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Efficacy of Non-Branded Cooking Oil Fortified with Carotene from RPO on Blood Retinol and IgG of Children Aged 7-9 Years

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Abstract: This research aimed to assess the efficacy of non-branded cooking oil fortified with carotene from red palm oil (RPO) on blood retinol and IgG level. Quasi-experimental pre- and post-treatment controlled trial design was applied in this study on 31 elementary school children aged 7-9 years, divided into control group (n = 16) and RPO group (n = 15). This research was conducted in Angsana Village, Leuwiliang Sub-district, located in Bogor Regency, Indonesia. The children's families received non-branded cooking oil once a week (1 kg/week) for eight weeks. Retinol concentration and IgG level in blood were assessed at baseline and after eight weeks. Results showed that serum retinol level increased by 5.31 µg/dL (50.9%) in the RPO group and 3.25 µg/dL (29.8%) in the control group. Statistical tests showed that serum retinol levels in both groups were not significantly different (p>0.05), either before or after the intervention. Similar results were shown in the delta of serum retinol level before and after the intervention between the RPO and control groups (p = 0.062). Based on the paired t-test, serum retinol levels in both groups were significantly different before and after the intervention (p = 0000). The average of vitamin A sufficiency increased in both groups, but only the RPO group that was categorized as sufficient. The average of IgG level in the control group was significantly different from the RPO group before the intervention (p<0.05), but there were no differences between control group and RPO group after the intervention. Intervention using non-branded cooking oil fortified with carotene from RPO tended to increase blood retinol level but did not increase IgG level.

Key words: Carotene, cooking oil, IgG, RPO, retinol

INTRODUCTION

Nutritional problem is a multifactorial issue that can affect the quality of life of each individual. UNICEF conceptual framework in 1998 illustrated that nutritional problems were directly caused by inadequate dietary intake and high rates of infectious diseases. Infection is one of the consequences of micronutrient deficiency. Micronutrients play a role in the formation of antibodies and the development of the immune system, one of which is vitamin A (Katona and Apte, 2008).

Vitamin A deficiency (VAD) is a public health problem in more than half of countries in the world, particularly in the African region and South East Asia. Children are prone to have this problem. WHO in 2016 reports that 250 million preschool children are estimated to have VAD and 250,000 to 500,000 children with VAD become blind every year, half of them were dying within 12 months of losing their sight. VAD may cause blindness in children and increase the risk of illness and death from infectious diseases (WHO, 2016). Based on the results of a study of micronutrient problems in Indonesia in 2006, VAD was not a public health problem anymore because its prevalence was below the cut-off of International Vitamin A Consultative Group (IVACG),

namely 15% (Departemen Kesehatan, 2009). However, it does not guarantee that VAD cases will not reappear in the following years. Therefore, alternative efforts are still required to maintain this condition. Fortification is one of the alternative strategies to achieve these goals. Vitamin A fortification of food is generally done by using commercial forms of synthetically-produced vitamin A. It is also can be done by using the natural form of vitamin A derived from plant-based foods. Crude palm oil (CPO) is a plant-based food that can be used to fortify due to the high content of carotene, particularly β-carotene (pro-vitamin A) which is equal to 643 ppm (Nagendran *et al.*, 2000). Beta-carotene is one of the hundreds of carotenoids which has the highest vitamin A activity with two interlocked retinol molecules (Almatsier, 2006).

Carotene content of CPO was 15 times higher than the one in carrots (Mukherjee and Mitra, 2009). CPO is quite abundant in Indonesia. According to the data from United States Department of Agriculture (USDA, 2016), the production of palm oil in Indonesia reached 33.000.000 MT in 2015. However, the CPO has not become a viable consumption material for humans. Refining processes of CPO in a minimum way can yield red palm oil (RPO) that still has a high carotene content,

i.e., 513 ppm (Nagendran *et al.*, 2000). According to Zeb and Mehmood (2004), carotene content derived from RPO was equal to 15 times of the carotene contained in carrot, 120 times of the β -carotene contained in tomatoes and 44 times of the β -carotene contained in green leafy vegetables.

Non-branded cooking oil is a foodstuff that has the potential to be fortified with vitamin A. In Indonesia, almost all people consume non-branded cooking oil. In 2014, the average consumption of cooking oil in Indonesia increased along with the increase in the average monthly per-capita income. The average consumption of cooking oil in Indonesia was 0.205 liters/capita/week in 2014 (BPS, 2016). Another survey reported that 77.5% of households in Indonesia used non-branded cooking oil for frying and the average consumption was 23 g/day (Martianto, 2005). Cooking oil was selected as the carrier of pro-vitamin A in order to increase energy intake, especially in the lower middle class. Fortifying the cooking oil with carotene could also provide other benefits, i.e. reducing the cost of imports of synthetic vitamin A. This condition was related to the government program, namely "Mandatory Fortification of Cooking Oil with Vitamin A". Hence, the efficacy of non-branded cooking oil fortified with carotene from RPO on the nutritional status and the status of vitamin A in children should be investigated. The general objective of this research was to assess the efficacy of non-branded cooking oil fortified with carotene from RPO on blood retinol and IgG of children aged 7-9 years.

MATERIALS AND METHODS

Design and location: This research used a quasi-experimental pre- and post-treatment controlled trial design. This study design was used to assess the effect of non-branded cooking oil fortified with carotene from RPO on blood retinol and IgG of children aged 7-9 years. The intervention was carried out over two months at Angsana 1 and 2 State Elementary Schools in Leuwiliang Sub-district, Bogor Regency, Indonesia. Analysis of retinol levels was performed in the laboratory of Center for Applied Health Technology and Clinical Epidemiology, the Ministry of Health of the Republic of Indonesia, located in Bogor. Meanwhile, the analysis of IgG was conducted in the laboratory of University of Indonesia, located in Jakarta. This study was approved by the Ethics Committee of the Ministry of Health of the Republic of Indonesia Number LB.02.01/5.2/KE235/2013 dated July 9, 2013.

Subjects: Subjects in this research were elementary school children aged 7-9 years, healthy (based on the results of the doctor's examination), had already received the explanation about the research, signed the informed consent and agreed to follow the research procedures. They were allocated into two intervention

groups, namely control group and RPO group. The main research variables examined in this study were serum retinol level and immune response (as measured by serum IgG level). Other variables were characteristics of elementary school children and their families, nutritional status (as measured by anthropometric index, namely weight-for-age Z-score/WAZ), food consumption and morbidity. Non-branded cooking oil was given to the family once a week (1 kg/week) for eight weeks. At the end of each week, the students had to bring the bottle of cooking oil to calculate and estimate the compliance. Retinol concentration and IgG level were assessed at baseline and after eight weeks.

Data collection and statistical analysis: The primary data collected in this research were data of the subjects (elementary school children) and their families, including the children's identity (name, gender, age, birth order of the child in the family and others), health status, food consumption, serum retinol level, results of the anthropometric measurements (weight and height) and morbidity. The data of children's identity were obtained through interviews with the children and their parents or caregivers by using questionnaires. Health status of the children (morbidity) was obtained through observations and interviews with the children and their parents (caregivers) and teachers by using questionnaires. Food consumption data were collected by food recall method, the data of serum retinol levels were collected through laboratory analysis by an extraction method (concurrent liquid-chromatographic assay of retinol) and serum IgG levels were obtained through laboratory analysis by enzyme-linked immunosorbent assay (ELISA) method. Children's weight data (W) were acquired by weighing with the analog of Stampede weight scale with an accuracy of 0.1 kg while children's height data (H) were obtained through measurements by using microtoise with an accuracy of 0.1 cm. The compliance level was assessed through interview with the children in the classroom by using a compliance form filled by the mothers. Identity and family characteristics of the subjects were analyzed using descriptive statistics and frequency statistics. Differences in serum retinol and IgG levels, as well as nutritional status of the control and RPO groups at the baseline and the end of intervention, were analyzed by t-test, Mann Whitney test and paired difference test.

RESULTS

Subjects' characteristics: Total number of subjects was 31 children, consisting of 14 boys (45.2%) and 17 girls (54.8%). There were 15 subjects in the RPO group, consisting of 9 boys (60%) and 6 girls (40%). Meanwhile, there were 16 subjects in the control group, consisting of 5 boys (31.3%) and 11 girls (68.7%). In both research groups, most of the subjects aged 8

years, namely 53.3% in RPO group and 50% in the control group. The average age of subjects in the RPO and control group were 7.8 ± 0.7 years and 8.1 ± 0.7 years, respectively. Statistical tests indicated that there was no significant difference in the average of age between the two groups ($p = 0.202$). In general, subjects were the first or second child in the family (29%). Most of the subjects in the RPO group was the first child (40%) while most of subjects in the control group was the second child in the family (50%). Two subjects in the RPO group (6.5%) were children with the birth order of ≥ 5 . According to the results of Mann Whitney test, the child's birth order between the RPO and the control groups was not significantly different ($p = 0.396$). Most of the subjects' mothers (61.3%) took care of their children by themselves while others had the other family members taken care their children (38.7%). Other family members included grandparents, uncles/aunts, sisters and other family members. Subjects were frequently raised by the older sister (19.4%). There was one child in the RPO group (3.2%) who was raised by other family members.

Energy, protein, fat and vitamin A intake: The average of energy intake in the RPO group increased by 12.6% but it tended to decrease in the control group (23.5%) after the intervention. An increase in energy intake in the RPO group was caused by an increase in the intake of protein (15.9%), fat (6%) and carbohydrates (12.4%). On the contrary, a decrease in energy intake in the control group after the intervention was the result of a decrease in the intake of carbohydrates (40.9%), protein (13.1%) and fat (21.8%). Results of paired difference test showed that the average intake of energy, protein and fat in the RPO group before and after the intervention were not significantly different ($p > 0.05$) while the average intake of vitamin A was significantly different ($p = 0.009$). Table 1 presented the average intake of energy, protein, fat and vitamin A of the subjects before and after the intervention.

Consumption of cooking oil: Consumption of cooking oil in the RPO group before the intervention was not significantly different from the control group ($p = 0.957$). The average consumption of cooking oil in the RPO and control groups were 14.4 ± 8.9 g and 14.2 ± 7.8 g, respectively. After the intervention, the consumption of cooking oil in the RPO group increased to 21.4 ± 4.1 g but it decreased slightly to 12.9 ± 4.5 g in the control group. The t-test showed that consumption of cooking oil in the RPO group was significantly higher than the control group after the intervention ($p = 0.000$). Data of subjects' cooking oil consumption during the intervention were presented in Table 2 and the data of cooking oil consumption during the intervention and vitamin A intake were presented in Table 3.

Serum retinol level: Serum retinol levels of the RPO group increased by $5.31 \mu\text{g/dL}$ (50.9%). Meanwhile, it increased by $3.25 \mu\text{g/dL}$ (29.8%) in the control group. The delta of serum retinol increment in the RPO group was higher than the control group. Results of statistical tests showed that the serum retinol level in the control group and RPO group was not significantly different ($p > 0.05$), either before or after the intervention. Similar results were shown in the delta of serum retinol level between the RPO group and the control group ($p = 0.062$). Based on the paired t-test, serum retinol levels in both groups were significantly different before and after the intervention ($p = 0.000$). Table 4 presented the average value of serum retinol level of the subjects.

Vitamin A status: Figure 1 presented the distribution of subjects by the category of vitamin A status before and after the intervention in the control and RPO groups. Before the intervention, vitamin A status in the control group was mostly categorized as marginal (62.5%) and the rest (37.5%) was categorized as deficient. Before intervention, subjects in the RPO group categorized as deficient were greater than the subjects categorized as marginal. There were 53.3% of subjects categorized as deficient and 46.7% categorized as marginal (with low vitamin A status). There was no subject categorized as sufficient or excessive in both groups. After intervention for the past eight weeks, there had been improvements in vitamin A status in both groups. Data in Fig. 1 showed the increase in percentage of subjects with sufficient vitamin A status and the decrease in percentage of subjects in both categories of deficiency. In the control group, the percentage in the sufficient category increased from 0 to 6.2%. In addition, there was also an increase in vitamin A status in the marginal category, from 18.7 to 81.2%. The increase was caused by the decrease in percentage by 25% in the deficient category. RPO group had the same change pattern as the control group but with a bigger increase, for example, the percentage in sufficient category increased by 13.3%. An increase in the marginal category was quite large (40%) and it was followed by a decrease in the percentage in the deficient category to 0%.

Nutritional status: The average of overall body mass index (BMI) before the intervention was $14.34 \pm 0.64 \text{ kg/m}^2$ and it increased to $14.84 \pm 0.90 \text{ kg/m}^2$ after the intervention. Similarly, in each intervention group, the average of BMI also tended to increase after the intervention although not significant (Table 5). T-test result showed that the average of BMI in the RPO group was significantly higher than the control group after the intervention. According to the data in Table 5, energy intake in the RPO group was higher than the control group. Before the intervention, 13.3% of subjects in the RPO group were thin and 86.7% were normal whereas

Table 1: Average intake of energy and nutrients before and after intervention in the RPO group and control group

Nutrient	RPO			Control		
	Before intervention	After intervention	p-value	Before intervention	After intervention	p-value
Energy (Cal)	1.123±398	1.264±214	0.243	1.184±288	906±198	0.004
Protein (g)	27.1±13.4	31.4±7.2	0.317	22.9±4.9	19.9±4.2	0.054
Fat (g)	36.4±16.5	38.6±7.5	0.642	31.7±7.6	24.8±6.9	0.007
Vitamin A (RE)	302.4±190.5	464.2±73.8	0.009	196.7±84.2	223.6±109.9	0.151

Table 2: Average consumption of cooking oil per day before and after intervention in the RPO and control groups

Group	Consumption of cooking oil (g)		
	Before intervention	After intervention	p-value
RPO	14.4±8.9	21.4±4.1	0.013
Control	14.2±7.8	12.9±4.5	0.598
p-value	0.957	0.000	

all subjects in the control group had normal nutritional status. After the intervention, all of the subjects (in RPO and control groups) had normal nutritional status. Distribution of the subjects in the RPO and control groups based on the nutritional status before and after the intervention were presented in Fig. 2.

Level of immunoglobulin (IgG): The level of IgG before the intervention in the control and RPO groups was significantly different ($p < 0.05$). This indicated that blood IgG levels in both groups were not homogeneous at baseline. Nutrient deficiency, such as zinc, vitamin A, selenium, iron, copper, vitamin C, vitamin E and folic acid, could reduce the immune response (Chandra 1997). The data of IgG level in both groups at the end of the research were not significantly different ($p > 0.05$). Table 6 presented the blood IgG levels of the subjects.

Score of morbidity: Morbidity in this research was defined as the number of subjects who were sick within two weeks prior to intervention and eight weeks after the intervention. Morbidity was known based on infectious diseases suffered by the children and the duration of illness through interview with the child and the mother. Score of morbidity was the result of frequency of illness multiplied by duration of illness. According to Sugiyono (2009), the scores were divided into three categories, namely low (< 4), medium (4-7) and high (≥ 8). Table 7 showed that the average score of morbidity was classified as mild before the intervention and it was decreased in intermediate 3. Morbidity scores in intermediate 1, 2, 3 and after the intervention were low. These results indicated that the majority of subjects were healthy during the intervention. After the intervention, the score in the RPO group increased to 0.67 ± 2.58 because one subject suffered from the mumps in the last two weeks of intervention.

DISCUSSION

The difference in the average intake of vitamin A before and after the intervention in the RPO group was due to

vitamin A content of the cooking oil consumed by the subjects. The average intake of energy and fat before the intervention in the control group were significantly different compared with the intake after the intervention ($p < 0.05$). Meanwhile, protein and vitamin A intakes were not significantly different ($p > 0.05$). The difference in the average energy intake was the result of differences in the average intake of fat and carbohydrates. Paired difference test showed that there were no differences in the average intake of energy, protein, fat and vitamin A between the RPO group and the control group before the intervention ($p > 0.05$). On the contrary, there were differences in the average intake of energy, protein, fat and vitamin A between the two groups after the intervention ($p < 0.05$). The differences occurred due to the increase in average intake of energy, protein, fat and vitamin A in the RPO group, in contrast to the control group.

In general, there was an increase in average sufficiency level of energy, protein, fat and vitamin A in the RPO group although the levels did not reach the normal threshold. Energy and protein sufficiency levels in the RPO group before the intervention were classified as severe deficiency but they were improved to the mild deficiency after the intervention. Fat sufficiency level increased from 58 to 62.1% but it was still classified as severe deficient. Vitamin A sufficiency level increased quite high after the intervention and it was categorized as sufficient ($\geq 77\%$).

The average level of energy, protein and fat sufficiency of control subjects decreased after the intervention, in contrast to the vitamin A sufficiency level. Overall, it could be concluded that the intake of energy and nutrients had not been sufficient. Before the intervention, the energy sufficiency level of control group was categorized as medium deficit ($> 70\%$), then it got worse after the intervention. It was similar with the protein and fat sufficiency levels. After the intervention, the vitamin A sufficiency level increased slightly (39.35 to 44.7%) but it was still categorized as deficient.

Low energy, protein, fat and vitamin A sufficiency levels were directly influenced by the lack of total intake of energy and nutrients from the food consumed. It was due to dietary changes of the subjects that tended to decline after the intervention, especially in the control group. The last month of intervention was the time of exam and school holidays where children usually had more playing time, so it was thought to affect their eating patterns. Another factor that might affect the sufficiency

Table 3: Average consumption of cooking oil per day and the average intake of vitamin A from the cooking oil

Group	Type of oil	Average of cooking oil consumption/day (g)	Average intake of vitamin A (RE)
Control	Control oil	12.9±4.7	0.00±0.00
RPO	RPO oil	21.4±4.2	53.4±10.5
	Non-intervention oil	0.2±0.6	0.00±0.00

Table 4: Average of serum retinol level before and after intervention in the RPO and control groups

Group	Before intervention (µg/dL)	After intervention (µg/dL)	Delta (µg/dL)	p-value
RPO	10.44±1.81	15.76±3.12	5.31±3.29	0.000
Control	10.88±2.53	14.12±3.41	3.25±2.64	0.000
p-value	0.589	0.175	0.062	

Table 5: Average of Body Mass Index (BMI) before and after intervention in the RPO and control groups

BMI (kg/m ²)	RPO	Control	Total	p-value
Before	14.51±0.64	14.18±0.62	14.34±0.64	0.166
After	15.19±0.84	14.50±0.85	14.84±0.90	0.032
p-value	0.006	0.007		

Table 6: Level of IgG before and after intervention in the RPO and control groups

Time	Level of IgG (mg/mL)		p-value
	RPO	Control	
Before intervention	313.73±148.99	185.83±179.47	0.031 ¹
After intervention	296.34±178.01	279.75±342.11	0.861 ²
Delta	-17.39±204.36	93.92±365.03	0.283 ²
p-value	0.730 ²	0.305 ²	

Table 7: Average score of morbidity in the RPO and control groups before, during and after intervention

Time	Control group	RPO group	p-value
Before intervention	4.44±5.10	4.87±5.79	0.828
Intermediate 1	0.38±1.02	0.33±0.90	
Intermediate 2	0.00±0.00	0.00±0.00	
Intermediate 3	0.00±0.00	0.00±0.00	
After intervention	0.00±0.00	0.67±2.58	0.302
p-value	0.005	0.032	

Table 8: Comparison of energy, protein, fat and vitamin A sufficiency in Indonesian and Philippine RDA for children aged 7-9 years

Nutrient	Philippine RDA (2002) (*)	Indonesian RDA (2012) (**)
Energy (Kcal)	1600	1850
Protein (g)	43	49
Fat (g)	-	72
Vitamin A (RE)	400	500

Sources: (*) Barba and Cabrera (2008), (**) Indonesian Ministry of Health (2013)

level of energy and nutrients was different standard of recommended dietary allowance (RDA) used in this research. When compared with other Asian countries, such as the Philippines, RDA in Indonesia was relatively higher. In the same age group (children aged 7-9 years), the energy, protein and vitamin A in Indonesian RDA were higher than the RDA used in the Philippines. Meanwhile, fat sufficiency has not been determined in that country (Table 8).

During the intervention period, the subjects' cooking oil consumption was mainly from the intervention cooking oil (fortified cooking oil and control oil), but it did not rule out the possibility of consuming other cooking oil circulating in the market. Consumption of non-fortified cooking oil outside home was due to the subjects' habit of snacking outside home, either at school or when playing outdoors during the school holidays. The types

of snacks that contributed to the amount of cooking oil consumed by the subjects were fried foods, such as vegetable fritter, fried tempeh, *cireng* (made from tapioca flour) and fried vegetable-stuffed tofu.

The average consumption of cooking oil in the RPO group was not significantly different from the average consumption of edible oil in adults, i.e., 23 g/day (Martianto, 2005). The small amount of non-intervention cooking oil consumption in the RPO group was due to the small amount of fried food consumed as snack. The fried food was substituted with other types of snacks, such as crackers, cheese ball, bread and instant noodles. The average consumption of cooking oil in the control group was relatively small because of the snacking habit and food consumption patterns. Subjects in the control group rarely bought fried food as a snack. The RPO fortified cooking oil contributed 53.4±10.5 RE

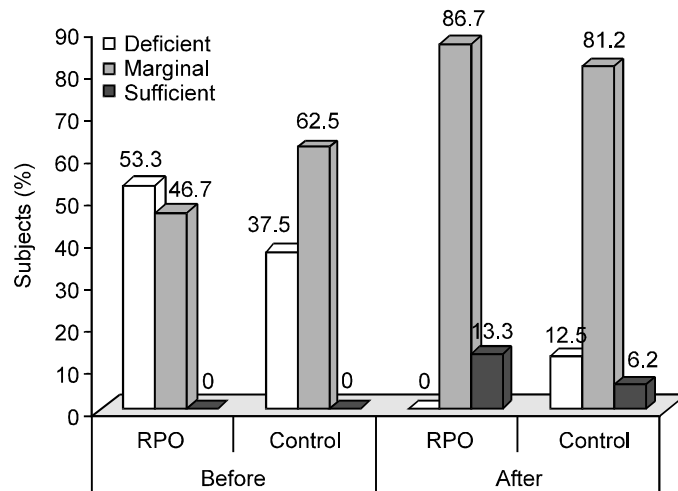


Fig. 1: Distribution of subjects in the RPO and control groups based on the vitamin A status before and after intervention

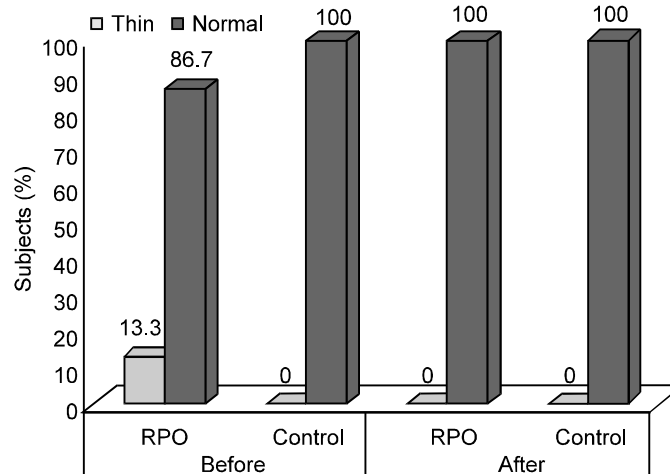


Fig. 2: Distribution of subjects in RPO and control groups based on the nutritional status before and after intervention

of vitamin A (10.7% of vitamin A sufficiency level in children aged 7-9 years).

The t-test results showed that the cooking oil consumption between the RPO group and the control group was not significantly different before the intervention ($p = 0.957$) but it was significantly different after the intervention ($p = 0.000$). This was due to the average consumption of cooking oil which increased substantially in the RPO group after the intervention. Therefore, it seemed that there was a significant difference in cooking oil consumption before and after the intervention in both groups ($p = 0.013$). The decrease in the average consumption of cooking oil in the control group was only in small quantity and it was not shown a significant difference ($p = 0.598$). The average consumption of cooking oil in the RPO group increased because the children liked foods that absorbed a lot of cooking oil, such as fish, chicken and tempeh.

Fortified cooking oil given to the subjects contributed 53.4 ± 10.5 RE per day (10.7% RDA) to meet the requirement of vitamin A. Increased serum retinol level in the RPO group was caused by the intake of vitamin A and carotene from other food sources, as well as from fortified cooking oil. Data of food consumption recall showed that most of the subjects, either in the control group or RPO group, liked eggs that were rich in vitamin A and instant noodles that had been fortified with vitamin A. Another source of vitamin A was from the vegetables containing pro-vitamin A, such as spinach, bean sprout and carrots. An increase in retinol level in the control group was caused by the increase in vitamin A intake. Retinol level in the subjects in the control group was 8.39-20.69 $\mu\text{g/dL}$.

IgG is the most common type of antibodies found in blood and covers about 80% of all blood immunoglobulins. IgG levels increase slowly during the

initial response to the antigen but increase rapidly with greater force in the second exposure (Corwin, 2001). In this study, carotene from RPO was fortified into the cooking oil and it was expected to boost the subjects' immunity which was indicated by the increased levels of blood IgG. Measurement of IgG level was performed at the beginning and the end of the study. In this study, the data of IgG level in both groups at the end of the research were not significantly different ($p > 0.05$). The IgG level in the RPO group tended to decrease after the intervention, in contrast to the control group. The results in this research were different from the previous research by Marliyati *et al.* (2014) which showed that after an 8-week intervention with fortified instant noodle, serum IgG level in the RPO group was significantly higher than the control and carrot groups ($p < 0.05$), as well as the delta value between those groups. Meanwhile, the serum IgG level in the carrot group was not significantly different from the control group ($p > 0.05$). The differences between these two researches might be due to the differences in the content of β -carotene in cooking oil and noodles. Besides that, the retention of β -carotene in cooking oil was not 100%. The content of β -carotene was 356 $\mu\text{g/g}$ in cooking oil (Wijaya, 2013) and 76.42 $\mu\text{g/g}$ in instant noodles (Marliyati *et al.*, 2010). Although β -carotene contained in cooking oil was higher, its retention in the first, second and third frying phases of non-branded cooking oil were 70.57%, 41.83% and 14.05%, respectively (Wijaya, 2013). Besides that, β -carotene in cooking oil was not all absorbed by the fried foods. Wijaya (2013) showed that the β -carotene content of fried tofu decreased from the first (0.57 $\mu\text{g/g}$) to the third frying (0.11 $\mu\text{g/g}$).

One of the benefits of vitamin A is to enhance the body's immune system. Enhancement of the immune system can be seen from the body's ability of the subjects to fight disease, especially infectious diseases. Villamor and Fawzi (2000) found that in hospital-based studies, vitamin A supplements had been consistently found to reduce the severity of measles infection, but no effect on non-measles respiratory infections had been observed. Study by Wieringa *et al.* (2010) showed that maternal supplementation with zinc and β -carotene affected the newborn's immune development in specific ways. Type of illness mostly suffered by the subjects were cold, cough, sore throat and fever. The duration of illness before the intervention ranged from two to seven days. In intermediate 1, the fever, cough and cold were only lasted for less than three days. However, after the intervention, one subject in the RPO group had mumps for 10 days. It was the longest duration of illness experienced by the subject. Results of paired difference test showed that there were no differences in morbidity scores between the control and RPO groups, before and after the intervention ($p > 0.05$). However, there was a significant difference in morbidity score among the subjects in each group before and after the intervention

($p < 0.05$). The morbidity scores after the intervention were lower than before intervention in both groups.

Conclusion: Serum retinol level in the RPO and control groups increased by 5.31 $\mu\text{g/dL}$ (50.9%) and 3.25 $\mu\text{g/dL}$ (29.8%), respectively. Statistical tests showed that the level of serum retinol between the control group and RPO group was not significantly different ($p > 0.05$), either before or after the intervention. Based on the paired t-test, serum retinol levels in both groups were significantly different before and after the intervention ($p = 0.000$). Vitamin A sufficiency increased in both groups, but only the RPO group that was categorized as sufficient. IgG level in the control group was significantly different from the RPO group before the intervention ($p < 0.05$), but there were no differences between the control and RPO groups after the intervention. There were no differences in morbidity scores between the control group and the RPO group, before and after the intervention ($p > 0.05$). Intervention with non-branded cooking oil fortified with carotene from RPO tended to increase blood retinol level but it did not increase IgG level and morbidity score.

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