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Efficacy of Red Palm Oil and Carrot Noodle on Blood Retinol, IgG and Nutritional Status of Children Aged 7-9 Years

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Abstract: The main objectives of this study were to analyze the efficacy of red palm oil (RPO) and carrot instant noodles in improving serum retinol levels, IgG levels and nutritional status compared to control group. RPO and carrot noodles were given to elementary school children 7-9 year of age (n = 11, respectively) once a day (50 g/ss) for 8 week. In quacy experimental design, the levels of serum retinol and IgG were measured twice in before and after intervention. Before intervention more than 60% children of control, RPO and carrot group had marginal vitamin A status, which had mean serum retinol of 17.57, 16.87 and 17.28 µg/dL, respectively. After intervention mean serum retinol were 21.32, 23.49 and 20.87 µg/dL, respectively and the percentage of marginal status of vitamin A were decreased. There were no significant differences in serum retinol levels among all groups before and after intervention. Mean IgG levels of control, RPO and carrot group were 2.05, 2.61 and 3.61 IU/mL at baseline and 5.30, 14.89 and 9.16 IU/mL as follow-up at end line, respectively. There were no significant differences in the IgG level among all group before intervention, while there were significant differences between IgG level of RPO versus control and carrot group after intervention. Mean nutritional status (weight for age) of control, RPO and carrot group were -1.73, -2.04 and -1,86 at baseline and -1.41, -1.77 and -1,46 as follow-up at end line, respectively. There were no significant differences in nutritional status among all groups before and after intervention.

Key words: Efficacy, RPO, carrot, noodle, retinol, IgG

INTRODUCTION

Over the past three decades, lack of Vitamin A (KVA) have been recorded as a public health problem and are the main cause of illness and death of preschool-aged children in developing countries (Maqsood, 2004). The WHO states that KVA suffered by about 40% of the world's population, especially pregnant or breast-feeding women and children under five years of age. More than 127 million children in the world experiencing the insufficiency of intake of Vitamin A (West, 2002).

Effects of lack (deficiency) of vitamin A in the body can cause a failure in the function, characterized by systemic abnormalities, anemia, fetal development and weak immune function. KVA may also cause keratinization of the mucous membrane on the lining of the respiratory tract, gastrointestinal tract, urinary tract, skin and epithelium on eyes (Mahan and Stump, 2004). One of the efforts to prevent the problem is fortification of vitamin A into food or with the addition of carotene as a precursor of vitamin A in the diet. In addition to using artificial fortificant, its may also use food as a source of provitamin A (especially β-carotene). The potential use of natural food is Red palm oil (RPO) and carrots. RPO has a high content of β-carotene which is 15-30 times higher than carrot and tomatoes (Ball, 1988). In addition to having a high content of provitamin A, the RPO is quite abundant in Indonesia. According to the BPS (2007), the amount of palm oil production in Indonesia until 2007 was approximately 11.8 million tons. Ketaren (2005) states that the high content of beta-carotene on RPO make RPO as a very potential as vitamin A fortificant.

Carrot is one of a group of horticultural commodities vegetables that potential for public health. Carrot production in Indonesia is quite abundant. According to data of the Central Bureau of statistics (2007) carrot production in Indonesia reached 409.465 tons with 192.964 tons of carrots is production from West Java province. The carrot is famous as a source of provitamin A is due to high content of beta-carotene. Content of carotene in carrot reach 2000 µg RE/g 100 g edible portion (Ball, 1988). Carotene in carrots accumulate and reach a maximum concentration after the plant was about 90-120 days (Rubatzky and Yamaguchi, 1997).

In Indonesia, one of the potential food for fortified is instant noodles. Instant noodle is food in every State in Asia and is the preferred food by all age, the price is affordable and easily obtainable. Instant noodles are very popular and widely consumed either as a main dish

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or as a food between meal (snack). SUSENAS Data for 1999-2002 indicate that consumption of noodle in Indonesia had increased from 3.1 kg/cap/yr to 4.3 kg/cap/yr. Through this study instant noodles was enriched with the addition of Red Palm Oil (RPO) and carrot flour as a natural source of provitamin A. The noodles that known as sources of carbohydrates can be improved as food alternatives for alleviate the problem of KVA.

The purpose of this study was to evaluate the effect of carrot and RPO instant noodles on serum retinol levels, immune response and the nutritional status of children aged 7-9 years.

MATERIALS AND METHODS

Design and location: The design of this research was Quasy Experimental pre post treatment controlled trial. The research design was used to evaluate the effect of carrot and RPO instant noodles on serum retinol levels, immune response and the nutritional status of children aged 7-9 years. Interventions were carried out over two months at SDN Angsana 1, Leuwiliang subdistrict, District of Bogor. Analysis of retinol levels and immune response (level of IgG) was done in the laboratory of Research and Development Centre for nutrition and food, the Ministry of Health of the Republic of Indonesia, Bogor. This study was approved by the ethics commission of the Ministry of Health of the Republic of Indonesia Number LB.03.02/KE/5393/2010 on July 9, 2010.

Subjects: Subjects in this research were children of elementary school aged 7-9 years, healthy (not suffer of secondary infections) based on the results of the examination from the doctor, have already received the explanation about the research, agreed upon informed consent and agreed to follow the research procedure. They were allocated into 3 (three) group of intervention, named Control Group, RPO Group and Carrot Group. The main research variables that was examined in this study were serum retinol levels and immune response as measured by serum IgG levels. Other variables in this study were the nutritional status (as measured by anthropometry with index W/A), characteristics of elementary school children and their families, food consumption and the morbidity. RPO and Carrot instant noodles were given to elementary school children 7-9 y of age (n = 11, respectively) once a day (50 g/ss) for 8 week. Total instant noodles given to each subject during intervention was 60 packs. Instant noodles are served, prepared by boiling. Provision of instant noodles do during recess at school (at 09.30 am), so that all subjects can consume noodles intervention at the same time, while at a holiday noodles was taken by subject to home. The levels of serum retinol and IgG were measured twice in before and after intervention and vaccinated for *Tetanus toxoid* (TT) at 2 week of intervention.

Data collection and statistical analysis: The primary data collected in this research were the data of subject and their family. The subject's data included the identity of the child (name, gender, weight, order of a child), health status, food consumption, level of retinol serum, level of serum IgG, Anthropometry (weight and height), morbidity level and the level of compliance. Child identity data obtained through interviews with the child and parents or care giver by using questionnaires, health status of children (morbidity) obtained through clinical examination and observations and interviews by medical personnel and researchers to the child, parents (care givers) and teachers use a questionnaire. Food consumption data obtained through a Food recall, while serum retinol levels obtained through laboratory analysis by the method of extraction (Concurrent Liquid Chromatographic Assay of Retinol) and the levels of serum IgG obtained through laboratory analysis by the method of ELISA (*Enzyme-Linked Immunosorbent Assay*). Data weight (W) obtained by weighing with scales weight Stampede analog, with accuracy of 0.1 kg, height data (H) obtained through measurements using microtus, with an accuracy of 0.1 cm, the compliance level and instant noodle consumption obtained through observation in the classroom and interview to the child.

Identity and family characteristics of subjects were analyzed using descriptive statistics and frequency statistics. Differences in levels of serum retinol and IgG, as well as nutritional status of the treatment group and the control group both at the beginning and end of the intervention, were analyzed using ANOVA.

RESULTS

Subjects characteristics: The subjects were students of 2nd elementary school with an average age of 8 years. Subjects in the control group, the RPO group and carrot group mostly a 2nd child with a number of 27.3% for the control group, 45.4% for the RPO group and 36.4% for the carrots. Subjects in control group and carrots group mostly have families in modest category with the proportion of 72.7% in the control group and 45.5% in the carrots, while in the RPO group most of the subjects are small families (45.4%).

By category of pursued education, mostly fathers in the control group, the RPO group and carrot groups only studying until complete of primary school with a proportion of 54.5% for the control group, 54.5% for the RPO group and 63.6% for carrots group. Based on the type of father's job, most of father worked as farmer and non-farmer. In the control group the number of families whose fathers worked as farmer was 45.5%, in the RPO group was 36.4% while in the carrot group was 54.5%. Father who worked as non-farmer in the control group was 27.3%, in the RPO group was 54.5% while in the carrot group was 18.2%. Based on income per capita of fathers in the control groups and carrots group, most of

the families were categories as poor families (63.6% for the control group and 81.8% for the carrots). In the RPO group, most of the families were categories as non-poor family (72.7%).

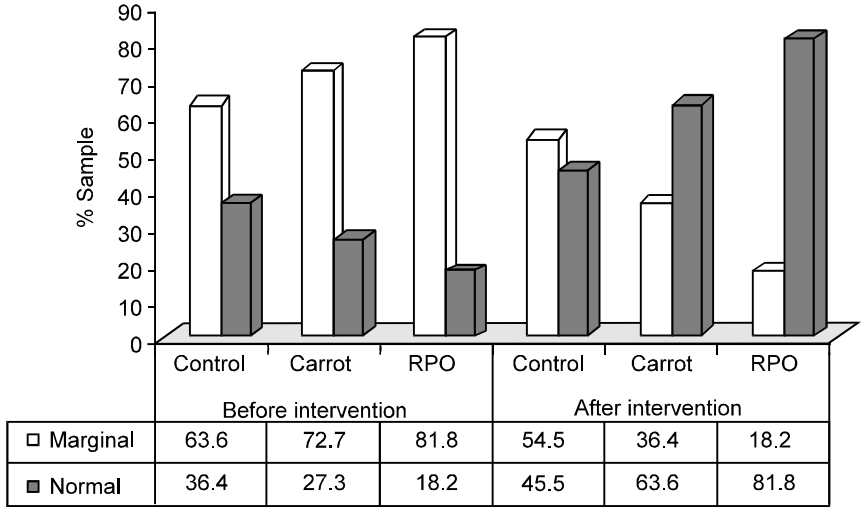
Serum retinol level: The range of serum retinol levels of subjects was 11.06-26.84 µg/dL before intervention. Average levels of serum retinol of control group before intervention was 17.57±4.83 µg/dL, carrot group was 17.28±3.77 µg/dL and RPO group was 16.88±4.86 µg/dL (Table 1). Average level of serum retinol of both the control group and treatment group (carrot and RPO) has increased after the intervention. Average of an increase in serum retinol levels in the control group was 3.75±5.50 µg/dL, the carrot group was 3.59±5.61 µg/dL and the RPO was 4.62±4.61 µg/dL. The difference test on each group to compare the serum retinol level between before and after intervention showed that serum retinol levels before intervention in control group, carrot and RPO group were lower significantly as compared to after intervention ($p<0.05$). But analysis of Variance (ANOVA) indicated that the level of serum retinol level among each group was not significantly different at before and after intervention period.

The distribution of subjects based on vitamin A status at before and after intervention period can be seen in Fig. 1. Before the intervention, most of the subjects in control group (63,6%) have marginal vitamin A status and about 36.4% were classified as having normal vitamin A status. Around 72,7% subjects of carrot group have marginal vitamin A status and 27.3% as normal. In RPO group around 81,8% of the subjects categorized in marginal vitamin A status and only two subject (18.2%) having the normal category. There were no subject with deficient and excess in vitamin A status in all groups.

Immunoglobulin levels (IgG): Before intervention, the average levels of IgG of control group, carrot group and RPO group were 2.05±2.92, 3.61±3.19 and 2.61±2.57 IU/mL, respectively. Serum IgG levels in both control group and treatment group were in the high category. Table 2 showed that after eight weeks of intervention, an elevation in the average of IgG level in control group was 3.25±2.44 IU/mL as well as in the carrot (5.55±5.84 IU/mL) and RPO group (12.28±6.18 IU/mL), respectively. Before the intervention, there was no significant difference between the levels of serum IgG of control group as compared to the treatment group. After the intervention the results indicated that the levels of IgG of RPO group differs significantly as compared to carrots group as well as the control group and also the delta. There was no significant difference between IgG level of the carrot group as compared to control group.

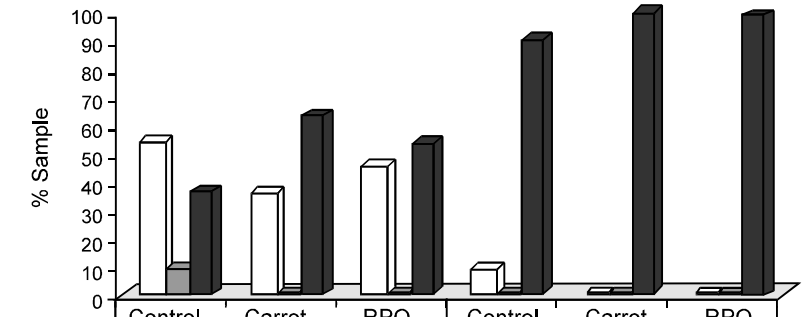
Figure 2 showed the distribution of subjects based on the categories of serum IgG levels. Before intervention, most of the subjects in the control group (54.5%) have low levels of IgG, 9.1% have sufficient levels of IgG and the rest (36.4%) have a high levels of IgG. The majority of carrot subjects (63,6%) have high serum IgG levels and 36.4% have low serum IgG levels and there were no subjects categorized as medium in the level of serum IgG. Mostly subjects (54.5%) in the RPO group have a high IgG level and 45.5% of the subject have low IgG level before intervention.

After intervention the percentage of subjects with high level of IgG in all groups were increased. In the control group an increase in percentage of the subject with high levels of IgG category was 54.5%, in carrot group was 36.4% and in the RPO group was 45.5%. There was only 9.1% of subjects in the control group that have low IgG level, while in treatment group all of the subjects have a high IgG levels after the intervention period.



	Before intervention			After intervention		
	Control	Carrot	RPO	Control	Carrot	RPO
Marginal	63.6	72.7	81.8	54.5	36.4	18.2
Normal	36.4	27.3	18.2	45.5	63.6	81.8

Fig. 1: Distribution of subjects based on vitamin A status at before and after intervention



	Before intervention			After intervention		
	Control	Carrot	RPO	Control	Carrot	RPO
Low	54.5	36.4	45.5	9.1	0	0
Normal	9.1	0	0	0	0	0
High	36.4	63.6	54.5	90.9	100	100

Fig. 2: Distribution of subjects based on IgG at before and after intervention

Table 1: Average of serum retinol levels of subjects before and after intervention

	Control	Carrot	RPO	p-value
Before intervention	17.57±4.83[a]	17.28±3.77[a]	16.88±4.86[a]	0.937
After intervention	21.32±5.02[a]	20.87±3.62[a]	23.49±5.71[a]	0.412
Delta	3.75±4.20[a]	3.59±5.61[a]	4.62±4.61[a]	0.273

*On the same line, number with the difference letter were shown statistically significant difference between groups (p<0.05)

Table 2: Average of IgG serum level of subject before and after intervention

	Control	Carrot	RPO	p-value
Before intervention	2.05±2.92[a]	3.61±3.19[a]	2.61±2.57[a]	0.449
After intervention	5.30±3.24[a]	9.16±5.74[a]	14.89±6.16[b]	0.001*
Delta	3.25±2.44[a]	5.55±5.84[a]	12.28±6.18[b]	0.001*

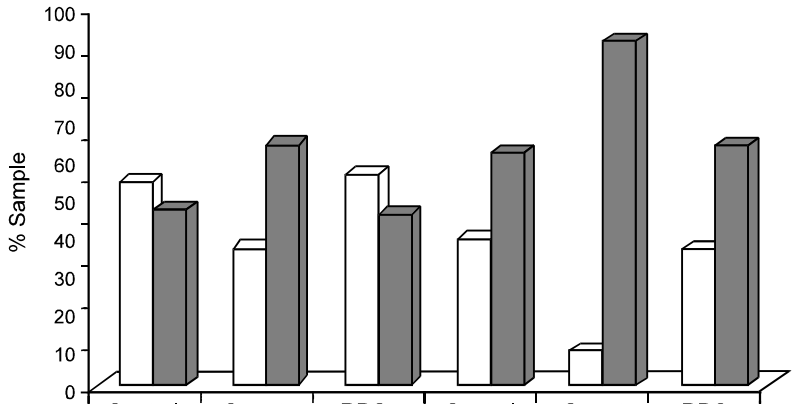
*On the same line, number with the difference letter were shown statistically significant difference between groups (p<0.05)

Body weight: Before intervention the body weight of subjects were around 15 kg up to 24 kg. Body weight is one factor that can be used to determine individual nutritional status. Before intervention, subjects of control group have an average body weight of 19.97±1.75 kg with the lowest body weight was 18 kg and the highest body weight was 23 kg. Subjects of carrot group have an average body weight of 19.50±1.79 kg with the lowest body weight was 16.5 kg and the highest was 22.5 kg, while in the RPO group, the average body weight was 18.45±2.43 kg with the lowest body weight was 15 kg and the highest body weight 22 kg (Table 3).

Statistical results by ANOVA showed that the average body weight of subjects in the control group was not differ as compared to the treatment group before intervention period ($p>0.05$). Within 8-week follow the intervention, the average body weight of subjects in control, carrots and RPO group were increased with γ value±SD: 1.48±0.73, 1.59±8.61 and 1.09±0.94 kg, respectively. There were statistically significant differences between the average body weights of control group as compared to the RPO group after the intervention ($p<0.05$). In contrast, there were no differences between control and carrot group ($p>0.05$).

Nutritional status: Before the intervention, neither subjects of carrot group nor control group have a normal nutritional status (z-score value of control group was -1.73±0.80 and carrot group was -1.86±0.39), whereas the average nutritional status within subject of RPO group was an underweight (z-score value was -2.04±0.52). Both before and after intervention, there were no subjects in the state of poor nutritional status and overweight. There were an increase in average z-scores value in neither control nor treatment group (carrot and RPO) (Table 4).

Statistical analysis showed that there was no significant differences in the nutritional status between control and treatment group ($p>0.05$) both before and after the intervention period. Delta z-score weight/age also showed no differences. Before the intervention, there were 53.9% of subjects in the control group categorized as underweight and 46.2% as normal nutritional status (Fig. 3). As well as control group, before intervention, the percentage of underweight in the RPO group was greater than normal nutritional status. There were 55.5% of subjects in the RPO group classified as underweight and the rest of 45.5% subjects classified as normal nutritional status. In contrast, carrot group has a greater percentage of normal nutritional status (63.6%) rather than underweight (36.4%).



	Before intervention			After intervention		
	Control	Carrot	RPO	Control	Carrot	RPO
Under weight	53.9	36.4	55.5	38.5	9.1	36.4
Normal	46.2	63.6	45.5	61.5	90.9	63.6

Fig. 3: Distribution of nutritional status among the subjects at before and after intervention

Table 3: Average body weight of subjects on before and after intervention (mean value±SD)

	Control	Carrot	RPO	p-value
Before intervention	19.97±1.75[a]	19.50±1.79[a]	18.45±2.43[a]	0.212
After intervention	21.45±1.81[a]	21.09±1.36[ab]	19.55±2.25[b]	0.049
Delta	1.48±0.73[a]	1.59±8.61[a]	1.09±0.94[a]	0.361

*On the same line, number with the difference letter were shown statistically significant difference between groups ($p<0.05$)

Table 4: Average of *z*-score value (body weight/age) of subject before and after intervention

	Control	Carrot	RPO	p-value
Before intervention	-1.73±0.80[a]	-1.86±0.39[a]	-2.04±0.52[a]	0.464
After intervention	-1.41±0.83[a]	-1.46±0.42[a]	-1.77±0.55[a]	0.347
Delta	0.32±0.21[a]	0.40±0.29[a]	0.27±0.30[a]	0.554

**On the same line, number with the difference letter were shown statistically significant difference between groups ($p<0.05$)

After the intervention, nutritional status in each group was increased. The nutritional status was improved with an increased percentage of subjects with normal nutritional status from 46.2% up to 61.5% in control group. In carrot group, the percentage of subjects with normal nutritional status were increased from 63.6% up to 90.9%, while in RPO group, were increased from 45.5 to 63.6% (Fig. 3). The carrot group has the highest percentage of subjects with normal nutritional status as compared to other group after the intervention period.

DISCUSSION

The elevation of serum retinol in RPO and carrot group after intervention was attributable to an increase in the intake of vitamin A from the intervention foods (RPO and carrot instant noodles). Carrot instant noodle has 398 RE of vitamin A or contributed for 79.6% of daily value, whereas RPO instant noodle has 653 RE of vitamin A or contributed for 127% of daily value. According to food intake analysis (24-h food recall method) indicated that another vitamin A-containing food in the diet were only contributed to a 6.5 and 20.5% of daily value in carrot and RPO group, respectively. In case of excess vitamin A intake of daily value, body will stores the conversion rate of provitamin A (β-carotene) to vitamin A (retinol) in the liver. It might be occurred over the homeostatic control of vitamin A in the body.

Analysis of Variance (ANOVA) indicated that the level of serum retinol level among each group was not significantly different at before and after intervention period. The rationale may be due to vitamin A derived from the conversion of provitamin A from RPO and carrot instant noodles were stored in the liver. Deposits of retinol in the treatment group (RPO and carrot group) were higher than in control group. Study on animal by Karlina (2011) showed that level of retinol in blood serum of control group was not significantly different as compared to RPO and carrot group, while level of retinol in the liver was significantly lower as compared to the RPO and carrot group. Another study conducted by Ribaya-Mercado *et al.* (2007) showed that Carotene-rich yellow and green leafy vegetables, when ingested with minimal fat, enhance serum carotenoids and the total-body vitamin A pool size and can restore low liver vitamin A concentrations to normal concentrations.

Before intervention, there was no significant difference between the levels of serum IgG in control group as

compared to the treatment group. After intervention, the results indicated that the levels of serum IgG in RPO group was significantly higher as compared to the carrot group as well as the control group. There was no differences between the carrot group as compared to a control group in IgG level. The elevation of IgG level in the RPO group after intervention may be due to the provision of RPO instant noodles which contain more carotene as compared to the carrot group and control group. RPO instant noodles might be proper as an alternative food product-containing β-carotene to improve vitamin A status particularly in primary school children. Van Stuijvenberg *et al*. (2001) indicated that biscuit with red palm oil as a source of b-carotene is as effective as a biscuit with synthetic β-carotene in improving the vitamin A status of primary school children. The additional qualities of red palm oil (no trans fatty acids; rich source of antioxidants) make it an excellent alternative fortificant for addressing vitamin A deficiency.

Several vitamins were confirmed have crucial role in the maintenance of immunocompetence. This includes vitamin A, beta-carotene, folic acid, vitamin B_6, vitamin B_{12}, vitamin C, vitamin E, riboflavin (Grimble, 1997; Chandra, 1994). Schmidt (1991) stated that micronutrient status of an individual can affect its immunocompetence. Beta-carotene is particularly effective in modulating immune functions and host defense against microorganisms or other invasive processes.

Before intervention, neither subject of carrot group nor control group had a normal nutritional status, whereas the average nutritional status within subject of RPO group was an underweight. Both before and after intervention, there were no subjects in the state of poor nutritional status and overweight. There were an increase in average z-scores value in neither control nor treatment group (carrot and RPO). After the intervention, all of the treatment groups have an average value of z-score for weight/age categorized as normal nutritional status. In the RPO group there was an improvement on nutritional status, from underweight to the normal nutritional status after the intervention period. Statistical analysis revealed that there were no differences between nutritional status of the subjects in the control group as compared to the treatment group ($p>0.05$) both at before and after the intervention period. The deltas of z-score value for body weight for age were also not different.

The increases of body weight were not significantly different among control, Carrot and RPO group. The same statistic results also in nutritional status (weight for age indices). This result indicated that food intervention used carrot and RPO instant noodle didn't affect the body weight and nutritional status of respondent. However after the intervention period nutritional status of children in each group was increased as compared to before intervention. Body weight and nutritional status (weight for age indices) are not affected by carotene directly, but intake of energy from instant noodle more contributable to the nutritional status. Each instant noodle contains energy as follow, carrot instant noodle contain energy of 369.5 kcal and RPO instant noodle contain energy of 408.6 kcal. The contribution to RDA for 7-9 years old children was 20.5% for carrot instant noodle and 22.7% for RPO instant noodle. The contribution of RPO noodle for energy slightly higher compare to carrot noodle because of using RPO as a fortificant (7.5% of wheat flour). The energy content of RPO has not been analyzed, but energy content of Palm oil around 902 kcal/100 g.

Conclusion: Analysis of Variance indicated that intervention of carrot instant noodle and RPO instant noodle didn't affect the average levels of serum retinol in each group ($p>0.05$). Both in control and treatment group serum IgG categorized as high level. Analysis of variance before intervention showed that no significant difference between serum IgG level in the control group as compared to the treatment group ($p>0.05$). After intervention, analysis of variance showed that serum IgG level in RPO group was significantly higher as compared to control and carrot group ($p<0.05$), as well as the delta value between those of group, while serum IgG level in carrot group was not significantly different as compared to control group ($p>0.05$). There were no significant differences in nutritional status among all groups before and after intervention

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