CHEMICAL, BIOLOGICAL AND ORGANOLEPTIC EVALUATION OF NEWLY FORMULATED THERAPEUTIC DIETS FOR PROTEIN CALORIE MALNUTRITION

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SUMMARY: Three supplementary food mixtures high in protein and calories were prepared using different percentage of locally produced cereals and legumes in addition to 10% skimmed milk powder in each formula to be used as therapeutic diets for infants suffering from protein energy malnutrition. These prepared mixtures were undergone chemical, biological and organoleptic evaluation. The data obtained revealed that 100g of each mixture provided 38, 28 and 20 g. protein and 382, 366 and 364 calories in formulae I, II and III respectively. The biological evaluation was carried out in comparison to three locally available food mixtures (Creacon, Gerber and Riri) comarable in protein percentage and a casein containing diet as a control group using experimental rats. The data obtained showed that body weight gain increased significantly only in case of rats fed creacon and formula II. The food and protein efficiency ratios for formulae I and II were higher compared to the control which was significant for formula II. Organoleptic evaluation revealed that formula I scored as very good, then comes formula II then at last formula III which were both scored as good.

Key words: Protein calorie malnutrition, therapeutic diets

INTRODUCTION

One of the key health problems in Egypt is malnutrition, particularly among young children (1). The decrease in infant and child mortality over recent years have not been paralleled by an equivalent improvement in nutrition. It might be that the improvement in survival rate have generated some degree of malnutrition, by preserving the life of infants and children who might have been died early in life. The major cases suffering from nutritional deficiencies were either protein or protein-calorie deficiency. These cases are in need of diet treatment, so great effort must be made to develop protein-rich food mixtures to alleviate protein calorie malnutition in Egypt.

The aim of the present work is to formulate and prepare high protein, high calories therapeutic diets of low price which may help as nutritional support for infants suffering from protein-energy malnutrition. From the economical point of view it was suggested to use careals such as rice, wheat and maize and legumes, such as soybeans, cowpeas, lentils, lupine, chickpeas and fenugreek which are produced locally and are available in low price. Skimmed milk powder was added to elevate the protein values of the suggested supplementary food mixtures.

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Table 1: Ingredients of the different prepared supplementary food mixtures (g/100g).

Formula I		Formula II		Formula III	
Ingredients	g	Ingredients	g	Ingredients	g
Lupine	50	Lupine	20	Lentils	20
Defatted soybean	30	Defatted soybean	20	Fenugreek	10
		Cowpeas	20	Chickpeas	20
Wheat	10	Maize	30	Rice	40
Skimmed milk	10	Skimmed milk	10	Skimmed milk	10
Total	100		100		100

MATERIAL AND METHODS

I. Formulation, preparation and chemical evaluation of therapeutic food mixtures

Materials

Ingredients of the supplementary food mixtures

Different cereals and legumes in addition to skimmed milk were used to prepare the different high protein, high calories food mixtures (Table 1). Lgumes and cereals were washed and treated differently through soaking and cooking to get rid of anti-nutritional factors, then dried in hot air oven (2).

Each of the legumes and cereals, were separately grinded in an electric grinder, then sieved through silk sieves. The dry powder was kept in sealed polythylene bags at 5°C in refrigerator until used in the formulation.

Methods

Formulation and preparation of supplementary food mixtures

In this study, three supplementary therapeutic diets were formulated. The ingredients of each supplementary food mixture were shown in Table 1. The ingredients of the different formulae were chosen so as to contain high protein and high calories contents according to food composition tables of the Near-East (3). Each formula mixture was blended together in the electrical mixer for full homogenization. Each dried formula was milled into powder form and sieved through a silk sieve.

Chemical evaluation of the supplementary food mixtures

Chemical evaluation of the protein content of each formula was carried out (4). Proximate analysis was done to determine the fat, crude fibre, calcium and iron contents of each formula (5). Phosphorus was determined according to stuffins (6). Available carbohydrates were calculated by difference amino acids composition of each diet has been calculated from food composition tables of the Near-East (3) and the amino acid score (chemical protein score) of each formula was calculated (7).

II. Biological Evaluation of the therapeutic food mixtures

Materials

a. Animals:

White male and female ablino rats of 40-59g. body weight were used in the present study. The animals were kept individually in wire bottomed cages at room temperature of 25 ± 2°C and a relative humidity of about 55%.

b. Diets:

1. Certain amounts of each of the previously prepared high protein, high calories formulae were weighed so as to contain l0 g. protein. The fat content was completed by corn oil to 15%. An amount of 3.5% salt mixture (8) and 1% vitamin mixture(9) were added to each diet. The diets were completed by corn starch to 100%.

2. Three high protein, high calories supplementary food mixtures available in the local market and having comparable percentage of protein and calories were chosen as reference formulae (Table 2). The diets were prepared as in the previous step.

3. A control diet containing 10% protein (from casein) was prepared.

The composition of the different diets are shown in Table 3.

Table 2: Chemical composition of the three references supplementary foods in the local market per 100g dried mixture (as written on packings).

Reference supplementary foods	Moisture	Protein	Fat	Total Carbohydrates (g)	Ash (g)	Minerals			Total energy (Kcal)
						Calcium (mg)	Phosphours (mg)	Iron (mg)	
Creacon	-	50.4	5.1	38.6	-	1066	880	16.67	401
Gerber*	2.5	25	6.2	59.0	-	1075	1075	50	391
Riri	5	18	-	74.9	2.1	1000	-	10	367

* Gerber contains 7.3% minerals.

Methods

Design of Biological Experiment:

This experiment was designed to evaluate the protein efficiency ratio of the formulated high protein, high calories food mixtures in comparison to reference diets and a casein diet as control.

Rats were divided into 7 groups, each included 6 rats. The rats of each group were fed one of the diets present in Table 3 for 4 weeks. During the period of the experiment, the food intake and the rats were weighed twice weekly. Growth curves were drawn representing the relationship between the rats body weights and time. After the end of experimental period, biological changes; food intake, body weight gain, food efficiency ratio (body weight gain/total food intake) and protein efficiency ratio (body weight gain/total protein intake) were calculated and tabulated. At the end of the experiment, rats were fasted 16-18 hours. The blood samples were drawn for determination of haemoglobin (10) concentration and haematocrit (11) %.

III. Organoleptic (Sensory) Evaluation

The three formulated mixtures were sensory evaluated according to Notter, *et al.* (12). The different formulae were prepared in pudding form. Ten mothers were asked to evaluate the different formulated mixtures. They were requested to score the appearance, texture, colour, taste and aroma. The mother's preference were made using sores form 1 to 10.

The data of the biological and organoleptic study were analyzed statistically using t-students test.

RESULTS AND DISCUSSION

The three formulated supplementary food mixtures in our study were chemically analyzed as shown in Table 4. It was noticed that, the moisture content ranged between (6.8-8.5%) in the formulated mixtures. This could be explained on the basis that, the analysis was carried out on the dry weight of the mixtures. The low moisture content could be of great privillege for good keeping quality of reasonable shelf-life time for the final products.

An infant food based on cereals, legumes and skimmed milk powder was found to contain 7% moisture. This moisture level was reported to have no effect on protein quality if the infant food was stored for 6 months (13).

Concerning the protein content of the formulated mixtures, it was found that the highest value was obtained with formulae I (38%) while the protein figure of the formula III was the lowest (19.6%), formula II contained intermediate value (27.6%). The obtained data for protein content of formula III were comparable with those reported by the Protein Advisory Group (14), who pointed out that the protein content of Superamine Products produced in Egypt, Algeria and Tunisia were 20.2%. Another baby food product in Ethiopia named Faffa had a protein content value of 20% (15). Five baby food mixtures were prepared through Baby Food Project in Egypt (16) showed that their protein contents ranged from 19.1-20.6. However current formulae I and II scored higher protein contents than that in the above mentioned reports.

The fat contents of the formulated supplementary food mixtures, were 8.83, 5.83 and 5.14% in formulae I, II and III respectively (Table 4). It could be also seen from Table 4 that the highest value for available carbohydrate was obtained from mixture III (59.94%) followed by mixture II (50.78%) then mixture I (47.69%).

The ash contents for the three formulated mixtures were 3.18, 3.15 and 4.42 for formulae I, II and III

Table 3: Composition of the different experimental diets (g/100g diet).

	Diets made from prepared formula			Diets made from reference local market formulae			Control
	Formula I	Formula II	Formula III	Creacon	Gerber	Riri	
Contents of supplementary foods*:							
Protein	10.00	10.00	10.00	10.00	10.00	10.00	-
Fat	2.33	2.11	2.63	1.01	2.48	-	-
Carbohydrate	9.92	18.40	30.58	7.66	23.60	41.60	-
Fibre	1.44	1.49	1.55	-	-	-	-
Casein	-	-	-	-	-	-	10.3
Fat	12.67	12.89	12.37	13.99	12.52	15.00	15.0
Starch	59.14	50.61	38.37	62.84	46.90	28.89	70.2
Salt	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Vitamin	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100	100	100

* 26.32g. of Formula I
36.23 g. of Formula II
51.02 g. of Formula III

*19.84 g. of Creacon
40 g. of Gerber
55.56 g. of Riri.

Table 4: Chemical composition of the three formulated food mixtures per 100 g dried mixture and their percentage amino acids scores (chemical protein score).

Mixture No	Moisture (g)	Protein (g)	Fat (g)	Available carbohydrate (g)	Crude fiber (g)	Ash (g)	Minerals mg			Total energy (Kcal)	Percentage amino acids scores
							Ca	Fe	P		
Formula I	6.82	38.0	8.83	37.69	5.48	3.18	288.6	7.3	595.4	382	50.7
Formula II	8.50	27.6	5.83	50.78	4.14	3.15	254.5	7.9	415	366	30.4
Formula III	7.86	19.6	5.14	59.94	3.04	4.42	201.3	9.6	553.4	364	29.6

(Each value is a mean of three determinations).
Formula I (50% Lupine, 30% Defatted Soybean, 10% wheat and 10% skimmed milk).
Formula II (20% Lupine, 20% Defatted soybean, 20% cowpeas, 30% Maize and 10% skimmed milk).
Formula III (20% lentils, 10% Fenugreek, 20% chikpeas, 40% Rice, and 10% skimmed milk).

respectively. The crude fibre contents of the formulated mixtures were 5.48, 4.14 and 3.04 for formulae I, II and III respectively. It was obvious that the crude fibre contents were high (specially in formulae I and II) this was due to presence of legumes. The figures of crude fibre in formulae I and II agreed with that of Moussa (17) Ohleson (18) assumed that the increase of fibres in baby foods might cause some irritation to the gastrointestinal tract. On the other hand, it was reported that the presence of fibres could be of some necessity for more efficient and normal peristaltic movements of the gastrointestinal tract (19).

The percentage of calories in the three formulated supplementary food mixtures (Table 4) ranged between 364-382 Kcals which agreed with those of Prasannappa (20) who produced two supplementary baby foods contain 388 Kcals and the Baby Food Project, in Egypt (16) which provided 374-386 Kcals.

Table 5: The means ± standard errors of the different biological parameters, blood haemoglobin and haematocrit of the different experimental groups (n=6 rats in each group).

	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total food intake (g)	Total protein intake (g)	Food efficiency Ratio	Protein efficiency ratio	Haemoglobin (g/dl)	Haematocrit (%)
Control	52.17 ±1.620	89.33 ± 3.740	37.17 ± 3.020	183.02 ± 4.510	18.30 ± 0.450	0.20 ± 0.010	2.11 ± 0.160	13.29 ±0.641	35.83 ± 1.167
Formula I	50.83 ± 2.550	91.67 ± 2.380	40.83 ± 2.300	161.75 ± 14.240	** 16.18 ± 0.580	0.27 ± 0.04	2.67 ± 0.370	14.73 ± 0.401	38.17 ± 1.014
Formula II	50.83 ± 2.550	* 100.83 ± 3.360	*** 50 ± 2.160	193.63 ± 8.560	19.36 ± 0.860	***** 0.26 ± 0.001	** 2.59 ± 0.065	14.53 ± 0.439	39 ± 1.096
Formula III	50.5 ± 2.470	76.5 ± 3.700	** 26 ± 1.890	*** 165.33 ± 2.450	16.53 ± 0.730	** 0.16 ± 0.01	** 1.58 ± 0.12	13.61± 0.491	33.33 ± 1.145
Creacon	50 ± 1.730	***** 132.25 ± 5.480	***** 82.25 ± 4.660	***** 252 ± 7.770	***** 25.20 ± 0.780	***** 0.33 ± 0.01	***** 3.27 ± 0.150	14.42 ± 0.335	40 ± 1.654
Gerber	49.83 ± 2.689	87.25 ± 3.354	37.42 ± 2.509	179.90 ± 9.252	17.99 ± 0.925	0.21 ± 0.001	2.27 ± 0.203	12.87 ± 0.345	39.67± 0.422
Riri	50.33 ± 1.299	96.33 ± 4.959	46.00 ± 4.116	*** 216.70 ± 9.517	*** 21.67 ± 0.952	0.21 ± 0 .013	2.11 ± 0.122	13.01 ± 0.296	39.50 ± 0.885

Values significantly differ from the control: * : P< 0.l05 **: P< 0.025 ***: P<0.01 *****: P< 0.001.

Table 6: Mean values ± standard error of the sensory characteristics of the formulated supplementary foods.

Supplementary Foods	Appearance	Texture	Colour	Taste	Aroma	Over all Average score
Formula I	8.4$^{(a)}$ ± 0.145	7.4 ± 0.183	8.5$^{(a)}$ ± 0.167	7.9 ± 0.163	8.1 ± 0.145	8.06
Formula II	7.9 ± 0.193	7.2 ± 0.248	8 $^{(c)}$ ± 0.189	7.5 ± 0.283	7.8$^{(c)}$ ± 0.112	7.68
Formula III	7.7$^{(b)}$ ± 0.170	7.1 ± 0.229	7.2$^{(b)}$ ± 0.260	7.1$^{(b)}$ ± 0.241	6.3 $^{(b)}$ ± 0.111	7.08

N=10 mothers in each group. Sensory characteristics that significantly differ on comparison: (a) When formula I was compared with II, (b) When formula I was compared with III, (c) When formula III was compared with II. a, b, c, : P< 0.05

As the different minerals play an important role in protein-energy malnutrition, therefore it was of interest to have an idea about the picture of the minerals contents of the formulated mixtures. The obtained data in Table 4 revealed that the highest calcium content was found to be in mixture I (288.6 mg/100 g) which contained 30% defatted soybean flour. Formula III contained the lowest value of calcium (201.3 mg/100g), while formula II contained intermediate amount (254.5 mg/100 g). The highest value of phosphorus was shown in mixture I (595.4 mg/100 g), while the lowest value was 415 mg/100 g in mixture II and intermediate (553.4 mg/100 g) in mixture III. The current study demonstrated that the caclium/phosphorus ratio in mixture I, II and III were 1:2, 1:1.6 and 1:2.7 respectively. This showed that mixture II met the ideal value (21) but mixture I and III met those of other authors (22). As for iron content of the formulated supplementary mixtures under study, it could be seen from Table 4, that the highest value of iron was for mixture III (9.6 mg/100 g.) which contained fenugreek. Formulae I and II contained nearly the same amount of iron (7.3 and 7.9 mg/100 g respectively).

Concerning amino acid scores (Table 4), it can be noticed that formula I scored the highest value when compared with formula II and III.

Biological evaluation of the three formulated mixtures was carried out to evaluate the nutritional values of the three prepared formulae. The biological results of pre-

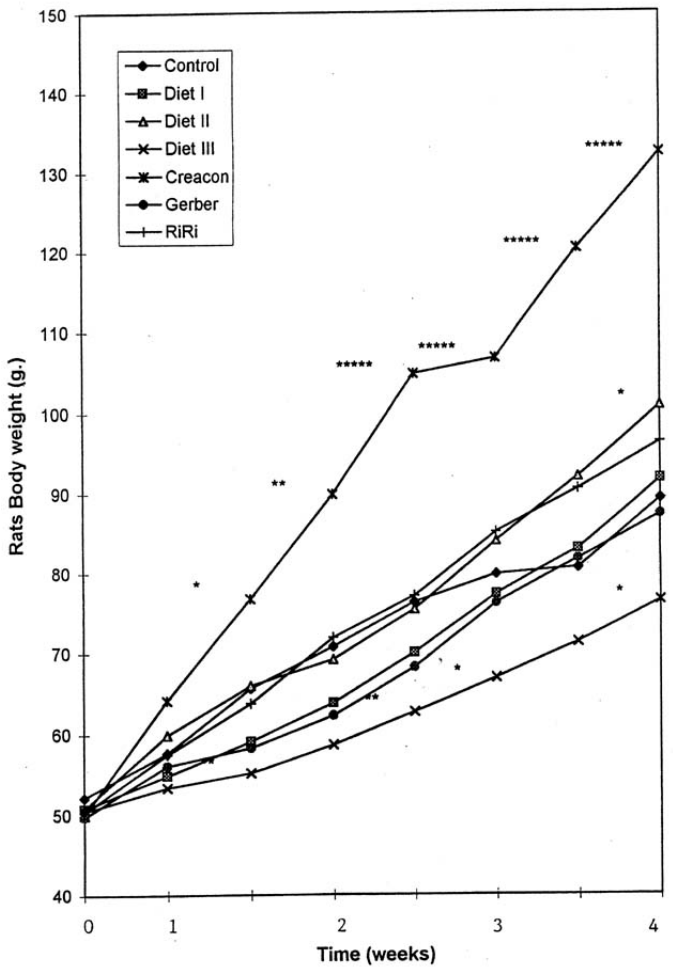

Figure 1: Growth curves of the different experimental groups. Values significantly differ from the control: *: P<0.05, **: P< 0.005, *****: P< 0.001

pared formulae I, II and III were compared with those of three references formulae present in the local market (Creacon, Gerber and Riri) and those of the six formulae were compared with the control diet of casein.

Figure 1 illustrated the growth curves of the rats fed the different diets. The animals fed on the Creacon had the best growth rate compared to all other diets. While the growth rate of rats fed formulae II, I and Riri were higher than the control, but Gerber and formula III were lower than the control.

Table 5 showed that the highest quantity of food and protein was consumed by rats fed Creacon diet and this may clarify the highest body weight gain on feeding this diet. With Riri diet it could be seen that the animals ate significant high quantity of food and consequently protein if compared to that of the control. It could be noticed here that in spite of the relatively high food consumption of Riri diet, the body weight gain did not change significantly. Feeding formula II showed non significant change of total food and protein intake but the body weight gain was significantly higher than the control. This may mean that the protein of this diet could be of high quality. On feeding Gerber diet no significant change was noticed in total food and protein intake which was reflected by no change in body weight gain. Although a significant decrease in total protein intake was noticed on feeding diet I, the body weight gain was increased non significantly. Feeding diet III produced significant reduction in total food intake and this was reflected by significant reduction in body weight gain if

compared to control rats but the rats still gained weight.

Food and protein efficiency ratios of formula II and Creacon were significantly higher than that of the casein diet. The food and protein efficiency ratios of diet I, Gerber and Riri were not significantly differ from the control. Diet III had food and protein efficiency ratios lower than that of the control. Formula I and II showed protein efficiency ratio comparable with those of other authors (23, 24).

When the three formulated mixtures were previously supplemented to marasmic infants (25), formula I promoted the best weight gain and restored reference of body composition followed by formula II; formula III induced increase in body weight but was the least if compared with formulae I and II.

Concerning the effect of the different experimental diets on blood haemoglobin and haemotocrit %, the data were presented in Table 5. No significant changes were noticed in either blood haemoglobin or heamatocrit % after feeding formulae I, II, III or Creascon. These results were expected because the rats under study were normal (not malnourished) and having normal concentration of haemoglobin and haematocrit %. The results of haemoglobin and haemotocrit % indicated that all the tested formulae were good enough to maintain the normal concentration of haemoglobin and haematocrit %.

Feeding Gerber or Riri diet produced significant increase in haematocrit % (where $P< 0.0025$ and <0.05 respectively) but had no significant effect on blood haemoglobin concentration.

Sensory evaluation of the formulated mixtures is one of the important tests to determine their acceptability. Table 6 illustrated the mean values of sensory characteristics scores such as appearance, texture, colour, taste, aroma and the overall average scores of the formulated mixtures. The obtained results indicated that the highest quality scores belonged to formula I for all the sensory characteristics followed by mixture II. Mixture III had the least quality scores. The overall average score for mixture I might be considered very good from the acceptability point of view. While mixture II and III were only good. Statistical analysis showed that the appearance scores for formula I was significantly higher than that of formula II and III. However, the appearance score of formula II did not significantly differ than that of III. No significant changes were noticed between the texture scores of the different formulae. This might be due to that all the formulae composed of legumes, cereals and 10% skimmed milk and that all were pretreated similarly, grinded after drying and contained more or less the same amount of crude fibres. The colour scores of formula I was significantly higher than that of formula II and III. Significant higher scores for colour of formula II was noticed when compared with that of formula III. The least score of colour that was found in formula III might be due to presence of lentils and fenugreek which gave the mixture slightly greenish yellow colour. It seems that the colour scores were high when it is more or less white. Concerning the taste scores, it was significantly higher in formula I when compared with formula III. While no significant changes were noticed between the taste scores of formulae I and II and between formula II and III. The aroma scores were significantly higher when either formula I or II was compared with formula III. This could be due to presence of fenugreek in formula III. Such differences in sensory characteristics scores were attributed to the different organoleptic properties of the supplementary items.

In conclusion, formula II proved to have significant higher protein and food efficiency ratios than the casin diet while those of formula I did not differ significantly from the casein diet. Formula III have the least protein and food efficiency ratios. Concerning sensory evaluation, formula I scored as very good followed by formula II that scored as good and which is better than formula III. The formulae prepared in the present study can be used successfully in underweight and protein calorie malnutrition states.

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