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THE STUDY OF NUTRACEUTICAL EFFECTS OF THE WHEY ZONAR IN THE MANAGEMENT OF OBESITY EXPERIMENTALLY INDUCED IN RATS

STUDIUL EFECTELOR NUTRACEUTICE ALE ZONARULUI ÎN MANAGEMENTUL OBEZITĂȚII INDUSE EXPERIMENTAL LA ȘOBOLANI

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ABSTRACT | REZUMAT

The aim of the study was to investigate the nutraceutical functional effects of lactoserum Zonar in the management of experimentally induced obesity in Sprague - Dawley rats. The study was performed on Sprague - Dawley male rats. Animals were randomly distributed into 6 groups, 5 animals per group: Negative control group (group 1), Positive control group (group 2). Hypercaloric food and Zonar group (group 3), Standard food and Zonar group (group 4), Obesity 1 Group (group 5), Obesity 2 Group (group 6), in the first 7 weeks, groups 5 and 6 received a diet based on hyperlipidic food, and in the last 4 weeks groups underwent changes in their diet, thus group 5 was passed on a standard diet and lactoserum Zonar, and group 6 remained on the hyperlipidic and lactoserum Zonar diet. The animals were subjected weekly to anthropometric measurements by calculating the body mass index (BMI) and the obesity index (IO). Group 2 gained significantly more body weight compared to all the other groups, thus the BMI and IO were significantly increased. It was observed that rats supplemented with Zonar for 10 and 4 consecutive weeks showed a significant decrease in serum triglyceride level compared to group 2. The oxidative stress parameters TOS and MDA were significantly higher in the group 2 than in groups on Zonar diet. The curent study showed that supplementing the diet with Zonar can reduce the increase in body weight, triglyceride and cholesterol, in rats supplemented with both, high fat diet or standard diet. It was also observed that oxidative stress levels were reduced in groups supplemented with Zonar for a longer period of time.

Keywords: lactoserum, Zonar, obesity, rats, body weight index, oxidative stress

Scopul acestui studiu îl constituie studiul efectelor nutraceutice funcționale ale lactoserumului Zonar în evoluția obezității induse experimental la șobolani Sprague - Dawley. Studiul a fost realizat pe șobolani Sprague - Dawley de sex masculin. Animalele au fost distribuite aleatoriu în 6 loturi, a câte 5 animale pe lot (n=5) după cum urmează: Lot control negativ (lot 1), Lot control pozitiv (lot 2), Lot hrană hipcalcalorică și Zonar (lot 3), Lot hrană standard și Zonar (lot 4), Lot Obezitate 1 (lot 5) și Lot Obezitate 2 (lot 6). În primele 7 săptămâni, loturile 5 și 6 au primit o dietă bazată pe hrană hiperlipidică, iar în ultimele 4 săptămâni au suferit modificări în dieta, astfel lot 5 a fost trecut pe o dietă standard și Zonar, iar lot 6 a rămas pe dieta hiperlipidică și Zonar. Animalele au fost supuse săptămânal la măsurători antropometrice, prin calcularea indicelui de masă corporală (IMC) și al indicelui de obezitate (IO). Lotul 2 a câștigat mult mai multă greutate corporală în comparație cu lotul 1, 3, 4, 5 și 6. IMC și IO au fost semnificativ crescute în lotul 2 comparativ cu celelalte loturi. S-a observat că șobolanii hrăniți cu Zonar timp de 10 și 4 săptămâni consecutive au arătat o scădere semnificativă a nivelului seric al trigliceridelor la loturile 4 3, 5, 6 în comparație cu lotul 2 Parametrii de stres oxidativ TOS și MDA au fost semnificativ mai mari în lotul 2 decât la restul loturilor.Rezultatele acestui studiu sugerează că suplimentarea dietei cu Zonar poate reduce creșterea greutății corporale, a trigliceridelor și a colesterolului la șobolanii suplimentați cu Zonar atât în dietă cu hrană standard cât și în dieta cu hrană hiperlipidică. De asemenea, s-a observat că nivelurile de stres oxidativ au fost reduse la loturile suplimentate cu Zonar pe o perioadă mai lungă de timp.

Cuvinte cheie: lactoser, Zonar, obezitate, șobolani, indice de masă corporală, stres oxidativ

Whey is a by-product that is obtained as a result of cheese processing. It is one of the components that separates from milk, after the milk coagulation process occurs. Whey is an excellent source of protein, peptides, lipids, vitamins, minerals and lactose, being considered a residual product until recently (25).

These components, especially proteins and peptides, due to their bioactive characteristics, make the whey a functional food with multiple applications in biomedicine (25).

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Also, whey proteins contain glycolmacropeptides (GMP), which are present due to the action of chemozine on casein in the first enzymatic step in the cheese making process. GMP is an excellent source of branched-chain amino acids (AAs) such as: leucine, isoleucine, valine, lysine (9).

These proteins possess important functional and biological properties, especially in terms of health promotion and disease prevention (12). Whey proteins are considered to be more suitable for use as a dietary supplement compared to casein, due to differences in amino acid composition (AA) and how they are absorbed (2). The proportion of AA with branched chain of whey is higher compared to casein. Whey proteins are more soluble in acidic environments, leading to faster digestion, being considered "fast proteins". Due to the increased solubility, faster digestion and higher plasma concentration of AA, whey proteins appear to be the most suitable for nutritional and functional benefits (15). Luhovyy et al., 2007 concluded that whey has anti-fatigue potential if added to dietary diets, which are aimed at controlling appetite and managing body weight as well as managing the metabolic consequences of excess adipose tissue.

The diet composition can have an intense effect on the expression of genes in the gut [(peptide YY (PYY) and glucagon-like peptides-1(GLP-1)] Whey proteins prevent GLP-1 de-gradation by inactivating the activity of dipeptidyl pep-tidase-4 (DPP4). After ingestion, whey is hydrolysed into bioactive peptides and amino acids, which are detected by the nervous system at different levels of the gastrointestinal tract. These detection systems act by satiation signals to reduce the amount of food ingested (13).

The aim of this study was to investigate the of nutraceutical effects of the lactoserum (whey) Zonar in the management of the experimentally induced obesity in rats.

MATERIALS AND METHODS

In the present study, we used a commercial formula of whey (lactoserum Zonar, SC Embrion, Satu Mare, Romania). The product was characterized regarding physico-chemical composition. The "Zonar" lactoserum was introduced into the freezer at -20 °C in special plastic pots, half filled, to eliminate the losses by a possible foaming, and then allowed to freeze. The plastic vessels were then placed on the freeze dryer stand where the temperature was set at 40-60°C and the pressure 1 bar, time of 35 hours. The lactose content was determined by High-performance liquid chromatography (HPLC) using HPLC Jasco Chromatograph (Japan), the method being described by Prodan et al, 2018. The total protein content was determined by electrophoresis, according to Prodan et al, 2018. Determination of minerals Ca, K, Mg, Na, P, Cu, Fe, Zn, Pb, Ca/P from the lactoserum samples was performed using the dry digestion method and the Optima 2100 inductively coupled optical emission spectrometer (ICP - AES) (Prodan et al, 2018).

The animals received two types combined granulated food, provided by the Cantacuzino Institute, Bucharest (Romania), the structure of the Standard food ration consists of: 18% protein, 1.5% fat, 5% fiber and the Hypercaloric food composition is 21% protein, 16% fat, 3.5% fiber. For this experiment there were used 30 animals, Sprague-Dawley rats, 5-month-old, male. They were distributed into 6 groups, 5 animals per group (n=5). The animals were purchased from the Cantacuzino Institute, Bucharest (Romania). The rats were housed in the Establishment for breeding and use of laboratory animals of UASVN Cluj-Napoca (Romania), on standard conditions, temperature 23ºC, humidity 55%, and light/dark cycle 12/12. They were fed on standard rodent pellets and ad libitum access to water for an acclimatization period of 7 days. The study was approved by the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Bioethics Committee and the State Veterinary Authority (aut. No. 48/29.03.2017).

The experimental groups are: Negative control group (group 1) received standard food and water, Positive control group (group 2) received hypercaloric food and water, Hypercaloric and Zonar group (group 3) received hypercaloric food and lactoser Zonar,Standard and Zonar group (group 4) received standard food and lactoser Zonar, Obesity 1 Group (group 5) and Obesity 2 Group (group 6) they both received as following, in the first 7 weeks, groups 5 and 6 received a diet based on hypercaloric food and in the last 4 weeks, groups underwent changes in their diet, thus group 5 was passed on a standard diet and lactoserum Zonar, and group 6 remained on the hypercaloric and lactoserum Zonar diet. The caloric requirement to maintain an adult animal is 160kcal /kg /day or 670kJ /kg /day (National Research Council (US) Subcommittee on Laboratory Animal Nutrition, 1995). Standard and Hypercaloric feed was given once a day orally, and whey Zonar was administered orally daily twice. The animals were weighed and measured weekly. The entire experimental period was 11 weeks. At the end of the experiment, they were euthanized and the abdominal fat, liver, kidney and kidney fat were harvested, the organs being also weighed.

Biochemical method and Oxidative stress marker.

Biochemical analysis was carried out using the UV-VIZ Screen master Touch spectrophotometry analyser (Hospitex diagnostics, Firenze, Italy). Triglycerides, Cholesterol, LDL, Glucose were measured.

The oxidative stress parameters determined within this experiment were: total antioxidant reactivity or capacity (TAR), total oxidant status (TOS), oxidative stress index (OSI) and Malondialdehyde (MDA).

All the parameters were measured using UV-Vis Jasco spectrophotometry analyser (Jasco V-630, Tokyo, Japan). TAR and TOS of serum samples were determined using an automated measurement method.

This method is essentially the same as that developed by Ozcan E., 2004. The ratio of total peroxide to total antioxidant potential was calculated in order to determine the OSI, an indicator of the degree of oxidative stress. The lipid peroxidation (MDA) has been determinate by thiobarbituric reaction, procedure reported by Mitev, 2010. Data analysis was represented by the mean and the standard deviation from the results obtained with a significance level of $p<0.05$.

The animals were subjected weekly to anthropometric measurements, such as weighing, length (nose-tail) and abdominal circumference. Calculating body mass index (BMI) is one of the most widespread methods of analysing obesity. Only the data regarding weight and height / length are needed and the result is obtained on the spot.BMI calculation is done as follows:

$$BMI = body\ weight\ (g)\ /\ length2\ (cm^2)$$

The following measurements were performed for the assessment of obesity index (OI): abdominal circumference, body mass and body length, which were considered as factors in the following formula:

$$OI = (Bm + Ac) / Bl$$

where: OI = obesity index; Bm = body mass (g); Ac = abdominal circumference (cm); Bl = body length (cm).

Body length was measured from the muzzle to the base of the tail, and abdominal circumference was measured at the navel (17).

RESULTS AND DISCUSSIONS

Following the investigations, the results were systemized, processed and presented in the form of tables. The whey Zonar compositions are described in Table 1.

Table 1

Protein and mineral content of the lactoserum (whey) Zonar

Chemical composition	ZONAR
Total protein (g/L)	1.517
BSA (g/L)	0.216
α-lactalbumin (g/L)	0.838
β-lactoglobulin B (g/L)	0.233
β-lactoglobulin A (g/L)	0.230
Lactose (g/L)	53.49
Ca (mg/kg)	190.85
K (mg/kg)	901.10
Mg (mg/kg)	47.49
Na (mg/kg)	250.42
Cu (mg/kg)	0.08
Fe (mg/kg)	3.395
Pb (mg/kg)	0.039
Zn (mg/kg)	0.21
P (mg/kg)	287.12
Ca/P (mg/kg)	0.66

Effects of the Zonar on anthropometric measurements (body weight, obesity index, body mass index). After the experimental period, the rats in the 2 group gained a higher body weight compared to the 1, 3, 4, 5 and 6 groups, which was statistically significant. Additionally, the mean body weight observed in group 4 was statistically significantly lower than in the group 3. The correlation between BMI and simple body

Table 2

Initial and final body weights, OI, BMI (mean ± standard error)

Parameters / Groups (n=5)	ANTHROPOMETRICS PARAMETERS			
	Initial weighing (g)	Final weighing (g)	BMI	OI
Group 1 Negativ control	422.4±22.2	581.2±14.6	0.78±0.03	22.1±0.6
Group 2 Pozitiv control	419.4±23.7	667.2±13.2***	0.87±0.05*	25.01±0.94***
Group 3 Hypercaloric+Z	414.2±17.5	606 ±36.1*	0.80±0.02*	22.8 ±0.98**
Group 4 Standard+Z	438±18.8	539.8±42.0**	0.70±0.04***	20.2±1.18***
Group 5 Obesity 1	431.4±30.3	564.4±67.3*	0.74±0.06**	21.34±2.09*
Group 6 Obesity 2	414.6±46.8	566.6±79.6*	0.74±0.06**	21.2±2.14*

Legend: Values are expressed as mean±SD (N=5); *statistically significant (P< 0.05) result between group; **statistically significant (P< 0.01) result between group; ***statistically significant (P< 0.001) result between group.

Table 3

The biochemical profile determined in experimental groups (mean ± standard error)

Parameters / **Groups** (n=5)	**BIOCHEMICAL PARAMETERS**			
	Triglycerides (mg/dl)	**Cholesterol** (mg/dl)	**LDL** (mg/dl)	**Glucose** (mg/dl)
Group 1 Negativ control	102.9±27.4	88.5±2.9	44.5±13.1	155.4±26.4
Group 2 Pozitiv control	173.4±11.9**	116.4±10.6**	37.2±5.2	231.2±30.1**
Group 3 Hypercaloric+Zn	77.6±16.2***	107.4±43.3	36.3±14.2	151.8±9.1**
Group 4 Standard+Zn	65.5±9.9***	93.4±27.3	41.8±16.4	152.7±40.4**
Group 5 Obesity 1	67.7±16.6***	82.1±12.5**	34.2±12.9	180.8±31.9*
Group 6 Obesity 2	95.6±7.2***	99.6±26.8	45.8±8.5	222.8±27.8

Legend: Values are expressed as mean±SD (N=5); *statistically significant (P < 0.05) result between group; **statistically significant (P< 0.01) result between group; ***statistically significant (P< 0.001) result between group.

weight is worth mentioning because the result is similar, the BMI being statistically significantly increased in group 2 compared to the rest of the groups (Table 2). Remarkably, the above-mentioned results are related to the obesity index, as well. Obesity is an important topic for both public health and preventive medicine. The obesity is characterized by an increase in the number and size of adipocytes at the cellular level in humans and animals (19). The effect of Zonar on obesity in rats feed with high fat diet was evaluated. This study showed that a high-fat diet for several weeks resulted in a significant increase in body weight. Supplementation with lactoserum / whey Zonar resulted in a remarkable reduction in the body weight, compared to the positive control group, showing the anti-obesity action of lactoserum Zonar (7).

According to Shi et al. (2012), β-lactoglobulin, which is one of the major fractions of whey protein, is a member of the lipocalin family, able to encompass hydrophobic molecules such as fatty acids, cholesterol, retinol and vitamin D. Therefore, it is possible that β-lactoglobulin participates in the reduction of the intestinal lipid uptake (5). Our experiment is consistent with these previous results (23; 7). The aetiology of obesity is multifactorial, and it has reached epidemic proportions in the last decades. Even at a low level, it has negative effects on health, and is associated with a shorter life expectancy. The adverse clinical consequences of obesity are so harmful that an increase of 20% over the ideal weight is associated with a 20% increase in the mortality rate in humans (26).

Measurement of lipid and serum glucose levels. Regarding the variation of the concentrations of glucose, triglycerides and cholesterol, the diet based on Zonar had a significant beneficial effect in lowering their concentrations and normalizing them in all groups, regardless of the continuous administration or administration in the diet for a fixed period (Table 3).

The most surprising results are observed in the case of changes made regarding the hyperlipidic diet, on a standard diet and Zonar for 4 weeks. Thus, we consider that it had a systemic anti-adipose effect, managing to reduce the cholesterol and triglyceride concentrations statistically significantly. Zonar diet has protective and preventive effects when consuming a normal or hyperlipidic diet, with regards to serum biochemical concentrations. The shock diet based on Zonar for 4 weeks, especially the one associated with caloric reduction denotes the best results in the case of already obese animals, thus it can be used as part of a weight loss diet (Table 3). No significant differences were observed in LDL-cholesterol levels in the six groups (Table 3). Dyslipidemia is another important symptom in pathogenesis of obesity, being characterized by hypertriglyceridemia, with low levels of LDL and VLDL (1). To investigate the effects of Zonar on improving lipid disorders in rats, triglyceride levels, total cholesterol and serum LDL were measured. Zonar supplementation was found to significantly reduce serum triglyceride and total cholesterol levels. These results were similar to the results reported by Jacobucci et al. (2001), where total cholesterol in blood and liver was significantly lower in rats receiving whey protein in the diet. Several studies (1; 6) have shown that in experimental models high-fat diets have disrupted glucose metabolism and caused glucose tolerance.

Table 4

Assessment of the oxidative stress (mean ± standard error)

Parameters / **Groups** (n=5)	**STRESS OXIDATIVE PARAMETERS**			
	MDA nanomoli /litru	**TOS** (µmol H2O2 eq/L)	**TAR** (µmol Trolox eq/L)	**OSI INDEX**
Group 1 Negativ control	3.99±0.22	7.76±1.68	1.09±0	7.10
Group 2 Pozitiv control	4.78±0.81	37.07±11.81**	1.10±0*	33.63
Group 3 Hypercaloric+Z	3.81±0.87	6.82±1.38**	1.10±0	6.18
Group 4 Standard+Z	2.78±0.38**	9.09±3.24**	1.09±0**	8.30
Group 5 Obesity 1	3.82±0.30	14.04±2.12*	1.09±0**	12.88
Group 6 Obesity 2	3.14±0.58**	14.59±5.62**	1.09±0**	13.35

Legend: Values are expressed as mean±SD (N=5); *statistically significant ($P < 0.05$) result between group; **statistically significant ($P< 0.01$) result between group; ***statistically significant ($P< 0.001$) result between group.

Similar to other studies, our study demonstrated a significant increase in blood glucose levels in the 2 groups, while the groups 5 ,4, 3 showed the lowest levels, these findings suggest that the lactoserum Zonar may reduce blood glucose levels.

These results coincide with other studies (20; 24) in which whey protein had a significant effect on blood glucose lowering (4).

Assessment of oxidative stress parameters:

There were differences between the OSI levels in the groups, group 2 having the highest OSI values (Table 4). These results correlate fairly well with Prigon et al., 2013, who suggested that TOS and OSI levels were higher in obese children with non-alcoholic fatty liver disease than in non-obese children. Also, Kilic et al., 2016, showed that markers of oxidative stress TOS and TAR were significantly higher in the obese groups compared with the control groups, and concluded that the increased level of TAR was the result of increased TOS in trying to balance oxidation, because young people have more active anti-oxidant systems (Table 4).

The diet based on the lactoserum Zonar had a significant beneficial effect in decreasing the concentrations, and normalizing the oxidative stress parameters (TOS and MDA) in all groups, regardless of the continuous administration or administration as diet for a fixed period. The most surprising results are observed in the case of changing the hyperlipidic diet on a standard diet with Zonar for 4 weeks.

Thus, we consider that it had a systemic antioxidant effect, managing to reduce the concentrations of TOS and MDA to a statistically significantly degree (Table 4).

CONCLUSIONS

The study's results suggest that diet supplementation with lactoserum Zonar can suppress the increase in body weight, BMI and OI. The levels of triglyceride, cholesterol and serum glucose decreased in rats supplemented with Zonar, even if the rats were subjected to a high fat diet or standard food. The parameters of the oxidative stress (TOS and MDA) were lower in the groups supplemented with lactoserum Zonar, and the longer the consumption of the lactoserum Zonar the lower the level of oxidative stress was, compared to the other experimental groups.

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