PREPARATION AND EVALUATION OF SUPPLEMENTED FOODS WITH FERMENTED CEREALS AND LEGUMES FOR INFANT

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ABSTRACT

The purpose of this study was to nutritionally evaluate supplement food formulated from fermented maize, peas and soybean. Maize, peas and soybean were the sole energy and protein sources. Experimental diets consist of dietary basal (A), fermented maize–soybean–pigeon peas 70:15:15 (B), fermented maize–soybean 70:30 (C), fermented maize–pigeon peas 70:30 (D) and Nutrend (E) a commercial foods supplement. A commercial product (Nutrend) manufactured by Nestle, Egypt and was used as standard diet. The formulated food supplement were fed albino rats for 28 days. The results showed that the average weight gained increased for dietary B, C, D and E (73.8-142.60, 73.8-98.26, 73.8-93.62 and 73.8-91.86, respectively), but diet A caused a decrease from 73.8 to 62.72 g. Protein qualities of dietary samples including biological value (BV; 94.26 and 98.67), true digestibility (TD; 72.55-80.46), net protein utilization (NPU; 71.72-76.53), protein efficiency ratio (1.63-3.49), feed efficiency (0.16-0.35), net protein ratio (2.49-4) and protein retention efficiency (46.38-61.24). The food supplement formulated from maize, pigeon peas and soybean supported animal growth and favorably compared with the standard. Meaning that, the application of multiple plant protein (peas and soybean) in the formulation of food supplement may be suitable for feeding of infants or children in developing countries to alleviate protein energy malnutrition.

Kay wards: supplemented foods, cereal, legumes

INTRODUCTION

Malnutrition has become one of the major world health problems facing developing countries. Throughout the developing world, malnutrition affects almost 800 million or 20% of the world population, (WHO, 2000; USAID, 2002). Clinically, malnutrition was characterized by inadequate or excess intake of protein, energy and micronutrients such as vitamins and minerals (Dutra-de-Oliveira, 1991). Protein energy malnutrition (PEM) generally occurs during the crucial transitional phase when children are weaned from liquid to semi-solid or fully adult foods. During this period, because of their rapid growth, children need nutritionally balanced, caloric-dense supplementary foods in addition to mother's milk (Fashakin and Ogunsola, 1982; WHO, 2000; Sajilata *et al.*, 2002). The formulation and development of nutritious weaning foods from under-utilized local and readily available raw materials has received a lot of attention in many developing countries, (Fashakin and Ogunsola, 1982; Ibironke *et al.*, 2012). The widespread problem of infant malnutrition in the developing world has stimulated efforts in research, development and extension by both local and international organizations. It has been discovered that legumes are largely replacing milk and other animal protein sources, which are expensive and not readily available, as suitable substitutes for high-quality protein, (Fashakin and Ogunsola, 1982; Ibironke *et al.*, 2012). The traditional weaning foods could be improved upon by combining locally available foods that could complement each other in such a way that new pattern of amino acids profile is created, by this combination, which is similar to what was previously recommended for infants' survival (Fashakin and Ogunsola, 1982). Traditional weaning foods in developing countries are known to be of low nutritive value and characterized by low protein, energy density and high bulk density. However, the high lysine content of legumes could improve the nutritional quality of cereals by complementing with sulphur containing amino acids that are limited in legumes but relatively high in cereals, whereas lysine is limited in cereals and high in legumes; therefore, a combination of legumes and cereals can be recommended to form a complete amino acid profile, (Fashakin and Ogunsola, 1982; Ekpenyong *et al.*, 1977). Protein quality depends on the amino acid profile, digestibility of the protein and the biological availability of its amino acids for the synthesis of tissue proteins, (Ekpenyong *et al.*, 1977; Fashakin and Ogunsola, 1982). Studies have highlighted the major dietary elements of peas such in terms of potassium, phosphorus, magnesium, calcium and minor dietary elements including iron, selenium, zinc, copper and manganese. Peas, in addition, is reported to be good sources of vitamin A, niacin and trace amount of thiamin, riboflavin, vitamin B_6, folate and pantothenic acid, which is highly medicinal effects (Srivastava *et al.*, 1988) and capable of preventing and cure a number of human ailments such as bronchitis, coughs, pneumonia, respiratory infections, dysentery, menstrual disorders, sores, wounds, abdominal tumors, tooth ache and diabetes (Saxena *et al.*, 2010). Although there are several studies on the subject, but data are lacking on nutritional evaluation of complementary food formulated from maize, soybean and pigeon peas (Saxena *et al.*, 2010; Srivastava *et al.*, 1988). However the combination of energy source from maize, protein sources with high lysine content from peas/soy bean could form a complete amino acids profile to form a nutritional adequate child's diet, (Saxena *et al.*, 2010; Srivastava *et al.*, 1988). Hence, the objective of this research is to nutritionally evaluate complementary foods formulated from maize, soybean and peas.

MATERIAL AND METHODS

Maize, soybean, peas, sugar, vegetable oil and cod liver oil were purchased from local market at Kafr Elsheikh Governorate, Egypt. While, the Nutrend (control diet), vitamins and minerals, were bought from Algomhoria Chemical Company, Tanta, Egypt. Thirty white albino rats of both sexes were obtained from Faculty of Medical Veterinary, Kafr Elsheikh University, Egypt. The maize, soybean and peas were cleaned, sorted and all extraneous materials carefully removed. The soybean and peas were first soaked in water for two hours, drained and then blanched for 20 minutes to inactivate enzymatic activities, de-hulled, sun dried and ground into fine smooth flour. The maize was also made into flour by first soaking the maize for a day, wet milled, made into dough and allowed to ferment for 48 hours, after that it was dried and milled into fine flour. The flours were packed into air-tight polyethylene bags and stored in the freezer. Mixing of the flour with the vitamin mix containing multivitamin was done using a Hobart (Fashakin *et al.*, 1989; Ibironke *et al.*, 2012). The basal diet is 100% carbohydrate, having no protein, (A), Fermented maize–soybean–peas 70:15: 15 (B), fermented maize–soybean 70:30 (C), fermented maize–peas 70:30 (D), Nutrend (E) a commercial dietary supplement (Ikujenlola and Fashakin, 2005; Fashakin and Ogunsola, 1982; Fashakin *et al.*, 1989).

Experimental animal procedure

The weights and ages of white albino rats ranged from 30-45 g, from three to six weeks old, respectively. Thirty white albino rats of both sexes were obtained from the Faculty of Medical Veterinary, Kafr Elsheikh University, Egypt. The experiment animals were weighed, randomly selected and distributed into six groups of five per group and housed in a metabolic cags. They were fed on pellets for seven days to acclimatize them to the new environment. The experimental animals were again reweighed and distributed into six groups of five per group, one group was sacrificed on the first day of the experiment and the liver, kidney and the plantar is muscle of the hind legs were stored in the freezer for nitrogen determination. The remaining experimental animals were placed on the experimental dietary for over a period of 28 days. Water and diets was administered *ad libitum*. During the period of the experiment, feed intake was recorded and the weights of the experimental animals were taken every three days. Seven days to the end of the experiment, the feces and urine of the experimental animals in the different groups were collected separately, urine was stored inside a bottle per groups containing 6N HCL to preserve it prior analysis, and the faces were dried in an oven at 60°C for 12 hours, cooled, weighed and stored inside a sealed polythene, per group. At the end of the 28 days, the animals were weighed, anaesthetized and sacrificed. Tissue samples from liver, kidney and plantar is muscles were removed, weighed and frozen until the nitrogen content was determined. Nitrogen in the faces and urine was determined by the micro Kjeldahl method (AOAC, 2000).

RESULTS AND DISCUSSION

Average weight gained for diets, fermented maize–soybean–peas 70:15:15 (B), fermented maize–soybean 70:30 (C), fermented maize–peas 70:30 (D) and Nutrend (E) a commercial diet, ranged from 73.85 to 142.60 g; 73.82 to 98.26 g; 73.83 to 93.62 g; and 73.80 to 91.86 g, respectively, but diet (A) 100% carbohydrates decreased from 73.80 to 62.72 g. The animals fed with fermented diets B-D compared favorably to diets E Nutrend. Experimental animals in groups B-D were looking healthy; this may be due to biological availability of amino acids for the synthesis of tissue proteins (Ekpenyong *et al.*, 1977). But animals in the basal diet group looked lean, weak and their skin was ruffled comparing with the other experimental animals; this may be due to lack of biological availability of amino acids for the synthesis of tissue proteins (Ekpenyong *et al.*, 1977). In addition, the animals fed with control diet ate large amounts of food comparing with other groups, while the basal group ate less quantity, and this reflected in the average weight gained of the animals during the experimental period as shown in Figure 1; this may be attributed to complete amino acids profile, sweet aroma which increases the appetite of the animal fed with control diet (Fashakin *et al.*, 1989; Ibironke *et al.*, 2012).

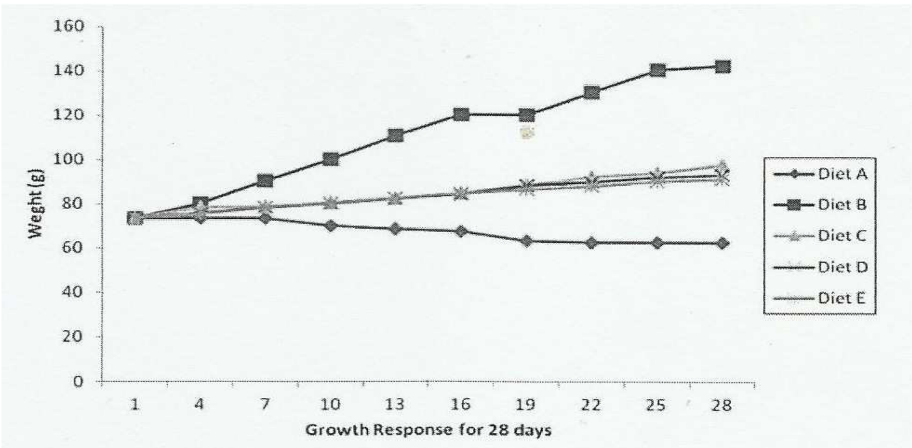

Figure 1: Average weight gained (g) of the animals during the experimental periods for 28 days

Notes: *100% carbohydrate (A); fermented maize–soybean–peas 70:15:15 (B); fermented maize–soybean 70:30 (C); fermented maize–peas 70:30 (D); and Nutrend (E) a commercial dietary supplement.*

Table (1) showed the weight of various tissues, after they were sacrificed; the tissue from the liver, kidney and the plantaris muscle collected from animals fed the basal diet were discovered to be smaller and dull in colour comparing with other animals fed on other diets and Nutrend; this may be linked to an incomplete amino acids profile in their diets, hence growth were retarded, (Fashakin *et al.*, 1989; Ibironke *et al.*, 2012).

Table 1: Weight of various tissues of experimental animals in grams

Formulated diets	Liver (g)	Kidney (g)	Plantaris muscle (g)
Diet A	2.75^{d}	0.73^{ab}	0.46^{c}
Diet B	5.42^{a}	0.87^{a}	0.78^{a}
Diet C	4.14^{b}	0.70^{b}	0.72^{a}
Diet D	4.76^{b}	0.66^{b}	0.69^{a}
Diet E	3.33^{c}	0.70^{b}	0.75^{a}
Control (at zero day)	2.64^{d}	0.42^{c}	0.68^{b}

Notes: The data are mean ± SD values of three determinations with different superscripts in a column are significantly different ($p<0.05$); 100% carbohydrate (A); fermented maize–soybean–peas 70:15:15 (B); fermented maize–soybean 70:30 (C); fermented maize–peas 70:30 (D); and Nutrend (E) a commercial dietary supplement.

Table 2 showed the total nitrogen in various tissues of experimental animals (mg)/ 100g, which include liver, kidney and Plantaris muscle. They ranged from 29,00-113,00; 11,00 –53,50 and 15,50-59,00. This is clearly reflected in the protein intake and confirmed in previous studies, (Fashakin *et al.*, 1989; Ibironke *et al.*, 2012). The combination of two proteins in the formulation of diet was reported previously to complement each other to form a complete amino acid profile (Fashakin *et al.*, 1989; Ibironke *et al.*, 2012).

Table 2: The total nitrogen in tissues of experimental animals (mg)/100g

Dietary samples	Liver (g)	Kidney (g)	Plantaris muscle (g)
Diet A	29.00^{f}	11.00^{f}	15.50^{f}
Diet B	113^{a}	53.50^{a}	59.00^{a}
Diet C	93.00^{b}	41.5^{b}	47.30^{b}
Diet D	84.00^{c}	37.00^{c}	42.00^{c}
Diet E	75.20^{d}	26.20^{d}	36.50^{d}
Control (at zero day)	53.45^{e}	27.67^{e}	23.63^{e}

Notes: *The data are mean ± SD values of three determinations with different superscripts in a column are significantly different ($p<0.05$); 100% carbohydrate (A); fermented maize–soybean–peas 70:15:15 (B); fermented maize–soybean 70:30 (C); fermented maize–peas 70:30 (D); and Nutrend (E) a commercial dietary supplement.*

The fermented maize–peas had the lowest weight among the three formulated diets; this may be linked to single plant protein incorporated into diets, this could be as a result of low appetite, incomplete amino acids profile and hence reduction in weight during the transformation, (Fashakin and Ogunsola, 1982; Fashakin *et al.*, 1989; Ibironke *et al.*, 2012). On the other hand, the tissues obtained from animals fed on Nutrend and maize–soybean–peas diets were big in weight comparing with those fed on other diet; the findings confirmed what had been previously reported, that the animals with big weight, produce big organs, (Fashakin *et al.*, 1989; Ibironke *et al.*, 2012). There were fat depositions around the tissues of animals fed on maize–soya bean and Nutrend; this may be attributed to the fact that most legumes have high content of fat, which in turns gets deposited into the diet during formulation, if not properly defatted, (PAG, 2007; Fashakin *et al.*, 1989).

Table 3 show the protein qualities of dietary samples including biological value (BV), true digestibility (TD), net protein utilization (NPU), protein efficiency ratio (PER), feed efficiency, net protein ratio (NPR) and protein retention efficiency (PRE). They ranged from 92.22 to 96.54; 72.13 to 82.34; 69.92 to 76.53; 1.51 to 4.27; 0.14 to 0.33; 2.39 to 4.10 and 44.32 to 62.22, respectively. The PER of the diet, which included Nutrend and the mixture of maize–soybean–peas, met the required standard of 2.1, while others were below the standard and this corroborated previous finding, that PER was influenced by weight gained and source of nitrogen, (PAG, 2007; Obizoba, 1990). The results obtained for PER and NPR were in accordance to what had observed previously, but the highest values were apparent in control diet (Nutrend) and compared favorable to all the formulated diets, the requirement value for NPU of 60% was met, this is synonymous to what others had been reported (PAG, 2007; Ikujenlola and Fashakin, 2005).

Table 3: Protein qualities of dietary samples in experimental animals

Diets	BV (%)	TD (%)	NPU (%)	PER	FE	NPR	PRE
B	96.43^{c}	82.34^{c}	76.53^{d}	4.27^{d}	0.33^{b}	4.10^{d}	62.22^{d}
C	94.36^{b}	77.41^{d}	74.62^{c}	2.23^{c}	0.21^{a}	2.55^{c}	50.07^{c}
D	92.22^{a}	72.13^{a}	74.23^{bc}	1.97^{b}	0.16^{a}	2.80^{b}	46.50^{b}
E	96.54^{c}	75.55^{b}	69.92^{a}	1.51^{a}	0.14^{a}	2.39^{a}	44.32^{a}

Notes: *The data are mean ± SD values of three determinations with different superscripts in a column are significantly different (p<0.05); 100% carbohydrate (A); fermented maize–soybean–peas 70:15:15 (B); fermented maize–soybean 70:30 (C); fermented maize–peas 70:30 (D); and Nutrend (E) a commercial dietary supplement*.

Table 4 show the proximate composition and energy kcal/100g of the dietary A-E; the crude protein, ash, crude fibre, ether extract, moisture (dry weight basis), nitrogen-free extract and energy kcal/100 g were ranged from 11.51 to 21.15; 2.31 to 3.01; 5.11 to 6.85; 5.16 to 9.23; 2.55 to 3.24; 57.06 to 69.43 and 369 to 564 kcal/100g, respectively. The diets were nutritionally adequate to prepare a complementary food and to meet the estimated daily nutrient requirements for complementary food (PAG, 2007; Ibironke *et al.*, 2012).

Table 4: Proximate composition of formulated diets in g/100g of the diets

Dietary sample	Crude Protein (%)	Ash (%)	Crude Fiber (%)	Ether Extract (%)	Moisture (dry basis) (%)	Nitrogen Free extract (%)	Energy (kcal/100g)
A	11.51^{a}	3.01^{b}	5.11^{a}	5.96^{a}	2.55^{b}	69.43^{e}	564^{e}
B	21.15^{e}	2.91^{e}	5.95^{d}	6.44^{d}	3.31^{d}	57.06^{a}	373^{c}
C	18.40^{d}	2.56^{d}	6.05^{c}	6.30^{c}	3.24^{e}	60.16^{b}	371^{b}
D	17.21^{c}	2.31^{c}	5.69^{b}	5.16^{b}	2.98^{c}	62.94^{c}	369^{a}
E	16.03^{b}	263^{a}	6.85^{e}	9.23^{e}	2.75^{a}	62.95^{d}	392^{d}

Notes: The data are mean ± SD values of three determinations with different superscripts in a column are significantly different (p<0.05); 100% carbohydrate (A); fermented maize–soybean–peas 70:15:15 (B); fermented maize–soybean 70:30 (C); fermented maize–peas 70:30 (D); and Nutrend (E) a commercial dietary supplement.

CONCLUSION

The study reveals that the application of mixed plant protein (cereal and legumes) sources is suitable in the formulation of complementary foods, in the reduction of protein energy malnutrition in developing countries. Supplementation of peas to other plant proteins, which is one of the underutilized legumes in Egypt, could be encouraged as a substitute or alternative to soybean. However, the results have shown that combination of energy source from maize and protein sources from peas/soy bean could form a complete amino acids profile that could translate to a nutritionally adequate child's diet to promote growth.

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الملخص العربى

إعداد وتقييم مدعم غذائي من الحبوب المتخمرة والبقول

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الغرض من هذه الدراسة هو إعداد وتقييم مدعم غذائى من خلطات من الذرة المتخمرة والبازلاء وفول الصويا المتخمرة كمصادر للطاقة والبروتين على التوالي. خلطات التجربة تتكون من الكنترول (A) (خالية من خامات التجربة) (A)، وخلطة الذرة المتخمرة + الصويا + البازلاء بنسبة خلط 70:15:15 (B)، وخلطة الذرة المتخمرة + الصويا بنسبة خلط 70:30 (C)، وخلطة الذرة المتخمرة + البازلاء بنسبة خلط 70:30 (D)، وخلطة تجارية (E). الخلطة التجارية مصنعة بشركة نستله مصر وتستعمل كخلطة قياسية. وضعت جميع الخلطات السابقة لتغذية الفئران لمدة 28 يوم. أظهرت النتائج ان متوسط الزيادة في الوزن لمجموعات الفئران المغذاه على خلطات B،C،D كانت (142,60-73,80) و(98,26-73,80) و(91,86-73,80) على الترتيب, لكن الخلطة A سببت انخفاض من (62,72-73,80). كما أوضحت مؤشرات جودة البروتين ان Biological Value (BV) (98,67-94,26) و True Digestibility (TD) (80,46-72,55) و Net Protein Utilization (NPU) (76,53-71,72) و Protein Efficiency Ratio (3,49-1,63) و Feed Efficiency (0,53-0,16) و Net Protein Ratio (4-2,49) و Protein Retention Efficiency (61,24-46,38).

لذا تكون خلطات المدعم الغذائي المكون من الذرة المتخمرة والبازلاء والصويا داعمة لنمو الحيوانات مقارنة بالخلطة القياسية، وهذا يعنى انه من الممكن تطبيق استخدام العديد من البروتينات النباتية (البازلاء والصويا) في إعداد مدعم غذائى للأطفال الرضع خصوصاً في بلدان الدول النامية للقضاء على سوء التغذية.