The Immune Function as Response to Level and Source of Protein in Pre-Mature and Mature Male Rats.

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Abstract: Dietary protein plays a significant and site-specific role in the developmental expression of the secretary immune system. In this sense, the aims of this study were as follows: 1) to evaluate in rats the severity effect of a protein-deficient diet (4%) on non specific cellular immune response (phagosytosis, killing and lymphocytes transformation index), immunoglobulins (IgG and IgM) and cytokine (IL-6). 2) to assess these parameters in rats re-fed on normprotein diet (20% casein) and rats re-fed on faba bean (20% raw bean) as alternative sources of protein. 3) to compare between premature and adult rats under the various levels and source of dietary protein. Forty eight rats were used in the present study, premature aging 40 days (weighted 85±5 gm) and adult aging 120 days (weighted 170 ±10 gm). Each animal age was divided into two groups, control (n=8) and experimental groups (n=16). Control group fed on a normoprotein chow, while experimental group fed on a diet having 4% protein (diet 1), for 3 weeks. After then, the experimental group of each age was divided into two groups. The first group received a normoprotein diet containing 20% casein (diet 2) and the second group received a diet containing 20% faba bean (diet 3) as alternative source of protein. The experiment was lasted for six weeks and the animal mortality and body weight were regularly recorded. At the end of experiment, the blood samples were collected through cardiac puncture from all animals under light anesthesia. The blood of each animal was split into two essay tubes, one heparinzed for the determination of the complete phagocytosis, killing and lymphocyte transformation index and the other wasn't; to obtain the serum for subsequent analyses of IgG, IgM and IL6 activity, total protein, albumin and thyroxin activity (T3 and T4). The results revealed that the body weight was reduced in protein-deficient rats as related to age associated with appearance of some cases of mortality. The rats re-fed on normoprotein chew (diet 2) showed increase in body weight more than the animal received diet 3 (20% faba bean) and this increase was more pronounced in premature rats. As a function of circulating levels of total protein, albumin and thyroxin, hypoproteic diet induced significant decrease in total protein content as well as T3 activity in both ages but the albumin and T4 level showed insignificant decrease. Nutritional recovery by diet containing 20% casein (diet 1) decreased the activity of T3 in premature rats. Regarding to rats re-fed on either diet 1 or diet 3, they showed significant increase in protein, albumin, T3 and T4 levels as compared to protein-deficient rats but this recovery was more pronounced in rats re-fed on a diet 1. With respect to immune function, low dietary protein induced disorder in the activity of phagocytosis, killing, lymphocytes transformation index (TL index) as well as IgG, IgM & IL6 in premature than in mature rats. But, these remarks of immune function were improved in both ages rat re-fed on diet 1 more than rats re-fed on diet 3. From the present results, it could be suggested that poor protein nutrition or inclusion of faba bean as the only source of protein in diet especially, young rats bring about a reduction in growth as well as impairment of undeveloped immune system because the absence of essential amino acids will comprise the ability of tissue to grow, be repaired or be maintained.
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Key words: hypoprotein diet, non cellular immune cells, immunoglobulin, cytokine, thyroxin, total protein.

1. Introduction

The scientific and technologic development of the biological and health areas taking place these last years has been trying to favour, directly or indirectly, the longevity of the human being. Protein malnutrition has long been recognized as a common problem, especially in children in the developing countries, whose inadequate nutritional intake is deficient for socio-economic reasons (Akinola et al. 2010). Within this scope it is important to consider that dietary protein deprivation during early life is known to have adverse effects on brain anatomy, physiology and biochemistry and even permanent brain damage (Torún & Chew, 1993) and also retards the differentiation of the morphological, metabolic, and contractile characteristics of skeletal muscle fibres in growing stage (Alves et al. 2008). Likewise, apart from protein undernutririon, deficiencies of iron and vitamin A which are together account for more than 75% of the deaths of infants and young children in some developing countries (Ou, 2002). Moreover, the impairment of immune function occurrence as a result of protein malnutrition correlated with the incidence of infection and the high morbidity of children younger than 5 years in developing countries (Santos et al. 1997). Generally, protein malnutrition disorders included growth failure, hypoproteinemia, oedema, fatty liver and reduced the antioxidant enzymes as well as hampered the immune defence in humans and animals (Sawosz et al. 2009).

Thus, the need for alternative protein sources has recently gained focus. Among the possible protein sources, lupins, peas and faba beans were successfully used in ruminants and non ruminants (Vandoni et al. 2007). Faba beans (*Vicia Faba*), in particular, show a high content of tannins, particularly in the coats, whilst the trypsin inhibitor activity is low in comparison with many other legumes (Volpelli et al. 2010). The nutritional value of beans has been traditionally attributed to it high protein content, which ranges from 25 to *35%,* despite the imbalance in sulphur amino acids. Most of these proteins are globulins (60%), albumins (20%), glutelins (15%) and prolamins. The chemical analysis of this legume revealed low saturated fat content and high in complex carbohydrates, fibres, micronutrients and phytochemicals such as anthocyanins, a variety of phenolic compounds, protease inhibitors, phytic acid, and saponins (Volpelli et al. 2010). Epidemiological (Brown et al. 1999), clinical (Fung et al. 2001) and animal (Rosa, 1998) studies proved that beans reduce blood cholesterol and the high amounts of resistant starch as well as high amounts of soluble and insoluble fibres contents in bean leads to favourable fermentation and possibly inhibits colon cancer (Hangen and Bennink, 2003). Greater consumption of beans by children in developing countries would significantly reduced morbidity and mortality in this age group.

Because qualitative or quantitative protein malnutrition suppresses the specific immune system (Chandra, 1983) and causes severe lymphopenia, while qualitative malnutrition could result in neutrophilia (Balkaya, 1999), the role of the non-specific immune system would be more important in this respect (Biyik et al. 2005). Therefore, the present study was focused on the effect of dietary protein deficiency (4%) followed by re-feeding on a diet containing normoprotin or faba bean as alternative sources of protein on some physiological markers such as animals survival, body weight, circulating total protein, albumin and thyroxin concentrations as well as on some immunological markers such as phagocytosis, killing, lymphocytes transformation index, IgG, IgM, IL6, in premature and adult rats.

2. Experimental methods

This experiment was carried out on forty eight male albino rats from two ages, premature aging 40 days (weighted 85±5 gm) and adult aging 120 days (weighted 170±10 gm) were used in the current study. All rats were kept in metabolic cages under controlled temperature (25°C±5) and light/dark cycles (12/12 hrs). During the whole experimental period water and chow were supplied ***ad-libitum.*** Each age was randomly allotted to two groups: control (n=8) and experimental group (n=16). The control group was fed with normoprotein chow (20 % casein, diet 1). This chow was bromatologically analyzed and from the results the components were calculated to be added so as to decrease the protein level to 4% casein (diet 2) or 4% casein and 16% faba bean (diet 3) while keeping the vitamin and mineral balance. The experimental animals fed on the prepared hypoproteic diet 2 for three weeks and then they divided into two groups. The first received basal diet 1 containing 20% casein and the second received diet 3 containing 4%casein and 16 % faba bean for other 3 weeks. Mortality and body weight of all groups were recorded throughout the experiment. At the end of experiment, the rats from both control and experimental groups of two ages were fasted for about 12 h and then the blood samples were collected from each animal through cardiac puncture under light anesthesia. The blood of each animal was divided into two essay tubes, one heparinzed and the other wasn't; the serum in this last tube was obtained through centrifugation at 3000rpm for 15 min., the obtained serum was kept at –20°C for subsequent analyses of IgG, IgM and IL6 activity using ELISA (Life Diagnosis, Inc.) according to Salauze et al. 1994 , total protein and albumin concentration according to Gornall et al. (1949) and Doumas et al.(1971) methods in addition to thyroxin activity (total T3 and T4) by radioimmunoassay kit purchased from (Institute of Isotopes Ltd. Budapest) according to Ratcliffe et al. (1974) technique. The non-clotted blood collected was used for the determination of the complete phagocytosis, killing according to **(**Woldehiwet and Rowan, 1990) and lymphocyte transformation index (Boyum, 1968 and Burrels and Wells, 1977).

Statistical analyses

All values were expressed as mean ± SE. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Duncan's test using SPSS program 17.0. *P* values < 0.05 were considered to be statistically significant.

Table (1): Percent composition of the chow offered to control and experimental groups.

Composition (g/100g)	Diet 1	Diet 2	Diet 3
casein	20	4	-
Faba bean	-	-	20
Fat	10	10	10
Carbohydrates	62.5	78.5	62.5
Cellulose	3	3	3
Salt mix.	3.5	3.5	3.5
Vitamin mix.	1	1	1

Source: Percent composition carried out by Laboratory of nutrition analyses of Analysis and Designer Department of Nutrition- National Research Centre, Cairo, Egypt.

3. RESULTS:

The present results showed a gradual decrease in body weight of the premature rats which received hypoproteic diet as compared to control (table 2). When the animals received alternative dietary casein or faba bean, their body weight returned to increase particularly, in the rats re-feeding normoprotein as compared to corresponding re-feeding on faba bean. It is worth noting that diminished daily food consumption from dietary casein in premature rats was more relative to daily consumption from dietary bean and such phenomena was probably the reason for mortality of 25% of the animals in this group. The loss in the body weight was overcome when the animals thereafter fed on dietary casein more than dietary faba bean.

Table (2): Effect of variable levels and sources of dietary protein on mortality and percentage of body weight change of premature rats.

	Control						Hypoproteic diet			Alternative diet					
										Casein			Faba bean		
Weeks	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**4th**	**5th**	**6th**
% of mortality	-	-	-	-	-	-	-	-	-	-	-	-	**12.5**	**12.5**	**12.5**
% of b.wt. Change	**+1.4**	**+2.7**	**+11.3**	**+15**	**+20.3**	**+32**	**-11.8**	**-11.5**	**-14**	**+42.7**	**+44**	**+50.**	**+26.5**	**+32**	**+25**

The results obtained in table (3) showed loss in the body weight of animals received hypoproteic diet, but when these animals received dietary casein or bean protein, the rate of body weight gain increased to reach its maximum level at the end of experiment. Although the weight gain of survived animals which consumed dietary casein was more than weight gain of animals consumed dietary faba bean, the mortality in this group was more.

Table (3): Effect of variable levels and sources of dietary protein on mortality and percentage of body weight change of adult rats.

Groups	Control						Hypoproteic diet			Alternative diet					
										Casein			Faba bean		
Weeks	1st	2nd	3rd	4th	5th	6th	1st	2nd	3rd	4th	5th	6th	4th	5th	6th
% of mortality	-	-	-	-	-	-	-	-	12.5	12.5	12.5	12.5	-	-	25
% of b.wt. Change	+11.9	+20.6	+9.2	+17.4	+22.5	+40.9	-0.03	-1.1	-6.3	+11.1	+19.2	+10.5	-0.8	-7.1	-1.3

Serum total protein level showed significant decrease (p<0.05) associated with insignificant decrease in albumin level in both ages of rats subjected to low dietary protein. But the protein malnutrition recovered when the animals re-fed on normal dietary casein more than the animals re-fed on dietary faba bean and this recovery was more pronounced in adult rats as shown in fig. (1&2).

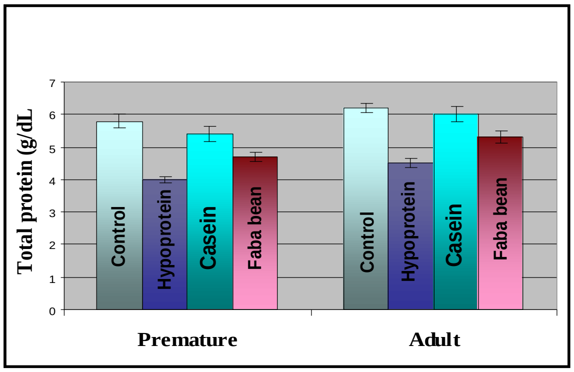

Fig.1. total protein level in premature and adult rats under effect of variable levels and sources of dietary protein

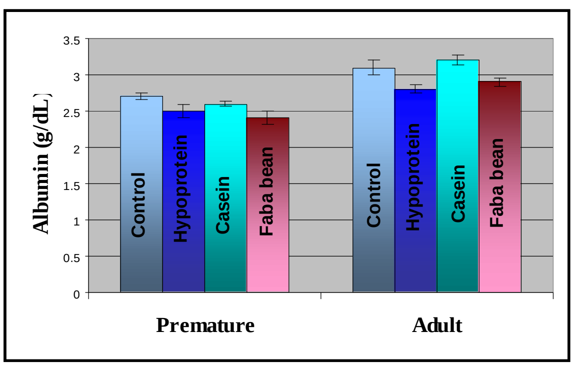

Fig.2. albumin level in premature and adult rats under effect of variable levels and sources of dietary protein

The activity of circulating T3 and T4 significantly (P<0.05) decreased in animals fed low-protein diet, returned to normal level by subsequent consumption of dietary casein or faba bean and the subsequent consumption of dietary casein or dry bean caused significant increase in T3 level particularly in adult rats re-fed on dry bean (Figs.3 & 4).

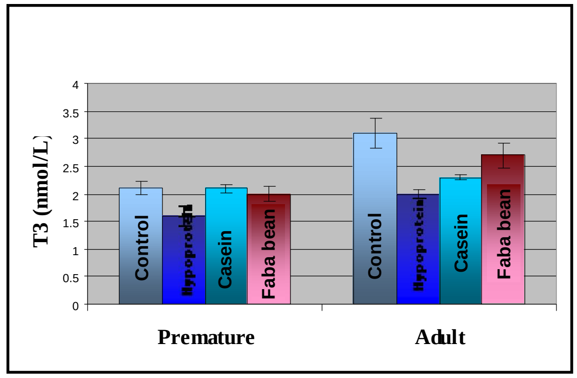

Fig.3. T3 in premature and adult rats under effect of variable levels and sources of dietary protein

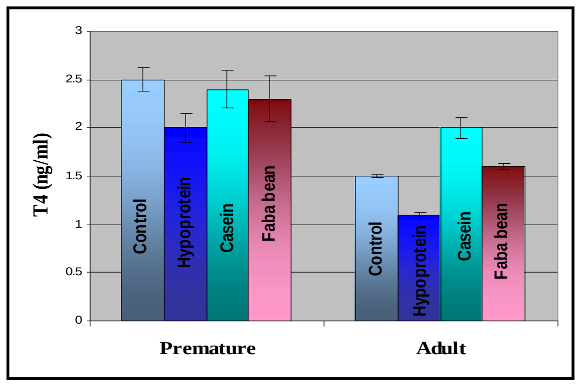

Fig.4. T4 level in premature and adult rats under effect of variable levels and sources of dietary protein.

As shown in figs. (5,6 and 7), hypoproteic diet (4%) induced significant decrease in the activity of phagocytosis and killing process as well as TL- index in premature rats as compared to corresponding adult or control rats. However, the following consumption of dietary casein had significant (p<0.05) effect on these non specific cellular immune functions more than the effect of dietary bean consumption and this effect was more pronounced in adult rats.

Figs. (8, 9 and 10) clarified that intake of hypoproteic diet 4% caused significant ($P< 0.05$) decrease in IgG, IgM and IL6 activity in premature and adult rats but the alternative dietary casein or faba bean modulated this decrease particularly in the adult rats which re-fed on dietary casein.

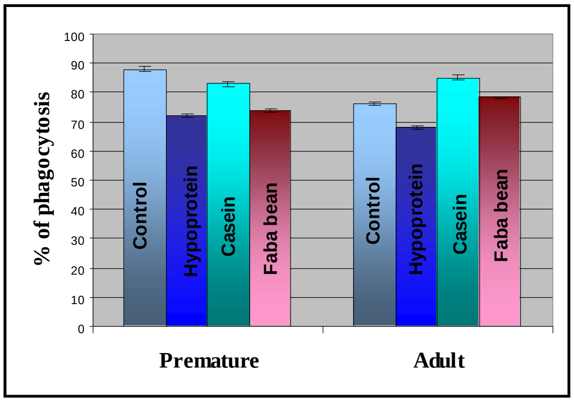

Fig.5. phagocytosis activity in premature and adult rats under effect of variable levels and sources of dietary protein

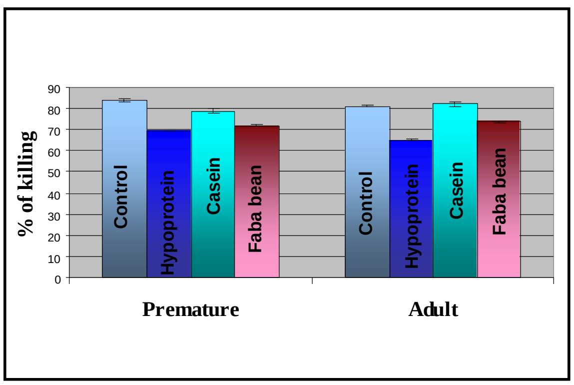

Fig.6. killing activity in premature and adult rats under effect of variable levels and sources of dietary protein

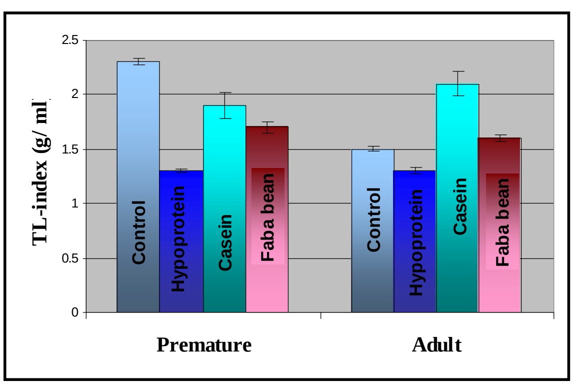

Fig.7. TL-index in premature and adult rats under effect of variable levels and sources of dietary protein

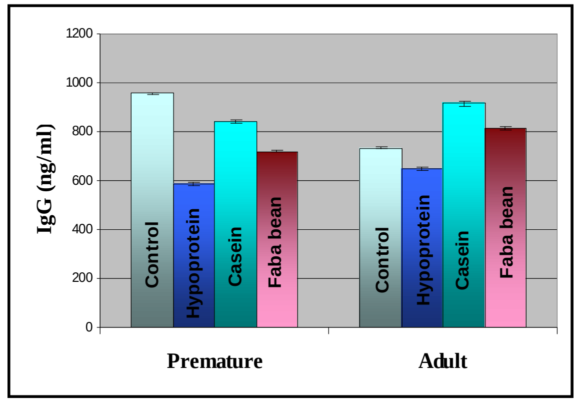

Fig.8. IgG concentration in premature and adult rats under effect of variable levels and sources of dietary protein

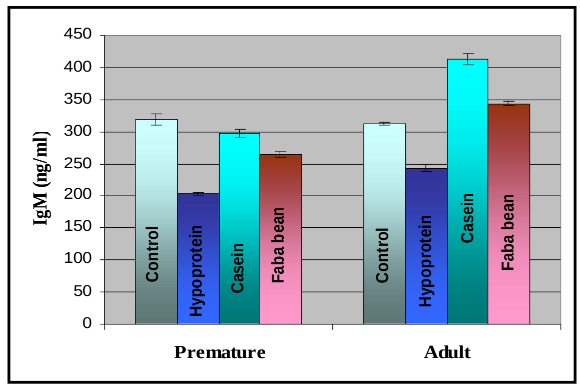

Fig.9. IgM concentration in premature and adult rats under effect of variable levels and sources of dietary protein

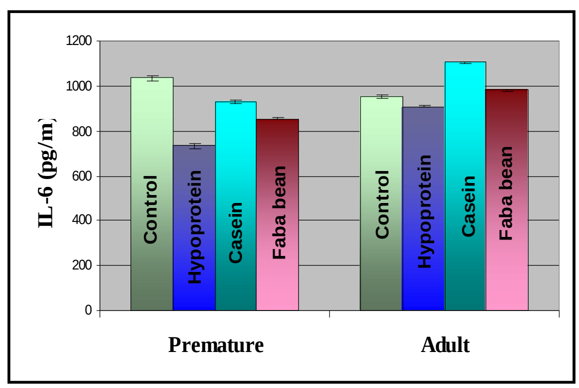

Fig.10. IL-6 concentration in premature and adult rats under effect of variable levels and sources of dietary protein

4. Discussion:

Protein deficiency is prevalent in underdeveloped countries and caused excessive morbidity and mortality in particular children during first years of life (Underwood, 2002). The earlier the malnutrition, the more severe the effects, resulted in greater severity and extent of damage to a range of organs and systems (Crogan and Pasvogel, 2003). Since, the sequential changes of malnutrition are altered cellular metabolism, impaired physiologic function, significantly affects growth and differentiation of tissues and cells and finally loss of body tissues (Briet et al. 2004). In the present study, hypoproteic diet 4% caused reduction in the body weight of premature and mature rats by 14% and 6.3% respectively, as compared to corresponding control. The significantly lower body weight of these groups was a result of a poor quality protein in the experimental diet due to the deficit in essential amino acids resulted in lower quantity of food consumed by the rats (Hernández et al. 2008). As expected, the effects of protein deficiency on body weights were more pronounced for the premature rats than for the adult rats because they able to select a protein diet appropriate to their bodily needs resulted in retardation in the differentiation of the morphological, metabolic, and contractile characteristics of their skeletal muscle fibres (Alves et al. 2008).

When the rats re-fed on the normal diet (20% casein) for three weeks, the increase in their body weight observed at the last phase of experiment suggesting that these animals recovered from malnutrition. In contrary, the animals allocated on faba bean diet as alternative source of protein showed decrease in their body weight at the end of experiment as compared to rats kept on normal diet and compared to rats re-fed with 20% casein. It was well noticed that survival of rats was correlated with percentage of increase in their body weights as well as the quantity of food consumed in both ages group. On other wise, the weight gain of adult rats was less than weight gain of premature and the weight gain of re-fed casein rats was more than rats re-fed on faba bean. Such impairment of growth in the animals fed on diets containing *Vicia faba* has been reported in rats (Martínez et al. 1987), mice (Martínez et al. 1992). This is because the digestibility and absorption of carbohydrates and proteins is adversely affected by the inclusion of faba bean in the diet (Sobrini et al. 1983). The possible involvement of several antinutritional factors such as polyphenols, tannins, protease inhibitors and lectins as well as the deficiency of sulphur amino acids in *Vicia faba* in the processes of metabolism and nutrient utilization has been the main reason of growth retardation (Larralde and Martinez, 1991). Since, dietary fibre may decrease digestibility of protein and amino acids by stimulating the production of bacterial protein, through adsorption of amino acids and peptides onto the fibre matrix, and by increasing the excretion of endogenous protein (Schulze et al. 1994). The mechanism by which bean exerts its effect in growing rats could be by substance present in it, which might either alter the electrolyte membrane permeability decrease the capacity of carrier system for the substrates (Santidrian, 1981). In this context, Goena et al (1988) established that feeding growing animals with faba bean as source of protein leads to a significant impairment of growth, which must be mainly attributed to a decrease in muscle mass (representing about 40% of body weight in mammals) achieved by a fall in muscle protein synthesis rather than to changes in protein breakdown. The mechanism seems to involve a reduction in muscle RNA activity associated with an increase in liver protein synthesis, which could be indirectly mediated by the flux of amino acids coming from the skeletal muscle (Kurpad, 2006). These facts emphasize the importance of nutritional recovery at early phases of life, in order to achieve normal growth and development (Gigante et al. 2007).

The dynamic balance among the serum thyroxin, circulating protein contents and the body weight was manifested in the present study in protein deficient rats by the depression in T3 and T4 activity associated with reduction in protein content and animal body weigh. Such relationship was depended on the stage of age and was considered as another remarkable feature of animals subjected to low dietary protein (Heard et al. 1977). The albumin was not significantly altered as well because the rats fed on low dietary protein for short run (Obatolu et al. 2003) and it may be decreases when the same diet employing with rats of the same strain, in the long run (Sant' Ana et al. 2001). The most intense effect of the hypoproteic diet employed in the current study was may be the effect on the activities of the complex enzymes of electron transport chain, which may be due to changes in mitochondrial protein synthesis, breakdown, or both (María & Carolina, 2007) or lack of vitamins B complex supplementation in the chow of the experimental group (Guyton & Hall, 2002) and/or association of malnutrition with concomitant sign of intestinal free radical damage and altered protein transport suggesting that oxidative stress is partly responsible for the intestinal dysfunction and depression of metabolic rate observed in malnutrition (Akinola et al. 2010). As related to last suggestion, Sidhu et al. (2004) indicating that malnutrition possibly caused alterations in antioxidants enzymes activities and increase lipid peroxidation. It has been proposed that free radical mediated tissue damage may be involved in malnutrition mainly because of the inadequate protective and repair mechanisms in protein-deficient animals or human. On the other hand, investigations of enzymes responsible for maintaining glutathione in the reduced state and studies in response to oxidative stress found increased activity of G6PD and showed that impaired antioxidant status and decreased proportions of red cell phospholipids were found in different types of malnutrition (Akinola et al. 2010). In spite of rats allocated on alternative dietary faba bean showed increase in serum protein and albumin levels, it was less than the level in rats re-fed on normal dietary casein. Such finding could be attributed to the tannins constituent in faba beans which increase the secretion of proline-rich proteins by the rat's parotid glands (Jansman et al. 1993) and interacting with both dietary and endogenously secreted proteins in the intestinal tract result in enhancement of fecal excretion of both sources of protein (Jansman et al. 1995). Moreover, the quality of faba bean protein appeared to be limited by the low content in sulfur-amino acids, tryptophan, valine, isoleucine, and threonine (Baudet and Mose, 1980). But the casein re-feeding, restored the activities of mitochondrial in the gastronomies muscle due to increased amino acid availability which important in restoring the activities of mitochondrial complexes (Briet & Jeejeebhoy, 2001).

As far as the relationship between thyroxin hormones (T3 and T4) and metabolic rate is concerned since, the thyroxin stimulated the transcription of genes that influence the metabolic rate of all cells, thus the greater the level of these hormones, the larger the metabolism (Guyton & Hall, 2002) depended on the protein content of the diet (Ahrens et al. 1990). Perhaps the decrease in thyroxin was due to adaptation of thyroid to smaller substrates availability in low dietary protein (Waterlow, 1996). Knowing that T4 is capable of increasing the rate of protein synthesis when there are adequate amounts of carbohydrates and lipids (Guyton & Hall, 2002), this essential cellular process should be impaired in the animals from hypoproteic group, especially in organs of greater cellular and/or protein turnover, due to the low level of the free hormone and also due to the presumptive lack of available amino acids caused by the hypoproteic diet (Araújo et al. 2005). On the other hand, the thyroxin level in both rats ages re-fed on dietary casein restored more than the rats re-fed on faba bean because some of the essential amino acids needed for bio-activity of thyroxin hormone not constituent in feba bean. It is of interest for future research to know how vegetable proteins raise levels of thyroid hormones. We are not aware of data showing that amino acids affect thyroid function, particularly plasma arginine and serine, which correlated best with thyroxin concentration.

The impairment in the immune function particularly, in children has been interpreted as a secondary outcome of inadequate dietary protein (Menezes et al. 2003). The aspects of immunity most consistently affected by protein malnutrition are cell-mediated immunity, phagocyte function, the complement system, secretary antibody, and cytokine production (Weller, 2001). In the present study, consumption of low dietary protein 4% induced significant decrease in processes of phagocytosis, killing and TL index as well as IL6, IgG and IgM in both age groups relative to control but this inhibitory effect was more pronounced in premature rats than in adult rats. Such results considerably attributed to a greatest lymphocyte blastogenesis in children than in adults, resulting from immaturity of immune system (Sasai et al.1981). In addition to that the time span of using protein-free diet played a key factor for the safety of this treatment (Sawosz et al. 2009). Hence, the experimental period of this study was not enough to reduce phagocytic activity clearly in peripheral blood of the adult malnourished rats. In this view, Borelli et al. (1998) have been reported that protein deficiency tended to decrease the number of lymphocytes and functions of T-helper cells, natural killer cells and peritoneal macrophages. Consequently, the low lymphocyte transformation rates in rats consumed hypoprpteic diet therefore indicated impairment of lymphocyte function. On the other hand, deficient of protein influenced the concentration of body potassium, an important intracellular ion which may play a role in contributing to the lymphocyte abnormality (Sellmeyer et al.1972). As a function of IL6, some experimental and clinical studies have suggested that protein malnutrition affect circulating IL-6 level (Agüero *et al*. 2006), which can have anti- and pro-inflammatory function (Lyoumi *et al*, 1998) and others established that protein malnutrition induces a low-grade inflammatory state in rats, as evidenced by elevated serum levels IL-6 and reduced serum levels of albumin (Suzuki et al. 2002 and Ling et al. 2004). Likewise, it was suggested that protein deficient animals present a failure in the regulatory mechanisms of the IgG and IgM resulted in decreased their total levels (Malavé and Layrisse, 1976). Abnormal low levels of IgG and IgM were demonstrated in children as a response to protein deficiency due to specific inhibition of their synthesis by dietary components (Kenrick and Smith, 1970).

Thus, it is reasonable to consider that decreased level of IL6, IgG and IgM observed in the present study, at least in part, reflected the Immunosuppressive effect consequent to a protein-deficient diet that exerted on cells mediated immunity to reduce its products.

In this way, other investigators suggested that protein deprival increased the intensity of oxidative burst in neutrophils. Reactive oxygen species released by neutrophils in an extended amount scan lead to cell, tissue, and organ damage and also may induce inflammation (Mitra and Abraham 2006). It seemed that deficit of protein-originated antioxidants, especially from sulphuric amino acids, might be a key factor explaining the present results. The increase of oxidative burst in neutrophils might be caused by reduced antioxidant capacity, resulting from deficiency of glutathione and other amino-derivative antioxidants as a sequence of low dietary protein (Sawosz et al. 2009).

The functional of the immune system competence was reduced in rats re-fed on faba bean diets for both cellular and humoral-mediated responses. It is well known that the response of the immune system has been widely recognized as an adequate index for the evaluation of the nutritional value of a diet (Stinnett, 1987), because it is sensitive to legume feeding (Sissons et al. 1988). Since zinc bioavailability is apparently reduced in animals fed on faba bean by the occurrence of phytates as indicated by the reduction in plasma zinc concentration (Martínez et al. 1986) and zinc is apparently involved in some immune mechanisms. The functional competence of the immune system was reduced in rats fed on faba bean diets for both cellular and humoral-mediated responses (Macarulla et al. 1989). Legume proteins contain considerable quantities of phytic acid, dietary fiber and other organic compounds, which may affect mineral bioavailability from the diet (Martínez et al. 1985). Zinc affects gene regulation within lymphocytes. This can dys-regulated intracellular killing, cytokine production, and phagocytosis (Shankar and Prasad, 1998). The macrophage, a pivotal cell in many immunologic functions, is adversely affected by zinc deficiency (Menezes et al. 2003). Also, zinc is crucial for the normal function of cells which mediate non-specific immunity, such as neutrophils and natural killer cell, B lymphocyte development and antibody production, particularly immunoglobulin G, is compromised by zinc deficiency (Larralde and Martinez, 1991).

5. Conclusion:

It could be shown for the first time that introduction of dietary proteins after weaning is important for a critical period in which both local and systemic aspects of the immune system undergo maturation. Also, the effects of early undernourishment are time-dependent and may cause irreversible changes in the regulation of metabolism. However, dietary protein depletion results in malnutrition at the whole body levels, indicated by reduction in body weight associated by impaired in circulating thyroxin hormone and decreased in total protein and albumin as well as suppression on the immune responses. In the light of present data, the reduction in immunological activity in these rats could be rather explained by a direct effect of the reduction of stimulation by dietary antigens. Consequently, it could be predict that protein malnutrition at an early age may have an unsuspected immunological impact in the children. Beans are not typically fed to the children, however, appropriate combinations of beans in adequate amount consumed with other sources of protein, will prevent protein malnutrition.

Further studies are needed to demonstrate whether protein supplementation can reverse the changes, at all health levels, induced by protein malnutrition.

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6. References:

1. Agüero G., Bioq J.V., Bioq S.R., Bioq C.H. and Alvarez A. (2006): Beneficial immunomodulatory activity of *Lactobacillus casei* in malnourished mice pneumonia: effect on inflammation and coagulation. Nutrition 22, 810-819.
2. Ahrens, S.K E., Hagemeister, H., Rgenunshelm, J., Agergaardf, N. and Barth, C. (1990): Response of Hormones Modulating Plasma Cholesterol to Dietary Casein or Soy Protein in Mini pigs. J. Nutr 120, 1387-1392.
3. Akinola, F.F., Oguntibeju, O. and Alabi, O.O. (2010): Effects of severe malnutrition on oxidative stress in Wistar rats .Scientific Research and Essays 5, 1145-1149.
4. Alves, A.P., Dâmaso, A.R. and Pai , V.D.(2008): The effects of prenatal and postnatal malnutrition on the morphology, differentiation, and metabolism of skeletal striated muscle tissue in rats . J.de Pediatr 84, 264-271.
5. Araújo, E.J.A., Sant'Ana, D.M.G.; Molinari, S.L. and Neto, M.H.M. (2005): Hematologic and biochemical parameters of rats subjected to hypoproteic and hipercaloric diet. Arq. ciên. Vet. Zool. Unipar 8, 139-146.
6. Balkaya, M.: The effects of feeding gelatin containing diet and following complete feeding on the counts of the peripheral white blood cells of the male female wistar albino rats. Tr. J. Vet. Anim. Sci 23, 417-429
7. Baudet,J.; Mose, J. (1980): Amino acid composition of different cultivars of broad

beans*(Vicia faba)*: Comparison with other legume seeds . In *Vicia faba:* Feeding Value, Processing and Viruses ; Bond, D.A., Ed.; Martinus Nijhoff : The Hague, p67.

8. Biyik, H., Balkaya, M, H., Meyra, N.S.A. and Cengiz, L. (2005): The effects of qualitative and quantitative protein malnutrition on cecal microbiota in wistar rats with or without neutrophil suppression. Turk J Vet Anim Sci 29, 767-773.
9. Boyum, A.(1968): Isolation of leucocytes from human blood. Further observations. Scand. Clin Lab Invest 97, 31-50.
10. Briet, F. and Jeejeebhoy, K.N. (2001): Effect of hypo-energetic feeding and refeeding on muscle and mononuclear cell activities of mitochondrial complex I-IV in enterally fed rats. Am J Clin Nutr 73, 975-83.
11. Briet, F., Twomey, C. and Jeejeebhoy, K.N. (2004): Effect of feeding malnourished patients for 1 mo on mitochondrial complex I activity and nutritional assessment measurements. Am J Clin Nutr 79, 787-794.
12. Brown, L., Rosner, B., Willett, W. W. and Sacks, F. M. (1999): Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr 69, 30-42.
13. Borelli, P., Souza, I. P., Borojevic, R., Dagli, M. L. & Kang, H. C. (1998) Protein malnutrition: some aspects of the in vitro adhesion of peritoneal mouse macrophages. Ann. Nutr. Metab 42, 367-373.
14. Burrells, C. and Wells, P.W. (1977): In vitro stimulation of ovine lymphocytes by various mitogens.Res.Vet.Sci 23,84-86.
15. Crogan, N.L. and Pasvogel, A. (2003): The influence of protein-calorie malnutrition on quality of life in nursing homes. J. Gerontol. Biol. Sci 58, 159-164.
16. Chandra, R.K. (1983): Numerical and functional deficiency in T helper cells in protein energy malnutrition. Clin. Exp. Immunol 51, 126-132.
17. Doumas, B.T., Watson, W. and Biggs, H.G. (1971): Albumin standards and the measurement serum albumin with bromocresol green. Clin Chim Acta 31, 87-96.
18. Fung, T. T. Willett, W., Stampfer, M.J. and Hu F.B. (2001): Dietary Patterns and the Risk of Coronary Heart Disease in Women. Archives of Internal Medicine 161, 1857-1862.
19. Gigante, D.P., Buchweitz, M., Helbig, E., Almeida, A.S., Neumann, N.A and Victora, C. G. (2007): Randomized clinical trial of the impact of a nutritional supplement “multimixture” on the nutritional status of children enrolled at preschool. J Pediatr (Rio J) 83, 363-369.
20. Goena, M., Santidrian, S., Cuevillas, F. and Larralde, J. (1988): Muscle and liver protein synthesis and degradation in growing rats fed a raw field bean (Vicia faba L.) diet. Rev. Esp. Fisiol 44, 345-352.
21. Gornall, A.G., Bardawill, C.J., David, M.M. (1949): Determination of serum protein by biuret method. J Biol Chem 117, 751-766.
22. Guyton, A. C. and Hall, J. E. (2002): Os hormonios adrenocorticois. In: Tratado de fisiologia médica.10 ed. Rio de Janeiro: Guanabara Koogan. 77, 818-822.
23. Hangen, L., and Bennink M. R. (2003). Consumption of black beans and navy beans (Phaseolus vulgaris) reduced azoxymethane-induced colon cancer in rats. Nutr Cancer 44, 60-65.
24. Heard, C. R. C., Frangi, S. M. and Wright, P. M. (1977): Biochemical characteristics of different forms of protein-energy malnutrition: an experimental model using young rats. Br J Nutr Londres 37, 1-21.
25. Hernández ,G.I., Cook ,J. H. and Sotelo, A .(2008): Effect of malnutrition on the pharmacokinetics of cefuroxime axetil in young rats. J Pharm Pharmaceut Sci 11, 9-21.
26. Jansman, A.J.M., Frohlich, A.A. and Marquardt, R. R. (1993): Production of proline-rich proteins by the parotid glands of rats fed diets containing tanninsfr om faba beans (*Vicia faba* L.). J. Nutr 124, 249-258.
27. Jansman, A.J.M., Verstegenf, M.W.A., Huisman, J .and Van den Berg, J.W.O. (1995): Effects of hulls of faba beans *(Vicia faba* L.) with a low or high content of condensed tannins on the apparent ileal and fecal digestibility of nutrients and the excretion of endogenous protein in ileal digesta and feces of pigs. J Anim Sci 73, 118-127.
28. Kenrick K. G. and Smith, W. J. A. (1970): Immunoglobulins and dietary protein antibodies in childhood coeliac disease .Gut 11, 635-640.
29. Kurpad, A.V.(2006): The requirements of protein & amino acid during acute &chronic infections. Indian J Med Res 124,129-148.
30. Larralde, J .and Martinez, J. A. (1991): Nutritional value of faba bean: effects on nutrient utilization, protein turnover and immunity. Options Méditerranéennes - Série Siminaires 10, 111-117.
31. Ling, P. R., Smith, R .J., Kie S, Boyce ,P .and Bistrian ,B .R. (2004): Effects of protein malnutrition on IL-6-mediated signaling in the liver and the systemic acute-phase response in rats American J. of Physiology, 287, R801-R808.

32. Lyoumi, S., Tamion, F., Petit, J., Déchelotte, P., Dauguet, C., Scotté, M., Hiron, M., Leplingard, A., Salier. J.P., Daveau, M., and Lebreton, J. P.(1998): Induction and Modulation of Acute-Phase Response by Protein Malnutrition in Rats: Comparative Effect of Systemic and Localized Inflammation on Interleukin-6 and Acute-Phase Protein Synthesis. J Nutr 128, 166-174.
33. Martínez, J.A., Macarulla, M.T., Marcos, R. and Larralde, J. (1992): Nutritional outcome and immunocompetence in mice fed on a diet containing raw field beans (Vicia faba, var. minor) as the source of protein. The British Journal of Nutrition 68, 493-503.
34. Malavé, I. and Layrisse, M. (1976): Immune response in malnutrition. Differential effect of dietary protein restriction on the IgM and IgG response to alloantigens. Cellular Immunology 21, 337-343.
35. María, C.M.S. and Carolina, E. (2007): Increased susceptibility to metabolic alterations in young adult females exposed to early malnutrition. Int J Biol Sci 3, 12-19.
36. Martinez, J.A. and Larralde, J. (1983): Correlation among growth rate and organ weights of rats fed on diets containing Vicia faba as source of protein. Growth 47, 26-34.
37. Martinez, J.A., Barcina, Y. and Larralde, J. (1985): Interrelationships between zinc supply and protein source in young and adult rats. Nutr Rep Int 32, 1037-1046.
38. Martinez, J.A., Barcina, Y. and Larralde, J. (1986): Zinc bioavailability from a faba bean diet to rats. Rev Esp Fisiol 42,123-124.
39. Martinez, J.A., Villanueva, M.R. and Larralde, J.(1987): Implicaci611 de los polifenoles en el bajo valor nutritivo de la *Vicia faba.* Arch Latinoam Nutr 37,324-332.
40. Menezes, J.S., Mucida ,D. S., Cara ,D .C., Leite, M.R., Vaz, N.M. And de Faria, A.M.C. (2003): Stimulation by food proteins plays a critical role in the maturation of the immune system. Int Immunol 15, 447-455
41. Mitra S. and Abraham E.(2006) : Participation of superoxide in neutrophil activation and cytokine production. BBA Mol Basis Dis 17, 732-741.
42. Obatolu, V. A.; Ketibu, A. and Adebowale, E. A.(2003): Effect of feeding maize/legume mixtures on biochemical indices in rats. Ann Nutr Metab Londres 47, 170-175.
43. Ou, B. (2002): Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. J Agric Food Chem 50, 3122–3128.
44. Ratcliffe,W.A.,Challand,G.S.& Ratcliffe,J.G.(1974): A critical evaluation of separation methods in radioimmunoassay for total triiodothyronine and thyroxin in unextracted serum.Ann clin Biochem 2, 224-229.
45. Rosa, C. O. B. (1998): The cholesterol lowering effect of black beans (Phaseolus Vulgaris, L) without hulls in hypercholesterolemic rats. Archivo Latino americanos De Nutricion 48, 299-305.
46. Salauze, D., Serre, V. and Perrin, C.(1994): Quantification of total IgM and IgG levels in rat sera by a sandwich ELISA technique. Comp. Haematol Int 4, 30-33.
47. Santidrian, S. (1981): Intestinal absorption of D-glucose, D-Galactose and L. lencine in male growing rats fed a raw field bean (Vacia Faba L) diet. J Anim Sci 53, 414-419.
48. Sant' Ana, D. M.G., Molinari, S. L. and Neto, M. H. (2001): Effects of protein and vitamin B de“ ciency on blood parameters and myenteric neurons of the colon of rats. Arq. Neuropsiquiatr. São Paulo, 59, 493-498.
49. Santos, M.A., R. Rosa, R. Curi R. and Barbieri, D.H.G.(1997): Effect of protein malnutrition on the glycolytic and glutaminolytic enzyme activity of rat thymus and mesenteric lymph nodes. Brazilian Journal of Medical and Biological Research 30, 719-722.
50. Sasai, K., Saito, M., Kataoka, N. and Kobayashi, K. (1981): Lymphocyte blastogenesis in normal and low birth weight infants and the effect of monocyte depletion on it. J Perinat Med 9,150-160.
51. Sawosz, E., Winnicka, A. Chwalibog, A., Oniemiec, T. Grodzik, M., and Sikorska, J.(2009): phagocytic and oxidative-burst activity of blood leukocytes in rats fed a protein-free diet . Bull Vet Inst Pulawy 53, 775-778.
52. Schulze, H., P. van Leeuwen, M.W.A. Verstegen, J. Huisman, W. B. Sellmeyer,E. ,Bhettay, A. S. Truswell,T O. L. Meyers, and Hjansen,J.D. L.(1994): Lymphocyte Transformation in Malnourished Children. Archives of Disease in Childhood 47, 429-438.
53. Shankar, A. H., Prasad, A .S.(1998): "Zinc and immune function: the biological basis of altered resistance to infection." Am J Clin Nutr., 68, 447S-463S.
54. Sellmeyer, E.,Bhettay, E. , Truswell, A.S., Meyers, O.L. and Hansen, J.D.L.(1972): Lymphocyte Transformation in Malnourished Children. Arch Dis Child 47, 429-435.
55. Sidhu, P., Garg, M.L. and Dhawan, D.K. (2004). Protective effects of zinc on oxidative stress

enzymes in liver of protein deficient rats. Nutr. Hosp 19, 341-347.

56. Sissons, J.W., Banks, S.M. and Miller (1988): Growth and immune responses in piglets fed soyabean meal. Seeds, pp. 359 -362 [J. Huissman, T. F. B. Van der Poel and I. E. Liener, editors]. Wageningen: Pudoc.
57. Sobrini, F.J., Martinez, J.A., Ilundain, A. and Larralde, J. (1983): The effects of *Vicia faba* polyphenols on absorption growth and metabolism in the rat. Pl Fds Hum Nutr 33, 31-235.
58. Stinnett, J.D. (1987): Nutrition and the immune response. CRC Press, Florida, USA.
59. Suzuki, K., Nakaji, S., Yamada, M., Totsuka, M., Sato, K. and Sugawara, K. (2002): Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. Exerc Immunol Rev 8, 6–48.
60. Torún, B. and Chew, F. (1993): Protein-energy malnutrition. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. 8th ed. Philadelphia: Lea & Febiger 2 , 951-976.
61. Underwood, B. A. (2002): Health and nutrition in women, infants, and children: overview of the global situation and the Asian enigma. Nutr Rev 60, S7-S13.
62. Vandoni, S.L., Vercelli, G., Borgo, G., Vitali, G., Gagliardi, R., Innocenti, M., Bassini, A. and Rossi, S.C.A., (2007): Il pisello proteico come fonte di fibra. L’Allevatore Magazine 63, 40-47.
63. Volpelli, L. A., Comellini, M., Masoero, F., Moschini, M., Fiego, D.P. and Scipioni, F. (2010): Faba beans (*Vicia faba*) in dairy cow diet: effect on milk production and quality. Italian Journal of Animal Science, 9, 27-35.
64. Waterlow, J. C. (1996): Malnutrición protéico-energetica. Washington: OPS. p.260-280
65. Weller, I. (2001): Secondary immunodeficiency. In: Roitt I, Brostoff J, Male DK, eds. Immunology. 6th ed. Edinburgh, Scotland: Mosby, 315–317.
66. Woldehiwet, Z. and Rowan,T.G.(1990): Some observations on the effect of age of calves on the phagocytosis and killing of staphylococcus aurous by polymorph -nuclear leucocytes. Immunology, 78, 308–317.

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