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Biological Studies of some High Protein Sources on some Parameters of Rats

Nehad,R. EL-Tahan, Doha elsied elsaied

Nutrition and Food Science Dep., Home Economics Faculty, Menoufia University.

Abstract

The present study investigated the effects of some high protein sources on liver functions, lipid profile and immunoglubin index (IgA,IgA,IgG) in rats . Twenty one male albino rats were divided into (3) groups (7) rats in each group. The first group was control which fed on basal diet only as a negative control. The other groups were received basal diet containing 10% from formula 1 which contained beef protein and formula 2 contained whey protein .Liver functions was assessed by estimation of plasma concentration of enzymes activities of aspartate amino transferase (AST), alanine amino transferase (ALT), lipid fraction (total cholesterol and triglyceride) and cholesterol fraction (HDL-c, LDL-c, VLDL-c). Results showed bad effect of these protein source on the tested parameters and the beef protein group was the most group which affected by high level of high protein source. So, this study concluded that high intake of high protein sources for a long time led to increase the liver enzymes , lipid profile and decrease the immunity status in rats

Key words: Beef protein, liver functions - cholesterol fractions.

Introduction

Protein is important for human beings and meat is known as the best source of animal protein. . The meat that is usually used for sources of protein. is poultry, beef and mutton. In Malaysia, beef is the third largest source of meat after poultry and pork (**Hoffman *et al*.,2004)**

Amino acid composition of meat products can play a significant role in meat identification; the ratios of amino acids arginine, histidine and lysine for the investigated species of animals have been obtained. These ratios do not depend on age or weight of the animal (**Weiss, *et al*., 2000).** The chemical and nutritional composition of each meat product is greatly varied from one product to another as it contains different kinds of tissues and sometimes a mixture of meat of various organs(**McClements *et al*. ,2010)**;

Whey proteins are easily and quickly digested. Whey protein is one of the 2 proteins of cow's milk. Whey proteins refer to the individual protein which is separated out from the casein while cheese making. These proteins are purified into different concentrations based on the end composition desired **(Mansour *et al*., 2000)**. The content of the whey protein may vary in carbohydrates, fat, immunoglobulins, lactose and minerals. Whey proteins are loaded with EAAs (Essential Amino Acids) including three BCAAs (Branched Chain Amino Acids) and also they contain subcomponents of micro fractions which provide the benefits of elemental nitrogen and amino acids. Whey protein is the one which is most useful for sports nutrition. In addition to these, whey products are evident in salad dressings, infant formulas, emulsifiers, baked goods and medical nutritional formulas **(Tietz*et al.*, 1976).** Whey protein is considered as an excellent protein for the choice of individuals of all ages for healthy diet and also to improve and maintain their health. Traditionally, Whey protein was only used by most of the athletes and bodybuilders to promote the muscle growth**.** But from past few years, whey protein is being used in some other applications. Some applications that using whey proteins are: cancer treatment, wound healing, infant health and weight loss.

According to (**Hoffman *et al*.,2004)** additional benefits of whey protein may include: Whey protein helps to increase the serotonin activity and helps to promote restful sleep (**McClements *et al*. ,2010)**; Whey protein helps enhance energy levels; it helps to decrease the

stress; it helps to keep the metabolic rate high; it helps to reduce body fat and build the lean body mass; and it helps to improve the memory loss under stress. In addition to these, some of the top benefits of whey protein may include: it provides immunity support, increase muscle mass, boost metabolism, and helps to improve overall health but the increasing intake of protein source may be caused bad effect on the functions of liver and kidney **(Layman. *et al*. , 2002).**

For that the paper aimed to explore the effects of feeding high protein sources for a long time on some vital parameters of normal rats.

Materials and methods

Source of materials

Whey protein and beef protein were obtained from international centre for research, Cairo, Egypt..

Chemical reagents

Reagent kits were purchased from Diamond Diagnostics (Egypt).

Experimental animals

Twenty one white male albino rats weighing about 120 ± 5g were used as experimental animals in the present investigation. They were obtained from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. They were kept under observation for one week (as adapted period) before the onset of the experiment. The animals were housed in stainless steel cages at normal atmospheric temperature (25 ± 5°C) and had a 12 h light-dark cycle. Food and water were consumed *ad libitum.*

Methods:

Animals diet

The basal diet was prepared according to **AIN (1993)**. The vitamin mixture was prepared according to **Campbell (1963)**, while salt mixture was prepared according to **Hegsted*et al.* (1941)**.

Experimental design

Twenty one male albino rats (120 ± 5g) were randomly divided into 3 equal groups (seven rats each). All rats were fed on basal diet for one week before starting the experiment for acclimatization. After the adapted period, the initial weight was 125 ± 5g. Groups of rats were as the follows:

Group (1): Rats (n=7) were fed on basal diet only as control negative group.

Group (2): Rats (n=7) were fed on formula 1 which contained 10% whey protein .

Group (3): Rats (n=7) were fed on formula 2 which contained 10% beef protein.

By the end of the experimental periods (28 days),rats were scarified using diethyl ether anesthesia at fasting state . Part of the blood was taken to determine the level of serum glucose and other portion of blood samples was collected and allowed to coagulate at room temperature; other portion of blood was added EDTA (ethylene diaminetetr acetic acid) and centrifuged at 3000r.p.mfor 15 minutes. Serum was carefullyaspirated and transferred into cleancovettubes and stored frozen at -20°Cuntilthe time of analysis.

Biochemical analysis:

Serum alkaline phosphatase (ALP)was determined according tothe procedure of **(IFCC methods., 1983)**.Aspartate amino transferase (AST) or (GOT)glutamic -oxaloacetic trans aminaseand glutamic pyruvic trans aminase (GPT) orAlanine amino transferase (ALT) werecarried out according to the method of **Henry(1974) and Yound (1975)**.Glucose was determined by enzymatic test according to**Tietz (1976)**and**Yound (1975)**. Enzymatic colorimetric determination of triglycerides was carried out according to **Fassati and Prencipe (1982)**. Total Cholesterol was determined by colorimetric method according to **Allain (1974)**.The determination of HDL was carried out according to the method of**Fnedewaid (1972)** and **GordonandAmer (1977)**. The determination of VLDL (very lowdensity lipoproteins) and LDL (low density lipoproteins) was carriedout according to the method of **Lee and Nieman (1996)** . Total immunoglobulin (IgG, IgM, IgE and IgA) determined by Radioimmunoassay as described by the method of **Patrono and Peskar (1987).**

Histopathological study: Livers of the scarified rats were dissected, removed, washed with normal saline and put in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. The tissue specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin (H and E) and then studied under an electronic microscope according to (**Carleton ,1979)**.

Statistical analysis

Statistical analysis were done using the Statistical Package for the Social Sciences (SPSS for WINDOWS, version 11.0; SPSS Inc, Chicago). Comparative analyses were conducted using the general linear models procedure (SPSS Inc). Values of $P<0.05$ were considered statistically significant.

RESULTS

1-Effect of feeding 10% tested formula on serum lipids in normal rats.

Administration of the tested formula at 10% level caused significant increases in serum of total cholesterol, triglycerides, LDL and VLDL compared to control group (Table 1). Serum HDL-c levels decreased but not significantly by the administration of the formula 1. Rats that were given formula 2 showed significantly lower levels of HDL compared to control group. The value of other lipid parameters of formula 2 were higher than control group. The obtained results in the same line of **(Crouse, (1999)** who found that high protein intake can increase LDL-c, total cholesterol and decrease the level of HDL-c. Also, **Gulfraz*et al., (2011)***reported that beef protein increase the absorption of lipids from diet .

Table (1): Effect of feeding 10% tested formula on serum lipids in normal rats

Serum lipids	G1	G2	G3
Total cholesterol	75.43^{a}±2.19	83.33^{a}±3.15	95.47^{a}±1.13
Triglycerides	76.48^{b}±0.13	80.8^{b}1.03±	89.4^{a}±3.01
HDL-C	53.94^{a}±0.12	40.87^{b}±1.15	33.29^{c}±0.04
LDL-C	20.2^{c}±1.17	37.9^{a}±4.34	42.5^{b}±0.74
VLDL-C	1.29^{b}±1.17	6.56^{a}±4.34	20.28^{b}±0.74

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at ($p\leq0.05$)

3-Effect of feeding 10% tested formula on liver function enzymes in normal rats.

From data presented in table (2) the administration of formula 2 (G3) significantly increase AST and ALT levels when compared with the control group. There is no significant differences between group 2 and 3. From the above results, it could be noticed that the high protein

source for long time had bad effect on the levels of liver enzymes especially in case of beef protein (**Roberfroid , 2000)**.

Table (2): -Effect of feeding 10% tested formulaon liver enzymes in normal rats

Parameters	G1	G2	G3
AST(U/L)	27.8^{b}±0.07	40.2^{a}±1.11	57.5^{a}±0.21
ALT(U/L)	29.8^{b}±1.91	48.9^{a}±1.41	67.4^{a}±0.5

4--Effect of feeding 10% tested formulaon immunological productions in normal rats

From table (3), it could be observed that administration of the formula 2 it is affect to rats activity (Group 3). The formula 2 induced significant decreases in serum levels of immunological profile compared to control group. The other tested formula decreased immunological profile but non significant changes in serum level of immunological productions when compared with control group. The bad effect of high protein sources caused bad effect on the main organs in the body which was liver which do The main functions to the body like lipid metabolism, build the main proteins which relate to immunity status and cardiovascular status (**Mallillin*et al.*, 2008 and Murty*et al.*, 2010)**

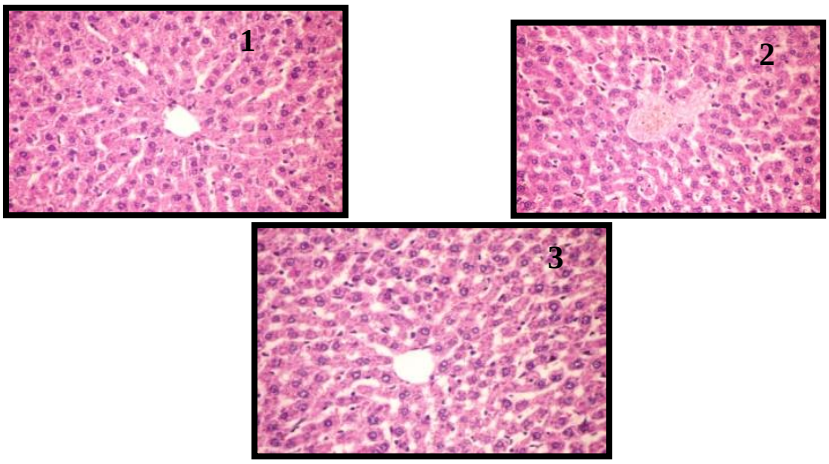
Table(3):Effect of feeding 10% tested formulaon immunological productions in normal rats.

Immunological Profile mg/dl	G1	G2	G3
IgE	59.87 a±1.34	55.5 a±0.2	44.17 b ±0.05
IgM	106.33 b±3.5	104.65b±5.65	97.2 b ±6.53
IgA	106.5 a ±1.5	100.5a ±0.5	78.1 b±0.1
IgG	1089.66 a ±25.16	1069 a±10.87	1000.05 b ±9.05

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different at ($p \leq 0.05$)

Histopathological examination of liver of the negative control rats fed on basal diet revealed normal histological picture of hepatic lobule which consists of central vein surrounded by normal hepatocytes as shown in (photo. 1). Examination of liver of group (2)showed of hepatocytes and infiltration of leucocytes in hepatic sinusoid (photo. 2). Liver and the third mixture showed fatty degeneration of hepatocytes and infiltration of leucocytes in hepatic sinusoid (photo 3).These results were according to **Mallillin*et al.(* 2008) and Murty*et al.(* 2010**) who

found that high protein diet can increase the accumulation of fat in liver tissue in normal status and caused fatty liver led to the disturbance which caused in liver enzyme .



Photos (1): Histopathological changes detected in the liver of negative control, formula 1 and formula 2.

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التأثير البيولوجي لبعض مركزات البروتين على الحالة الصحية للفئران

نـهاد رشاد ،ضحى السيد السعيد أحمد
أستاذ التغذية وعلوم الأطعمة- كلية الإقتصاد المنزلي -جامعة المنوفية.

الملخص العربى

تم تحقيق في هذه الدراسة آثار بعض مصادر البروتين عالية على وظائف الكبد ، والدهون الشخصي ومؤشر المناعة (IgA، IgA، IgG) في الفئران.

تم تقسيم اربعه وعشرون الفئران البيضاء الذكور

المجموعة1 تعذت الفئران على الوجبه الغذائيه الضابطه.

المجموعة2 تغذت الفئران على الوجبه الغذائيه الضابطه مع10%من بروتين الشرش.

المجموعة 3 تغذت الفئران على الوجبه الغذائيه الضابطه مع10%من بروتين الصويا.

المجموعة 4 تغذت الفئران على الوجبه الغذائيه الضابطه مع10%من بروتين لحم

وقدرت أوزان الكبد والرئة والقلب والكلى والأمعاء الدقيقة لمعرفة تأثير تركيزات البروتين المختلفه على وزن الأعضاء الداخلية المستهدفة بالتأثيرودراسة كيموحيوية والتى عنيت بدراسة سيريولوجية انزيمية لتقدير الانزيمات التى تدل على رد فعل الكبد نتيجة لتأثير التعامل مع تركيزات الجلاتين حيث قدرت انزيمات كبدية ناقلة لمجموعة الأمين حيث تزداد معدلاتهما عند حدوث التهاب بخلايا الكبد- كان السبب كميائيا او عدوى فيروسية طبقا للدراسات المشار إليها فى متن الرسالة وهما انزيم الجلوتامين بيروفات ناقل الأمين والثانى الجلوتامين اكسالواسيتات ناقل الأمين . ودعمت الدراسة بدراسة هيماتولوجيا الدم حيث قدرت عدد كرات الدم الحمراء والهيموجلوبين وعدد الصفائح الدموية كصورة دم كاملة حيث تعد من الدلائل القطبية على تأثير تركيزات البروتين محل الدراسة على الحيوية العامة والجهاز المناعى ومدى حدوث التهاب للحكم على استخدام تركيزات البروتين محل الدراسة .وتمت الدراسة الهستوباثولوجية للوقوف على حالة أنسجة الكبد والكلى والأمعاء الدقيقة لتعزز كل من الدراسات الكيموحيوية وتأتى الدراسة الغذائية كمحصلة نهائية لرد فعل تركيزات الجلاتين محل الدراسة على الجسم.