3 (21.4)	4 (28.6)	4 (28.6)	3 (21.4)
Not Important	0 (0)	1 (100)	0 (0)	0 (0)
Nutrient content				
Most important	23 (28.7)	31 (38.8)	17 (21.3)	9 (11.3)
Important	10 (11.5)	30 (34.5)	34 (39.1)	13 (14.9)
Least important	5 (17.2)	9 (31)	10 (34.5)	5 (17.2)
Not Important	1 (25)	0 (0)	1 (25)	2 (50)
Ingredients				
Most important	26 (25.2)	42 (40.8)	22 (21.4)	13 (12.6)
Important	10 (13.5)	24 (32.4)	31 (41.9)	9 (12.2)
Least important	3 (13.6)	4 (18.2)	9 (40.9)	6 (27.3)
Not Important	0 (0)	0 (0)	0 (0)	1 (100)
Packaging				
Most important	24 (33.8)	19 (26.8)	17 (23.9)	11 (15.5)
Important	9 (11.7)	32 (41.6)	25 (32.5)	11 (14.3)
Least important	6 (12.5)	19 (39.6)	17 (12.5)	6 (12.5)
Not Important	0 (0)	0 (0)	3 (25)	1 (25)
Expiry date				
Most important	36 (19.9)	66 (36.5)	55 (30.4)	24(13.3)
Important	1 (6.7)	4 (26.7)	6 (40)	4 (26.7)
Least important	2 (66.7)	0 (0)	1 (33.3)	0 (0)
Not Important	0 (0)	0 (0)	0 (0)	1 (100)

Table 4 shows the reasons why adolescents read the food label. More than half (51%) of adolescents read food labels due to considerations of taste and cost. Followed by being to be able to comprehend the information presented on food labels (49.5%), to control energy intake (38%), for health and beauty concerns (31%), and due to food allergy (20.5%). Nearly half of the adolescents understood each food label informations, yet only 18.2% "often" use the food labels.

Meanwhile, Table 5 depicts attitudes towards not using or consulting food labels. The results indicate that 45.5% of adolescents cited

Table 4. Frequency and factors referring to the food label

Refer to the food label	Often (%)	Sometimes (%)	Rarely (%)	Never (%)
Understand each info on the food label				
Yes	18 (18.2)	41 (41.4)	31 (31.3)	9 (9.1)
No	21 (20.8)	29 (28.7)	31 (31.3)	20 (19.8)
Experience food allergy				
Yes	10 (24.4)	16 (39)	12 (29.3)	3 (7.3)
No	29 (18.2)	54 (34)	50 (31.4)	26 (16.4)
To control energy intake				
Yes	17 (22.4)	27 (35.5)	25 (32.9)	7 (9.2)
No	22 (17.7)	43 (34.7)	37 (29.8)	22 (17.7)
For health or beauty				
Yes	14 (22.6)	32 (51.6)	9 (14.5)	7 (11.3)
No	25 (18.1)	38 (27.5)	53 (38.4)	22 (15.9)
Concern about taste and price				
Yes	15 (14.7)	33(32.4)	35 (34.3)	19 (18.6)
No	24 (24.5)	37 (37.8)	27 (27.6)	10 (10.2)

Table 5. Frequency and factors of not referring to the food label

Do not refer to food label	Use of food label			
	Often (%)	Sometimes (%)	Rarely (%)	Never (%)
Do not know how to use food label				
Yes	10 (18.5)	13 (24.1)	20 (37)	11 (20.4)
No	29 (19.9)	57 (39)	42 (28.8)	18 (12.3)
Time constrain/limited time				
Yes	19 (20.9)	39 (42.9)	22 (24.2)	22 (24.2)
No	20 (18.3)	31 (28.4)	40 (36.7)	18 (16.5)
Label was not attractive/confusing				
Yes	10 (13.9)	31 (43.1)	19 (26.4)	12 (16.7)
No	29 (22.7)	39 (30.5)	43 (33.6)	17 (13.3)
There is no label on certain food				
Yes	14 (21.2)	26 (39.4)	20 (30.3)	6 (9.1)
No	25 (18.7)	44 (32.8)	42 (31.3)	23 (17.2)
No health problem				
Yes	9 (14.1)	26 (40.6)	21 (32.8)	8 (12.5)
No	30 (22.1)	44 (32.4)	41 (30.1)	21 (15.4)

time constraints as the main reason for not using food labels, followed by unattractive/confusing labels (36%), absence of labels on certain foods (33%), no health concerns (32%), and lack of knowledge on how to use food labels (27%).

Other practices regarding food label use

According to the data presented in Table 6, more than half of the adolescents (51%) reported that they sometimes read the list of ingredients on food labels. Among the items listed, sugars were the most frequently referred to by adolescents,

Table 6. Association between practices and food label use

Practices items	Use of food label			
	Often (%)	Sometimes (%)	Rarely (%)	Never (%)
Read: List of ingredients				
Often	19 (35.8)	19 (35.8)	13 (24.5)	2 (3.8)
Sometimes	12 (11.8)	40 (39.2)	38 (37.3)	12 (11.8)
Rarely	7 (20.6)	8 (23.5)	11 (32.4)	8 (23.5)
Never	1 (9.1)	3 (27.3)	0 (0)	7 (63.6)
Read: Serving size				
Often	14 (34.1)	19 (46.3)	5 (12.2)	3 (7.3)
Sometimes	13 (17.8)	29 (39.7)	26 (35.6)	5 (6.8)
Rarely	9 (13.6)	21 (31.8)	25 (37.9)	11 (16.7)
Never	3 (15)	1 (5)	6 (30)	10 (50)
Read: Health claim				
Often	18 (32.1)	24 (42.9)	9 (16.1)	5 (8.9)
Sometimes	14 (16.1)	29 (33.3)	33 (37.9)	11 (12.6)
Rarely	5 (13.2)	15 (39.5)	15 (39.5)	3 (7.9)
Never	2 (10.5)	2 (10.5)	5 (26.3)	10 (52.6)
Read: Calories/energy				
Often	16 (34)	21 (44.7)	8 (17)	2 (4.3)
Sometimes	17 (18.9)	31 (34.4)	34 (37.8)	8 (8.9)
Rarely	4 (8.5)	16 (34)	17 (36.2)	10 (21.3)
Never	2 (12.5)	2 (12.5)	3 (18.8)	9 (56.3)
Read: Calories from fat				
Often	18 (35.3)	22 (43.1)	8 (15.7)	3 (5.9)
Sometimes	15 (17.4)	32 (37.2)	32 (37.2)	7 (8.1)
Rarely	4 (9.1)	14 (31.8)	17 (38.6)	9 (20.5)
Never	2 (10.5)	2 (10.5)	5 (26.3)	10 (52.6)
Read: Total fat				
Often	19 (38.8)	20 (40.8)	7 (14.3)	3 (6.1)
Sometimes	12 (14.6)	29 (35.4)	34 (41.5)	7 (8.5)
Rarely	6 (12.2)	19 (38.8)	15 (30.6)	9 (18.4)
Never	2 (10)	2 (10)	6 (30)	10 (50)
Read: Trans fat				
Often	18 (39.1)	17 (37)	8 (17.4)	3 (6.5)
Sometimes	14 (16.9)	31 (37.3)	31 (37.3)	7 (8.4)
Rarely	5 (10.4)	19 (39.6)	15 (31.3)	9 (18.8)
Never	2 (8.7)	3 (13)	8 (34.8)	10 (43.5)
Read: Saturated fat				
Often	17 (41.5)	17 (41.5)	6 (14.6)	1 (2.4)
Sometimes	15 (17.6)	31 (36.5)	32 (37.6)	7 (8.2)
Rarely	5 (9.6)	20 (38.5)	16 (30.8)	11 (21.2)
Never	2 (9.1)	2 (9.1)	8 (36.4)	10 (45.5)

Continue from Table 6

Table 6. Association between practices and food label use

Practices items	Use of food label			
	Often (%)	Sometimes (%)	Rarely (%)	Never (%)
Read: Cholesterol				
Often	21 (42)	19 (38)	7 (14)	3 (6)
Sometimes	11 (13.6)	32 (39.5)	32 (39.5)	6 (7.4)
Rarely	5 (10.4)	17 (35.4)	17 (35.4)	10 (20.8)
Never	2 (9.5)	6 (28.6)	6 (28.6)	10 (47.6)
Read: Sodium				
Often	15 (33.33)	19 (42.2)	7 (15.6)	4 (8.9)
Sometimes	16 (20)	29 (36.3)	30 (37.5)	5 (6.3)
Rarely	4 (7.4)	19 (35.2)	21 (38.9)	10 (18.5)
Never	4 (19)	3 (14.3)	4 (19)	10 (47.6)
Read: Carbohydrate				
Often	19 (38.8)	20 (40.8)	7 (14.3)	3 (6.1)
Sometimes	12 (14.5)	31 (37.3)	33 (39.8)	7 (8.4)
Rarely	6 (11.8)	17 (33.3)	18 (35.3)	10 (19.6)
Never	2 (11.8)	2 (11.8)	4 (23.5)	9 (52.9)
Read: Protein				
Often	22 (36.7)	24 (40)	10 (16.7)	4 (6.7)
Sometimes	10 (12.7)	31 (39.2)	30 (38)	8 (10.1)
Rarely	5 (11.4)	13 (29.5)	19 (43.2)	7 (15.9)
Never	2 (11.8)	2 (11.8)	3 (17.6)	10 (58.8)
Read: Dietary Fibre				
Often	19 (40.4)	21 (44.7)	5 (10.6)	2 (4.3)
Sometimes	11 (12.9)	33 (38.8)	34 (40)	7 (8.2)
Rarely	6 (12.5)	14 (29.2)	19 (39.6)	9 (18.8)
Never	3 (15)	2 (10)	4 (20)	11 (55)
Read: Sugars				
Often	18 (27.7)	15 (19.2)	15 (23.1)	6 (9.2)
Sometimes	26 (40)	28 (35.9)	28 (35.9)	7 (9)
Rarely	15 (23.1)	28 (35.9)	18 (39.1)	9 (19.6)
Never	6 (9.2)	7 (9)	1 (9.1)	7 (63.6)
Read: Vitamin and mineral				
Often	20 (37)	22 (40.7)	7 (13)	5 (9.3)
Sometimes	10 (12.5)	29 (36.3)	33 (41.3)	8 (10)
Rarely	6 (12.2)	17 (34.7)	19 (38.8)	7 (14.3)
Never	3 (17.6)	2 (11.8)	3 (17.6)	9 (52.9)

with 32.5% reporting that they often refer to this information. Protein (30%) and health claims (28%) were the next most commonly referred to items that adolescents reported referring to often. In this study, practices of reading all labels were significantly associated with using food

labels when making decisions when buying food products.

Nutrition knowledge is understanding the concepts and processes regarding nutrition and health, such as the relationship between diet and health, diseases, food nutrients, dietary guidelines, and recommendations (Miller & Cassady 2015). Studies have shown that nutrition knowledge significantly impacts a person's understanding and use of information on food labels (Shireen *et al.* 2022). The utilisation of food labels among adolescents in this study was not significantly associated with their level of nutrition knowledge. This is consistent with Evelyn *et al.* (2020), which suggests that even if a person is aware of health, it may not be enough to encourage them to read food labels during purchasing. Adolescents in this study had moderate knowledge of general nutrition information, but only 84.5% could explain or interpret information on the Nutrition Information Panel (NIP). This may be due to a need for more knowledge of serving size (Shah *et al.* 2010) or need for clarity between calories per serving and calories per packaging. Adolescents reported needing help to answer questions about which nutrient provides the most calories, with most thinking carbohydrates provide more calories than fat. This may be due to poor knowledge of energy intake and expenditure. Additionally, low nutrition knowledge among young adults in Malaysia may be due to a lack of interest in healthcare issues (Jefrydin *et al.* 2019).

This study found that 90.5% of adolescents were concerned about the product's expiration date and often read the information. This is similar to previous research by Saha *et al.* (2013) that reported adolescents prioritising product safety and shelf life over other information. Despite their concern for expiry dates, only 19.9% often used food labels during purchasing, indicating no significant relationship between concern for expiration dates and use of food labels (Shireen *et al.* 2022). Adolescents reported that taste (51%) and price (51%) were the main reasons for referring to food labels, while time constraints (45.5%) were the reason for not referring to them. The semi-structured focus group discussion in Jerfydin *et al.* (2019) found that some adolescents had limited time to read all the information while grocery shopping and only bought desired foods. However, the relationship between attitudes and use of food labels varied in different studies. Adolescents view the importance of various nutritional components listed on food labels, with some believing that information like fat content will help them lose weight (Jefrydin *et al.* 2019). Among the components, sugar content is the most frequently referred to (32.5%). Health education, awareness, and trust in low-fat/calorie diets significantly influence label use behaviour. About 51% of adolescents reported "sometimes" reading ingredient lists on food labels as they prefer products with better quality ingredients (Miller & Cassady 2015). The ingredients list can provide information on a food's healthfulness as the ingredients are listed in descending order of proportion by weight (Miller & Cassady 2015).

CONCLUSION

Adolescents have inconsistent attitudes towards food labeling, as they understand the significance of the expiration date but only sometimes check it during food purchases. This is due to time constraints, cost, and taste preferences. However, consistently reading food labels can help individuals make informed choices about their portion intake. Awareness campaigns including topic on food label use can aid adolescents in making healthier choices in their daily lives.

ACKNOWLEDGEMENT

We would like to thank all adolescents who took part in this study. The article processing fee is funded by Pembiayaan Yuran Penerbitan Artikel Berindeks (PYPA), Research Management Centre (RMC), Universiti Teknologi MARA (UiTM), Malaysia.

DECLARATION OF INTERESTS

The authors have no conflict of interest.

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Volume 18, Number 1, March 2023

ISSN 1978-1059
EISSN 2407-0920

JURNAL GIZI DAN PANGAN
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Articles

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Jurnal Gizi dan Pangan

Volume 18, Number 1, March 2023

9 771978 105189

Jurnal Gizi dan Pangan
(Indonesian Journal of Nutrition and Food)

ISSN : 1978-1059 **(printed)**
EISSN : 2407-0920 **(electronic)**

Publication Fee : IDR 1,500,000 (120 US$ Full amount)
SWIFT Code: BNINIDJABGR

Publication Frequency : Three times a year (March, July, and November)

Subscription Rate : IDR 250,000 /year (not including shipping rate and handling)
Bank BNI account number: 0266948576
Account name: Dodik Briawan

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