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THE BIOLOGICAL STUDIES ON HIGH PROTEIN AND ENERGY FOODS TO IMPROVE THE DECREASING OF ALBINO RATS WEIGHT

El-Sayed, M. M.; El-Tahan, Nehad, R.; El-Banna, Samah, M. S. and Khalifa, Heba, A. H.*

Department of Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Egypt

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ABSTRACT: some parameters of biological and biochemical of rats with low weight as affected by three protein sources (soybean protein, whey protein and meat protein) combined with chocolate, coconut, oat, and glucose syrup were studied. Twenty-four adult male albino rats were divided into 4 groups, their weight was an average 80±5g, there was one group kept as a control group, and the other three groups were given daily tested combined at the level 10% for 4 weeks. Body weight gain (BWG), feed intake (FI), feed efficiency ratio (FER), liver enzymes (ALT and AST), lipid profile (HDL-c, LDL-c and VLDL-c) and thyroid hormones (T3, T4 and TSH), were estimated. Results showed that body weight gain was markedly increased ($P \leq 0.05$) especially in the group (4) fed on combined which contained meat protein, oat, chocolate, and glucose syrup followed by group (3) fed on whey protein, oat, chocolate, and glucose syrup combined when compared to control group. Meat protein combined caused significantly ($P \leq 0.05$) increasing total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VLDL-c), mean levels and increased activity of aspartate aminotransferases (AST) and alanine aminotransferases (ALT) enzymes while soybean protein and whey protein combined caused significantly ($P \leq 0.05$) decreased the above parameters as compared to group (4) and their mean values near to control group. From that, this study recommended that intake of the whey protein and soybean protein had good effect on the biological and biochemical of rats with low weight so, it may be useful for improving the low weight.

Key words: Whey protein, weight gain, T3, T4 and TSH hormone, formula.

INTRODUCTION

Low weight was defined as people with a body mass index under 18.5 or a weight 15% to 20% below that normal for their age and height group. Also, described person's body weight is considered too low to be healthy. (Mahan and Kathleen, 2000).

A person's low weight may be caused by genetics, metabolism, nutritional deficiencies (often due to poverty), or disease. Being underweight has been linked to some medical conditions, such as hyperthyroidism, cancer or tuberculosis. People with digestive system or liver problems mayn't be able to adequately absorb nutrients. People with certain eating disorders can also experience low weight due to nutrient deficiencies or excessive exercise. (Milas and Kresimira, 2012).

Low weight individuals may be advised to gain weight by increasing their calorie intake. This can be done by eating high-calorie foods, such as dried fruits, cheese and nuts. Body weight can also be increased by consuming liquid nutritional supplements. Other nutritional supplements may be recommended for person who do not consume adequate amounts of vitamins or minerals. Protein can promote muscle growth and repair, while eating a high-carb source can lead to weight gain. Protein is important for person's health, and meat is known as the best source of animal protein. Meats commonly used for protein sources are poultry,

*Corresponding author: heba.khalifa.2023@gmail.com

beef, pigs and lamb. (Ministry of Agriculture, 2009).

Irina *et al*. (2011) stated that the amino acid type of meat products can play an important role in determining the identity of meat. The ratios of the amino acids arginine, histidine, and lysine were determined for the animals under studies. These percentages don't depend on the age or weight of the animals. The chemical and nutritional composition of a meat product varies greatly from product to another as it contains different types of tissues and sometimes a mixture from different organs. (Pitti, 2013).

Soybean protein products are used in food to treat malnutrition due to their functionality, highly nutritional value, and low cost. To achieve their ideal nutritional and functional properties as well as flavour, different treatments were used. modification of Soybean proteins can be by treated with physical, chemical, and enzymatic. Various heat treatments are commonly used, while the most convenient method to modification soybean proteins from a safety point of view is their limited proteolysis. These treatments cause physical and chemical changes that effect on their functional properties (Pesicm, 2003).

Whey protein is a mixture of proteins isolated from whey, the liquid substance created as a byproduct of cheese production. Proteins consist of α-lactalbumin, β-lactoglobulin, serum albumin and immunoglobulin. Whey protein is commonly marketed as a nutritional supplement, and has various health claims. Whey protein improves body composition and increases weight (Baer, 2006). Whey protein is the best protein that affects the hormones that control appetite and hunger. A diet containing whey protein increases energy expenditure and obesity in low-weight mice, helps control blood glucose levels and has been shown to be beneficial for weight management. A meal containing α-lactalbumin maintains fat oxidation and quickly provides amino acids for use during exercise, resulting in improved athletic training efficiency. (Belobrajdic *et al.*, 2004*).*

For that, this study aimed to investigate the effect of combined from some protein sources with chocolate, coconut, oat and glucose syrup on the biological, and biochemical parameters of rats with low weight.

MATERIALS AND METHODS

Materials

The chocolate, coconut, oat and glucose syrup were purchased from local market, in Menoufia Governorate. Protein sources (soybean protein, whey protein, meat protein, and casein), starch, salt combined, cellulose and vitamin combined obtained from Gommhoryia Co. Giza, Egypt. Albino rats, mean weight was 80±5g were obtained from **Institute of Ophthalmology, Medical Analysis Dep., Giza, Egypt**.

Methods

Biological Experiments

The basic diet is prepared with quality ingredients per 100 grams. The diet composition was as follows: cornstarch 67%, casein 13%, corn oil 10%, fiber 5%. (AIN, 1993), Salts combined 4% (Hegsted *et al.*, 1941), and vitamin combined 1% (Campbell, 1963).

Biological experiments were conducted in the central laboratory of the Faculty of Home Economics, Menoufia University. Twenty-four albino rats, average weight 80 ± 5g, were individually housed in wire cages in a room at 25 ± 2°C and kept under normal hygienic conditions. All rats were fed the basal diet for one week before the start of the adaptation experiment. After that, they were divided into four groups, each group consisting of six mice, as follows:

Group (1): Rats fed on basal diet as a control group.

Group (2): Rats fed on basal diet and 10%the first combined (60 %soybean protein, 20%oat, 10%chocolate and 10%glucose syrup).

Group (3): Rats fed on basal diet and10% the second combined (60%whey protein, 20%oat, 10%chocolate and 10%glucose syrup).

Group (4): Rats fed on basal diet and 10% the third combined (60%meat protein, 20%oat, 10%chocolate and 10%glucose syrup).

Biological evaluation

During the experiments period (28 days), the amounts of feed consumed and/or wasted daily were recorded. In addition, the weight of rats was recorded weekly. Body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER) were determined according to (Chapman *et al.* (1959) using the equations:

Feed intake=total amount of diet- waste amount

BWG (g) = final weight (g) - initial weight (g)

FER = weight gain (g)/feed intake (g)

Biochemical evaluation

At the end of the experiments period, rats were fasted overnight and blood samples from each rat were collected and centrifuged to get serum. The serum was carefully separated, transferred into clean, dry Eppendorf tubes and kept frozen at -20°C for analysis as described by **Schermer (1967)**.

Hematological analysis

Serum total cholesterol, triglyceride and High density lipoprotein cholesterol were calorimetrical method determined according to AIN, (1993), Fossati *et al.* (1982) and Kikuchi *et al.* (1998), respectively. (HDL-c) was determined calorimetrical method according to. Calculated Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) was according to Lee and Nieman (1996) as follows:

LDL-c = TC-[HDL-c + (TG/5)] VLDL-c

The activity of aspartate aminotransferases (AST) and alanine aminotransferases (ALT) enzymes were assigned by the method of Yound (1975). The determination of T3, T4 and thyroid stimulating hormone (TSH) carried out according to Uotila *et al*. (1981).

Statistical analyses

Statistical analysis was carried out using (SPSS), Version 20. The results were expressed as mean ± Standard deviation (mean ± S.D.). one way classification analysis was used for variance analysis (**ANOVA**). The differences between means were tested for significance using least significant difference (LSD) test at $P \leq 0.05$ (SAS, 2002).

RESULTS AND DISCUSSION

Effect of tested combined on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of low weight rats.

Data in Table (1) showed that group (4) which fed on combined contained 60% meat protein 20% oat, 10% chocolate and 10% glucose syrup led to increase ($P \leq 0.05$) the feed intake which was higher than the control group and other tested groups. There were significant ($P \leq 0.05$) differences among tested groups and control one. For body weight gain, there were significant ($P \leq 0.05$) differences among all groups. Group (4) was the highest body weight gain followed by group (3) and the last effect was group (2). By regarding feed efficiency ratio, the fourth group was the best group with significant ($P \leq 0.05$) changes to the other groups. The lowest group for all biological parameters was the control group. These results were matched with **Baer (2006)** who found that whey protein improved the feed intake and body weight of rats. Meat which had high content of protein enhanced the digestive system functions, including those that control transit time, bowel habits, and mucosal motility as well as those that modulate epithelial cell proliferation (Irina, 2011). Firew *et al.* (2021) found that oat can significantly increase upper- and lower-body strength, reduce body fat and improve overall body composition.

Table (1): Effect of tested combined on feed intake (FI), body weight gain (BWG) and feed efficiency ratio (FER) of low weight rats.

Parameters	Groups				LSD
	G1	G2	G3	G4	
Feed intake g/day	8.48^{d}± 0.03	9.15^{c}±0.09	10.03^{b}±0.15	10.58^{a}±0.01	0.29
BWG g/28days	20.28^{d}±2.01	22.58^{c}±0.32	25.58^{b}±0.72	29.17^{a}±1.42	2.07
FER	0.085^{d}± 0.002	0.088^{c}±0.002	0.091^{b}±0.001	0.098^{a}± 0.001	0.002

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

Effect of tested combined on serum lipids of low weight rats

Administration of the combined contained 60% meat protein and 20% oat, 10% chocolate and 10% glucose syrup, which used in feeding group 4 caused significant ($P \leq 0.05$) increases in serum of total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) compared to control group and the other groups (Table 2). But serum high density lipoprotein cholesterol (HDL-c) levels significant ($P \leq 0.05$) decreased in this group as compared to the others. The other tested groups which were given combined 1 and 2 showed non-significant ($p > 0.05$) as compared to control group for the other lipid parameters.

The obtained results in the same line of Irina (2011), who found that the combined of group 4 increased the values of lipid profile and this led to contain meat protein with oat and glucose syrup. While, combined of groups 1and 2 can increase the level of HDL-c because of reducing the absorption of lipids by activity phytochemical from combined components (Firew *et al.*, 2021).

Effect of tested combined on liver enzymes of low weight rats.

Results were presented in Table (3) the administration of combined (1) caused significant ($P \leq 0.05$) difference as compared to control group. Group 3 and 4 significantly ($P \leq 0.05$) had the highest values of aspartate aminotransferases (AST) when compared to group 2 and control group. For alanine aminotransferases (ALT), adding different combined to the diet of low weight rats significantly increased the activation of ALT enzyme when compared to control group, although, there were significant differences between each other. From the above results, it could be noticed that high content of vitamins (A & E) and vital minerals (zinc, selenium iron and calcium) in all combined components can improve the function of the alimentary tract and also lower blood glucose, cholesterol levels and liver functions (Firew *et al.*, 2021). Enzymes are a special form of proteins that catalyze of chemical reactions in biological systems, whereas, in humans, animals and plants there are many metabolic of decomposition and synthesis. Liver enzymes which are examining liver function are: ALT, AST, LDH-lactate dehydrogenase, γ-GT-gamma glutamyl transferase, alkaline phosphatase ALR, iron, copper, ceruloplasmin and others. In the liver, ALT catalyzes the transfer of α-amino nitrogen of the alanine to α- ketoglutarate to form pyruvate, which is used in gluconeogenesis (Jerry Kaneko *et al.*, 2008). Whey consists of several proteins, including beta-lactoglobulin, alpha-lactalbumin, serum albumin and glucomacro peptides. Whey proteins contain all the essential amino acids in higher concentrations compared with some vegetables that are sources of protein such as soybean, corn and wheat (Walzem *et al.*, 2002). Previous studies have shown that high amount of meat protein consumption seems to be associated with insulin resistance, type 2 diabetes, metabolic syndrome, and oxidative stress (Zelber-Sagi *et al.*, 2018).

Effect of tested combined on T3, T4 and TSH hormones of low weight rats

The effect of tested combined on T3, T4 and TSH hormones of low weight rats were tabulated in Table (4). For T3 and TSH hormones, it could be noticed that the highest ($P \leq 0.05$) mean value was in the group (4) and the lowest ($P \leq 0.05$) mean was in the control group. There were significant ($P \leq 0.05$) differences between groups (2, 3 and 4) and the control (group1). For T4 hormone, it could be observed that the highest ($P \leq 0.05$) heights value was in the control group while the lowest mean value was in the fourth group. There were significant ($P \leq 0.05$) among combined groups and control group.

Table (2): Effect of tested combined on serum lipids of low weight rats

Parameters	Groups				LSD
	G1	G2	G3	G4	
Total cholesterol	86.44^{b}±2.67	88.21^{b}±2.95	88.76^{b}±1.99	94.98^{a}±2.21	3.54
Triglycerides	76.68^{c}±3.93	76.84^{b}±4.01	81.46^{b}±1.03	115.96^{a}±2.99	4.62
HDL-cholesterol	47.92^{a}±0.99	47.87^{a}±1.02	48.89^{a}±1.24	43.90^{b}±1.19	1.54
LDL-cholesterol	23.18^{b}±1.77	24.97^{b}±0.65	23.58^{b}±0.77	27.89^{a}±0.03	1.84
VLDL-cholesterol	15.34^{b}±0.21	15.37^{b}±0.17	16.29^{b}±0.06	23.19^{a}±0.28	0.95

a,b,c letters in the same row are not statistically significantly different ($P \leq 0.05$).

Table (3): Effect of tested combined on liver function enzymes in normal rats.

Parameters	Groups				LSD
	G1	G2	G3	G4	
AST(U/L)	28.81^{d}±0.57	32.52^{c}±0.61	36.93^{b}±1.06	43.12^{a}±1.50	1.86
ALT(U/L)	29.81^{d}±0.91	37.93^{c}±0.55	39.13^{b}±0.92	44.4^{a}±1.01	1.22

a,b,c letters in the same row are not statistically significantly different ($P \leq 0.05$).

Table (4): Effect of tested combined on T3, T4 and TSH hormones of low weight rats.

Parameters	Groups				LSD
	G1	G2	G3	G4	
T3 (pmol/L)	0.95^{c} ±0.03	0.95^{c}±0.09	2.77^{b}±0.05	2.97^{a} ±0.08	0.11
T4 (pmol/L)	30.01^{a}±0.90	29.97^{a}±0.26	28.11^{b} ±0.63	24.76^{c}±0.35	0.93
TSH (mIU/L)	4.01^{c} ±0.002	4.05^{c}±0.02	4.93^{b}±0.03	5.78^{a} ±0.21	0.22

a,b,c letters in the same row are not statistically significantly different ($P \leq 0.05$).

Thyroid hormones are involved in many vital functions in the body including the use of total energy, cellular breathing, tissue growth, metabolism balance of nutrients, and thermal formation. In order to maintain all these functions, thyroid hormones must be within a specific domain in the blood. The thyroid hormone level (T3 and T4 levels in the blood is controlled by the thyroid axis in the pituitary gland. The thyroid stimulation hormone (TSH) increases, increased stress, disease, increased past demand, and low levels of T3 and T4. Hormones such as somatostatin, dopamine, growth factor, and glucocorticoids can cause hypothyroidism (Wier and Farley, 2006). Low weight and low nutrients in the diet one reason caused low in thyroid hormones. This result was agreed with Milas and Kresimira (2012), who study the effects lack of nutrients for 4-5 weeks on thyroid activity and found that it caused a marked hypertrophy of brown and white adipose tissue, but no change in the weight of thyroid, while there was a significant decrease in the thyroid hormones. The evidence regarding the impact of soybean on thyroid health is limited, and some studies do not show a link between soybean and TSH or thyroid function. At the current time, there is no clear consensus regarding how much or how little soybean is safe for thyroid function (Bell and Ovalle, 2001). Whey protein is commonly used due to its antioxidant, antitumoral, and immunity increasing features and its effect on protein synthesis. There are few studies about the relationship between the use of whey protein supplements and thyroid functions. In a study by Carvalho *et al*. (2000), showed that uptake of exogenous amino acids, especially tryptophan, inhibit thyroid peroxidase activity. It was thought that this effect may occur due to the competition of exogenous amino acids with normal substrates for binding sites or reducing their oxidized forms. In the study of Passos *et al.* (2002) reported that adult fed a diet containing over 10% protein, significantly higher TSH and T3 as compared to the control group.

Conclusion

From the above results, it could be concluded that combined contained meat protein had the best effect on some biological parameters such as BWG, FI and FER. The combined contained whey protein and soybean protein caused low biological parameters with better biochemical parameters as lipid profile, liver enzymes and thyroid hormones of low weight rats when compared the meat protein combined. So, the consumption of whey protein and soybean protein had safe effect on the organs functions and improvement of body weight.

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الدراسات البيولوجية على الأطعمة الغنية بالبروتين والطاقة لتحسين إنقاص وزن فئران الألبينو

محمد مصطفي السيد، نهاد رشاد الطحان، سماح محمود البنا، هبه علي حسن خليفه

قسم التغذية وعلوم الأطعمة - كلية الاقتصاد المنزلي- جامعة المنوفية

الملخص العربى

أجريت دراسة على تأثير توليفات مصادر البروتين الثلاثة (بروتين الصويا، بروتين شرش اللبن، وبروتين اللحم) مع الشوكولاتة وجوز الهند والشوفان وشراب الجلوكوز على بعض المعايير البيولوجية والكيمو- حيوية للفئران منخفضة الوزن. تم استخدام ٢٤ فأر من ذكور فئران الألبينو تم تقسيمها على أربع مجموعات، وكان متوسط أوزانها ٥±٨٠ جرام . كانت إحدى هذه المجموعات كمجموعة ضابطة بينما أعطيت المجموعات الثلاث الاخري تركيبة مختبرة يومياً عند مستوى ١٠% لمدة ٤ أسابيع. تم قياس الزيادة في وزن الجسم، المأخوذ الغذائي، نسبة كفاءة التغذية، إنزيمات الكبد، مستوى الدهون وهرمونات الغدة الدرقية. أظهرت النتائج الي زيادة وزن الجسم بشكل ملحوظ خاصة في المجموعة (٤) التي تغذت على تركيبة تحتوي على بروتين اللحوم والشوفان والشوكولاتة وشراب الجلوكوز تليها المجموعة (٣) التي تغذت على بروتين شرش اللبن والشوفان والشوكولاتة وشراب الجلوكوز عند المقارنة بالمجموعة الضابطة. تسببت تركيبة بروتين اللحوم في زيادة كبيرة في متوسط مستويات الكوليسترول الكلي والدهون الثلاثية وLDL-c وVLDL-c وزيادة نشاط إنزيمات AST وALT بينما تسببت تركيبات بروتين الصويا وبروتين شرش اللبن في انخفاض ملحوظ في المعلمات المذكورة أعلاه مقارنة بالمجموعة (٤) وقيمهم قريبة من المجموعة الضابطة. توصلت هذه الدراسة بأن تناول بروتين مصل اللبن وبروتين الصويا له تأثير جيد على الخصائص البيولوجية والكيميائية الحيوية للفئران ذات الوزن المنخفض، لذلك قد يكون مفيداً لتحسين الوزن المنخفض.

الكلمات المفتاحية : بروتين شرش اللبن ، زيادة الوزن، هرمون T3، توليفات.