مجلة دراسات وبحوث التربية النوعية

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المجلة العلمية المحكمة لدراسات وبحوث التربية النوعية

المجلد الثالث – العدد الأول – مسلسل العدد (٥) – الجزء الثاني، يناير ٢٠١٧

رقم الإيداع بدار الكتب ٢٤٢٧٤ لسنة ٢٠١٦

### ISSN-Print: 2356-8690 ISSN-Online: 2356-8690

موقع المجلة عبر بنك المعرفة المصري https://jsezu.journals.ekb.eg

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### Role of Whey, Soy Protein Concentrates and Skimmed Milk on Obese Rats

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#### Abstract:

The effect of whey and soy protein concentrates, skimmed milk powder as well as their combination on body weight, serum lipid profile, kidney and liver functions, leptin and thyroid hormones levels was investigated in obese rats. Thirty six adult male albino rats were divided into two main groups, the first main group (6 rats) was fed on basal diet and served as the control negative group, while the second main group (30 rats) was fed on high fat diet for four weeks to induce obesity then was divided into five subgroups, as follow: subgroup (1) was fed on high fat diet and served as the control positive group, subgroups (2, 3 and 4) were fed on high fat diet containing 30% protein from whey protein concentrate (WPC), skimmed milk powder (SMP) and soy protein concentrate (SPC), respectively and subgroup (5) was fed on high fat diet containing 30% protein from mixture (WPC, SMP and SPC) at equal ratios. The results indicated that supplementation with WPC, SPC, SMP and/or their mixture significantly decreased ( $P \le 0.05$ ) the elevated body weight of obese rats compared with the control positive group. Moreover, serum cholesterol, triglycerides, LDL-c, VLDL-c, urea, creatinine, uric acid, ALP, ALT and AST, leptin hormone and TSH concentrations were significantly decreased ( $P \le 0.05$ ) in all treated groups, while serum HDLc, FT<sub>3</sub> and FT<sub>4</sub> were significantly increased ( $P \le 0.05$ ) compared with the control positive group. The obtained results suggest that supplementing with WPC, SPC, SMP and their mixture may cause favorable effects in body weight reduction as well as improving serum lipid profile, liver and kidney functions, leptin and thyroid hormones in obese rats.

## Key words: Serum lipid profile, kidney and liver functions, leptin hormone, thyroid hormones.

#### **Introduction:**

Obesity has become a serious problem that increases health costs and reduces healthy life years (Aronne, 2001 and Carnethon *et al.*, 2004). In addition, there is a strong association between obesity and the development of comorbid conditions, including insulin resistance and/or type 2 diabetes mellitus, hypertension, dyslipidemia and metabolic syndrome (Wilson *et al.*, 2005). Obesity is a multi-factorial disease characterized by a positive energy balance that results from excess energy intake and/or insufficient energy expenditure, it is manifested by an excess of adipose tissue (Del *et al.*, 2011).

Much attention is given nowadays to the importance of high protein diets in weight management (Schaafsma, 2006). Dairy foods not only provide a good source of high quality protein, but also a rich assortment of micronutrients (Lutsey *et al.*, 2008).

Whey protein is a high biological value protein, has received attention because of reported benefits in the field of sports nutrition, including its effects on body composition and physical performance (**Ha and Zemel, 2003 and Haraguchi** *et al.,* **2011**). Low-fat, high-carbohydrate dietary trends are being eschewed in favor of higher-protein, lower-carbohydrate diets. For individual seating high-protein diets, whey is an attractive source of dietary protein. Whey has made a significant commercial impact in the weight-loss industry for its protein content alone. The essential and non-essential amino acids in whey act as substrates for protein synthesis and may improve body mass index in individuals participating in exercise programs (**Burke** *et al.,* **2001**). The bioactive components in whey are thought to act synergistically with calcium to attenuate lipogenesis, accelerate lipolysis, and effect nutrient partitioning between adipose tissue and skeletal muscle (**Zemel, 2003**).

Soybeans provide one of the most abundant plant sources of dietary protein. The protein content of soybeans varies from 36% to 56% (Grieshop *et al.*, 2003). A number of studies on animals and humans suggest that consumption of soy protein have favorable effects on obesity and lipid metabolism (Velasquez and Bhathena, 2007). Dietary calcium plays a pivotal role in the regulation of energy metabolism. High-calcium diets attenuate adipocyte lipid accretion and weight gain during over consumption of an energy-dense diet and increase lipolysis and preserve

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thermogenesis during caloric restriction, thereby markedly accelerating weight loss. The main source of calcium components is dairy products such as skimmed milk (Kolars *et al.*, 1985 and Bammi *et al.*, 1998). Skimmed milk is low in fat, yet it is rich in protein, calcium, and lactose, meaning its nutritional value is high (Eller *et al.*, 2012).

This study was conducted to investigate the effect of WPC, SPC, SMP and their mixture on body weight, lipid profile, kidney and liver functions, leptin hormone and thyroid functions of obese rats.

#### **Materials and Methods:**

#### Materials:

Casein, vitamins, minerals and cellulose were purchased from El-Gomhoria company for trading drugs, chemicals and medicine instruments, Cairo, Egypt. WPC and SPC were obtained from Agriculture Research Center, Cairo, Egypt. While, SMP was obtained from Arab International Company for Commercial Business, Cairo, Egypt. Beef tallow was obtained from local market. Adult male albino rats (Sprague- Dawley strain) weighting  $180 \pm 5$  g were purchased from Helwan Experimental Animals Station, Ministry of health, Cairo, Egypt. Kits for blood analysis were purchased from Gama Trade Company for Chemicals, Cairo, Egypt.

#### **Methods:**

This experiment was carried out at the Animal House of the Faculty of Home Economics, Helwan University.

#### **Chemical composition:**

Protein, carbohydrates, fats, calcium and phosphorus content in WPC, SPC and SMP were determined according to the official methods (A.O.A.O., 2005).

#### Induction of obesity:

Rats were fed for four weeks on basal diet according to **Reeves** *et al.* (1993) with some modification include (high fat diet containing: casein 14%, cellulose 5%, vitamin mixture 1%, mineral mixture 3.5%, sucrose 10%, beef tallow 19% + corn oil 1%), 1-cystine 0.18, choline bitartrate 0.25% and the reminder was starch to induce obesity in rats (Min *et al.*, 2004).

#### **Biological study:**

Thirty six adult male albino rats (Sprague- Dawley strain were housed in well aerated cages under hygienic conditions and were fed on basal diet for one week for adaptation. All diets were formulated to cover the nutrient requirements of the rats following the recommendations of the American Institute of Nutrition (AIN-93M) (**Reeves** *et al.*, **1993**). After this week rats were divided into two main groups as follows:-

The first main group (6 rats) was fed on basal diet (as a control negative group). The second main group (30 rats) was fed on high fat diet for four weeks then were divided as follows: Subgroup (1) was fed on high fat diet (as the control positive group). Subgroups (2, 3 and 4) were fed on high fat diet containing 30% protein from WPC, SPC, SMP, respectively. Subgroup (5) was fed on high fat diet containing 30% protein from mixture (WPC + SMP+ SPC) at equal ratios.

All rats were observed each day, their feed intake (FI) was determined daily and body weights were obtained every week. Feed Efficiency Ratio (FER) and body weight gain (BWG) were calculated. At the end of the experimental period (8 weeks), rats were fasted overnight before sacrifying and the blood samples were collected from each rat then were centrifuged to obtain serum.

#### **Biochemical analysis :**

Serum was analyzed to determine the following parameters:

- Kidney functions: serum urea, serum uric acid and creatinine were measured according to Kaplan (1984); Patton and Crouch (1977) and Murray (1984), respectively.
- Liver functions: (AST, ALT, ALP) were determined according to Bergmeyer *et al.* (1978).
- Lipid profile: serum total cholestrol, triglycerides, high density lipoprotein (HDL-c), low density lipoprotein (LDL-c) and very low density lipoprotein (VLDL-c) were calculated according to Richmond (1973);
   Wahlefeld (1974); Albers *et al.* (1983) and Fridewald *et al.* (1972), respectively.
- Leptin hormone was determined using ELISA (Enzyme-Linked Immunosorbent Assay) (Xiong et al., 2005).

# - FT3 (Rat FT3 ELISA Kit), FT4 (Enzyme-linked immuneosorbent assay Kit) and TSH (**TSH rat ELISA**, 2012) concentrations.

#### **Statistical Analysis:**

The obtained results were analyzed using SPSS program. ANOVA test was used to compare results among groups, P-value of 0.05 was considered statistically significant (SPSS, 1986).

#### **Results and discussion:**

The results in Table (1) indicated that rats fed on high fat diet revealed significant (P $\leq$ 0.05) increase in the levels of initial body weight and final body weight compared with the control negative group. Rats fed on diet supplemented with either WPC or SPC or SMP or their mixture had significant (P $\leq$ 0.05) decrease in the levels of final body weight compared with the control positive group. The highest reduction in levels of final body weight was recorded in the mixture group.

This study supported the report of Coker et al. (2012) and Hursel et al. (2010) that studied on 12 elderly persons and indicated that whey protein supplement together with essential amino acids reduce fat accumulation in tissues and prevent adverse effects resulting from obesity. The mechanism responsible for increased fat loss refers to calcium intake. Studies have shown that high levels of calcium intake causes limiting the levels of parathyroid hormone (PTH), 1.25dihydroxy vitamin D and can prevent the obesity by discouraging the body from fat storing (Zemel et al., 2000 and Norman et al., 2001). The same results were obtained by (Baer et al., 2011) that stablished that Free-living overweight or obese individuals consuming a daily supplementation of whey proteins (56 g protein/day) had a decrease in body weight and fat mass after 23 weeks. Also, Iritani et al., (1996) obtained similar results when studied the effects of dietary soy protein on body weight of obese Wistar fatty rats. Wistar fatty rats and their lean litter mates were fed casein or soy protein diet containing hydrogenated fat (4% hydrogenated fat plus 1% corn oil) or corn oil (5%) for 3 weeks. After 3 weeks of feeding, the fatty rats fed soy protein had lower body weight than those fed casein. In addition, the obtained findings agreed with Skinner et al., (2003) who noticed an adverse association between calcium consumption and percent

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of body fat in a group composed of 8-year old children. Results of **Drapeau** *et al.* (2004) showed that calcium and milk products consumption triggered positive changes in whole body composition. **Zemel** *et al.* (2004) in their intervention study of obese individuals who were fed low calorie diet for 24 weeks, revealed that high calcium diets either from milk or calcium supplements considerably reduced body weight and body fat mass when compared with the control group.

	Initial	Final	BWC	Weight	Feed	
Group of rats	BW	BW	DWG (04)	reduction	intake	FER
	(g)	(g)	(70)	(%)	(g/day/rat)	
Control ( vo)	149.83 ±	183.16±	22.24±	-22.24 18	10	$1.85 \pm$
	1.24 <sup>b</sup>	2.08 <sup>b</sup>	0.85 <sup>a</sup>		0.07 <sup>a</sup>	
Control (100)	$229.83 \pm$	268.16±	16.76	-16.67 23	1.66 ±	
Control (+ve)	3.15 <sup>a</sup>	2.66 <sup>a</sup>	±1.67 <sup>b</sup>		0.14 <sup>a</sup>	
	233.33 ±	177.16±	-24.04±	24.07	19.6	$-2.86 \pm$
WPC at 30%	5.04 <sup>a</sup>	3.47 <sup>bc</sup>	0.59 <sup>c</sup>	24.07		0.11 <sup>b</sup>
SDC at 200/	$233.66 \pm$	160.83±	-31.17±	21.16 16.8	$-4.33 \pm$	
SPC at 50%	2.82 <sup>a</sup>	2.56 <sup>d</sup>	0.55 <sup>d</sup>	51.10	16.8	0.08 <sup>c</sup>
SMP at 30%	231.00±	172.83±	-25.15±	25 10 10 2	-3.17 ±	
	3.00 <sup>a</sup>	2.32 <sup>c</sup>	0.83 <sup>c</sup>	25.18	18.5	0.12 <sup>b</sup>
30% from	231.00±	$153.16 \pm$	-33.68±	22.60	16	$-4.86 \pm$
(WPC+SPC+SMP) sw	1.06 <sup>a</sup>	1.77 <sup>e</sup>	0.9 <sup>d</sup>	33.09	10	0.14 <sup>d</sup>

Table (1): Effect of WPC, SPC and SMP and their mixture on body weight status, FI and FER.

Data are expressed as mean  $\pm$  SE. Means with different superscript letters in the same column are significantly different at P  $\leq$  0.05.

The results in Table (2) illustrated that feeding rats on high fat diet caused significant (P $\leq$ 0.05) increase in the levels of TG and TC compared with the control negative group. Rats fed on basal diet supplemented with WPC, SPC, SMP and their mixture had significant (P $\leq$ 0.05) decrease in the mean value of TG compared with the control positive group. In addition, significant (P $\leq$ 0.05) decrease in the levels of TC was observed in the groups fed on WPC, SPC, SMP and their mixture compared with the control positive group. The highest decrease in the levels of TG and TC was recorded in the group fed on the mixture of WPC, SPC and SMP compared with the other treated groups.

The obtained results were in agreement with Kawase *et al.* (2000); Zhang *et al.* (2007) and Farhad *et al.* (2012) who suggested that WPC reduces TG and TC levels due to having bioactive components such as Lactalbumine, angiotensin conversion enzyme inhibitor and branched amino acids. In another study it was demonstrated that WPC supplements may lead to decrease TG levels in diabetes mellitus patients (Mortensen *et al.*, 2009).

It is well established that SPC consumption reduces serum TC, LDL-C, and TG as well as hepatic cholesterol and TG. Studies in animals indicate that soy protein ingestion exerts its lipid-lowering effect by reducing intestinal cholesterol absorption and increasing fecal bile acid excretion, thereby reducing hepatic cholesterol content and enhancing removal of LDL (**Wright and Salter, 1998 and Greaves** *et al.*, **2000**). Also, these results were in the line of **Hidaka** *et al.* (**2012**) that stablished that consumption of skimmed milk (500 mL/d) for 2 weeks was shown to reduce fasting TG and TC concentrations compared with whole-milk consumption.

The results in Table (3) showed that rats fed on high fat diet caused significant ( $P \le 0.05$ ) increase in the levels of LDL-C and VLDL-C compared with the control negative group. But, it caused significant  $(P \le 0.05)$  decrease in the levels of HDL-C compared with the control negative group. Rats fed on WPC, SPC, SMP and their mixture had significant ( $P \le 0.05$ ) decrease in the levels of LDL-C and VLDL-C compared with the control positive group. Whereas, rats fed on WPC, SPC, SMP and their mixture had significant (P<0.05) increase in the levels of HDL-C compared with the control positive group. The highest decrease in the level of LDL-C and VLDL-C was found in the mixture group. Whereas, the highest increase in the level of HDL-C was recorded in the mixture group. The obtained results agreed with the finding of several human Cribb et al. (2007) and Cribb and Hayes (2008) and rodent studies of Belobrajdic et al. (2004); Pichon et al. (2008) and **Baer** et al. (2011) that have demonstrated the ability of whey protein to improve body composition. The same results were obtained by Han et al., (2014) who demonstrated that the whey protein significantly increases HDL-C in rats fed with a high-fat diet. The supplementation of whey protein and whey-related peptides has beneficial effects on lipid metabolism-related markers including TG, TC, LDL-C and HDL-C.

Dietary soy protein has also been shown to directly affect hepatic cholesterol metabolism and LDL-C receptor activity (Lovati et al., 1985 and Kirk et al., 1998). Also, Lovati et al. (1985) demonstrated an binding of VLDL-C liver membranes increased to of hypercholesterolemic rats fed a diet containing soy protein, suggesting altered hepatic metabolism with increased LDL-C and beta-VLDL-C removal by hepatocytes. Another study by Lovati et al. (1987) have shown that soy protein diet consistently increased degradation of LDL-C by mononuclear cells from patients with hypercholesterolemia, even in the presence of an elevated cholesterol intake. In addition, Steinmetz et al. (1994) conducted a randomized controlled trials in which subjects consumed skimmed milk or whole-fat milk (236 mL/1000 kcal) for 6 weeks. Post intervention LDL-C concentrations were significantly lower in the skimmed milk group than in the whole-fat milk group. Furthermore, maintaining usual milk and dairy consumption was associated with an increase in HDL-C (Barr et al., 2000).

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Crown of rots	TG	TC	
Group of rats	(mg/dl)	(mg/dl)	
Control (-ve)	$64.2 \pm 1.73^{\circ}$	$78.02 \pm 1.21^{d}$	
Control (+ve)	$86.64 \pm 2.15^{a}$	$104.82 \pm 3.04^{a}$	
WPC at 30%	$71.50 \pm 1.19^{b}$	$92.28 \pm 1.48^{b}$	
SPC at 30%	$69.62 \pm 2.67^{bc}$	$86.18 \pm 2.04^{bc}$	
SMP at 30%	$71.16 \pm 1.07^{b}$	$88.44 \pm 3.05^{bc}$	
<b>30% from (WPC + SPC+ SMP )</b>	$67.18 \pm 2.44^{bc}$	$81.81 \pm 2.98^{cd}$	

Data are expressed as mean  $\pm$  SE.

Means with different superscript letters in the same column are significantly differences at  $P \le 0.05$ .

Group of rats	LDL-C	VLDL-C	HDL-C
	(mg/dl)	(mg/dl)	(mg/dl)
Control (-ve)	$8.14 \pm 2.52^{d}$	$12.84 \pm 0.34^{\circ}$	$57.04 \pm 1.40^{a}$
Control (+ve)	$46.01 \pm 3.69^{a}$	$17.32 \pm 0.43^{a}$	$41.48 \pm 1.75^{\circ}$
WPC at 30%	$27.26 \pm 2.39^{b}$	$14.30 \pm 0.23^{b}$	$50.72 \pm 2.15^{b}$
SPC at 30%	$21.27 \pm 2.52^{bc}$	$13.92 \pm 0.53^{bc}$	$50.98 \pm 2.56^{b}$
SMP at 30%	$25.52 \pm 3.43^{b}$	$14.23 \pm 0.21^{b}$	$48.68 \pm 1.23^{b}$
30% from (WPC + SPC+ SMP )	$15.66 \pm 4.08^{cd}$	$13.43 \pm 0.48^{bc}$	$52.72 \pm 2.03^{ab}$

 Table (3): Effect of feeding rats on WPC, SPC, SMP and their mixture on serum lipoproteins in obese rats.

Data are expressed as mean  $\pm$  SE.

Means with different superscript letters in the same column are significantly differences at  $P \le 0.05$ .

The results in Table (4) indicated that feeding rats on high fat diet caused significant (P<0.05) increase in levels of serum leptin hormone compared to control negative group. Rats fed on WPC, SPC, SMP and their mixture had significant (P<0.05) decrease in levels of leptin hormone compared with the control positive group. The highest decrease in the level of leptin hormone was recorded in the mixture group compared with other treated groups.

The obtained results were in agreement with **Korpela** *et al.* (2008) who showed that, mice that consumed a high-fat diet (60% of total calories from fat) with 18% protein (whey protein) and 1.8% calcium carbonate for 12 weeks had not only an inhibition in the accumulation of fat mass but also an increase in gene expression in the visceral adipose tissue of leptin and  $\beta$ 3-adrenergic receptor when compared to another group of mice that received a diet with similar fat and protein contents, that instead used casein instead of whey and only 0.4% calcium carbonate. Thus, the authors suggest that the whey protein may reduce obesity via improvement of leptin sensibility. Also, **Yamashita** *et al.* (1998) compared the effects of a meat-based diet with a plant-based diet

in 36 overweight or obese women, age  $40\pm9$  yrs. Both diets were designed to provide similar energy intake but one contained red meat and the other soybeans as the major protein source. After 16 weeks on the diet, subjects in both diet groups lost weight (9% of body weight) and showed similar decreases in plasma leptin levels.

In contrast with these results, **Larnkjær** *et al.* (2014) published that Leptin increased significantly in the skimmed milk group compared with the pre-test control group in a randomized study to investigate the effect of milk proteins and water on body weight and risk markers of metabolic syndrome.

 Table (4): Effect of WPC, SPC, SMP and their mixture on serum leptin.

Group of rats	Leptin hormone (pg/ml)
Control (-ve)	$35.25 \pm 3.15^{d}$
Control (+ve)	$107.95 \pm 3.65^{a}$
WPC at 30%	$63.95 \pm 3.95^{b}$
SPC at 30%	$48.30 \pm 2.70^{\circ}$
SMP at 30%	$52.20 \pm 4.00^{\circ}$
<b>30% from (WPC + SPC+ SMP )</b>	$45.00 \pm 2.00^{cd}$

Data are expressed as mean  $\pm$  SE.

Means with different superscript letters in the same column are significantly differences at  $P \le 0.05$ .

The results in Table (5) showed that feeding rats on high fat diet caused significant (P $\leq$ 0.05) increase in the levels of uric acid, creatinine and urea compared with the control negative group. The results in this table revealed significant (P $\leq$ 0.05) decrease in the mean values of uric acid in rats fed on WPC or SPC or SMP and their mixture with the compared to control positive group. The data indicated significant (P $\leq$ 0.05) decrease in levels of creatinine in rats were fed on WPC, SPC, SMP and their mixture compared with the control positive group. Likewise, there were significant (P $\leq$ 0.05) decrease in the mean values of

urea in WPC group, SPC group, SMP group and mixture group compared with the control positive group. The highest decrease in the level of uric acid was found in the SPC group, while the highest decrease in the levels of serum creatinine and urea was recorded in the mixture group compared with other treated groups.

The results were in the line of Abdel-Wahhab et al. (2016) that revealed that rats treated with WPC had significant decrease in the levels of serum creatinine, urea and uric acid compared with normal control group and compared with tienilic acid treated group. WPC may increase the endogenous antioxidant capacity of the kidney to overcome oxidative stress induced by tienilic acid; this in turn improves kidney integrity and function and consequently enhance renal excretory function of urea and creatinine as well as enhances the kidney to maintain electrical neutrality and improve body homeostasis. Also, these results were in accordance with Al-Ahdab (2014) who investigated that feeding diabetic rats on diets containing 20% Soy protein (SP) or 15% SP + 5% casein protein (CP) or 10% SP +10% CP or 5% SP +15% CP for 6 weeks significantly (P<0.05) decreased the elevated serum urea, uric acid and creatinine compared with control positive group. The nephroprotective effect of soy protein also was reported by Anderson et al. (1998) and Chen et al. (2006) that reported that Consumption of soy protein reduced levels of blood urea nitrogen, Serum and urinary creatinine and serum uric acid compared with animal protein. The nephroprotective effect of Soy protein intake could be due to preventing the inflammation of kidney and increasing the renal flow via facilitating synthesis of nitric oxide (Lopez et al., 2007).

	Uric acid	Creatinine	Urea
Group of rats	(mg/dl)	(mg/dl)	(mg/dl)
Control (-ve)	$1.85 \pm 0.17^{c}$	$0.69 \pm 0.04^{\circ}$	$26.94 \pm 1.35^{d}$
Control (+ve)	$3.30\pm0.09^a$	$1.97\pm0.08^{a}$	$42.74 \pm 1.75^{a}$
WPC at 30%	$2.61 \pm 0.15^{b}$	$1.15\pm 0.14^{b}$	35.44± 1.56 <sup>b</sup>
SPC at 30%	$2.51 \pm 0.17^{b}$	$1.05 \pm 0.13^{b}$	$32.40 \pm 1.28^{\rm bc}$
SMP at 30%	$2.61 \pm 0.17^{b}$	$1.03 \pm 0.07^{b}$	$35.08 \pm 1.19^{b}$
30% from (WPC + SPC+ SMP )	$2.53 \pm 0.22^{b}$	$0.89 \pm 0.04^{bc}$	$30.54\pm 0.95^{cd}$

 Table (5): Effect of WPC, SPC, SMP and their mixture on serum kidney functions of rats.

Data are expressed as mean  $\pm$  SE.

Means with different superscript letters in the same column are significantly differences at  $P \le 0.05$ .

The results in Table (6) showed that rats fed on high fat diet had significant (P $\leq$ 0.05) increase in the levels of serum ALP, ALT and AST compared with the control negative group. Rats fed on basal diet supplemented with WPC, SPC, SMP and their mixture had significant (P $\leq$ 0.05) decrease in the mean value of levels of serum ALP compared with the control positive group. These results indicated significant (P $\leq$ 0.05) decrease in levels of ALT in rats that fed on diet supplemented with WPC, SPC, SMP and their mixture compared with the control positive group. The data in the same table revealed that rats fed on WPC or SPC or SMP or their mixture had significant (P $\leq$ 0.05) decrease in the mean value of serum AST compared with the control positive group.

The mixture group caused the highest decrease in the level of ALP and AST, while the highest decrease in the level of ALT was recorded in the group fed on SPC compared with other treated groups.

The obtained results agreed with the findings of **Gad** *et al.* (2011) who studied the effect of spirulina and WPC on serum AST, ALT and ALP in rats treated with CCl4 and investigated that the rats treated with WPC had significant (P $\leq$ 0.05) decrease of levels of ALT, AST and ALP compared with CCl4 group.

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Also, these results were in the line with those reported by **Tovar** *et al.* (2005) who concluded that feeding Soy protein compared to casein reduces effects of diabetes on the liver of diabetic rats as it reduces high liver enzymes (AST and ALT) and decreases hepatic lipotoxicity in hyperinsulinemic obese rats. The hepatoprotective effect of Soy protein was in accordance with that reported by that demonstrated by **Torres** *et al.* (2006) and **Zhou** *et al.* (2014). Also, these findings agree with **Al-Ahdab** (2014) who reported that feeding diabetic rats on diets containing 20% Soy protein instead of casein or more Soy protein and less casein produced

hepatoprotective effect. This effect was evident from significant decreases in the elevated serum levels of liver enzymes (AST and ALT) in diabetic rats. The decrease in serum liver enzyme levels was found to be dependent upon the percent of soy protein added to diets.

In addition, these findings were in accordance with **Soltan (2013)** who stablished a high decrease in levels of serum AST, ALT and ALP in osteoporotic rats fed on basal diet containing 20% skimmed milk compared with the control positive group and compared with osteoporotic rats fed on basal diet containing only 10% skimmed milk.

Group of rats	ALP	ALT	AST
	(U/I)	(U/I)	(U/I)
Control (-ve)	$1.36 \pm 0.10^{\rm c}$	$27.50 \pm 1.85^{\circ}$	$52.22 \pm 2.39^{\rm c}$
Control (+ve)	$2.74 \pm 0.13^{a}$	$44.52 \pm 1.63^{a}$	$72.48 \pm 2.61^{a}$
WPC at 30%	$2.07 \pm 0.09^{b}$	$33.86 \pm 2.70^{b}$	$61.60 \pm 2.58^{b}$
SPC at 30%	$1.94 \pm 0.24^{b}$	$32.74 \pm 1.53^{bc}$	$62.28 \pm 2.37^{b}$
SMP at 30%	$2.06 \pm 0.11^{b}$	$34.48 \pm 1.32^{b}$	$64.40 \pm 2.11^{b}$
30% from (WPC + SPC+ SMP )	$1.89\pm0.18^{b}$	$35.14 \pm 1.81^{b}$	$59.48 \pm 1.99^{b}$

Table (6): Effect of WPC, SPC and SMP on liver functions of rats.

Data are expressed as mean  $\pm$  SE.

Means with different superscript letters in the sme column are significantly differences at  $P \le 0.05$ .

The results in Table (7) illustrated that feeding rats on high fat diet caused significant (P $\leq$ 0.05) decrease in the levels of FT4 and FT3 compared with the control negative group, whereas, there was significant (P $\leq$ 0.05) increase in the level of TSH compared with the control negative group. The rats fed on diets supplemented with WPC or SPC or SMP and their mixture had significant (P $\leq$ 0.05) increase in the mean value of levels of FT4 and FT3, but they had significant (P $\leq$ 0.05) decrease in the levels of TSH compared to control positive group.

The highest increase in the levels of FT4 and FT3 was found in the mixture group compared with other treated groups. Also, the highest decrease in the level of TSH was recorded in the combination group. These results agreed with Layman et al. (2003) and Belobrajdic et al. (2004) who postulated that WPC consumption may reduce body fat storage and weight gain by stimulating the release and activity of hormones that increase metabolic rate. Thyroid hormone (T3 and T4) concentrations increase in subjects consuming a high-protein diet, compared to a high-carbohydrate diet. In addition, these results agreed with Forsythe (1995) and Potter et al. (1996) who stablished that soy protein elevated plasma T4 concentrations. This may have been due to an increased glandular production of thyroid hormones or to an elevation of T4 subsequent to inhibition of the peripheral conversion of T4 to T3. Findings also indicated T3 was higher among casein-fed animals and lower among animals fed an equivalent amount of SPC. Soy protein consumption also was found to contribute to age related alterations in thyroid hormone in animals. Mitsuma et al. (1998) indicated that, soy protein consumption might be capable of generating a thyroid hormone profile similar to that found in low T3 syndrome; in other words, soy protein consumption might cause a shift in thyroid hormone profiles toward unchanged or increased T4 and rT3 at the expense of T3 production. The effects of 30 grams of soybeans fed daily for 1-3 months to 37 healthy adults was investigated. Soybean consumption resulted in a significant increase in TSH levels, although levels remained within normal limits (Ishizuki et al., 1991).

In conclusions, the diet supplemented with WPC, SPC, SMP and their mixture resulted in favorable effects in body weight reduction as well as improving lipid profile, liver and kidney functions, leptin hormone and thyroid hormones. It could be suggested that WPC, SPC, SMP and their mixture could be used a suitable supplementation therapy for obesity.

Group of rats	FT4	FT3	TSH
	(ng/dl)	(pg/dl)	(ng/ml)
Control (-ve)	$5.87\pm0.13^{a}$	$70.34\pm2.96^a$	$1.67 \pm 0.10^{d}$
Control (+ve)	$2.57\pm0.20^{\rm c}$	$39.99 \pm 0.29^{\circ}$	$5.70 \pm 0.40^{a}$
WPC at 30%	$3.75 \pm 0.25^{b}$	$52.60 \pm 1.00^{b}$	$4.65 \pm 0.35^{b}$
SPC at 30%	$3.94 \pm 0.16^{b}$	$56.70 \pm 2.70^{b}$	$4.08 \pm 0.08^{bc}$
SMP at 30%	$3.56\pm0.27^{\text{b}}$	$50.94 \pm 3.94^{b}$	$4.84 \pm 0.17^{b}$
30% from (WPC + SPC+ SMP )	$4.10\pm0.30^{b}$	$59.75 \pm 2.75^{b}$	$3.39\pm0.07^{c}$

 Table (7): Effect of WPC, SPC, SMP and their mixture on thyroid hormones.

Data are expressed as mean  $\pm$  SE.

Means with different superscript letters in the same column are significantly differences at  $P \le 0.05$ .

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دور مركز البروتين لشرش اللبن، الصويا واللبن المنزوع الدسم علي الفئران البدينة

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#### الملخص:

تم دراسة تأثير كل من مركزات البروتين لشرش اللبن، الصويا واللبن البودرة (المجفف) المنزوع الدسم وخليطهم؛ على وزن الجسم، وصورة دهون الدم، وظائف الكلى والكبد وهرمون اللبتين وهرمونات الغدة الدرقية في الفئران البدينة. تم تقسيم عدد (٣٦) فأر إلى مجموعتين رئيسيتين: المجموعة الرئيسية الأولى (٦ فئران) تم تغذيتها على الوجبة الأساسية فقط ومثلت المجموعة الضابطة السالبة، والمجموعة الرئيسية الثانية (٣٠ فأر) وتم تغذيتها على نظام غذائي عالى في محتواه من الدهون وذلك لمدة أربعة أسابيع وذلك لاحداث السمنة للفئران، بعد ذلك تم تقسيم المجموعة الرئيسية الثانية الى خمس مجموعات فرعية على النحو التالي: المجموعة الفرعية (١) تم تغذيتها على نظام غذائي عالى الدهون (كمجموعة ضابطة موجبة)، والمجموعات الفرعية (٢،٣،٤) تم تغذيتهم على نظام غذائي عالى الدهون يحتوي على ٣٠% بروتين من (مركز شرش اللبن ومركز الصويا واللبن المنزوع الدسم)؛ على التوالي ، والمجموعة الفرعية (٥) تم تغذيتها على نظام غذائي عالى الدهون يحتوى على ٣٠% من مخلوط مركز بروتين شرش اللبن + مركزبروتين الصويا + اللبن المجفف المنزوع الدسم بنسب متساوية. لقد أشارت النتائج إلى أن التدعيم بمركزات البروتين لشرش اللبن، الصويا واللبن المنزوع الدسم وخليطهم يؤدى الى نقص معنوى (p<0.05) في وزن الجسم مقارنة بالمجموعة الضابطة الموجبة. علاوة على ذلك، تم تسجيل انخفاض مستويات الدهون الثلاثية والكوليسترول الكلي والليبوبروتينات منخفضة الكثافة والليبوبروتينات منخفضة الكثافة جدا واليوريا والكرياتينين وحمض اليوريك، وكذلك فان انزيمات الكبد AST, ALT, ALP وهرمون اللبتين وهرمون الغدة الدرقية (TSH) تتاقصوا بشكل معنوى (P<0.05) في كل المجموعات المعالجة، بينما الليبوبروتينات عالية الكثافة وهرمونات  $FT_3$  و  $FT_4$  تزايدت بشكل معنوى (P $\leq 0.05$ ) مقارنة بالمجموعة الضابطة الموجبة. ويتضبح من هذه النتائج أن التدعيم بمركزات البروتين لشرش اللبن

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والصويا واللبن المنزوع الدسم وخليطهم يؤدى الى انخفاض فى وزن الجسم للفئران البدينة وكذلك تحسين مستوى دهون الدم ووظائف الكلى والكبد وهرمون اللبتين وهرمونات الغدة الدرقية .