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Comparative Study of Nutritional Recovery with Soybean and Casein Meals in Malnourished Rats

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Abstract: The purpose of this research was to comparatively investigate the effect of soybean protein and casein on serum Dehydroepiandrosterone-sulfate (DHEA-s), thyroxine (T3 and T4), immunoglobulins (IgG, IgM), interleukin-6 (IL-6), triglyceride, cholesterol, total protein and albumin in a 30 days feeding after malnutrition. A control group feed on balanced diet was included. Forty female rats were used in the present study (150± 10 gm body weight). The animals were divided into 4 groups. Group 1, served as controls. Group 2, included malnourished rats (5% casein). Group 3, included rats re-fed on casein after malnutrition. Group 4, included rats re-fed on soya bean after malnutrition. As a function of malnutrition and in relation to the control group, the malnourished rats showed significant decrease in TAG, total protein and T3 but DHEA-s, cholesterol, albumin and T4 levels were not changed. On the other hand, low dietary protein induced disorder in the activity of IgG, IgM and IL-6. Nutritional recovery by diet containing 20% casein and soya bean increased the activity of DHEA-s and thyroxine, cholesterol, total protein and albumin as well as the levels of immunoglobulins and IL-6. But the level of TAG was decreased by supplemental protein either casein or soya bean. The recovery was more pronounced in rats re-fed on casein diet.

Key words: Soybean protein · Dehydroepiandrosterone-sulfate · Thyroxine · Immunoglobulins · Interleukin-6 · Lipids · Proteins · Malnutrition

INTRODUCTION

Protein malnutrition has long been recognized as a common problem. So, shortage of animals protein for human consumption and the prevalence of protein malnutrition in developing countries probably necessitate the need for plant protein substitution and/or supplementation in the diet.

The amount and nature of dietary protein affects the levels of plasma cholesterol. Likewise, apart from protein under-nutrition, deficiencies of iron and vitamin A [2]. Generally, protein malnutrition disorders include growth failure, oedema, fatty liver and hampered immune defense in human and animals [3]. Thus, the need for alternative protein sources has recently gained focus.

Soy proteins are derived from soy beans, which have high protein content (35-40% of dry weight). Approximately, 90% of the proteins in soybeans exist as storage proteins, primarily ß-conglycinin, a glycoprotein composed of three sub-units and glycinin, a hexameric protein, where each sub-unit is composed by acidic and basic polypeptides linked together by a disulfide bond [4]. Also, isoflavones (e.g., genistin, daidzin and glycitein) are major soy phytoestrogens present in soy foods [4]. Soy proteins are closely associated with minor components such as isoflavones, saponins, trypsin inhibitors, phytic acid, lectin, fibres and others which influence the reproductive system [5], glucose metabolism and causing cancer and cardio-vascular diseases [6].

Casein, as major protein in bovine milk, contains substantial amount of phosphorous and calcium in micellar form. Many bio-active components including flavonoids, lectins, saponins, phytates, cysteine proteases and trypsin inhibitors contribute to the physiological functions of plant proteins [7].

Because qualitative or quantitative protein malnutrition suppresses the specific immune system [8] and causes sever lymphopenia, while qualitative malnutrition results in neutrophilia [9], the role of the non-specific immune system would be more important in this respect [10]. therefore the present study was focused on the effect of dietary protein deficiency (4%) followed by re-feeding on a diet containing right amount of protein or soya bean as alternative sources of protein on some

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physiological markers such as circulating protein, albumin, lipid profile, DHEA-s and thyroxine concentrations as well as on some immunological markers such as IgG, IgM and IL-6 in adult rats.

MATERIALS AND METHODS

Animal Feeding and Management: A total number of forty female albino rats (4 months age and 150+10 g body weight) were kept in wire-bottom cages with controlled ambient temperature (26-32°C) and lighting alternating 12 hr period of light and dark for an adaptation period of 10 days. During the adaptation period, the animals were allowed free access of balanced diet and water was provided ad-libitum. The animals were randomly assigned in two groups:

- Control group(G1): (n=10) fed on a balanced diet (Table 1) containing 20% casein for 60 days after which blood samples were drawn from them.
- Malnourished group(G2) (n=30) fed on a low protein diet containing 5% casein for 30 days after which blood samples were taken from 10 rats. For the remaining rats, they divided into two equal subgroups.

The first group(G2a): received basal diet containing 20% casein. The second group(G2b): received newly formulated diet containing 20% soy bean instead of casein. Animals in the two subgroups were fed on the diets for another 30 days.

At day 60th of the experiment, blood samples were collected by cardiac venipuncture from all groups after overnight fasting, sera were separated and stored at-20°C pending biochemical and hormonal assays.

The salt mixture provided the following amounts (g/kg diet): calcium 4.0, potassium 2.4, sodium 1.6, magnesium 0.4, iron 0.12, manganese 0.032, copper 0.005, zinc 0.018, cobalt 0.0004, iodine 0.002.

The vitamin mixture provided the following amounts (mg/kg diet): retinol 12, chole-calciferol 0.125, thiamin 40, riboflavin 30, nicotinic acid 140, pyridoxine 20, pyridoxal 300, cyanocobalamin 0.1, ascorbic acid 1600, a-tocopherol 340, calcium pantothenate 200, choline 2720, folic acid 10, p-amiobenzoic acid 100, biotin 0.6.

Biochemical and Hormonal Assays: Analysis of IgG, IgM and IL-6 activity were performed using ELISA (Life Diagnosis, Inc.) [11]. Total protein and albumin concentration [12], Serum cholesterol and triglycerides (TAG) [13,14] were estimated.

Table 1: Composition of control and low-protein diets (g/kg)

Ingredients	Balanced diet	Low-protein
Milk casein	200	50
Wheat	597	747
Saccharose	50	50
Maize oil	40	40
Agar-agar (Fibers)	50	50
Salt mixture	40	40
Vitamin mixture	20	20
Methionine	3	3

Serum DHEA-s and thyroxin activity were assayed by radioimmunoassay techniques using commercial kits purchased from DSL (diagnostic system laboratories, Webster, Texas, USA) based on the techniques described by David and Wang [15] for DHEA-s and (Institute of isotopes Ltd. Budapest) according to Ratcliffe *et al*. [16] for thyroidal.

Statistical Analysis: All values were expresses as mean + SE. Statistical analyses was performed with one way analysis of variance (ANOVA) followed by Duncan's test using SPSS program 17.0. *p* values < 0.05 was considered to be significant.

RESULTS

Effect of Malnutrition: The results obtained in Fig. (1-3) showed that protein deficient diet 5% caused significant (P< 0.05) decrease in IgG, IgM and IL-6 activity in rats while alternative dietary casein (diet 2) or soya bean modulated this decrease which ore pronounced in the animals fed dietary casein(G2a) than the animals fed dietary soya bean(G2b). As shown in Fig. (4), total protein level showed significant decrease (p<0.05) associated with insignificant decrease in albumin level in rats subjected to low dietary protein in rats Fig. 5. The protein malnutrition recovered when the animals re-fed on normal dietary casein more the animals re-fed on dietary soya bean and this was more pronounced in adult rats.

As shown in (Fig. 6), low dietary protein induced significant (P<0.05) decrease in T3 level in adult rats and consumption of dietary casein or dry bean subsequently caused significant increase in T3 level particularly in animals fed dry bean. As a function of T4, the level not affected by low dietary protein consumption but it increase significantly (P<0.05) when the animals received dietary casein or dry bean Fig. 7.

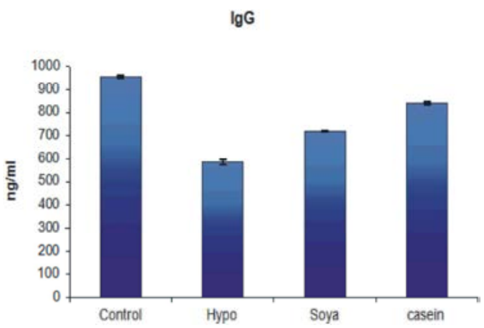
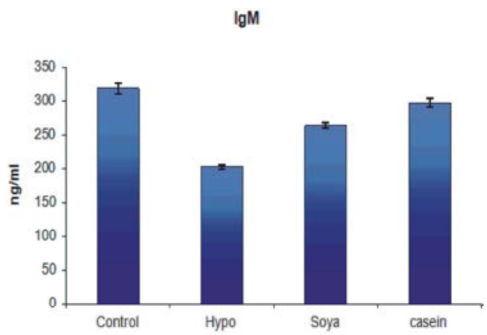


Fig. 1:



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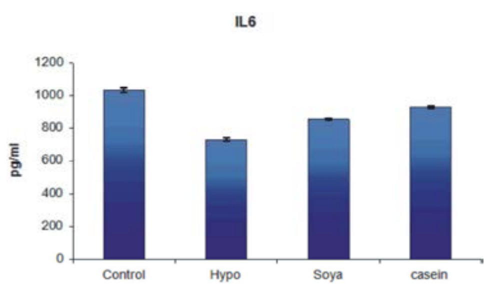

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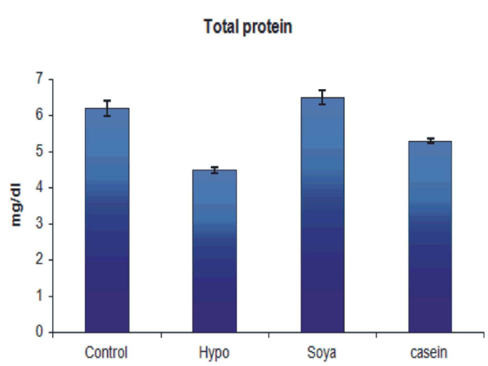
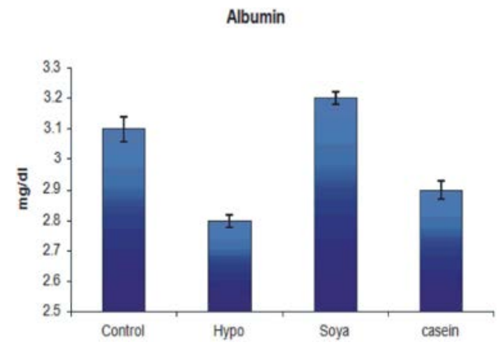


Fig. 4:

As shown in (Fig. 8 and 9), low dietary protein induced significant ($P<0.05$) decrease in TAG and cholesterol levels respectively in adult rats and consumption of dietary casein or dry bean subsequently caused significant increase in TAG and cholesterol levels



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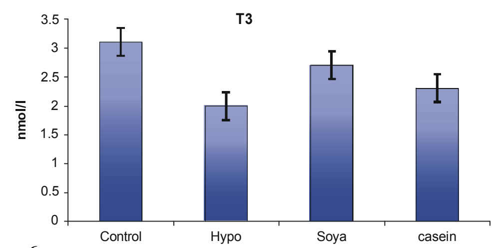
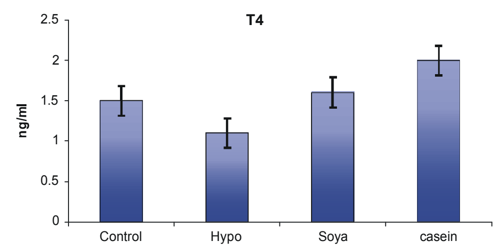


Fig. 6:



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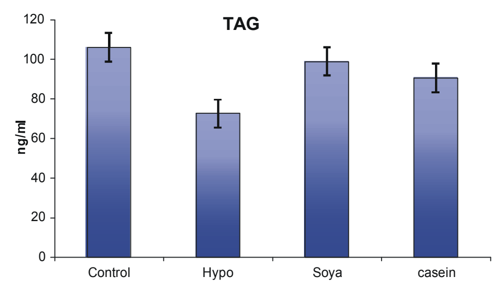

Fig. 8:

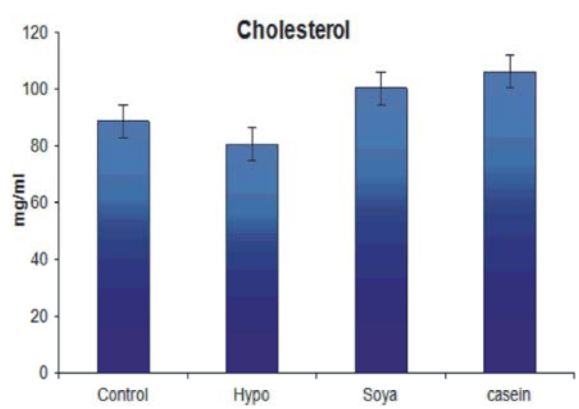

Fig. 9:

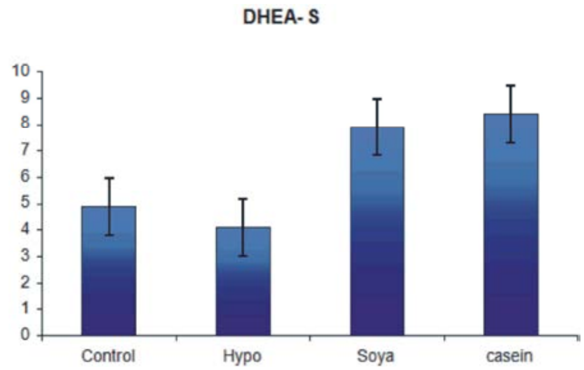

Fig. 10:

when the animals received dietary casein or dry bean. Similarly, low dietary protein induced significant ($P<0.05$) decrease in DHEA-s level in adult rats and consumption of dietary casein or dry bean subsequently caused significant increase in DHEA-s level the animals (G2a)showed elevated values of DHEA-s compared to the animals in G2b.

DISCUSSION

Protein deficiency is prevalent in developing countries due to malnutrition [17]. The sequential changes of malnutrition are altered cellular metabolism, impaired physiologic function, retarted growth and loss of body condition [18].

Results of the present study demonstrated significantly lower serum TAG in protein malnourished rats as compared to control. These results are in agreement with Jekle *et al.* and Kavanagh *et al.* [19, 20]. In contrast to what has been observed in those subjected to protein restriction during growth phase after weaning which showed increases in the proportion of body fat, in this study we verified decreases in the total fat mass and percentage of body fat, but no alteration in the proportion of body protein [21].

Low protein diet promotes a rapid and drastic decrease in serum TAG with concomitant increased deposition of TAG in liver. In rats fed low protein diet, circulating very low density lipoprotein (VLDL) and low density lipoprotein (LDL) are decreased and many authors have postulated that the impairment of lipid transport was due to decreased synthesis of the corresponding apolipoprotein [22, 23]. Soybean diet has been associated to reduction in the fat deposits due to the action of isoflavone that increases energy expenditure by altering the brown-fat activity and the thyroid function [24, 25]. In this study, soybean diet was more effective than the casein diet in reducing the proportion of fat deposits, although the animals maintained on soybean diet had eaten proportionally the same amount of diet and had showed lower energy expenditure than those fed the casein diet. It has been reported that genistein, an isoflavone of soy, decreases lipogenesis, enhances lipolysis and counteracts the antilipolytic action of insulin in isolated rat adipocytes genistein [26, 27]. Recently, we reported that our rats fed with the soybean diet show considerable amount of genistein in the sera [28] and express less mRNA of acetyl-CoA carboxylase in the liver [29]. Thus, the reduced adiposity seen in our animals maintained on soybean diet could be an effect of this isoflavone on lipid metabolism. Furthermore, Soy protein consumption with a median of 30 g/d was associated with a significant improvement in lipoprotein risk factors for coronary heart disease (CHD). Compared with crossover randomized controlled trials (RCTs,) parallel RCTs had significantly higher quality grades and were associated with significantly greater improvements in serum LDL-cholesterol values. Regular consumption of soy protein daily (15 to 30 g) has a significant favorable impact on serum lipoprotein risk factors for CHD [30].

The present finding demonstrated that cholesterol levels were not affected by protein malnutrition, a finding, which was previously reported, as the main affected on lipid were reported to be on serum TAG and not cholesterol [31]. The present finding of no difference in serum cholesterol between animal protein and plant protein supplemented rations may be due to the non-affect of dietary protein on intestinal cholesterol absorption [32]. However, other reports indicated hypercholesterolemia in animal protein fed rats than plant protein fed ones [33]. Teixeira *et al.* [34] showed that consuming as little as 20 g soy protein/d instead of animal protein for 6 wk reduces concentrations of non-HDL cholesterol by ˜2.6%.

The results of the present study indicated that plant proteins can influence some parameters of lipid metabolism and steroid hormones namely DHEA-s. The elevated serum levels of DHEA-s were quite evident in soybean only.

Most of studies carried out on adrenal steroid hormones, DHEA-s, dealt with effect of treatment using this hormone on varies biochemical parameters, however, there is a lack of data as regards to their serum level under the condition of malnutrition and or the substitution of animal protein with plant protein. DHEA-s may act as a large plasma reservoir of hormone [35], thus obviating the need for tight regulation of adrenocortical production of DHEA-s. The long half-life of DHEA-s in blood supports this hypothesis [36].

In addition, the steroid hormone, DHEA-s was found to be unaffected by protein malnutrition. It may hypothesized that this is secondary to unchanged cholesterol level in protein malnourished rats, as the cholesterol nucleus is the precursor of the steroid hormone synthesized by the adrenal glands. Differences in DHEA/DHEA-s ratio were not fed under various physiological and pathological conditions [37, 38].

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. Soybean diet has been associated to reduction in the fat deposits due to the action of isoflavone that increases energy expenditure by altering the brown-fat activity and the thyroid function [39]. Malnutrition is more prevalent in the adults and is often a result of energy imbalance from various causes. Dietary supplementation to promote positive energy balance can reverse malnutrition [40].

The albumin was not significantly altered as well because the rats fed on low dietary protein for short run [41, 42] and it may be decreases when the same diet employing with rats of the same strain, in the long run [43]. 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 (ETC), which may be due to changes in mitochondrial protein synthesis, breakdown, or both [44] or lack of vitamins B complex supplementation in the chow of the experimental group [45] 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 [46]. As related to last suggestion, Sidhu *et al.* [47] 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 [46]. In spite of rats allocated on alternative dietary soya 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 soya beans which increase the secretion of proline-rich proteins by the rat's parotid glands [48] and interacting with both dietary and endogenously secreted proteins in the intestinal tract result in enhancement of fecal excretion of both sources of protein [49]. Moreover, the quality of soya bean seeds contain high quantity of protein and its amino acid composition is approximate to composition of animal proteins, therefore is often used as replacement component of meat protein.

Decline in nutritional status is related with the loss of protection to infectious diseases, caused by decreased humoral and cellular immunity.

[50, 51]. The aspects of immunity most consistently affected by protein malnutrition are cell-mediated immunity, phagocyte function, the complement system, secretary antibody and cytokine production [52]. In the present study, consumption of low dietary protein 4% induced significant decrease in processes of IL6, IgG and IgM in rats relative to control. The capacity for response to IL-6 is preserved, suggesting adaptive preservation of acute-phase responsiveness during malnutrition [53]. Such results considerably attributed to a greatest lymphocyte blastogenesis in malnourished rats [54 and 55]. In addition to that the time span of using protein-free diet played a key factor for the safety of this treatment [3]. 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.* [56] and McCarter *et al.* [57] 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 [58, 59]. As a function of IL6, some experimental and clinical studies have suggested that protein malnutrition affect circulating IL-6 level [60-61], which can have

anti-and pro-inflammatory function [62]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 [63]. Likewise, it was suggested that protein deficient animals present a failure in the regulatory mechanism(s) of the IgG and IgM resulted in decreased their total levels [64]. References and further reading may be available for this article. To view references and further reading you must purchase this article. 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 [65].

The functional of the immune system competence was reduced in rats re-fed on soya-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 [66, 67].

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