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Effect of Feeding Palm Sugars Enriched with CPO and RPO on Liver Retinol and IgG Concentration of Vitamin A Depletion Rats

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Abstract: A sixteen weeks experiment was conducted to evaluate the effect of feeding CPO and RPO enriched palm sugar on liver retinol and immunoglobulin G (IgG) concentration in vitamin A depletion rats. Thirty-four-weeks old-male Sprague Dawley rats were received the standard diet (AIN-93M) for two weeks as adaptation period, after that 24 rats followed by vitamin A-free diet, for 10 weeks as a depletion period and of six remaining rats were given the standard diet as a control group. The depleted rats were then divided into 3 groups and received daily for 4 weeks as repletion period either a red palm oil (RPO) or crude palm oil (CPO) enriched palm sugar, or retinyl palmitate (RE) which each treatment contain approximately 40 µg beta carotene. Rats were blood-sampled at week 2 and week 4 and liver retinol and immunoglobulin G (IgG) concentration were determined. The body weight was taken every 5 days. Increasing on liver retinol concentration in rat administered retinyl-palmitate was higher than in rat administered CPO or RPO enriched palm sugar ($p = 0.0136$) which the increased in RE, CPO and RPO groups of 115.4, 86.4 and 61.7%, respectively. However, administering of enriched palm sugar both of CPO and RPO for 2-4 weeks can improve vitamin A status to above cut off point which increase from 0.063 µmol/g liver up to 0.107-0.117 µmol/g liver in CPO group and 0.079-0.102 µmol/g liver in RPO group. On the other hand, serum immunoglobulin G (IgG) level of animal administered palm sugar containing CPO or RPO were higher than in rat administered retinyl palmitate ($p = 0.0073$), which is indicated by increasing levels of serum IgG in both groups of CPO and RPO, that is equal to 225 and 216.01 percent, respectively compared to RE group of 59.98 percent. From this research we conclude that coconut palm sugar enriched with CPO or RPO were potentially as food-based intervention to overcome vitamin A deficiency.

Key words: CPO, RPO, palm sugar, liver retinol, IgG

INTRODUCTION

Vitamin A deficiency (VAD) is a major public health nutritional problem in many developing countries. Globally, night blindness affects 5.3 million preschool age children and 9.8 million pregnant women. An estimated 250,000 to 500,000 vitamin A deficient children become blind every year, half of them dying within 12 months of losing their sight (WHO, 2009).

Vitamin A plays an essential role in a large number of physiological functions that encompass vision, normal growth, reproduction, hematopoiesis, differentiation of epithelial and mesenchymal cells and is required in variety of processes related to immunity including maintenance of epithelial barriers and acquired cellular mediated and humoral immunity (Sommer and West, 1996). Vitamin A up-regulates the T-helper type 2 (Th2) cells and promotes humoral immunity, antibody production, Ig maturation and deactivation of macrophages (Garcia, 2012).

Palm oil, both crude palm oil (CPO) and red palm oil (RPO), is a rich source in provitamin A carotenoids alpha and beta-carotene, and generally contains a total of 500-800 mg of provitamin A carotenoids/kg oil, which is ~15 times higher than the carotenoid content of carrots. In addition to being a very rich source of beta-carotene, the carotenoids in RPO are easily absorbed during digestion because they are already dissolved in oil (Rice and Burns, 2010).

Because of its exceptionally high pro-vitamin A carotenoids content, RPO has been proposed as a food-based intervention to prevent vitamin A deficiency (VAD) and has been tested for this purpose in various small-scale human feeding studies (Lietz *et al*., 2001; Zagre *et al*., 2003; Canfield *et al*., 2001; Radhika *et al*., 2003; Stuijvenberg *et al*., 2001; Zeba *et al*., 2006). These interventions have almost always been successful, increasing or at least maintaining indices of vitamin A status (Rice and Burn, 2010). Indeed, a study on vitamin A supplementation through giving different dosage of

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RPO or retinol palmitate to preschool children by Sivan *et al*. (2002), showed that RPO at 5 and 10 mL daily for the 7-month long field trial was much better than the corresponding dose of synthetic vitamin A and resulted in a much higher mean gain in plasma beta-carotene level in the children. Red palm oil may play a double role in improving vitamin A status by providing both pro-vitamin A carotenoids and oil that promotes the absorption of vitamin A and pro-vitamin A carotenoids. Vitamin A deficiency is caused by insufficient intake of food rich in vitamin A or provitamin A carotenoids to meet the requirement for growth, infection, pregnancy and lactation (WHO, 2009).

Various interventions have been proposed to address vitamin A deficiency. One of these interventions is food fortification, which generally involves the addition of preformed vitamin A to commonly consumed staple foods or condiments. Vitamin A fortification is a central strategy for vitamin A deficiency reduction in many countries (Burri, 2012). Food fortification, especially of oil or sugar, has also been successful in some communities (Klemm *et al*., 2010).

Palm sugar made from coconut palm juice (toddy), which is evaporated until it is over saturated and then it is molded. In connection with effort to improve vitamin A status, palm sugar is suitable as nutrition vehicle, because it is used in the household and by the food industry as a food ingredient. The red-yellow color of red palm oil will improve the appearance of a brown sugar product. In recent study, 30 mL of red palm oil (RPO-CPO) which added at saturated phase of coconut sugars processing, yields a palm sugar which had a highest sensory acceptability and had the carotene content of 1337-3946 µg/100 g and carotene retention of 25.30-63.38% (Hidayah *et al*., 2014).

Although there have been many reports on human feeding studies of red palm oil as a food-based intervention to prevent vitamin A deficiency, there appears to be no available reports on the impact of feeding palm sugar enriched with palm oil on vitamin A status and immune. Therefore, this research aimed to evaluate the effect of feeding palm sugar enriched with CPO and RPO on improving liver retinol and IgG concentration of vitamin A depletion rats.

MATERIALS AND METHODS

Site of experiment: The study was conducted at Animal Hospital, Bogor Agricultural University, Bogor, West Java, Indonesia, since October 2012 up to March 2013. This study was approved by the Animal Ethics Committee of the Bogor Agricultural University, number: 04-2012 IPB.

Experimental diets: coconut sugar enriched with red palm oil was produced by evaporating purified coconut palm juice (toddy) to which 30 ml of Red Palm Oil (RPO) or Crude Palm Oil (CPO) had been added per 10 L of coconut juice (toddy). Resulted products were placed in air-tight, light protected plastic containers and stored at 4°C until being analyzed for beta-carotene (UPLC), total carotene (spectrophotometry), FFA, total lipid (Shoxlet), moisture, reduced sugar and total sugar (Nelson Somogyi) or used in the bioavailability experiment. Experimental diet were prepared by dissolve coconut sugar in plain water to make the sugar solution which contain approximately 40 µg beta-carotene per 1,5 mL of sugar solution. Detail of chemical composition of coconut sugar enriched with red palm oil were shown in Table 1.

Experimental animal: Thirty-4 weeks-old male Sprague Dawley rats (*Rattus norvegicus*) were obtained from the Department of Food and Drugs Supervision, Jakarta, Indonesia. Rats received the standard diet AIN-93M (Reeves, 1993) for two weeks as adaptation period and then twenty four rats were given a vitamin A-free diet (modified of AIN-93M) to deplete their vitamin A levels, of the remaining six rats which received the standard diet as a control group in depletion period. Rats were sacrificed in week 4, week 8 and week 10 of the depletion period and liver retinol and IgG concentration were determined. The remaining of 18 rats were divided into three groups: (1) RPO group which received palm sugar enriched with red palm oil, (2) CPO group which received palm sugar enriched with crude palm oil (1.5 mL/d) and 3) and RE group which received retinyl palmitate (0.6 mL/d). Each treatment contains approximately 40 µg beta carotene/day. All received vitamin A deficient diet during the repletion period. Composition of vitamin A deficient diet and vitamin A

Table 1: Chemical and physic characteristic of experimental brown sugar

Component	RPO coconut sugar	CPO coconut sugar
Moisture (%, grafimetri)	7.8	8.21
Total carotene (µg/100 g, spectrophotometry)	1337	3946
beta-carotene (µg/100 g, HPLC)	291	487
Total sugar (%) (Nelson Somogyi)	61.17	62.3
Reduce sugar (%, Nelson Somogyi)	2.22	2.61
Total lipids (%) (Shoxlet)	1.09	1.73
FFA (%, titration)	0.122	0.142
Texture (mm/g/sec, pnetrometer)	0.0023	0.0031

Table 2: Composition of vitamin A-free diet (modification of AIN 93 M) (Reeves *et al*., 1993)

Ingredient	g/kg diet
Casein (>85% protein)	140
Corn oil	50
Corn starch	615
Sucrose	100
Mineral Mix	35
Vitamin A free-vitamin mix	10
Fiber (CMC)	50
BHT	0.005

Table 3: Composition of free vitamin A-vitamin mix (modified of AIN-93M-VX)

Vitamin	g/kg mix
Nicotinic acid	3.0
D-calcium panthotenat	1.6
Pyridoxine HCl	0.7
Thiamine-HCl	0.6
Riboflavin	0.6
Folic acid	0.2
D-biotin	0.02
Vitamin B_{12} (0,1% triturated in manitol)	2.5
Vitamin E Alpha tocopherol powder (250 U/g)	30
Vitamin D_3 (400,000 U/g)	0.25
Phylloquinone	0.75
Powdered sucrose	961.255

free- vitamin mix were shown in Table 2 and Table 3, respectively. Each treatment contains approximately 40 µg beta-carotene and was administered daily for two weeks by force feeding. Content of energy, protein and fat per kg of the diet were 3425.7 Kal, 120.87 g and 49.95 g, respectively. Rats were weighed every five days during the whole experiment. At week 2 and week 4 of repletion period, rats were sacrificed and blood-sampled and had the liver removed, then the liver retinol and immunoglobulin G (IgG) levels were determined.

Serum and liver samples preparation: Blood and liver samples were collected after 4, 8 and 10 weeks of feeding in depletion period and after 2 and 4 weeks of feeding in repletion period. Blood were collected by cardiac puncture, under ketamine and xylazine anaesthesia. Blood was left at room temperature for 4 h and then centrifuged at 1200 g for 5 min to separate the serum which was then kept at -80°C. The livers were excised, weighed and stored at -80°C until assayed.

Serum retinol analysis: Serum retinol was analyzed by HPLC according to AOAC Official method (AOAC, 2005). Retinol extraction from serum were carried out by mixing 100 µL of serum samples with 100 µL of sodium diodezyl sulfate. The mixture was added 200 µL ethanol absolute and vortexed for 60 second at 2000 rpm. A milliliter of heptanes containing 0.5 g BHT/L was added as extraction solvent. Samples were vigorously vortexed during 1 min and then centrifuged at 1500 rpm for 10 minute in Damon/IEC Division Centrifuge. The heptanes was recovered. The washing process with extraction solvent was done three times. The heptanes extract were mixed and dried under nitrogen gas flow and re-dissolved in 200 µL of mobile phase, filtered through a 0.45 µm Millipore filter and 50 µL was injected onto the HPLC. A 10 µm µ Bondapak C18 (3.9x150 mm) column was used with methanol:acetonitrile:dichloromethane (235:235:30) as mobile phase at 1 mL/min flow rate and 5 min run time using a diode array detector at 325 nm. A calibration curve using a range of concentrations of retinol dissolved in methanol was constructed.

Liver retinol analysis: Liver retinol was analyzed by HPLC according to AOAC Official method (AOAC, 2005). Retinol extraction from liver was carried out by homogenizing 1 g liver with 10 mL 25% sodium ascorbate and vortexed for 20 sec. A saponification step was done with 2 mL KOH (10 M) and incubation at 80°C for 30 min in a water-bath. The solution was then cooled in a refrigerator for 5 min. Three mL of hexane containing 0.5% BHT was added as an extraction solvent, the tube was vigorously vortexed for 20 sec and then centrifuged at 1500 rpm for 10 min. The hexane layer was recovered. The extraction process was repeated twice more and the pooled hexane volumes were dried under nitrogen gas flow and re-dissolved in 200 µL of mobile phase, filtered through a 0.45 µm Millipore filter and 50 µL was injected onto the HPLC.

Serum immunoglobulin G (IgG) analysis: serum IgG were analyzed by rat IgG ELISA core Kit (Komabiotech, Inc.). The component of kit content were: coating antibody (goat anti-rat IgG), detection antibody (HRP conjugated goat anti-rat IgG), standard protein and prestained color development-reagen (TMB solution).
The materials used were: ELISA microplates, coating buffer (50 mM carbonate-bicarbonate buffer, pH 9.6), assay iluents (PBS, 1% BSA, pH 7.4), washing solution (PBS, 0.05% tween-20, pH 7.4), stop solution (2 M H_2SO_4), blocking solution (PBS, 1% BSA, pH 7.4). A hundred micro liter of diluted coating antibody were coat in each Basic-Life plate well and incubate at 4°C for overnight and then washed using washing solution for 3-5 times. A 200 µL of blocking solution was added to each well and incubate at room temperature for at least 1 h and were aspirated and washed with washing solution. Furthermore, 100 µL of standard or sample were transferred to assigned wells, aspirated and washed. The detection antibody was added of 100 µL and after aspirated and washed, 100 µL of TMB color development reagent were applied and mixed slightly the well, allow for about 30 min then 100 µL of stop solution were added and read at 450 nm on microtiter plate reader.

Statistical analysis: Results are expressed as the means±SE. The significance of differences between

means values was determined by one-way analysis of variance (ANOVA) and when ANOVA indicated significant differences ($p<0.05$), group means were compared by Duncan Multiple Range Test (DMRT).

RESULTS

Chemical and physic characteristic of experimental palm sugar: Chemical composition and texture both of CPO and RPO coconut sugars were presented in Table 1. The total carotene and beta carotene content of CPO coconut sugars were higher (3946 µg/100 g and 487 µg/ 100 g, respectively), than the RPO sugar (1337 µg/100 g and 291 µg/100 g, respectively). Coconut sugar enriched with CPO had the moisture content and reduce sugar namely 8.21 and 2.61%, respectively, while the RPO sugar had 7.8 and 2.22%, respectively, which were still in range with the Indonesian National Standard (SNI) for coconut sugar (<10%). Both products also had a similar texture, however coconut sugar which enriched with RPO had a tougher texture (0.0023 mm/g/sec) than the CPO sugars (0.0031 mm/g/sec).

Changes in body weight, liver retinol and immunoglobulin G (IgG) concentrations during the depletion and repletion period: Rats underwent depletion period for 10 weeks which given vitamin A-free diet (modification of AIN 93 M) (Reeves, 1993), to deplete their liver retinol level with cut off point of <007 µmol/g liver (Gropper *et al*., 2009). On the onset of depletion period up to week 5, delta increase of body weight was 33.55 g (15.76%) and from week 6 to 10 delta decrease of body weight was 3.75 g (1.79 %) from initial weight or 29.8 g (21.23%) from optimal weight (Fig. 1). Body weight loss was followed by hair loss on the start of week 7 and from week 9-10 some rats excreted the abnormal or mushy feces which indicating rats in infected state.

After 10 weeks without vitamin A intake, hepatic retinol levels of rats in depletion group decreased under cut off point (0.07 µmol/g liver), namely 0,063 µmol/g liver, indicating the vitamin A deficient rats (Fig. 2).

After received provitamin A obtained from CPO or RPO enriched coconut sugar in CPO and RPO groups, respectively, or vitamin A (retinyl palmitate) in RE group, which given as much as 40 µg beta-carotene/day for two weeks, the hepatic retinoid level increased in groups of CPO, RPO and RE from 0.063 µmol/g liver to 0.117 (85.55%), 0.102 (50.98%) and 0.204 (174.72%) µmol/g liver, respectively. The following two weeks' uptake (four week repletion) however showed decreased hepatic retinoid level in all groups, CPO, RPO and RE as much as 0.0101, 0.0234 and 0.0018 µmol/g liver, respectively (Fig. 3).

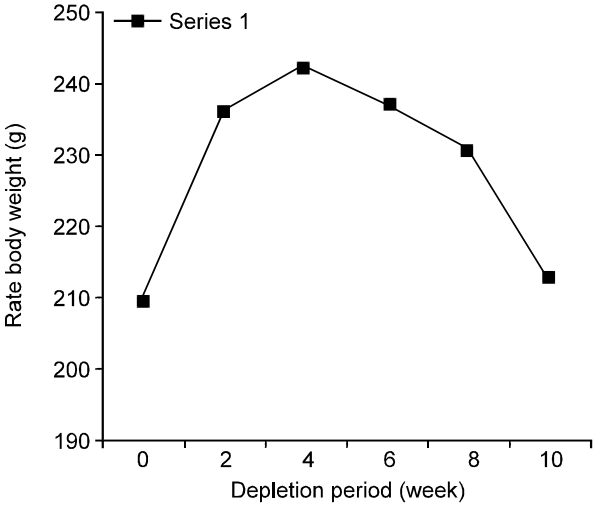

Fig. 1: Body weight changes of rats during depletion period (mean±SE)

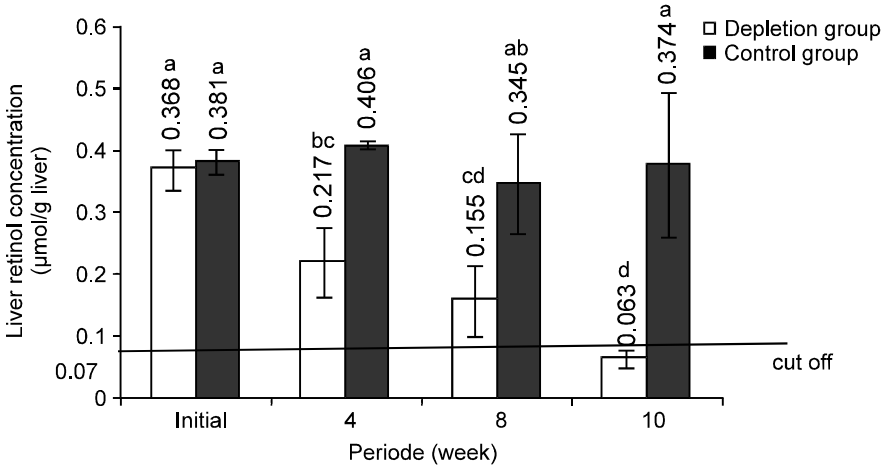

Fig. 2: Changes on liver retinol concentration during depletion period in depletion group and standard control group (mean±SE)

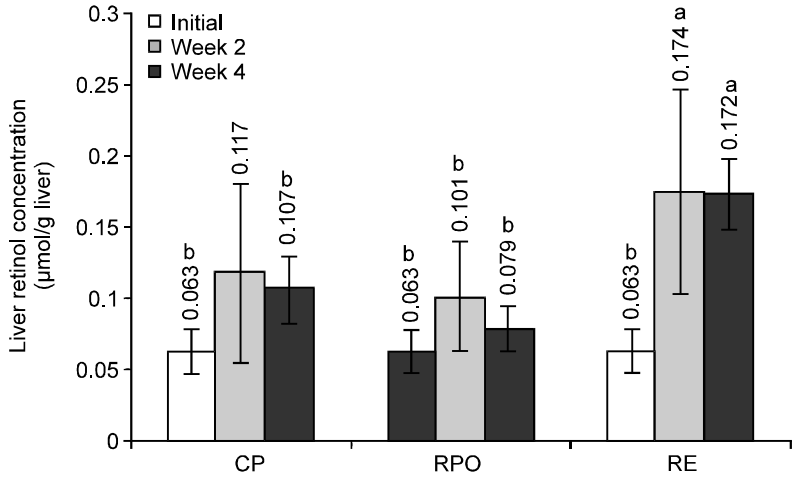

Fig. 3: Liver retinol concentrations changes in CPO, RPO and RE groups during repletion period ($p<0.05$)

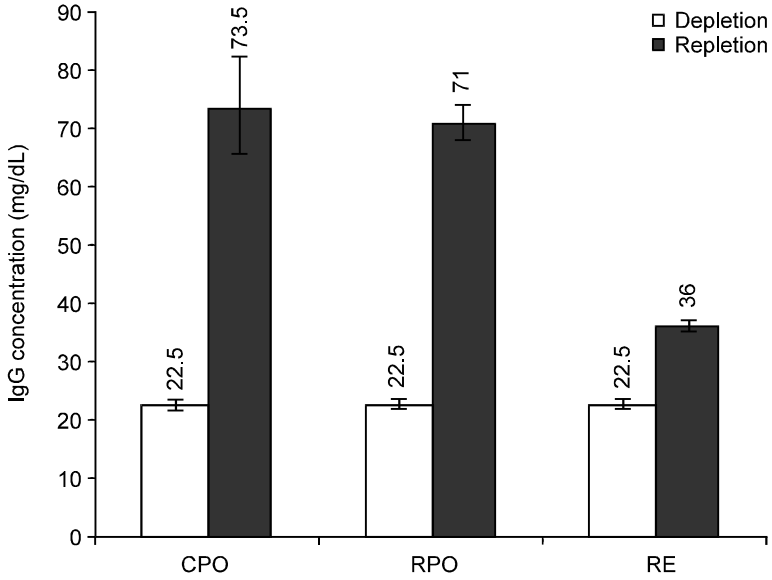

Fig. 4: Immunoglobulin G concentration during the depletion and repletion period

Vitamin A deficiency influenced body immune as observed from the low immunoglobulin G (IgG) during depletion period. The average of IgG in depletion period was 22.5±0.707 mg/dL. Vitamin A deficiency would restrain humoral and cellular immunity (Yang *et al*., 2011). Administering of CPO and RPO enriched coconut sugar containing provitamin A significantly resulted in better immunity than retynil palmitate (RE) as observed from the high level of IgG in CPO and RPO groups as much as 73.5 mg/dL (225.99%) and 71 mg/dL (216.01%), respectively. RE group showed lower IgG level of 36 mg/dL or increasing percentage of 59.98% (Fig. 4).

DISCUSSION

From the initial of depletion period to week 5, rats' body weight increased despite the absence of vitamin A intake because rats can be use the reserved liver retinol to meet the normal demand amount for metabolism. Liver has the capacity to reserve vitamin A supply for months (Semba and Bloem, 2003) so when vitamin A intake in absent, body can use the reserved vitamin A in the liver. This is shown from the ever decreasing liver retinol reserve. Comparatively, liver retinol level in control group was stable due to sufficient vitamin A intake in daily diet. The means level of liver retinol at the end of depletion period of depletion rats group and standard control group were 0.063±0.0144 µmol/g liver and 0.374±0.115 µmol/g liver, respectively (Fig. 2). Liver retinol level in depletion group significantly decrease from 0.217 to 0.063 µmol/g liver (depleted 70,97% or decreased 3.43 times) ($p<0.05$). Research by Lopez *et al*. (2010) showed a more slightly decrease of 64% during 12 week of depletion period.

Vitamin A and provitamin A intake of 40 µg beta-carotene/day showed increasing liver retinol level in all treatment groups. Based on statistic analysis by ANOVA showed that hepatic retinoid level at week 2 and week 4 in group administered with retinyl palmitate was significantly higher than that of CPO and RPO enriched coconut sugar ($p = 0.0136$; alpha = 0.05) (Fig. 3). Retynil palmitate performed faster response in improving vitamin A status of rats namely 2.72-2.75 times compared to CPO or RPO group with increased hepatic retinoid level as many as 1.7-1.86 times and 1.24-1.62 times, respectively.

This is because retinyl palmitate is a form of vitamin A was easily metabolized than carotene and more efficient in returning vitamin A reserve in liver. Carotene in CPO and RPO will initially turn into retinol through a chain reaction of oxidation and reduction (Gropper *et al*., 2009). Human intestines has as well a limited capacity to absorb beta-carotene. Many studies showed that only 37% from 40 mg beta-carotene was converted into retinol (O'Neil and Thurnham, 1998), while 64% from 15mg beta-carotene was converted into retinol (Vliet *et al*., 1995). Another studies reported that carotenoid bioavailability from RPO was 37% (Huang *et al*., 2000), while Burri (2012) further reported that retinol equivalence from RPO was 6.7:1 (carotenoid: retinol). Feeding CPO and RPO enriched coconut sugar as source of provitamin A for 2 to 4 week however had improved vitamin A status of deficient rats back to normal up to cut off point of 0.07 µmol/g liver. This result in our research is in line with Stuijvenberg *et al*. (2000, 2001), which administering RPO enriched biscuit with 34% RDA for six months to students could improve vitamin A status by increasing 46.7% retinol serum, while three-month treatment only resulted in 15.4% increase. Similarly, 5 mL RPO daily uptake to students for one year increased retinol serum from 33.45 to 63.75 µg/dL (Sivan *et al*., 2002).

Administering 40 µg beta-caroten/day provitamin A or vitamin A for four weeks in this research was proved to improve immune status of depletion rats. All groups had the IgG level increased after received the experiment diet. The groups given CPO and RPO enriched palm sugar had significantly increased better immmune responses than that of retinyl palmitate group as observed from higher immunoglobulin G (IgG) ($p = 0.0073$). The IgG concentrations of CPO, RPO and RE group during 4 weeks of intervention increased from 22.5 mg/dL up to 73.5, 71 and 36 mg/dL, respectively (Fig. 4). Vitamin A up-regulates the T-helper type 2 (Th2) cells and promotes humoral immunity, antibody production, Ig maturation and deactivation of macrophages (Garcia, 2012).

High level of IgG in CPO and RPO groups might due to other components than carotenoid in CPO and RPO to maintain optimal health as antioxidant or immunostimulant, mainly vitamin E and lycopene. Red palm oil contains 560-1000 ppm vitamin E consisting of 18-22% tocopherol and 78-82% tocotrienol (Bester *et al*. 2010; Sundram, 2003), 18.5-38 ppm lycopene (Benade 2013; Aziz, 2006). Vitamin A, beta carotene and vitamin E are co-factors in immune response (Chunningham *et al*., 2005). Vitamin E performs immune-enhancing effect by stimulating cell proliferation in immunopoietic, increases the number of antibody produced by plasma cells and induces antibody production shift from IgM to IgG (Tengerdy *et al*., 2003). Gabriel *et al*. (1984) reported that in three strains of vitamin A deficient rats, administering 200 mg/kg diet significantly increased lymphocyte proliferation. Similarly, vitamin E and lycopene synergize in building immune response and well protection against prostate cancer (Pryor *et al*., 2000).

Conclusion: Feeding palm sugar which enriched with CPO or RPO for 2-4 weeks was proved to improve liver retinol concentration above cut off point (>0.07 µmol/g liver) and had the level increased of immunoglobulin G of vitamin A depletion rats. From this research we conclude that palm sugar enriched with CPO or RPO were potentially as food-based intervention to overcome vitamin A deficiency.

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REFERENCES

AOAC, 2005. Official Methods of analysis. Association of Official Analytical Chemists, Washington DC,2005. Methode 2001.13. p: 50-53.

Aziz, A.A., 2006. Development of HPLC analysis for detection of lycopene in tomato and crude palm oil. Malaysia (): Faculty of Chemical Engineering and Natural Resources. University College of Engineering and Tecnology Malaysia, 2006.

Benade, A.J.S., 2013. Red palm oil carotenoids. Potential role in disease prevention. In: Watson, R.A., V.R.Preedy, editor. Bioactive Food as Interventions for Cardiovascular Disease. Elsevier. London, p: 333-343.

Bester, D., A.J. Esterhuyse, E.J. Truter and J. van Royen, 2010. Cardiovascular effects of edible oil: a comparison between four popular edible oils. Nut. Res. Rev., 23: 334-348.

Burri, B.J., 2012. Evaluating global barriers to use of red palm oil an as an intervention food to prevent vitamin A deficiency. Comprehensive Rev. in Food Sci. and Food Safety, 11: 221-232.

Canfield, L.M., R.G. Kaminsky, D.I. Taren, E. Shaw and J.K. Sander, 2001. Red palm oil in the maternal diet increases provitamin A carotenoids in breast milk and serum of the mother-infant dyad. Eur. J. Nutr., 40: 30-38.

Chunningham, R.S., D.F. Mc Neeley and A. Moon, 2005. Mechanism of nutrient modulation of the Immune response. Am. Academy of Allergy, Asthma and Immunology. Doi:10.1016/j.jaci., 5.04.036.

Gabriel, E., A. Bendich and L.J. Machlin, 1984. Strain difference in testes degeneration, myopathy and the lymphoyte mitogen response in vitamin E deficient rats. Proceed. of the Soc. for Exp. Biol. and Med., 176: 378-383.

Garcia, O.P., 2012. Effect of vitamin A deficiency on the immune response in obesity. Proceed. of the Nutr. Soc., 71: 290-297.

Gropper, S.S., J.L. Smith and J.L. Groff, 2009. Advanced Nutrition and Human Metabolism. 5th ed. Wadsworth, Cengange Learning, Canada, 373-391.

Hidayah, D., H. Riyadi, R. Rimbawan, E. Damayanti, A. Sulaeman and E. Handharyani, 2013. Addition of Red Palm Oil as Source of Provitamin A on Carotene Retention, Physical and Sensory Properties of Brown Sugar. J. TIP, forthcoming.

Huang, C.J., Y.L. Tang, C.Y. Chen, M.L. Chen, C.H. Chu and C.T. Hseu, 2000. The bioavailability of beta-carotene in stir or deep-fried vegetables in men determined by measuring the serum response to a single ingestion. J. Nutr., 130: 534-540.

Klemm, R.D., K.P. West, A.C. Palmer, Q. Johnson, P. Randall, P. Ranum and C.C. Northrop, 2010. Vitamin A fortification on wheat flour: considerations and current recommendations. Food Nutr. Bull., 31: S47-61.

Lietz, G., C.J.K. Henry, G. Mulokozi, J.K.L. Mugyabuso, A. Ballart, G.D. Ndossi, W. Lorri and A. Tomkins, 2001. Comparison of the effects of supplemental red palm oil and sunflower oil on maternal vitamin A status. Am. J. Clin. Nutr., 74: 501-9.

Lopez, A.C., E.M. Yahia and P.G.K. Ramirez, 2010. Bioconvertion of caroteoids in five fruits and vegetables to vitamin A measured by retinol accumulation in rat livers. Am. J. Agric. and Biol. Sci., 5: 215-221.

O'Neil, M.E. and D.I. Thurnham, 1998. Intestinal absorption of beta-carotene, lycopene and lutein in man and women following a standard meal; response curves in the tryacilglycerol-rich lipoprotein fraction. Br. J. Nutr., 79: 149-159.

Pryor, W.A., W. Stahl and C.L. Rock, 2000. Beta carotene: from biochemistry to clinical trials. Nutr. Rev., 58: 39-53.

Radhika, M.S., P. Bhaskaram, N. Balakrishna and B.A. Ramalakshmi, 2003. Red palm oil supplementation: A feasible diet-based approach to improve the vitamin A status of pregnant women and their infants. Food and Nutr. Bull., 24: 208-217.

Reeves, P., F.H. Nielsen and Fahey Jr., 1993. AIN 93 Purified diet for laboratory rodent: Final report of the American Institute of Nutrition ad hoc Writing Committee on Reformulation of the AIN 76A Rodent Diet. J. Nutr., 123: 193-1951.

Rice, A.L. and J.B. Burns, 2010. Moving from efficacy to effectiveness: Red Palm Oil's role in preventing vitamin A deficiency. J. Am. Coll. Nutr., 29: 302S-313S.

Semba, R.D. and M.W. Bloem, 2003. The anemia of vitamin A deficiency: Epidemiology and pathogenesis. J. Clin. Nutr., 56: 271-281.

Sivan, S.Y., Y.A. Jayakumar, C. Arumughan, A. Sundaresan, A. Jayalekshmy, K.P. Suja, D.R.S. Kumar, S.S. Depa, M. Damodaran, C.R. Soman, V.R. Kutty and P.S. Sarma, 2002. Impact of vitamin A supplementation through different dosages of red palm oil and retinol palmitate on preschool children. J. Trop. Pediatrics, 48: 24-28.

Sommer, A. and K.P. West, 1996. Vitamin A deficiency: Health, Survival and Vision. Oxford University Press, New York.

Stuijvenberg, M.E., M. Faber, M.A. Dhansay, C.J. Lombard, N. Voster and A.J.S. Benade, 2000. Red palm oil as source of beta-carotene in a school biscuit used to address vitamin A deficiency in primary school children. J. Food Sci. and Nutr., 51: S43-S50.

Stuijvenberg, M.E., M.A. Dhansay, C.J. Lombard, M. Faber and A.J.S. Benade, 2001. The effect of a biscuit with red palm oil as a source of beta-carotene on the vitamin A status of primary school children: a comparison with beta-carotene from a synthetic source in a randomized controlled trial. Eur. J. Clin. Nutr., 55: 657-662.

Sundram, K., R. Sambanthamurthi and Y.A. Tan, 2003. Palm fruit chemistry and nutrition. Asia Pac. J. Clin. Nutr., 12: 355-362.

Tengerdy, R.P., D.L. Meyer, L.H. Lauerman, D.C. Lueker and C.F. Nockels, 2003. Vitamin E enhances humoral antibody response to Clostridium perfringens type D in sheep. J. Vet., 139: 147-152.

Van Vliet, T., W.H.P. Schreurs and H. Van Den Berg, 1995. Intestinal beta-carotene absorption and cleavage in man: response of beta-carotene and retynil ester in the triglyceride-rich lipoprotein fraction after a single oral dose of beta-carotene. Am. J. Clin. Nutr., 110-116.

WHO, 2009. Global prevalence of vitamin A deficiency in populations at risk 1995-2005. WHO Global Database on Vitamin A Deficiency. Geneva, Switzerland.

Yang, Y., Y. Yajie, T. Yuehong and W. Weiping, 2011. Effect of vitamin A deficiency on mucosal immunity and response to intestinal infection in rat. J. Nutr., 27: 227-232.

Zagre, N.M., F. Delpeuch, P. Traissac and H. Delisle, 2003. Red palm oil as a source of vitamin A for mothers and children: impact of a pilot project in Burkina Faso. Pub. Health Nutr., 6: 733-742.

Zeba, N.A., P.Y. Martin, S.T. Issa and H.F. Delisle, 2006. The positive impact of red palm oil in school meals on vitamin A status: Study in Burkina Faso. Nutr. J., 5: 1-10.