

## Effect of different protein sources on the biological and biochemical parameters of rats

Hoda M. El Gezery<sup>1</sup> and Amal. H. Emara<sup>2</sup>

1-Studies and Research Department- National Nutrition Institute, Cairo, Egypt

2-Chemistry and Metabolism Department- National Nutrition Institute, Cairo, Egypt

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### ABSTRACT

The current study verified the impacts of beef, whey and protein of soy sources at the levels of 10 and 15% on biological and biochemical parameters of normal rats. Forty-nine male Sprague Dawley rats, each weighing approximately 120±5g were randomly divided into seven groups each of seven rats. The initial set of subjects had only a basic diet and served as the control group. The basal diet was given to the other six groups and a supplementation of 10 and 15% different protein sources for 28 days. The biological parameters (BWG, FI and FER) and biochemical parameters (blood glucose, insulin hormone and insulin resistance, ALT, AST, AIP, albumin, urea, uric acid, creatinine, lipid profile, atherogenic ratios, antioxidant enzymes and interleukins productions) were determined. The findings implied that protein type affected the mean values of biological parameters of normal rats and the high levels of animal sources led to reduce the biological parameters when compared to plant source and control group. The protein sources led to increase the organs weight especially in animal protein sources, the organs weight was increased by rising the protein intake level. The mean values of blood glucose, insulin hormone and insulin resistance of the control group were substantially lower than the other groups. The highest lipid profile recorded significant increasing in group fed on basal diet with 15% as animal protein sources followed by 10% protein except HDL-c. The highest kidney and liver functions values were found in groups fed with basal diet and 15% beef protein followed by the same level of whey protein. Administration of high protein from different sources showed substantial increase in serum PRL, estradiol and the serum interleukin (IL1, IL6 and TNF-a) activities, but showed significantly reduction levels of serum testosterone as in relation with the control group and soy protein source. Thus, it important to regulate protein consumption especially from animal sources for long time and to make a balance between animal and plant protein sources to mitigate the risk of conditions such as infertility, liver and kidney disease, blood vessel disorders, and cardiovascular disease.

**Keywords:** Protein intake, biological effect, biochemical parameters.

### INTRODUCTION

Protein is common nutrient that acts as a major building block of the human body concerned with growth and maintenance. The tolerance of human bodies for the variability of acidity is very narrow and variations from the norm can be highly dangerous. Protein is one of the so called “buffer solutions”, it helps to attract and maintain water where necessary to help pump the blood through

blood vessels to keep different fluids in motion. Meats, fish, eggs, poultry and cultured milk products are foods containing animal protein, protein-rich plant foods include beans, nuts, and grains. Presently, adults should consume 0.8 g/kg of body weight per day, children should consume 1.5 g/kg, and teenagers should consume 1 g/kg. To give just one example, some bodybuilders advocate for consuming more protein than is

recommended by the current RDA for muscle mass building and/or fat loss by athletes (Hoffman and Flavo, 2004; Maryam *et al.*, 2022).

Whey protein derived from cheese, natural source, and it is almost complete protein which contains all EAA as needed. Immunoglobins, bovine serum albumin, alpha lactalbumin, and beta-lacto globulin make up whey protein. Actually, of all the natural foods, Whey protein has the most BCAAs. Unlike other necessary amino acids, BCAAs are absorbed directly into muscles and used initially during exercise and resistance training, making them crucial for athletes. In order to replenish lost BCAAs and start regenerating lean muscle mass, the body uses whey protein. Shakes, meal replacements, and protein bars often use this powder, which is available in a variety of flavors (Hayes & Cribb., 2008; Ryan *et al.*, 2022; Serena *et al.*, 2023).

Soybean, as well as its by-product okara represents a rich dietary fiber source protein and oil. Cultivated soybeans have a protein content of about 40 percent and oil of about 20 percent. Soybeans have been an important protein source, and it has good balance in amino density since all the essential amino acids are present in the product, and is known to contain physiologically helpful constituents such as soluble and insoluble fiber, isoflaventh the genistein, daidzein and glycitein and lecithins which have been shown to decrease cholesterol levels and lessen the likelihood of hyperlipidemia and blood vessel disease (Hong *et al.*, 2010; Nishinari *et al.*, 2014). Soy protein is a great way of getting protein in our diet. It is equally nutritious as casein but has a short digestion time of 2-4 hours on ingestion hence should be incorporated in meals. It has good antioxidant properties since soy products are often rich in other healthy vitamins, and good processability including gelation and emulsification

properties and water and oil absorption (Sanchez *et al.*, 2011; Phoon *et al.*, 2014; Pabich and Materska, 2019).

Beef is a natural source of protein that has all the necessary amino acid chains in one product. A beef protein powder is convenient to take than bulky mass of meat the body requires for proteins. Meat and bone meal protein powders are particularly common in supplementing shakes since the latter is easy to transport anywhere regardless of the contingency. Protein powdered from beef comes from a variety of bovine organs and tissues, including the skeleton and other connective tissues that help keep muscles in place. At times, it looks almost identical to collagen. On top of that, it's absorbed by the body with relative ease. So, it's safe to say that beef protein isolate is packed with protein. Many other protein powder products contain carbohydrates and fats, but this one doesn't. Along with being gluten-free, lactose-free, and soy-free in it (Hsu and Sun, 2006; Pedro *et al.*, 2021).

The current study aimed to assess the impact of 10 and 15% different protein sources on biological and normal albino rats' biochemical parameters.

## MATERIALS AND METHODS

### Materials

**Protein sources:** Soy protein, Whey protein and beef protein were acquired from Gomhoryia Co., Dokki, Giza, Egypt.

**Chemicals:** The commercial kit were purchased from the Technogene Chemical Company in Dokki, Egypt, Giza. Casein, minerals mixture, vitamins mixture, starch and cellulose were purchased from the Cairo Corporation for Chemical Trade, Cairo, Egypt.

**Animals:** Forty-nine male Sprague Dawley rats, approximately 120±5g, were acquired from Research Department of Institute

Ophthalmology Medical Analysis, Dokki, Cairo, Egypt.

## Methods

### The Experimental design

The basal diet was described by Reeves *et al.* (1993). This involved using vitamin mixture components according to the methods of Campbell (1963). According to the instructions supplied by Hegsted *et al.* (1941) the mixture of salt was prepared

Every biological experiment was conducted at experimental animal unit of National Nutrition Institute. The rats were rehoused in clean, well-ventilated cages with controlled humidity, light/dark cycles of 12 hours and standard filtered feed. They were also given unlimited access to water. Rats were given basal diet for one week prior to the start of the experiment to allow for acclimatization. Subsequently, the rats were split into seven groups (7 rats each):

**Group 1:** (negative control group) fed on basal diet for a 28-day trial period

**Groups 2 and 3:** (beef protein groups) were fed on basal diet containing 10 % and 15% beef protein, respectively

**Groups 4 and 5 :**(whey protein groups) were fed on basal diet containing 10 % and 15% whey protein, respectively.

**Groups 6 and 7:** (soy protein groups) were fed on basal diet containing 10 % and 15% soy protein, respectively.

At the end, animals were weighed, fasted overnight, and then sacrificed under very light ether anesthesia. Blood samples were collected from hepatic portal vein of each rat into dry clean centrifuge tubes. Serum was carefully separated by centrifugation of blood samples at 3500 rpm (round per minute) for 15 minutes at room temperature, transferred into dry clean Eppendorf tubes, then kept frozen at - 20°C for latter determinations. Liver, kidney and heart has been removed from rats by careful dissection, washed in saline solution (0.9%),

dried using filter paper and independently weighed

This study was approved by the Research Ethics Committee (REC) at National Hematology and Tropical Medicine research Institute (NHTMRI)- Cairo -Egypt (Approval protocol number: A2-2024).

### Biological assessment

We tracked the diet and weight on a daily and monthly basis. We assessed the weights of each organs and computed the feed efficiency ratio (FER) and body weight gain (BWG) using the following formulas from Chapman *et al.* (1959):

Body weight gain = Final weight (g) - Initial weight (g)

Feed efficiency ratio (FER) = Body weight gain (g) / Feed intake (g).

### Biochemical analysis

Blood glucose levels were assessed using the enzymatic colorimetric method according to Tietz (1976). Uric acid, urea and creatinine were assessed regarding to the enzymatic method of Patton and Crouch (1977), Henry (1974) and Schultz (1984). ALT, AST, ALP and albumin determination were conducted corresponding to the method of Tietz (1976), Henry (1974) Belfield and Goldberg, (1971) and Doumas *et al.* (1971), respectively. Triglycerides, total cholesterol and high-density lipoprotein cholesterol (HDL-c) were measured according to Fassati and Prencepe (1982), Allain (1974) and Lopez (1977).

VLDL (very low-density lipoproteins) and LDL were estimated according to the method of Friedwald *et al.* (1972) as follows:

VLDL (mg/dl) = Triglycerides/5

LDL (mg/dl) = (Total cholesterol – HDL) – VLDL

The ratio of LDL- c/ HDL- c was calculated corresponding to Kikuchi *et al.* (1998).

CRI (Coronary Risk Index) = TC/HDL-C (Bhardwaj *et al.*, 2013).

CRR (Cardiac Risk Ratio) = LDL-C/HDL-C (Bhardwaj *et al.*, 2013).

Atherogenic Index of serum (AI) was calculated by the methods of NCEP, 2002.

The thyroid stimulating hormone (TSH), free T4 and free T3 determination were carried out according to the methods of Uotila *et al.* (1981) and Patrono and Peskar (1987).

Leptin, insulin, serum testosterone (T) and Estradiol hormones were determined according to Cosidine *et al.* (1996), Defronzo *et al.* (1979), Tietz (1995) and Considine and Siha (1996), respectively.

Malondialdehyde (MDA), Glutathione – S – transferase (GST) activity and catalase levels were analyzed by the method of Buege and Aust (1978), Habig *et al.* (1974) and Aebi (1984), respectively.

Levels of serum cytokines (IL1, IL6 and TNF-a) were determined according to the method of Smith (1988), Van (1990) and Maury (1986), respectively.

### Statistical analysis

The methods of statistical analysis are based on **Snedecor and Cochran (1972)**. All results were presented as the mean± standard deviation. SPSS, version 11.0 (Chicago, DL-USA), was used as a statistical tool for the social sciences, to conduct the current studies.

## RESULT AND DISCUSSION

After 28 days of feeding with the different protein sources at the levels of 10 and 15%, the feed intake of control group rats in the current study was nearly like that of rats fed 10% as whey protein (G4) with no significant difference, the beef protein at level of 10% recorded the highest mean value and the feed intake in soy protein at the level 15% was lower than the others (Table 1). The mean body weight values were higher in 10% beef and whey protein groups than that of rats fed control diet and other protein sources. Rats fed the same soy protein level had a

lower body weight than the other groups with substantial differences ( $P \leq 0.05$ ). Furthermore, the FER was significantly smaller in the soy protein group, compared to the other groups (table 1). There were no substantial differences among animal protein source and that of control at the level 10%. This indicated that protein type affected the mean values of biological parameters of normal rats and the high levels of animal sources led to reduce the biological parameters when compared to plant source and control group.

The current results agreed with those reported by Emily *et al.* (2021) who found that whey protein at the rate of 10 g/day immediately before or during a meal led to increase their mean weight and can reduce adipocyte numbers and the contribution of adipose tissue to body mass. Also, Fei and Feifan (2022) reported that feeding on whey protein enhances satiety and suppresses food intake in humans and reduces food intake more than does casein, egg albumin, or soy protein. When whey is consumed with carbohydrate, it reduces the subsequent glycemic response, as do other proteins. However, Jeddiah and Patrick (2019) indicated that whey protein supplements have extra carbohydrates in the form of sugars. There are some that have fats as well. This means that weight can be gained in fat form, which is unhealthy.

Soy protein is usually described as a complete protein due to the fact that, like animal protein, it is usually a source of most of the essential amino acids. It contains almost the same nutritional merit as animal protein of biological value. For example, isolated soy protein has a PDCAAS of 1.0, the same value as casein and egg protein, however calculated methionine/glycine and lysine/arginine of soy protein were lower than animal source (Keenan *et al.*, 2007).

Reduction in feed intake and body weight was observed when the protein source

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level especially animal source was increased. Bray *et al.* (2012) found that even if a person takes high protein, it does not lead to an additional increase of fat mass. Also, Antonio *et al.* (2016) supported that the high-protein diets can promote weight loss because high protein foods tend to promote a feeling of fullness, helping reduce hunger cravings and overeating.

The effects of various protein sources on the weight of normal rats' livers, spleens, and kidneys at levels of 10% and 15% were illustrated in Table (1). It was obvious that the organs weight was increased by rising the protein intake level especially in animal protein sources. On the other hand, the source of soy protein had the lowest effect of increasing the organs weight and the level 10% recorded no significant changes compared to the control group. Liver was the highest sensitive organ to high protein intake followed by kidney and spleen.

Although intaking protein is necessary for the development and maintenance of muscles, organs, and bones, yet a diet rich in

protein can aid in weight loss, fat reduction, increased satiety (the sensation of being full), and muscle retention. Also, the excess nitrogen in protein's building blocks can cause damage to the kidney and liver. The increased nitrogen and metabolic waste products caused by the excessive protein consumption have put a strain on the kidney and liver, which might be hazardous in filtering out ammonia and other nitrogen sources such as glutamine from the blood to synthesize urea, albuminuria, or fluid and electrolyte balance (Groziak *et al.*, 2014). High protein intake, as a result of the liver's conversion of waste protein to triglycerides, which are then stored in fat cells and can end up in the liver itself, insulin resistance can contribute to an increase in triglycerides and an increase in the uptake of fatty acids by the liver, leading to even more buildup of triglycerides in the liver. Also, high protein diet is a cause of splenomegaly and may cause an enlarged spleen (Dong *et al.*, 2013; Emily *et al.*, 2021).

**Table (1): Body weight gain, feed intake, feed efficiency ratio and Some organs weight of rats as affecting of feeding different protein sources**

| Groups                         | Parameters                |                           |                          |                        |                         |                         |
|--------------------------------|---------------------------|---------------------------|--------------------------|------------------------|-------------------------|-------------------------|
|                                | FI (g/day)<br>Mean ±SD    | BWG (g/28d)<br>Mean ±SD   | FER<br>Mean ±SD          | Liver (g)              | Spleen (g)              | Kidneys (g)             |
| <b>(G1):<br/>Control group</b> | 14.07±0.24 <sup>b</sup>   | 37.89 ± 1.23 <sup>b</sup> | 0.096±0.002 <sup>a</sup> | 4.12±0.09 <sup>d</sup> | 0.44±0.027 <sup>d</sup> | 1.36±0.01 <sup>d</sup>  |
| <b>G2</b>                      | 15.11±0.21 <sup>a</sup>   | 40.11 ± 1.41 <sup>a</sup> | 0.094±0.001 <sup>a</sup> | 4.93±0.03 <sup>b</sup> | 0.58±0.032 <sup>b</sup> | 1.58±0.042 <sup>b</sup> |
| <b>G3</b>                      | 13.22±0.17 <sup>c</sup>   | 32.05 ± 1.54 <sup>c</sup> | 0.087±0.002 <sup>b</sup> | 5.53±0.11 <sup>a</sup> | 0.64±0.039 <sup>a</sup> | 1.94±0.035 <sup>a</sup> |
| <b>G4</b>                      | 14.28±0.41 <sup>b</sup>   | 38.21 ± 1.61 <sup>b</sup> | 0.096±0.003 <sup>a</sup> | 4.42±0.13 <sup>c</sup> | 0.53±0.009 <sup>c</sup> | 1.51±0.042 <sup>c</sup> |
| <b>G5</b>                      | 13.01±0.23 <sup>c</sup>   | 31.41 ± 1.43 <sup>c</sup> | 0.086±0.003 <sup>c</sup> | 5.45±0.06 <sup>a</sup> | 0.62±0.023 <sup>a</sup> | 1.89±0.047 <sup>a</sup> |
| <b>G6</b>                      | 13.13 ± 0.39 <sup>c</sup> | 33.11 ± 0.09 <sup>c</sup> | 0.090±0.001 <sup>b</sup> | 4.19±0.13 <sup>d</sup> | 0.46±0.031 <sup>d</sup> | 1.39±0.032 <sup>d</sup> |
| <b>G7</b>                      | 12.48 ± 0.17 <sup>d</sup> | 29.16 ± 0.23 <sup>d</sup> | 0.083±0.002 <sup>c</sup> | 4.32±0.06 <sup>c</sup> | 0.51±0.022 <sup>c</sup> | 1.46±0.021 <sup>c</sup> |
| <b>LSD</b>                     | 0.43                      | 1.72                      | 0.003                    | 0.15                   | 0.04                    | 0.05                    |

Values are mean ±SD. The same letter means there was no significantly between groups, while the difference letter means that there were significantly differences between groups ( $p \leq 0.05$ ).

It was observed from data in Table (2) that the control group had substantially lower mean values for insulin hormone, insulin resistance, and blood glucose in comparison to the other groups. No substantial

differences were found among groups fed on 15% animal protein sources, also, the same effect was found between control group and the group fed 10% soy protein. Groups of 15% soy protein and 10% whey protein

recorded no significant differences. The obtained results were in accordance of those of Hoffman and Flavo (2004) who found that the bioactive peptides and amino acids produced by whey protein's digestion in the intestines raise blood glucose levels through the actions of bioactive peptides and amino acids which produced during digestion in the gastrointestinal tract. A number of gut hormones like cholecystokinin, peptide YY, and the incretins (gastric inhibitory peptide and glucagon-like peptide 1), are triggered by these amino acids and peptides and it was found that they have synergizing effects on insulin secretion from  $\beta$ -cells and have an effect on regulating food intake. The bioactive peptides derived from whey protein can also act as DPP-4 inhibitors within proximal gut to allow none of this degradation to happen. The current results are also in accordance with Johnson *et al.* (2019) who indicated that due to higher digestibility and higher concentration of BCAA, insulin level rises rapidly.

Proof to the fact that the branched-chain amino acids were the active component mediating the effect of whey protein has thus emerged by observation of a comparably low

impact on plasma insulin and glucose concentrations by intact whey, hydrolysed whey protein and branched-chain amino acids. Several studies also described that WP rapidly enhances insulin and incretins, also slow gastric discharge, therefore effectively magnify the effect of insulin, GLP-1 (Wu *et al.*, 2016).

It is proposed that the insulin-releasing activity of SP could be due to the protein fraction of the preparation. The exact working model which SP could bring about hyperinsulinemia is related with more level of amino acid alanine and arginine, thus enhancing secretion of glucose-dependent insulinotropic polypeptide (GIP). Enhanced leucine concentrates in SP resulted from its higher branched-chain amino acid (BCAA) levels can increase glucagon-like peptide-1 (GLP-1) response thereby raising insulin secretion (Sun *et al.*, 2017; Hamley *et al.*, 2019).

Beef protein which contained more authentic amounts of amino acids caused the pancreas to secrete the hormones glucagon and insulin. Insulin makes muscle cells take up amino acids while, glucagon makes liver release sugar in serum (Piatti, 2013).

**Table (2): Blood glucose and insulin resistance of normal rats as affecting of feeding different protein sources (mg/dl).**

| Groups              | Parameter                |                            |                            |
|---------------------|--------------------------|----------------------------|----------------------------|
|                     | Blood glucose (mg/dl)    | Insulin hormone (mIU/L)    | Insulin resistance (mg/dl) |
| (G1): Control group | 96.43±7.83 <sup>d</sup>  | 10.50± 0.36 <sup>d</sup>   | 2.50±0.28 <sup>d</sup>     |
| G2                  | 157.82±4.82 <sup>b</sup> | 11.55± 0.42 <sup>b</sup>   | 4.50±0.006 <sup>b</sup>    |
| G3                  | 187.65±7.31 <sup>a</sup> | 14.61 ± 0.27 <sup>a</sup>  | 6.77±0.03 <sup>a</sup>     |
| G4                  | 141.77±6.92 <sup>c</sup> | 11.10± 0.26 <sup>c</sup>   | 3.89±0.44 <sup>c</sup>     |
| G5                  | 180.31±2.91 <sup>a</sup> | 14.01 ±0.41 <sup>a</sup>   | 6.24±0.51 <sup>a</sup>     |
| G6                  | 101.39±3.65 <sup>d</sup> | 10.65 ± 0.009 <sup>d</sup> | 2.67±0.43 <sup>d</sup>     |
| G7                  | 146.55±5.32 <sup>c</sup> | 10.94 ± 0.301 <sup>c</sup> | 3.96±0.33 <sup>c</sup>     |
| LSD                 | 9.55                     | 0.43                       | 0.53                       |

Values are mean ±SD. The same letter means there was no significantly between groups, while the difference letter means that there were significantly differences between groups ( $p \leq 0.05$ ).

The different sources of protein at the levels 10 and 15% on lipid profile, CRI, CRI

and AI presented had notable different impacts relative to the group of control

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(Table 3). The highest lipid profile was documented significant increasing in group fed on basal diet with 15% as animal protein sources followed by 10% protein except HDL-c. Feeding on different levels of soy protein led to decrease the lipid profile level in all groups, however there were no significant differences with control group except LDL-c and HDL-c.

Cardiovascular risk was predicted utilizing the CRI, CRI and AI which were higher in all groups than the control group. This effect was significantly amplified by rising the protein source level especially animal protein source as compared to plant source.

The current investigation indicated that rats' lipid profiles were altered when given whey protein and beef protein, which led to elevated serum cholesterol and triglyceride levels compared to the control group. Compared to the other groups that consumed animal protein sources, rats feed soy protein sources performed marginally better. There was a difference in the impact that dietary proteins have on plasma cholesterol levels. It was found that animal proteins especially casein raised the plasma total cholesterol level than plant proteins especially soya. Thomsen *et al.* (2020) found that soy protein after six weeks reduced the concentration of triglycerides more by 12.4% when compared to animal protein.

Wolfe *et al.* (2018) found that most dietary protein above the ranging of 10-35% increases cardiovascular disease risk factors and all-causes of mortality are linked to TEE. According to certain NHANES III data, middle-aged people (50–65 years old) who eat more protein had a greater risk of dying from cardio metabolic diseases than those whose diets are lower in protein. This

supports suggestion of eating dietary protein at RDA especially for midlife adults increasing dietary protein intake for older adults than the RDA in order to reduce cardiometabolic related mortality. The association among the high-protein diet and the increasing of cardiovascular disease risk exists due to the fact that this macronutrient increases levels of bad LDL cholesterol while decreasing levels of good HDL cholesterol (Piatti, 2013). On the other hand, Dong *et al.* (2013) and Levine *et al.* (2014) mentioned that the diets with high protein and low carbohydrate cause reduction in blood pressure and enhancing glycemic control as well as the blood lipid profile. The positive impact on cardio metabolism by high protein diets seem to be at least the same or even greater than those of low fat. Lagiou (2012) reported that it was ascertained that most proteins in food such as fatty cuts of meat, whole dairy products and other foods high in fat raise cholesterol levels and hence the risk of heart diseases.

Proteins found in soy contain phytoestrogens and is flavones, which inhibit the body's ability to absorb cholesterol and produce new cholesterol. Soy is also a good source of protein, fiber as well as heart healthy omega-3, though not the same type found in salmon or tuna, however, it has cholesterol free and low in saturated fat, hence it has a positive impact on reduction in bad cholesterol (Suh *et al.*, 2001). However, it was indicated that these compounds for long time stimulate growth of some types of cancer cells, affected female fertility and interfered with the thyroid hormone to boost cholesterol and LDL level (Sonia *et al.*, 2019).

**Table (3): Effect of feeding protein sources on lipid fractions, CRI, CRR and AI for rats.**

| Groups              | Parameters                |                          |                         |                         |                         |                         |                        |                         |
|---------------------|---------------------------|--------------------------|-------------------------|-------------------------|-------------------------|-------------------------|------------------------|-------------------------|
|                     | Total cholesterol (mg/dl) | Triglyceride             | HDL-c (mg/dl)           | LDL-c (mg/dl)           | VLDL-c (mg/dl)          | CRI                     | CRR                    | AI                      |
| (G1): Control group | 91.71±0.73 <sup>d</sup>   | 109.76±1.21 <sup>c</sup> | 51.78±1.79 <sup>a</sup> | 17.98±0.92 <sup>f</sup> | 21.95±0.37 <sup>c</sup> | 1.77±0.013 <sup>c</sup> | 0.35±0.05 <sup>c</sup> | 0.77±0.010 <sup>c</sup> |
| G2                  | 102.02±2.83 <sup>b</sup>  | 123.45±0.72 <sup>c</sup> | 41.55±1.56 <sup>c</sup> | 35.78±0.74 <sup>c</sup> | 24.69±1.44 <sup>b</sup> | 2.46±0.05 <sup>c</sup>  | 0.86±0.04 <sup>c</sup> | 1.46±0.07 <sup>c</sup>  |
| G3                  | 108.11±2.91 <sup>a</sup>  | 131.05±0.65 <sup>a</sup> | 35.12±1.74 <sup>d</sup> | 46.78±0.91 <sup>a</sup> | 26.21±0.82 <sup>a</sup> | 3.07±0.14 <sup>a</sup>  | 1.33±0.03 <sup>a</sup> | 2.07±0.10 <sup>a</sup>  |
| G4                  | 99.39±0.69 <sup>e</sup>   | 120.34±0.99 <sup>d</sup> | 42.09±0.39 <sup>c</sup> | 33.32±1.58 <sup>d</sup> | 24.07±0.26 <sup>b</sup> | 2.36±0.07 <sup>c</sup>  | 0.79±0.04 <sup>c</sup> | 1.36±0.09 <sup>c</sup>  |
| G5                  | 102.79±1.94 <sup>b</sup>  | 127.01±1.74 <sup>b</sup> | 36.18±0.89 <sup>d</sup> | 41.21±0.32 <sup>b</sup> | 25.40±0.25 <sup>a</sup> | 2.84±0.15 <sup>b</sup>  | 1.14±0.07 <sup>b</sup> | 1.84±0.05 <sup>b</sup>  |
| G6                  | 92.97±2.04 <sup>d</sup>   | 105.90±1.77 <sup>d</sup> | 46.58±0.45 <sup>b</sup> | 25.21±0.56 <sup>c</sup> | 21.18±0.08 <sup>c</sup> | 1.99±0.09 <sup>d</sup>  | 0.54±0.05 <sup>d</sup> | 0.99±0.11 <sup>d</sup>  |
| G7                  | 93.94±1.32 <sup>d</sup>   | 108.12±2.03 <sup>c</sup> | 46.64±1.02 <sup>b</sup> | 25.68±0.87 <sup>c</sup> | 21.62±0.65 <sup>c</sup> | 2.01±0.14 <sup>d</sup>  | 0.55±0.06 <sup>d</sup> | 1.01±0.07 <sup>d</sup>  |
| LSD                 | 3.07                      | 2.98                     | 1.96                    | 2.07                    | 1.08                    | 0.15                    | 0.07                   | 0.11                    |

AI: Atherogenic Index of serum = AI=(TC-HDL)/H D L-c)

CRI: (Coronary Risk Index (TC/HDL-c)

CRR: (Cardiac Risk Ratio (LDL-C/HDL-c).

Values are mean ±SD. The same letter means there was no significantly between groups, while the difference letter means that there were significant differences between groups (p≤0.05).

The kidney function values were directly proportional to dietary protein types (Table 4). The highest kidney functions values were found in groups fed with basal diet and 15% beef protein followed by the same level of whey protein. The group of control indicated no significant difference with the group fed 10% soy protein. There was no significant difference between the mean values for kidney functions was groups 3 and 5 as well as groups 4 and 7.

In comparison to the control group, there was a more substantial negative impact on uric acid, urea nitrogen, and creatinine levels caused by the whey portion and meat protein, suggesting a decline in kidney function.

Proteins are used by the body and ammonia is produced as a by-product which then converted to urea. If a person takes high quantities of protein, high quantities of urea are produced which puts higher pressure on the kidneys. A high-protein diet may increase the risk for high blood pressure because when blood pressure inside the kidneys increases, the kidneys may become worked up and filter

more blood than normal. High-protein diet usually consists of high amount of purine which may induce the uricosuric effect and leads to low serum acid levels in urine and also leads to high levels of uric acid in blood. Thus, in the high protein diet fed animals the activity of all the enzymes of urea cycle is considerably elevated. Arginine is limiting the conversion to urea of blood ammonia resulting from the peed deamination of the sent surplus amino acids (Hayes and Cribb, 2008).

The risks of developing kidney stone include decreased fluid consumption and high protein consumption which promotes renal acid elimination, while acid challenges may be quenched partially by bone, which supplies calcium for elimination through the kidneys. Thus, the essential protein ingestion maybe enhanced hypercalciuria. Calcium renal calculi may develop for this reason. The amino acids found in meat and other animal products are rich in purines, which are building blocks of uric acid. Therefore, it can be concluded that hyperuricosuria, a symptom observed in some uric acid stone



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formers, is caused by animal protein. There is a significant pH dependence of the solubility in urine. Below pH 5.5 to 6.0, uric acid itself begins to precipitate and crystallization proceeds even when hyperuricosuria is not present. A notable study about the effects of protein overload and its link to stone-forming abilities revealed that a daily high protein diet for 6 weeks is able to supply a large quantity of acid load to the kidney and enhance the likelihood of producing stones (authors found lower urinary citrate level and higher urinary undissociated uric acid saturation). A second adverse outcome associated with whey protein intake is an increase in pH levels in blood. But when there is more protein in your blood, the kidney has difficulties in breaking it down, and leads to the rise in blood acidity (Piatti, 2013 and Nilsson *et al.*, 2017). Feeding on tested formula which content animal and plant source of protein and also soy protein as a plant source led to increasing the levels of kidney functions but the elevation of these levels was in normal range (5 to 23 mg/dl for urea, 0.7-1.3 mg/dl for creatinine and 2.14-7.9 mg/dl for uric acid) (Peterson *et al.*, 2011; Hamley *et al.*, 2019).

Liver enzymes in control demonstrated the minimal value, while the highest enzymes levels occurred in group (3) followed by group (5) with no significant differences. There was no significant between G4 and G7. The same statically effect was showed between groups fed on basal diet with 10% protein of soy and control group. From the obtained results, animal protein sources had high significant effect on liver functions when compared to plant protein source. The study of Nestle *et al.* (2007) was in the same line of obtained results who found that a high protein intake can lead to liver injury in normal, nonsmoking adults. Ammonia, a product of protein breakdown, is toxic to the brain when it accumulates in the blood stream in large

quantities. They also discovered that intake of animal protein increases the risk of developing fatty liver disease particularly among the elderly and overweight persons.

While consuming high protein food for long time, positively affects the necrosis factor, interleukin 1 and Il -6 which are the cytokines of pro-inflammatory. These sites are liberated in systemic circulation in case of substantiated aggression, for example, in postoperative conditions and in infections, and the intensity of this reaction depends upon the intensity of aggression. Experiments have shown that hepatocellular apoptosis is responsible for the chief function in chronic hepatic diseases (Levine, 2014).

The switch to the high protein diet was accompanied by reductions in liver glycogen, as well as enhancement of the activities of enzymes involved in amino acid metabolism and gluconeogenic enzymes; however, there were reductions in malice enzyme and phosphorylase activities. In this respect, there are various kinds of enzymes, however the three most usually reported are aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphates' (ALP). When any disturbance accrued in liver due to imbalance in the obtained diet as increase protein intake especially for athletic people led to liver damage, which is demonstrated by highly elevated AST and ALT levels (Hamley *et al.*, 2019).

Most liver, bowel, and prostate carcinomas are directly related to diet with high meat protein showing positive correlation in the global comparisons. The association, however, appears to be more robust when measured using meat protein or processed meat and colorectal cancer. Among these include production of heterocyclic amines in meat when it is cooked. P450 enzymes are necessary for the acetylating of these heterocyclic amines; individuals with a fast-acetylating genotype who eat an unhealthy amount of animal

protein may be at increased risk for developing colorectal cancer. Notably, saturated fat—known to promote malignancies of the colon and breast—is mostly found in red meat. Heptacyclic amine metabolism and chemically induced NOC large bowel tumors are associated with NH<sub>3</sub>,

although N-nitroso compounds produced by large bowel bacteria are also probable contributors. Several chromosomal alterations have been observed in human colorectal cancer responses to NOCs (Messia and Marconi, 2011).

**Table (4): Effect of feeding protein sources on kidney functions and liver functions for rats**

| Animal Groups | Parameters             |                         |                        |                         |                         |                          |                        |
|---------------|------------------------|-------------------------|------------------------|-------------------------|-------------------------|--------------------------|------------------------|
|               | Uric acid              | Urea                    | Creatinine             | AST                     | ALT                     | ALP                      | Albumin (mg/dl)        |
| G1            | 2.71±0.34 <sup>d</sup> | 16.63±1.43 <sup>d</sup> | 0.84±0.09 <sup>d</sup> | 35.16±1.21 <sup>d</sup> | 33.31±1.88 <sup>d</sup> | 72.93±2.81 <sup>d</sup>  | 4.09±0.03 <sup>a</sup> |
| G2            | 3.94±0.21 <sup>b</sup> | 27.93±0.95 <sup>b</sup> | 1.29±0.08 <sup>b</sup> | 44.87±3.22 <sup>b</sup> | 42.79±2.76 <sup>b</sup> | 94.73±3.81 <sup>b</sup>  | 3.71±0.07 <sup>c</sup> |
| G3            | 6.02±0.27 <sup>a</sup> | 36.64±2.21 <sup>a</sup> | 1.76±0.07 <sup>a</sup> | 53.02±3.56 <sup>a</sup> | 51.11±1.77 <sup>a</sup> | 122.73±4.82 <sup>a</sup> | 3.15±0.09 <sup>d</sup> |
| G4            | 3.51±0.10 <sup>c</sup> | 24.79±0.93 <sup>c</sup> | 1.13±0.08 <sup>c</sup> | 40.13±4.01 <sup>c</sup> | 39.51±2.07 <sup>c</sup> | 89.15±3.73 <sup>c</sup>  | 3.92±0.11 <sup>b</sup> |
| G5            | 5.73±0.18 <sup>a</sup> | 33.49±1.43 <sup>a</sup> | 1.71±0.10 <sup>a</sup> | 50.22±2.65 <sup>a</sup> | 47.95±1.74 <sup>a</sup> | 118.23±1.96 <sup>a</sup> | 3.26±0.10 <sup>d</sup> |
| G6            | 2.99±0.29 <sup>d</sup> | 19.78±1.12 <sup>d</sup> | 0.94±0.09 <sup>d</sup> | 38.02±2.06 <sup>d</sup> | 37.11±1.87 <sup>d</sup> | 78.43±5.22 <sup>d</sup>  | 4.01±0.12 <sup>a</sup> |
| G7            | 3.34±0.22 <sup>c</sup> | 24.54±0.72 <sup>c</sup> | 1.12±0.10 <sup>c</sup> | 40.07±3.22 <sup>c</sup> | 40.62±1.32 <sup>c</sup> | 89.11±2.87 <sup>c</sup>  | 3.91±0.11 <sup>b</sup> |
| LSD           | 0.32                   | 3.15                    | 0.11                   | 4.01                    | 2.07                    | 5.53                     | 0.13                   |

Values are mean ±SD. The same letter means there was no significantly between groups, while the difference letter means that there were significantly differences between groups ( $p \leq 0.05$ ).

Table (5) results presented the different protein sources impact on some metabolic hormones of rats. The groups that consumed a lot of animal protein had the highest mean values for leptin and TSH, and the groups that consumed the least amount of animal protein had the lowest mean values for T4 and T3.

The group fed on 10% soy protein had nearly values to control group with non-significant differences. HPD-fed rats had significantly increased leptin and TSH hormones concentration during the study. While the higher the diet of beef protein result into reduction the levels of insulin, T3 and T4. In specific, it has been demonstrated that the plasma concentration of leptin corresponds to the body fat mass since leptin is involved in a critical physiological process of informing the brain about the amount of energy reserve for satiety and energy utilization (Nilsson *et al.*, 2017). Furthermore, the results yielded here were validated with that of Binder *et al.* (2014)

who confirmed that, with elevated dietary protein intake, leptin sensitivity is enhanced. The published data of the 24-h studies on human subjects informed that an acute elevation of oral protein intake results in leptin plasma concentration (Van *et al.*, 2013).

According to Clifton (2012), proteins from the diet. Includes a property that increases secretion of insulin hence increasing glucose uptake which is a fact from the literature. In the long run, nevertheless, a high dietary protein intake was found to be a determinant of type 2 diabetes meaning this with the obtained results. Through in vivo studies, the following effects have been demonstrated: Insulin is able to stimulate transport of short side chain amino acid intracellularly, increase RNA transcription and translation, increase gene expression of albumin and other proteins and the insulin also inhibits the breakdown of proteins in the liver. The

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results have revealed that high protein diet especially animal protein such as processed meats boosts the risks of type 2 diabetes and reduces the level of insulin hormones (Song *et al.*, 2014).

Although short-term consumption of proteins has been linked with a protective effect as regards risk of type 2 diabetes, excessive levels of protein in the diet in the long-term has been linked with a negative effect on the risk of type 2 diabetes. In addition, a group of amino acids, namely BCAA, one of the most recognized categories of amino acids and an essential component of the dietary protein were recently identified as significantly linked to diabetes. There is no clear and consistent trend as to the influence of protein consumption on insulin sensitivity and diabetes using both observational data and interventional data (Schwingshackl and Hoffmann 2013).

TH are involved in almost all tissues' differentiation, growth, development and function processes. They have been known for a long time as one of the principal controllers of oxygen demand, and basal metabolic rate. These effects have been primarily to the pharmacological effects of TH on the heart and obligatory working tissues such as liver, WAT and BAT and skeletal muscles. Both Thy3 and Thy4 are also involved with the central regulation of energy balance at the level of the hypothalamus. The total T4 in adults is normally expected to be between 5.0 to 12.0 $\mu$ g/dl. Normal level of Total T3 in adult is 80-220 ng/dl and TSH was 0.4-4.0 milli-international units/liter (Azadbakht *et al.*, 2013).

Normal rats (control group) showed significant decrease in serum levels of

prolactin and estradiol accompanied with marked increase in serum testosterone. Administration of high protein with different sources to normal animals showed significantly increase in serum PRL, and estradiol, but showed significantly reduction levels of serum testosterone. For testosterone the control group showed the highest level, followed by the group that received a basal diet supplemented with 10% soy protein. Group 3 (G3), which received a basal diet plus 15% beef protein, had the lowest value. Prolactin levels were substantially higher in the groups that consumed the protein sources but the beef protein had the high effect on the hormone level followed by whey protein and soy protein. From the previous studies, it was found that the normal of sexual hormones in men were 270-1070 ng/dl for testosterone, (2.0–5.5 ng/dl) for estradiol and 20 ng/ml for prolactin (Valek *et al.*, 2017).

Oomizu and Sarkar (2020) found that animal protein can cause inflammation in the body that damages the gut leading to blood sugar issues and a disordinate men hormones. High protein meals and a high protein to carbohydrate ratio have been connected with modifying resting testosterone and cortisol and insulin-like growth factor concentrations. High blood prolactin further inhibits testicular function, resulting to reduced synthesis of testosterone-the major male sex hormone-and sperm. Low testosterone leads in diminished energy, sex desire, muscle mass, loss of strength, and blood count (=anemia). If continued over numerous years, low blood testosterone levels can cause a loss of bone strength (osteoporosis). High blood prolactin also has claims of interfering with sexual libido.

**Table (5): Effect of feeding protein sources on Thyroid hormones and sexual hormones on rats.**

| Animal Groups | Leptin (ng/ml)               | Thyroid hormones              |                              |  | sexual hormones                |                              |                               |
|---------------|------------------------------|-------------------------------|------------------------------|--|--------------------------------|------------------------------|-------------------------------|
|               |                              | T3 (ng/dl)                    | T4 ( $\mu$ g/dL)             | TSH (milli-international units /liter) | Serum Testosterone (ng/dl)     | Estradiol (ng/dl)            | Prolactin (ng/ml)             |
| G1            | 3.76 $\pm$ 0.08 <sup>d</sup> | 89.54 $\pm$ 1.76 <sup>a</sup> | 1.22 $\pm$ 0.01 <sup>a</sup> | 3.55 $\pm$ 0.23 <sup>d</sup>           | 595.2 $\pm$ 8.83 <sup>a</sup>  | 3.56 $\pm$ 0.03 <sup>f</sup> | 16.53 $\pm$ 0.90 <sup>f</sup> |
| G2            | 5.23 $\pm$ 0.19 <sup>b</sup> | 83.34 $\pm$ 2.54 <sup>c</sup> | 1.02 $\pm$ 0.02 <sup>c</sup> | 5.25 $\pm$ 0.12 <sup>b</sup>           | 529.67 $\pm$ 6.40 <sup>d</sup> | 4.27 $\pm$ 0.04 <sup>b</sup> | 24.14 $\pm$ 0.43 <sup>b</sup> |
| G3            | 6.45 $\pm$ 0.23 <sup>a</sup> | 76.03 $\pm$ 1.72 <sup>d</sup> | 0.93 $\pm$ 0.03 <sup>d</sup> | 5.99 $\pm$ 0.54 <sup>a</sup>           | 498.54 $\pm$ 4.88 <sup>f</sup> | 4.38 $\pm$ 0.08 <sup>a</sup> | 26.14 $\pm$ 0.56 <sup>a</sup> |
| G4            | 4.15 $\pm$ 0.09 <sup>c</sup> | 86.01 $\pm$ 1.47 <sup>b</sup> | 1.13 $\pm$ 0.03 <sup>b</sup> | 4.63 $\pm$ 0.27 <sup>c</sup>           | 533.31 $\pm$ 9.21 <sup>c</sup> | 4.01 $\pm$ 0.05 <sup>d</sup> | 20.11 $\pm$ 0.72 <sup>d</sup> |
| G5            | 6.18 $\pm$ 0.21 <sup>a</sup> | 79.01 $\pm$ 1.98 <sup>d</sup> | 1.05 $\pm$ 0.05 <sup>c</sup> | 5.87 $\pm$ 0.42 <sup>a</sup>           | 513.31 $\pm$ 8.03 <sup>e</sup> | 4.13 $\pm$ 0.07 <sup>c</sup> | 22.63 $\pm$ 0.82 <sup>c</sup> |
| G6            | 3.91 $\pm$ 0.12 <sup>d</sup> | 89.80 $\pm$ 1.84 <sup>a</sup> | 1.20 $\pm$ 0.04 <sup>a</sup> | 3.73 $\pm$ 0.42 <sup>d</sup>           | 587.67 $\pm$ 9.59 <sup>a</sup> | 3.62 $\pm$ 0.08 <sup>f</sup> | 17.03 $\pm$ 0.92 <sup>f</sup> |
| G7            | 4.19 $\pm$ 0.11 <sup>c</sup> | 86.38 $\pm$ 3.33 <sup>b</sup> | 0.04 $\pm$ 0.04 <sup>b</sup> | 4.69 $\pm$ 0.43 <sup>c</sup>           | 554.65 $\pm$ 6.87 <sup>b</sup> | 3.86 $\pm$ 0.09 <sup>c</sup> | 19.04 $\pm$ 1.01 <sup>e</sup> |
| LSD           | 0.27                         | 3.45                          | 0.05                         | 0.54                                   | 10.02                          | 0.09                         | 1.01                          |

Values are mean  $\pm$ SD. The same letter means there was no significantly between groups, while the difference letter means that there were significantly differences between groups ( $p \leq 0.05$ ).

Data in Table (6) showed that malonildiadehide (MDA) concentrations was significantly high in the group fed on beef protein followed whey protein source. There are no substantial differences among group of control and 10% soy protein. The other tested protein groups were significantly higher than the group of control. Superoxide dismutase and monoaldehyde levels were substantially increased by increasing the levels of protein source especially animal sources when compared to plant source. For glutathion -S-transferase, Glutathione peroxidase and catalase were significantly reduced by increasing the animal protein sources followed by plant protein source. All parameters of the control group recorded mean values nearly to the group fed 10% soy protein with no significant differences. So, this proved that the rate of oxidation is significantly lower in the control group in comparison to rats fed on protein sources especially animal sources.

The provision of high protein intakes results in fat accumulation as well as an increase in body mass index. Although there is an association between fat storage and decreased oxidative stress, metabolic syndrome is thought to have several causes, including an imbalance in the control of adipocytokine synthesis and an increase in

reactive oxygen species (ROS) generation at certain sites. Together, the rise of NADPH oxides and the fall of antioxidant enzymes caused by fat buildup contribute to oxidative stress. Insulin resistance and thrombosis are both exacerbated by oxidative stress, which in turn increases the production of plasminogen activator inhibitor-1 and TNF- $\alpha$ , two adipocytokines. Adiponectin plasma levels are affected by oxidative stress, while thermogenesis remains unaffected; adiponectin is insulin-sensitizing and anti-atherogenic. The deposition of fat triggers a chain reaction of systemic oxidative stress, thereby increasing the levels of ROS in the vascular wall and leading to the onset of atherosclerosis, which finally contributes to cardiovascular disease (Ramezanipour *et al.*, 2014).

Dietary regimens, as a mainstay in weight management, have been shown to promote shift in pro-oxidant – antioxidant balance due to the reduction of oxidative stress markers. A high protein intake leads to increases in thermogenic response, when combined with an excess, causes oxidative stress and an increase in electron flow throughout the mitochondrial respiratory chain. No matter where the oxidized amino acids originate from during protein catabolism, they will end up in the Krebs

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cycle, where they will be used to fuel the mitochondrial respiratory chain and generate ATP. Along with the oxidation of amino acids as substrates, the process will also produce reducing equivalents, which will be reoxidized in the mitochondrial electron transport chain. Coenzyme Q's superoxide and other reactive oxygen species are produced in the mitochondria as a result of an increase in electron transport via the respiratory chain. Endogenous and exogenous antioxidants might not be able to effectively counteract the rapid formation of ROS. The consequence of these rapidly synthesized responses is a detrimental effect from ROS cascade into oxidative stress. A potential mechanism of cell damage via oxidative stress may be via lipid peroxidation of the cellular membrane with MDA as the

chain reaction end product (Jutamulia *et al.*, 2018). Van *et al.* (2013) indicated that high protein diet (raw soy flour) administered over a period of 30 days caused significantly higher lipid peroxidation and lower levels of antioxidant enzymes including SOD, catalase, and glutathione peroxidase in pancreatic tissue. Valek *et al.* (2017) found that rats given a high-protein diet for 15 weeks did not exhibit a discernible change in protein distribution throughout organs and there was no variations in plasma GSH levels among protein consumption. Nevertheless, compared to the other diets, the HP-to had substantially lower total blood GSH concentrations ( $p < 0.05$ ). Furthermore, as compared to the other diet groups, those with an appropriate protein intake had much lower liver GSH levels (Pedro *et al.*, 2021).

**Table (6): Effect of feeding protein sources on Malonildeldehyde (MDA), serum glutathione-S-transferase, glutathione peroxidase, superoxide dismutase and catalase for rats.**

| Animal Groups | Parameters             |                                |                                 |                             |                         |
|---------------|------------------------|--------------------------------|---------------------------------|-----------------------------|-------------------------|
|               | MAD (umol mg-1)        | Glutathion transferase(umol/L) | -S Glutathion peroxidase(mU/ml) | Superoxide dismutase (U/mg) | Catalase (IU/ml)        |
| G1            | 0.99±0.03 <sup>e</sup> | 1.95±0.05 <sup>a</sup>         | 8.35±0.24 <sup>a</sup>          | 4.94± 0.22 <sup>g</sup>     | 67.93±0.76 <sup>a</sup> |
| G2            | 2.22±0.05 <sup>c</sup> | 1.57±0.06 <sup>c</sup>         | 7.07±0.18 <sup>c</sup>          | 6.65±0.21 <sup>c</sup>      | 56.85±1.84 <sup>c</sup> |
| G3            | 2.92±0.09 <sup>a</sup> | 1.29±0.07 <sup>c</sup>         | 6.45±0.21 <sup>c</sup>          | 7.18±0.24 <sup>a</sup>      | 50.35±0.76 <sup>c</sup> |
| G4            | 2.09±0.06 <sup>d</sup> | 1.62±0.08 <sup>c</sup>         | 7.32±0.16 <sup>c</sup>          | 6.39±0.18 <sup>d</sup>      | 58.62±0.98 <sup>c</sup> |
| G5            | 2.45±0.11 <sup>b</sup> | 1.42±0.07 <sup>d</sup>         | 7.01±0.25 <sup>d</sup>          | 6.91±0.07 <sup>b</sup>      | 54.12±1.34 <sup>d</sup> |
| G6            | 1.26±0.08 <sup>c</sup> | 1.89 ±0.02 <sup>a</sup>        | 8.12±0.22 <sup>a</sup>          | 5.45±0.12 <sup>f</sup>      | 65.05±1.23 <sup>a</sup> |
| G7            | 1.09±0.10 <sup>f</sup> | 1.72±0.04 <sup>b</sup>         | 7.57±0.09 <sup>b</sup>          | 6.11±0.19 <sup>c</sup>      | 61.85±1.99 <sup>b</sup> |
| LSD           | 0.11                   | 0.08                           | 0.25                            | 0.24                        | 2.43                    |

Values are mean ±SD. The same letter means there was no significantly between groups, while the difference letter means that there were significant differences between groups ( $p \leq 0.05$ ).

Data in Table (7) indicated that the activities of serum interleukin IL1, IL6, and TNF- $\alpha$  for the investigated groups compared with the control group were substantially increased by intaking high protein. Although the activity was still greater than the control value, it was noticeable that the activities of the previous parameters level increased with animal protein sources in the basal diet. There was no statistically substantial difference between groups (6) and the control group, although the animal protein group had

the highest values for Tumor Necrosis Factors, Interleukin 6, and Interleukin 1. Jutamulia *et al.* (2018) indicated that at high levels, animal protein induced cytokines enzymes such as tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1b, interleukin (IL)-6 and interleukin (IL)-10. Pabich and Materska (2019) reported that soy protein was considered to have anti-inflammation effect when compared with animal protein.

**Table (7): Effect of feeding protein sources on serum interleukin (IL1, IL6 and (TNF-a) for rats.**

| Animal Groups | Interleukin 1 IL1 (pg/ml) | Interleukin 6 IL6 (pg/ml) | Tumor necrosis factors TNF (pg/ml) |
|---------------|---------------------------|---------------------------|------------------------------------|
| G1            | 66.60±4.03 <sup>f</sup>   | 82.67±4.91 <sup>f</sup>   | 116.67±5.21 <sup>f</sup>           |
| G2            | 101.58±5.22 <sup>c</sup>  | 106.91±0.77 <sup>c</sup>  | 155.86±2.96 <sup>c</sup>           |
| G3            | 120.01±1.94 <sup>a</sup>  | 124.03±1.25 <sup>a</sup>  | 175.06±3.62 <sup>a</sup>           |
| G4            | 93.23±2.87 <sup>d</sup>   | 99.27±2.32 <sup>d</sup>   | 140.95±5.29 <sup>d</sup>           |
| G5            | 111.85±5.43 <sup>b</sup>  | 113.27±3.77 <sup>b</sup>  | 164.95±3.88 <sup>b</sup>           |
| G6            | 73.63±5.08 <sup>f</sup>   | 86.96±5.02 <sup>f</sup>   | 121.33±5.53 <sup>f</sup>           |
| G7            | 83.99±4.76 <sup>c</sup>   | 92.79±3.97 <sup>c</sup>   | 130.17±6.74 <sup>c</sup>           |
| <b>LSD</b>    | <b>6.01</b>               | <b>5.42</b>               | <b>7.89</b>                        |

Values are mean ±SD. The same letter means there was no significantly between groups, while the difference letter means that there were significantly differences between groups ( $p \leq 0.05$ ).

### Conclusion

Proteins are part of the structure in animal tissues. Most organs and tissues in animals need proteins amongst other components as their structural units. Thus, proteins in animal nutrition are required for the development and repair of tissues. However, this study showed that chronic excessive intake of proteins-particularly those of animal origin-increases the chance of developing diseases such as cardiovascular disease, disorders of blood vessels, liver and kidney problems, and seizures compared to the plant source.

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### تأثير مصادر البروتين المختلفة على الصفات البيولوجية والكيميائية للجرذان

هدى مسعود الجزيري<sup>1</sup> ، امال حامد عمارة<sup>2</sup>

- 1- قسم الكيمياء والتمثيل الغذائي – المعهد القومي للتغذية، القاهرة، مصر  
2- قسم الدراسات والبحوث – المعهد القومي للتغذية – القاهرة – مصر

#### المستخلص

تهدف هذه الدراسة إلى تقييم تأثير مصادر مختلفة من البروتين (بروتين اللحم البقري ومصل اللبن وفول الصويا) بمستويات 10 و 15% على الصفات البيولوجية والكيميائية للفئران الطبيعية. وقد تم استخدام تسعة وأربعين ذكراً من الفئران تم تقسيمهم إلى سبع مجموعات: تم تغذية المجموعة الأولى على الوجبة الأساسية كمجموعة ضابطة بينما تم تغذية المجموعات الست الأخرى على الوجبة الأساسية مضاف إليها مصادر البروتين بنسبة 10 و 15% لمدة 28 يوم. وفي نهاية التجربة تم ذبح الفئران وسحب عينات الدم منها وعمل التحاليل الكيميائية المطلوبة كما تم تقدير وزن الجسم للفئران المتناول من الطعام ومعدل كفاءة الغذاء ونسبة الجلوكوز في الدم الصائم ومقاومة الأنسولين وهرمون الأنسولين وإنزيمات الكبد ووظائف الكلى ودهون الدم والإنزيمات المضادة للأكسدة وإنتاج الإنترلوكينات. أشارت النتائج إلى أن نوع البروتين أثر على متوسط قيم المعايير البيولوجية للجرذان السليمة وأن المستويات العالية من المصادر الحيوانية أدت إلى انخفاض المعايير البيولوجية بالمقارنة مع المصدر النباتي والمجموعة الضابطة. وكان متوسط قيم الجلوكوز في الدم وهرمون الأنسولين ومقاومة الأنسولين في المجموعة الضابطة أقل بكثير من المجموعات الأخرى. أعلى نسبة دهون سجلت زيادة معنوية في المجموعة التي تغذت على العليقة الأساسية بنسبة 15% كمصادر بروتين حيواني. وارتفعت قيم وظائف الكلى والكبد في المجموعات التي تغذت على نظام غذائي أساسي و 15% من بروتين اللحم البقري يليها نفس المستوى من بروتين مصل اللبن. أظهر إعطاء نسبة عالية من البروتين من مصادر مختلفة زيادة ملحوظة استراديول وأنشطة الإنترلوكين. ولكن أظهرت انخفاضاً معنوياً في مستويات هرمون التستوستيرون في الدم مقارنة مع مجموعة السيطرة ومصدر بروتين الصويا.

الكلمات الدالة: المتناول من البروتين -التأثير البيولوجية - التحاليل الكيميائية.