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ORIGINAL ARTICLES

Nutritional Evaluation of Weaning Food Blends Fortified with Some Carotenoids- Rich Vegetables

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ABSTRACT

In the present study, we evaluated the effect of adding carrot (2 %), parsley (4%) and spinach (4%) in a blend of infant weaning foods based on whole-wheat grains, soaked, germinated, fermented and cooked cowpea, faba and white beans. Sixty-six weanling male Sprague-Dawley rats fed on basal diet for one week then fed on vitamin A free diet for six weeks. After the vitamin A depleting period, rats were divided to eleven dietary groups: one group (6 rats) was fed on basal diet as a positive control group, second group was continued feeding on vitamin A free diet and used as negative control and nine groups fed with different weaning food blends. After six weeks, the animals were anaesthetized with diethyl ether. Blood samples and organs were collected. Food intake, body weight gain, and organ weights were recorded. Plasma and liver vitamin A, protein patterns, hemoglobin, lipid profile, liver and kidney functions were determined using the standard methods. Results for the nutritional parameters showed that the groups fed with carrot and parsley-weaning blends recorded the highest level of food intake, body weight gain and feed conversion efficiency and the values were close to the values recorded in the rat group that fed on positive control diet. For plasma and liver vitamin A also the highest values were recorded for the rat groups fed on the carrot and parsley blends compared with the rat group fed on positive control diet. For lipid parameters, protein profiles, hemoglobin, liver and kidney functions, all values for all of the nine tested weaning food blends were safe and not significantly different from the values recorded in the rat group fed with the positive control diet. Our nutritional evaluation for the tested weaning blends indicated that adding of the pro- vitamin A rich vegetables to legumes and cereals based weaning foods can cover from 75 to 85 % of the recommended dietary allowance for infant (age 6 to 24 months). The present study also recommends that using of these blends as a good and cheap source of vitamin A for the infants in developing countries.

Key words: vegetables, legumes, weaning food, vitamin A, carotene, rats.

Introduction

Breast-feeding is very important and essential for the infant's health, growth and for actual survival in developed and developing countries.

Many positive qualities of breast milk were reported by Mata (1978) including that, breast milk is available with no fees, is safe and provide a number of protective qualities for the infant during the first two years. In many developing countries, it is well confirmed that between the 4th and the 6th months of the infant age feeding on breast milk alone is the main reason for the low weight relative to the infant age. Poor women in some developing countries in her best breast-feeding period can provide from 500 to 600 ml of breast milk / day. For ideal growth infant at age 3 to 4 months with average weight 5 kg requires more than 850 ml of the breast milk daily as reported by Whitehead (1976). Therefore, the infants in such regions did not receive sufficient breast milk to meet their nutritionals needs.

WHO (1998) reported in a special review the importance of the breast feeding for the infants for two years with additional complementary foods at the same time when the infants reach 6 months of age. The amount of nutrients provided by complementary foods should be covered the daily nutritional requirements beside the human milk. There were about 21 studies reviewed by WHO (1998) on complementary feeding in developing countries and concluded that average breast milk consumption was 674 ml/day, 616 ml/day, and 549 ml/ day for both of infants at age 6-8 months, 9-11 months, and young children at age 12-23 months, respectively. The WHO review confirmed that the children after six moths of age should have a high quality complementary food. Therefore, there was a need to provide some weaning foods that were usually used during the transition period between breastfeeding and total oral intake. Weaning foods should be nutritionally balanced and easily digestible (Hansen *et al*., 1981).

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Malnutrition in developing countries is very serious problem between the young children, particularly during and after weaning. The child during this time enters the family eating pattern and eats not only small amounts of foods, but also unsuitable kinds of food for weaning purposes (Bressani, 1981).

Vitamin A deficiency among infants and preschool children is a major health problem. Its symptoms rose after the liver vitamin A storage depleted. Vitamin A deficiency most commonly affects the eye of infants and preschool children. Internally, then, impairing dark adaptation, leading to night blindness and externally by disrupting the epithelia of the cornea leading to a condition known as xeropthalmia (WHO, 1982). Young children with vitamin A deficiency are usually vulnerable for infection, particularly those of diarrhea, measles complications and respiratory infections (Guthrie & Picciano, 1995).

Vitamin A in the human diet derived from animal sources as retinol and from plants as a pro-vitamin A (carotenoids). The carotenoids, ranging from red to yellow in color, found in dark green leafy vegetables and orange colored fruits and vegetables (Lanchance and Fisher, 1990).

β-carotene posses the highest vitamin A activity and is also the most widely distributed in nature (Mercandante and Rodriguez, 1990). Fruits and vegetables are the cheapest sources of pro-vitamin A in the developing countries. One way to increase vitamin A intake of infants is through the incorporation of high carotenoids food in their diets. Such as carrot that considers one of the richest vegetables in carotenoids (5.6 mg/100 g), and some leafy vegetables such as spinach and parsley were reported to contain considerable amounts of carotenoids (Podsedck, 2000 and Edenharder, *et al*., 2002).

Cereals are often used as the main ingredient in weaning foods because they are highly digestible, low in anti-nutritive factor, and dose not cause allergic reactions. Cereal grains are rich in carbohydrates but deficient in essential amino acids such as lysine, thus making their protein quality poorer than that of animals (Horn & Schwartz, 1961).

Adding carrot or dark green vegetables will increase the amount of vitamin A in cereals based weaning food blends. The level of protein in cereals is also below the requirement needed for child growth (Hansen *et al*., 1981) but the nutritional value of cereals can be greatly enhanced when mixed with legumes such as cowpea, white and faba beans which reported to be of high nutritious values (Roy, *et al*., 2010). Some treatments were usually taken place in the preparation of legumes such as soaking, germination, fermentation and cooking in order to get rid of protein inhibitors and enhancing the protein digestibility to provide a good property to digest the legume's protein. In a previous study done by Dalia and Magda (2006), it was possible to formulate some blends of cereals (whole wheat grains), legumes (soaked, germinated, fermented and cooked cowpea, white and faba beans) and some carotenoids rich-vegetables (carrot (2%), spinach (4%) and parsley (4%)) in an attractive color and taste for the infants. These blends were formulated to me*et al*l the infants' requirements between the ages of 6 to 24 months. In addition, these different blends were covered the vitamin A daily needs for these infants with low fees. The present investigation, a biological evaluation using rats was done to assess the nutritional effect and health value of these blends expressed by their effects on the growth, different organ weights, plasma and liver retinol (vitamin A), lipid profiles and organs (liver and kidney) functions.

Materials and Methods

2.1. Materials:

Cereals, legumes, and vegetables:

Whole wheat grain (*Tritium aeritinum, L.),* var. Sakha 69, cowpea (*Vigna Unguiculato, L.),* var. Cream 7, faba beans (*Vicia faba, L.)* var. Giza Blanca and white beans (*Phaseolus vulgaris, L.),* var. Giza 6 were obtained from the Agriculture Research Center, Giza, Egypt. Yellow carrots (*Caucus carota, L.*), parsley (*Petroselinum crispum*), spinach (*Spinacia olaeracea*), skimmed milk powder, corn oil, salt and vanilla were purchased from local market.

The analytical kits used for biochemical analysis were obtained from Stanbio Laboratory (Texas-USA).

2.2. Methods:

2.2.1. Preparation of weaning food blends:

Whole wheat and legumes were cleaned and washed with tap water. Wheat was soaked in tap water (1:3 w/v) for 18 h at ambient temperature (30 – 32 ºC). The wheat grains were dried at 50 ºC and milled. Legumes were soaked and germinated separately according to the method described by Sanni *et al* (1999) and cooked in boiling water (cowpea, 10 min; faba beans and white beans, 30 min), dried at 50 ºC and milled. Wheat and legumes were fermented separately using *Saccharomyces cerevisiae* and *L. plantarum* ATCC as starter cultures for 36 h and then dried at 65 $\pm$ 1 ºC for 15 h. (Egounlety, 2002). Vegetables (carrots, parsley and spinach) were washed. Carrots were peeled and cut into thin slices. The vegetables were dried completely in freeze drier and

grinded. All weaning food blends contained wheat flour (52.87 – 54.87 %), corn oil (5%), skimmed milk powder (8%), salt (0.1 %) and vanilla (0.03 %) as constant substances. Carrot (2%), parsley (4%) and spinach (4%) were added to blends as a source of *pro*- vitamin A (carotenoids). The constituents of all weaning food blends under study are shown in Table (1).

Table 1: Ingredients of the expremintal blends.

Ingredients	Blends								
	1	2	3	4	5	6	7	8	9
Wheat flour	54.87	54.87	54.87	52.87	52.87	52.87	52.87	52.87	52.87
Cowpeas	30.00	-	-	30.00	-	-	30.00	-	-
Faba beans	-	30.00	-	-	30.00	-	-	30.00	-
White beans	-	-	30.00	-	-	30.00	-	-	30.00
Skimmed milk powder	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Carrots	2.00	2.00	2.00	-	-	-	-	-	-
Parsley	-	-	-	4.00	4.00	4.00	-	-	-
Spinach	-	-	-	-	-	-	4.00	4.00	4.00
Salt	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vanilla	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

1- Wheat cowpea carrots 2-Wheat faba bean carrots 3- Wheat white bean carrots
4- Wheat cowpea parsley 5- Wheat faba bean parsley 6- Wheat white bean parsley
7- Wheat cowpea spinach 8- Wheat faba bean spinach 9-Wheat white bean spinach

2.2.2. Animal experiment:

Sixty-six weanling male Sprague-Dawley rats weighing 58 g ± 4 g were obtained and housed in the National Research Center, Giza, Egypt. The rats were kept under normal health laboratory conditions and fed on basal diet for one week. Water and basal diet were provided *ad libitum*. After one week, the rats were divided into two groups as follows:

The first group (6 rats) was kept as positive control and continued fed on the basal diet AIN-93 (Reeves, 1993). The second group (6 rats) was fed on the free vitamin A depletion diet (vitamin A deficient diet) for 6 weeks. Then, rats were divided into 10 groups (each of 6 rats). One group was fed on the free vitamin A diet and 9 groups (from 3 to 11) fed on the tested blends for 6 weeks. The numbers of the rat groups are presented in table (2).

Table 2: Numbers of rat groups used in the experiment.

Numbers	Rat group
1	Positive control
2	Negative control
3	Wheat, cowpea and carrots
4	Wheat, faba beans and carrots
5	Wheat, white beans and carrots
6	Wheat, cowpea and parsley
7	Wheat, faba beans and parsley
8	Wheat,white beans and parsley
9	Wheat, cowpea and spinach
10	Wheat, faba beans and spinach
11	Wheat, white beans and spinach

2.2.3. Growth of rats:

The rats were weighed twice a week, total feed intake of each rat was weighed and Feed Conversion Efficiency (F.C.E.) was calculated according to Proll *et al,* (1998). At the end of the experiment, rats were weighed and anesthetized by diethyl ether. Liver, kidney, lung, heart and spleen were collected, washed in saline solution, dried with filter paper and weighed. The organs weight percentage was calculated as follows (Weight of organ / total body weight x 100).

2.2.4. Biochemical assays:

Blood was withdrawn by heart puncture under slight diethyl ether anesthesia. The blood sample was divided in 3 sample tubes, 2 tubes containing heparin (one for blood and the other for separation of plasma). Blood samples were left for 15 min at room temperature, then the tubes were centrifuged for 15 min at 3000 rpm and the clean supernatant plasma samples were frozen kept at -20 ºC until analysis.

Plasma and liver retinoid were extracted, dissolved in methanol : ethanol (1:1, v:v) according to the method of Esteban-Pretel *et al.,* (2010).Vitamin A then measured using HPLC method using the method of Arnaud *et al.,*(1991). HPLC system (Agilent 1100 series) coupled with UV–Vis detector (G1315B) and G1322A DEGASSER. Sample injections of 10 µl were made from an Agilent 1100 Series auto-sampler; the chromatographic separations were performed on ZORBAX -Eclipse XDB-C_{18} column (4.6×250 mm, particle size 5 um).

Gradient elution was employed using 84% of methanol-isopropanol (55:45, V/V) as solvent A and 16% water as solvent B for 1 min, with a linear gradient decreasing from 16 to 0% B, followed by an isocratic elution (0% B). Vitamin A was detected at 325 nm.

Plasma alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed by the method of Bergmeyer and Harder (1986). Alkaline phosphatase (Al P; EC 3.1.3.1) and gamma-glutamyl tranferase (γ-GT) activities were measured using the methods describe by Varley *et al.,* (1980) and Szasz (1969), respectively. Plasma total proteins (TP) and albumin concentrations were analyzed using the methods of Lowry *et al.,* (1951) and Doumas *et al.,* (1977), respectively. Globulin concentrations were determined by difference (TP - albumin). Blood haemoglobin was determined using the method described by Wintrobe (1967).

Lipid parameters including plasma total lipid, triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol were measured according to the methods described by Knight *et al*., (1972), Fossati and Prencipe (1982) and Waston (1960), Assmann (1979) and Wieland and Seidel (1983), respectively.

Plasma uric acid, urea and creatinine to assess kidney function, were determined according to Caraway (1955), Patton and Crouch (1977) and Larsen (1972) respectively.

2.2.5. Statistical analysis:

Each parameter analyzed separately by using one-way analysis of variance (ANOVA). For determining differences between groups, the Duncan test was used. All p values of ≤ 0.05 were considered to be significant, Rukhin, (2012).

3- Results:

3.1. Food intake, body and organ weights:

Table (3) shows that the rat groups fed on carrot and parsley blends recorded the highest values for the body weight gain and for the feed conversion efficiency(FCE) as the same as positive control rat group recorded (139.11 g and 0.26, respectively). In addition, rat groups fed on spinach blends recorded the lowest value in FCE than the other rat groups fed with the tested blends (0.23). The negative control rat group recorded the lowest value than all of the other groups in body weight gain (94.09 g) and in the FCE (0.17).

Table 3: Body weight gain, total feed intake and feed conversion efficiency (FCE) of rats fed on tested blends.

Rats groups	Initial weight (g.)	Final weight (g.)	Weight gain (g.)	Total feed intake (g.)	FCE*
1	57.91 ± 2.35 ^{a}	197.02± 5.35 ^{a}	139.11±3.52 ^{a}	532.91±16.29 ^{c}	0.26 ± 0.009 ^{a}
2	57.08 ± 5.21 ^{a}	151.17 ±3.48 ^{d}	94.09±2.01 ^{d}	540.75±9.68 ^{bc}	0.17 ± 0.005 ^{d}
3	58.21 ± 3.69 ^{a}	191.90 ± 9.64 ^{ab}	133.67±6.81 ^{ab}	533.17±11.19 ^{c}	0.25 ± 0.015 ^{ab}
4	57.78 ± 3.37 ^{a}	190.93 ± 7.61 ^{ab}	133.15±4.47 ^{abc}	531.67±13.49 ^{c}	0.25 ± 0.007 ^{ab}
5	58.13 ± 4.41 ^{a}	194.77 ± 5.77 ^{ab}	136.64±3.77 ^{a}	541.68±3.95 ^{bc}	0.25 ± 0.006 ^{ab}
6	58.34 ± 2.94 ^{a}	187.04 ± 4.71 ^{abc}	128.70±1.84 ^{bc}	537.67±23.04 ^{bc}	0.24 ± 0.007 ^{b}
7	57.18 ± 3.22 ^{a}	194.70 ± 3.91 ^{ab}	137.52±0.72 ^{a}	545.92±13.92 ^{bc}	0.25 ± 0.005 ^{ab}
8	57.07 ± 1.04 ^{a}	192.52 ± 5.17 ^{ab}	135.45±4.33 ^{ab}	545.68±9.96 ^{bc}	0.25 ± 0.004 ^{ab}
9	57.79 ± 2.59 ^{a}	197.46 ± 4.97 ^{a}	139.67±2.42 ^{a}	570.63±11.57 ^{a}	0.24 ± 0.001 ^{b}
10	57.54 ± 3.80 ^{a}	177.74 ± 8.06 ^{c}	126.86±4.39 ^{c}	550.44±8.90 ^{abc}	0.23 ± 0.008 ^{c}
11	56.42 ± 2.09 ^{a}	184.41 ± 4.56 ^{bc}	126.76±2.55 ^{c}	561.17±7.84 ^{ab}	0.23± 0.007 ^{c}
LSD($p\leq0.05$)	5.653	10.207	6.279	21.544	0.0126

*Feed Conversion Efficiency (F.C.E.) = gain of rat weigh / total feed intake, g

Any two means have the same letter in the same column did not significantly different at $P \leq 0.05$

As presented in table (4), no significant differences were found between the positive control rat group and all of the tested groups in relative organ weights except for the negative control group that recorded the lowest value for the relative liver weight (1.86).

3.2. Vitamin A in plasma and liver of rats fed with different food blends:

Table (5) shows the level of vitamin A in plasma and liver of rats fed with different tested blends. The values for plasma vitamin A of rats fed on the positive control diet recorded the highest value (98.39 µg/ml).

While the rat group fed on the negative control diet recorded the lowest value (34.53 µg/ml). For the rest of rat groups fed with the different tested blends, the values for plasma vitamin A of rats fed on the carrot blends recorded the highest value (average value : 88.3 µg/ml). While the plasma vitamin A of rat groups fed on parsley and spinach blends recorded non-significant lower values compared with both of positive and carrot blends fed rat groups (average values: 84.3 and 84.9 µg/ml, respectively). In addition, the level of liver vitamin A of rat groups fed on different tested blends indicated that, liver vitamin A in rat groups fed on carrot blends was 57.9 µg/g. while the rat groups fed on parsley and spinach recorded the average values : 56.9 and 56.4 µg/g, respectively . The rat group fed on the negative control diet recorded a significant decrease in liver vitamin A level (14. 57µg/g) than the other tested rat groups and the positive control rats group that recorded the highest value 62.07µg/g.

Table 4: Relative organs weights of rats fed on tested blends.

Rats groups	Relative liver weight	Relative kidney weight	Relative lung weight	Relative heart weight	Relative spleen weight
1	2.63 ± 0.44 ^{a}	0.62 ± 0.05 ^{ab}	0.47 ± 0.03 ^{a}	0.23 ± 0.04^{bc}	0.22 ± 0.02 ^{a}
2	1.86 ± 0.04 ^{b}	0.43 ± 0.04^{d}	0.62 ± 0.04 ^{b}	0.18 ± 0.03^{c}	0.19 ± 0.03 ^{ab}
3	2.27 ± 0.23 ^{ab}	0.47 ± 0.02^{cd}	0.44 ± 0.05 ^{a}	0.26 ± 0.04 ^{ab}	0.17 ± 0.01 ^{ab}
4	2.60 ± 0.15 ^{a}	0.59 ± 0.02 ^{abc}	0.44 ± 0.08 ^{a}	0.21 ± 0.03^{bc}	0.20 ± 0.02 ^{ab}
5	2.17 ± 0.17 ^{ab}	0.48 ± 0.07 ^{bcd}	0.50 ± 0.05 ^{a}	0.23 ± 0.03^{bc}	0.20 ± 0.06 ^{ab}
6	2.68 ± 0.26 ^{a}	0.60 ± 0.05 ^{abc}	0.47 ± 0.10 ^{a}	0.25 ± 0.01^{bc}	0.20 ± 0.02 ^{ab}
7	2.37 ± 0.51 ^{ab}	0.57 ± 0.13 ^{abcd}	0.45 ± 0.08 ^{a}	0.32 ± 0.02 ^{a}	0.17 ± 0.01 ^{ab}
8	2.23 ± 0.28 ^{ab}	0.45 ± 0.05^{cd}	0.45 ± 0.06 ^{a}	0.22 ± 0.02^{bc}	0.16 ± 0.01 ^{b}
9	2.31 ± 0.50 ^{ab}	0.46 ± 0.07^{cd}	0.41 ± 0.05 ^{a}	0.22 ± 0.05^{bc}	0.16 ± 0.04 ^{b}
10	2.45 ± 0.19 ^{ab}	0.54 ± 0.02 ^{abcd}	0.43 ± 0.01 ^{a}	0.20 ± 0.05^{bc}	0.19 ± 0.02 ^{ab}
11	2.56 ± 0.36 ^{a}	0.64 ± 0.18 ^{a}	0.48 ± 0.05 ^{a}	0.32 ± 0.05 ^{a}	0.20 ± 0.03 ^{ab}
LSD(p≤0.05)	0.5406	0.1331	0.1024	0.0604	0.0448

The organs weight percentage was calculated as follows (Weight of organ / total body weight x 100).
Any two means have the same letter in the same column did not significantly different at $P \leq 0.05$

Table 5: Vitamin A in plasma and liver of rats fed on tested blends.

Rats groups	Plasma vitamin A (µg/ml)	Liver vitamin A (µg/g)
1	98.39 ± 3.52 ^{ab}	62.07 ± 3.30^{ab}
2	34.53 ± 2.46 ^{d}	14.57 ± 1.60 ^{d}
3	87.37 ± 5.08 ^{ab}	54.90 ± 3.16 ^{c}
4	85.63 ± 2.95 ^{bc}	53.27 ± 3.62 ^{c}
5	91.97 ± 4.20 ^{a}	65.60 ± 3.08 ^{a}
6	80.23 ± 4.70 ^{c}	58.40 ± 3.82 ^{bc}
7	89.27 ± 4.75 ^{ab}	57.90 ± 4.20 ^{bc}
8	83.33 ± 5.75 ^{bc}	54.43 ± 1.90 ^{c}
9	81.90 ± 4.28 ^{c}	57.63 ± 4.30 ^{bc}
10	85.37 ± 5.56 ^{bc}	54.93 ± 3.74 ^{c}
11	87.51 ± 4.42 ^{ab}	56.60 ± 4.25 ^{bc}
LSD(p≤0.05)	7.525	5.876

Any two means have the same letter in the same column did not significantly different at $P \leq 0.05$

3.3. Liver functions:

Table (6) presents the activities of the liver enzymes ALT, AST, ALP and γ GT. Non-significant changes were recorded between the values of ALT, AST , ALP and γ GT in the different rat groups fed on the different diets and the positive control diet. Except for the rat group fed with the negative control diet that recorded a significant increase in the ALT, AST, ALP and γ GT activities (42.93, 53.13, 93.17 and 6.72, respectively).

3.4. Kidney functions:

In general, no statistically significant differences were recorded between the different rat groups fed with the tested blends in the kidney functions indicators (plasma uric acid, urea or creatinine). Only in the plasma, creatinine value in the rat group fed on the negative diet that recorded a significant increase for creatinine (1.44 U/l) than the other group fed with different blends as presented in table (7).

3.5. Lipid patterns:

As shown in table (8), the plasma total lipids of rats groups fed on different blends were significantly higher than that of rats fed on positive control diet. The plasma total lipids of the rat group fed with positive control diet was 444.45 mg/dl, while the average values for the rat groups fed in carrot, parsley and spinach blends were 476.5, 526.0 and 529.3 mg/dl, respectively. The plasma total lipids of rat group fed on the negative diet were significantly higher than the other tested blends; the level was 615.8 mg/dl. The same results were recorded on

the plasma triglycerides, total cholesterol and LDL- cholesterol values of rat groups fed on the different tested blends. Whereas, the values of HDL-cholesterol indicated that both of the rat fed on the tested blends and the rat group fed on the positive control diet were higher than the negative control diet.

Table 6: Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (γ-GT) of rats fed on tested blends.

Rats groups	ALT (U/L)	AST (U/L)	ALP (U/L)	γ GT (U/L)
1	31.47 ± 2.78 [a]	40.41± 4.28 [a]	71.17 ± 4.36 [a]	3.82 ± 0.24 [a]
2	42.93 ± 3.63 [b]	53.13 ± 2.31 [b]	93.91 ± 4.09 [b]	6.72 ± 0.48 [b]
3	34.72 ± 1.71 [a]	39.94 ± 1.95 [a]	77.51 ± 4.48 [a]	3.85 ± 0.43 [a]
4	29.56 ± 2.09 [a]	42.60 ± 3.39 [a]	76.65 ± 5.10 [a]	3.70 ± 0.13 [a]
5	30.51 ± 1.97 [a]	40.98 ± 1.59 [a]	72.40 ± 1.46 [a]	3.64 ± 0.21 [a]
6	32.83 ± 6.42 [a]	41.34 ± 3.14 [a]	73.98 ± 1.60 [a]	3.84 ± 0.32 [a]
7	34.84 ± 4.14 [a]	42.72 ± 3.40 [a]	77.31 ± 1.84 [a]	3.87 ± 0.27 [a]
8	33.89 ±3.69 [a]	42.59 ± 4.46 [a]	73.71 ± 1.31 [a]	3.98 ± 0.32 [a]
9	32.13±4.68 [a]	40.11± 3.17 [a]	74.87 ± 4.93 [a]	3.85 ± 0.11 [a]
10	35.82 ± 2.83 [a]	42.77 ± 3.49 [a]	76.01 ± 4.03 [a]	3.81 ± 0.10 [a]
11	34.57 ±3 .57 [a]	42.23 ± 3.53 [a]	76.18 ± 2.82 [a]	3.81 ± 0.29 [a]
LSD(p≤0.05)	6.134	5.532	6.046	0.488

Any two means have the same letter in the same column did not significantly different at $P \leq 0.05$

Table 7: Plasma uric acid, urea and creatinine of rats fed on tested blends.

Rats groups	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
1	3.79 ± 0.17 [a]	31.75 ± 2.22 [a]	1.19 ± 0.09 [b]
2	3.82 ± 0.30 [a]	30.80 ± 2.64 [a]	1.44 ± 0.04 [a]
3	3.69 ± 0.25 [a]	32.99 ± 4.57 [a]	1.18 ± 0.07 [b]
4	3.87 ± 0.15 [a]	32.41 ± 2.97 [a]	1.28 ± 0.07 [b]
5	3.71 ± 0.24 [a]	32.20 ± 3.20 [a]	1.23 ± 0.05 [b]
6	3.72 ± 0.29 [a]	32.29 ± 4.92 [a]	1.22 ± 0.07 [b]
7	3.77 ± 0.19 [a]	31.78 ± 5.03 [a]	1.23 ± 0.08 [b]
8	3.60 ± 0.16 [a]	31.80 ± 1.51 [a]	1.26 ± 0.09 [b]
9	3.76 ± 0.17 [a]	33.14 ± 3.52 [a]	1.27 ± 0.12 [b]
10	3.77 ± 0.13 [a]	31.97 ± 3.00 [a]	1.27 ± 0.09 [b]
11	3.70 ± 0.18 [a]	32.70 ± 3.13 [a]	1.21 ± 0.07 [b]
LSD(p≤0.05)	0.3396	5.9283	0.1279

Any two means have the same letter in the same column did not significantly different at $P \leq 0.05$

3.6. Protein patterns:

Plasma total proteins, albumin (A), globulin (G), the ratio of A/G and the blood hemoglobin are presented in table (9). The values for plasma total proteins were non significantly different from the rat group fed on the positive control (7.8 mg/dl) and the rat groups fed on carrot, parsley and spinach , the average values were 7.7, 7.9 and 7.8 mg/dl , respectively. While, the plasma total proteins of rats fed on the negative diet recorded the lowest significant value (5.2 mg/dl). The plasma albumin, globulin and the ratio of albumin / globulin were non significantly different from the rat groups fed on the positive control diet. A significant increase in blood hemoglobin concentration was found in the rat groups fed on carrot, parsley and spinach blends while, the rat groups fed on the negative and positive diets recorded 14.02 and 7.8 g/dl, respectively.

Table 8: Lipid profile of rats fed on tested blends.

Rats groups	Total lipid (mg/dl)	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL-chol. (mg/dl)	LDL-chol. (mg/dl)
1	444.45± 39.43 [a]	156.08 ± 5.03 [a]	83.14 ± 4.9 [c]	47.03 ± 2.12 [a]	4.89 ± 3.73 [c]
2	615.79 ± 24.66 [ab]	183.60 ± 13.47 [b]	102.49 ± 3.76 [a]	40.54 ± 2.18 [b]	25.23 ± 1.45 [a]
3	469.39 ± 25.73 [ab]	161.19 ± 19.89 [a]	84.14 ± 3.52 [c]	43.78 ± 1.33 [ab]	8.12 ± 6.76 [bc]
4	471.89 ± 12.96 [ab]	156.27 ± 6.26 [a]	82.98 ± 3.31 [c]	42.53 ± 2.26 [ab]	9.19 ± 0.16 [bc]
5	488.13 ± 23.95 [bc]	163.38 ± 7.51 [a]	84.45 ± 7.37 [c]	43.73 ± 3.16 [ab]	8.04 ± 5.69 [bc]
6	482.77 ±20.95 [cd]	162.87 ± 3.69 [a]	92.76 ± 4.72 [b]	47.58 ± 1.71 [a]	12.60 ± 4.23 [b]
7	525.13 ± 12.45 [cd]	161.32 ± 6.03 [a]	85.84 ± 3.21 [bc]	47.52 ± 1.09 [a]	6.06 ± 5.02 [bc]
8	570.04 ± 46.48 [cd]	168.87 ± 5.42 [ab]	88.07 ± 1.33 [bc]	45.74 ± 3.20 [ab]	8.55 ± 3.66 [bc]
9	460.08 ± 23.23 [cd]	161.18 ± 1.52 [a]	84.48 ± 3.27 [c]	42.87 ± 2.46 [ab]	9.37 ± 0.86 [bc]
10	571.13 ±76.07 [cd]	162.70 ± 8.07 [a]	82.84 ± 3.86 [c]	42.47 ± 3.75 [ab]	7.83 ± 1.88 [bc]
11	556.82± 25.10 [d]	162.70 ± 8.07 [a]	88.45 ± 3.31 [bc]	42.82 ± 4.67 [ab]	12.36 ± 1.63 [b]
LSD (p≤0.0)	58.829	15.285	6.989	4.635	6.408

Any two means have the same letter in the same column did not significantly different at $P \leq 0.05$

Table 9: Plasma total proteins, albumin (A), globulin (G), A/G ratio and blood heamoglobin of rats on tested blends.

Rats groups	Total protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	A/G ratio	Blood heamoglobin (g/dl)
1	7.79 ± 0.24 ^{ab}	3.87 ± 0.18 ^{ab}	3.92 ± 0.29 ^{a}	0.99 ±0.11 ^{ab}	14.02 ± 0.18 ^{e}
2	5.19 ± 0.56 ^{c}	2.49 ± 0.12 ^{c}	2.70 ± 0.63 ^{b}	0.95 ±0.23 ^{ab}	7.81 ± 0.26 ^{g}
3	7.76 ± 0.30 ^{ab}	3.89 ± 0.13 ^{ab}	3.87 ± 0.22 ^{a}	1.05 ± 0.13 ^{ab}	14.51 ± 0.39 ^{de}
4	7.83 ± 0.54 ^{ab}	4.13 ± 0.18 ^{ab}	3.71 ± 0.48 ^{a}	1.12 ± 0.14 ^{ab}	16.89 ± 0.09 ^{ab}
5	7.61 ± 0.38 ^{ab}	3.89 ± 0.57 ^{ab}	3.79 ± 0.70 ^{a}	1.03 ± 0.28 ^{ab}	14.71 ± 0.59 ^{d}
6	8.05 ± 0.55 ^{a}	3.89 ± 0.36 ^{ab}	4.16 ± 0.57 ^{a}	0.95 ± 0.17 ^{ab}	16.46 ± 0.72 ^{bc}
7	7.80 ± 0.26 ^{ab}	3.60 ± 0.32 ^{b}	4.20 ± 0.12 ^{a}	0.86 ± 0.11 ^{b}	17.46 ± 0.10 ^{a}
8	7.89 ± 0.12 ^{ab}	4.08 ± 0.24 ^{ab}	3.81 ± 0.23 ^{a}	1.09 ± 0.13 ^{ab}	16.89 ± 0.21 ^{ab}
9	7.49 ± 0.39 ^{ab}	3.98 ± 0.13 ^{ab}	3.50 ± 0.39 ^{ab}	1.14 ± 0.15 ^{ab}	16.08 ± 0.19 ^{c}
10	7.94 ± 0.46 ^{ab}	3.70 ± 0.36 ^{ab}	4.23 ± 0.15 ^{a}	0.88 ± 0.07 ^{ab}	13.04 ± 0.06 ^{f}
11	7.91 ±0.72 ^{ab}	4.26 ± 0.27 ^{a}	3.65 ± 0.95 ^{a}	1.23 ± 0.35 ^{a}	16.28 ± 0.38 ^{bc}
LSD(p≤0.05)	1.225	0.502	0.841	0.315	0.596

Any two means have the same letter in the same column did not significantly different at $P \leq 0.05$

4- Discussion:

Breast-milk satisfies the nutrient and energy requirements of the infant for the first 6 months. As the child grows, the nutrient composition of milk increasingly becomes inadequate to meet the infant's requirements. The nutrients most affected are some essential minerals and vitamins especially vitamins A. Therefore, to be able to meet the changing requirements of the infant's development, there is the need to supplement the breast milk with a nutritious diet, which could be a proprietary formula or locally prepared at home, while breastfeeding continues for at least two years (Okoye, 1992). The vitamin A deficiency is a major public health problem affecting an estimated 190 million preschool–age children, mostly from Africa and South-East Asia. Inadequate intakes of vitamin A at infants developing age could lead to vitamin A deficiency, which, when severe, may cause visual impairment (night blindness) or increase the risk of illness and mortality from childhood infections such as measles and those causing diarrhea (WHO, 2011).

The present work carried out to provide different food blends that can use as a complementary weaning food for kids after the six months. Using whole-wheat grain as a cereal base, some legumes as a protein sources including cowpea, faba and white beans. Some treatments such as soaking, germination, fermentation and cooking were done to remove the legume inhibitors and to make optimum benefits of whole-wheat grain and legumes.

Some freeze-dried carotenoids- rich vegetables were including in these different weaning food blends as a main source of vitamin A. These vegetables were carrot (2%), spinach (4%) and parsley (4%) that contain high levels of β- carotene (8836, 5597 and 2109 mg/100g, respectively) (García, *et al*., 2012 and Magda, 1997). The preparation, treatments, the chemical evaluation and the panel test for these different tested weaning food blends were previously published by Dalia and Magda, 2006.

The amounts of β- carotene in the three blends supplemented with carrots were 2152.2, 2146.8 and 2154.6 µg/100 g that equal 358.7, 357.8 and 359.1 µg retinol equivalent. Meanwhile, the amount of β- carotene in the three blends supplemented with parsley were 1801.2, 1789.8 and 1849.2 µg/100 g that equal 300.2, 298.3 and 308.2 µg retinol equivalent. Whereas, the amount of β- carotene in the three blends supplemented with spinach were 2105.4, 2098.2 and 2109.6 µg/100 g that equal 350.9, 349.7 and 351.6 µg retinol equivalent. Manar (1999) prepared some weaning food blends containing cereals, fruits and vegetables. The results of her study indicated that addition of oven dried carrot (10%) to the weaning food blends 440 µg/100 g of vitamin A. In our study the freez dried carrot blends (2%) provided about 300 µg of vitamin A /100 g.

Since the recommended dietary allowances (RDA) for infants based on the content and volume of human milk is 375 – 420 RE until the age of six months and 375 – 400 retinol equivalent (RE) thereafter, until the first year (Guthrie, 1989).These different blends covered about 75-87% of the RDA for infants at the age from six months to one or two years.

The biological evaluation for nine different blends were done using nine rat groups and two more groups for positive and negative control groups in a vitamin A depletion – repletion experiment. The nutritional parameters such as body weight gain, the feed conversion efficiency ratio (FCE) and the organ weight were calculated. The values of the tested nutritional parameters indicated that these blends were accepted and could provide a healthy growth. These results suggest that vitamin A deficiency impairs rat development and that the carotenoids from the different blends may supply enough vitamin A to maintain rat growth. Our results were in agreement with what Tee *et al*., 1996 and Siqueira *et al*., 2007 found in some similar studies.

The organs functions such as kidney and liver enzymes activities were measured, the values were in the safe range and were accepted. Lipid pattern, protein profiles and the hemoglobin were studied and all values were safe and in agreement with a study done by Mariam, 2005.

Vitamin A in plasma and liver levels were assessed. The values illustrated that the adding of carotenoid- rich vegetables to the cereal based and legumes weaning food blends provide acceptable values for vitamin A in

plasma and liver as the same as the positive diet did and better than what the negative diet did also. That means that adding the carotenoid-rich vegetables made a good vitamin A level from all of the tested blends. The bioavailability of carotenoids is affected by several factors including the type of carotenoids in foods, the matrix in which carotenoids are incorporated and the diet composition where carotenoids are consumed (De Pee, 1996 and De Pee &West, 1996).

The liver retinol decreased during the vitamin A depletion period (15 weeks) from 62.07 ± 3.30 µg down to 14.57± 1.60 µg/g liver. According to Green *et al*. (1987), liver retinol was 43 µg and low liver retinol was 2.2 µg/g liver during the vitamin A depletion period.

Tee *et al*. (1996), studied the biological utilization of carrot and some leafy vegetables that rich in different types of carotenoids in rat. This study proved that the bioavailability of the major carotenoids in the tested vegetables was high, as estimated by the accumulation of retinol in liver and in plasma of rats. The results of Tee *et al*., (1996) study are in agreement with the results found in our study.

Zakaria-rungkat *et al*. 2000 studied the effect of the food matrix(such as carbohydrates) and some carotenoids-rich vegetables (leafy and tubers) in the accumulation of vitamin A in the liver of vitamin A depleted rats compared with a rat group supplemented with of synthetic vitamin A . The study confirmed that the liver vitamin A level of the rat groups fed on the tested diets were close to that rats supplemented with a synthetic vitamin A or higher. That was in agreement with the results of present study.

Using of whole-wheat grains and the tested legumes after soaking, germinating, fermenting and the cooking treatments provided a good and a suitable matrix for the carotenoides bioavailability from the tested blends (Mensa-Wilmot *et al*., 2001). Our biological evaluation of the nine complementary weaning food blends confirmed the nutritional and healthy effect of these blends.

5-Conclusion:

This study concluded that, using mixtures of some legumes (white, kidney and faba beans) after doing the soaking, germination, fermentation and the cooking treatments with the cereal base blends (whole wheat grain) and with the addition of some carotenoid-rich vegetables (carrot, spinach and parsley) provided a good and healthy complementary weaning foods blends cowpea,faba and white beans. These different blends will cover about 75-87% of the RDA for infants at the age from six months to two years with no health problems. This study recommends the importance of using the carotenoids rich vegetables in the complementary weaning food mixtures as a healthy, safe and economic way for the controlling of vitamin A deficiency in the developing countries.

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