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CLINICAL AND LABORATORY FINDINGS REGARDING THE BIOLOGICAL EFFECTS OF LACTOSERUM CONSUMPTION IN WISTAR RATS

CERCETĂRI CLINICE ȘI DE LABORATOR PRIVIND EFECTELE BIOLOGICE ALE CONSUMULUI DE LACTOSER LA ȘOBOLANI WISTAR

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ABSTRACT | REZUMAT

The motivation behind the study consists in the promotion of lactoserum (from comercial product, Zonar, noted in this work with letter Z) as aliment, with functional nutritional properties. The purpose of the study consists in the evaluation of the biological effects of Z, administered to Wistar rats, as supplement on top of regular rations and exclusive food source. The study consisted in the daily clinical monitoring of animals during the experiment (weekly body mass determination, eating behavior, general appearance), and paraclinical, haematological determinations: E, Hb ,Ht, VEM, HEM, CHEM, leukocytes), biochemical (carbohydrates, lipids, proteins, serum enzymes).

The rats were slaughtered and the tissue samples (liver, duodenum) were used for histopathological tests, in order to evaluate the tisular reaction to Z consumption. The experiment was carried out using 15 adult rats, males, clinically healthy,randomly assigned in 3 equal lots. The reference lot (n=5) was fed with standard nourishments: combined granulated fodder; the 2nd lot (n=5) was fed with Z + combined granulated fodder 20 g/day; the 3rd lot (n=5) was fed only with Z. For 21 days, body weight was closely evaluated, as well as the metabolic status of the rats. Blood samples were taken for haematological and biochemical analysis. The consumption of Z caused a decrease in the body mass of the rats from the experimental lots. As for the haematological parameters, there have been no major changes. The consumption of Z as exclusive nutrient has caused hypoglycemia, hypotriglyceridemia and hypocholesterolemia.

Keywords: whey, Zonar (Z), Wistar rats, haematological investigations

Prezentul studiu evaluează efectele biologice ale lactoserului (din produsul comercial Zonar, codificat în această lucrare cu litera Z) administrat la șobolani Wistar, ca supliment la rația alimentară și ca sursă exclusivă de nutrienți. Studiul a constat în monitorizarea clinică zilnică a animalelor pe parcursul experimentului (masă corporală, comportament alimentar, aspectul general), și paraclinică: determinări hematologice (E, Hb, Ht, VEM, HEM, CHEM, și leucocite), biochimice (glucide, lipide, proteine, enzime serice).

Animalele au fost sacrificate, iar din probele biologice tisulare (ficat, duoden) s-a efectuat examen histopatologic, pentru a evalua răspunsul tisular la consumul de Z. Experimentul a fost realizat pe 15 șobolani adulți, masculi, clinic sănătoși, distribuiți randomizat în 3 loturi egale. Lotul martor (n=5) a fost furajat cu hrană standard; lotul 2 (n=5) furajat exclusiv cu Zonar + nutreț combinat granulat 20 g/zi; Lot 3(n=5) la care s-a administrat exclusiv Z. Timp de 21 de zile s-a urmărit evoluția masei corporale și statusul metabolic al animalelor. Consumul de Z a avut ca efect scăderea masei corporale a șobolanilor din loturile experimentale. În ceea ce privește parametrii hematologici, nu s-au decelat modificări majore ale valorilor hematologice. Consumul de Z ca sursă exclusivă de hrană are efect hipoglicemiant, hipotrigliceridemiant, hipocolesterolemiant.

Cuvinte cheie: lactoser, Zonar (Z), șobolani Wistar, investigații hematologice

The structure and properties of whey-based beverages are similar in composition to whey: water and solid components. Protein components are bioactive food ingredients that classify whey as a dietary product beneficial for animals and human health [7, 8]. Whey proteins contain thermosensitive fractions like β-lactoglobulin, α-lactalbumin and bovine serum albumin, immunoglobulins and thermostable fractions (peptones and other protein fractions). Due to the rich content in essential amino acids (lysine, cysteine, methionine, cystine) whey proteins have a high biological value compared to other animal protein [11].

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Cystine / methionine ration is 10 times higher in whey proteins than in casein [3]. Denaturized proteins (e.g., lactalbumins) in whey are 100% absorbed by the human digestive system, compared to 75% of casein proteins [4].

A way of preventing catabolism of endogenous proteins during exercise, maintaining high endogenous use of lipid consists of providing a dietary source of amino acids [6]. This kind of meal has the advantage of producing small amount of insulin and thus does not impair fat mobilization, having also the potential to bring to the muscles branched chain amino acids and glucose, produced through gluconeogenetic pathway [6].

In a study on Wistar rasts fed with three different preexercise food (one meal enriched with glucose, one enriched with whole milk protein and one with alpha lactalbumin enriched whey protein), it was demonstrated that the body weight of the rats has increased. This resulted in an increase of fat mass due to meals enriched in glucose and whole milk protein, while the meals enriched with alpha-albumin whey protein caused an increase in lean body mass and muscle. Bouthegourd et al [6] believes that whey protein enriched food has the potential to rapidly deliver amino acids for use during exercise and decreased adiposity. Also high-calcium diets attenuate adipocyte lipid accretion and weight gain, increase lipolysis and preserve thermogenesis during caloric restriction.

Consumption of dairy sources of calcium attenuates weight and fat gain to a greater degree than supplemental sources of calcium [15].

These concepts are demonstrated in clinical trials on animals and humans. Obese subjects fed with 3 daily dairy products present significant reduction in adipose tissue mass without caloric restriction and accelerated weight and body fat loss secondary to caloric restriction [14]. The whey also contains lactose that enhances its digestibility. Lactose from whey is an important source of energy and has several beneficial effects such as stimulation of peristalsis, increase of calcium and phosphorous in the body and the establishment of slight acidity in the gut that inhibits pathogen growth and development [2, 7]. Soluble vitamins are present in milk, passing in the whey products. The most significant are folic acid, cobalamin and riboflavin [7].

The purpose of the study consists in the evaluation of the biological effects of Z[1)], administered to Wistar rats as supplement, on top of regular rations and as exclusive food source.

MATERIALS AND METHODS

The experiment was conducted on 15 clinically healthy, adult, female Wistar rats. The investigation was conducted with the approval of the Bioethics Committee of Veterinary Medicine Faculty, Cluj-Napoca.

The Wistar rats were housed in a facility under standard conditions: temperature 23°C, humidity 55%. The animals were fed with standard rodent granular food, containing:18% crude protein, 1.5% crude fat, 5% crude fiber and water ad libitum. Rats were randomly assigned into 3 equal groups as follows: group 1 (n=5) Control; group 2 (n=5) Z + standard food (H+Z); group 3 (n=5) fed exclusively with Z.

The rats were housed separately in 15 cages so Z intake could be monitored. The diet for the control group was based on standard food, group 2 was fed with 20 g of standard food and Z, 10 ml three times a day, and group 3 was fed exclusively with Z 10 ml three times a day. The chemical composition of Z was determined with Milkoscan, an infrared milk analyzer. This is attested by an analysis from a certified laboratory of animal production and food safety, LSVSA, Cluj-Napoca. At the same laboratory, was determined the total metal waste: Cadmium, Lead by atomic spectrometry, and the total aflatoxins and medical waste were determined by ELISA testing. The chemical composition of Z was determined using spectrometry at the biochemical laboratory, Faculty of Veterinary Medicine, Cluj-Napoca (Table 1, 2, 3).

The experiment lasted three weeks. Each rat was housed separately in order to better assess the intake of Z. At the end of each day the total amount of Z consumed was measured. Body weight of the rats was evaluated, every week from the beginning to the conclusion of the experiment. In the 3rd week, blood samples were taken from the rats under anesthesia with Xylasine and Ketamine, for the hematological and biochemical examinations. Complete Blood count was determined using the automatic Abacus junior Vet - hematology counter. Biochemical analysis was carried out using the spectofotometry analyzer at the bioche-

1) ZONAR (Z) is a whey based beverage produced by S.C EmbryOm Capital Investment SRL, from Satu Mare city. It is developed from cow milk and it's similar to sweet whey with a few particularities in the manufacturing process.

mical laboratory of the Faculty of Veterinary Medicine Cluj-Napoca,which monitorized: parameters of carbohydrate metabolism (glucose), protidic metabolism (total protein, albumin), lipidic metabolism (tryglicerides, cholesterol) and enzymatic parameters (ALT, AST, UREA, Creatinine).

Table 1

The chemical composition of product Z

Parameters	The value obtained from Z	Reference value from cow milk	Values for whey from cheese
Density (g/cm^3)	1.0285	1.028	1.04
Protein (g/100g)	0.92	3.2	1.10
Casein (g/100g)	0.6	2.7	-
Fat (g/100g)	-0.10	3.5	0.86
Lactose(g/100g)	4.82	4.55	4.88
Total solids(g/100g)	6.47	12.5	7.60
Fat solids (g/100g)	6.51	8.5	6.63
Freezing point (° Celsius)	-0.380	-0.525	-0.411
pH	6.27	6.4 - 6.7	-
Acidity (° Thorner)	3.85	15 - 19	15.82

Table 2

Metal and micotoxins in product Z

Parameters		Results
Metal waste	Cadmium	Undetectable value
	Lead	Undetectable value
Micotoxin and medical waste	Aflatoxin M1	Undetectable value

Table 3

The chemical composition of product Z

Parameters	Value from Z	Value from Cow Milk
Ca	31.83 mg/dl	112 mg/dl
P	28.9 mg/dl	89 mg/dl
Mg	6. 71 mg/dl	11 mg/dl
Fe	0.12 mg/dl	0.05 mg/dl
Cl	151.38 mg/dl	97 mg/dl
Na	4.44 mg/dl	53 mg/dl
K	166 mg/dl	136 mg/dl

In statistical analysis, all date were reported as mean ± standard deviation. GraphPad Prism 5.0 was the programmed used in order to process the data. Following statistical observation we obtained the following results. The distribution of data was normal and evaluation of difference was performed by ANOVA one way test, followed by Tukey – HAD (Honestly Significance Difference) test in order to correct any errors.

RESULTS AND DISCUSSION

Clinical examination was performed weekly in order to assess the general condition, appetite, maintenance, body mass oscillation of experimental animals.

The animals in the experimental groups consumed an average of 25 ml of Z daily, similar with water consumption (20-50 ml/day). During 21 days the average body weight fluctuated, seeing a decrease in body mass in both experimental groups.

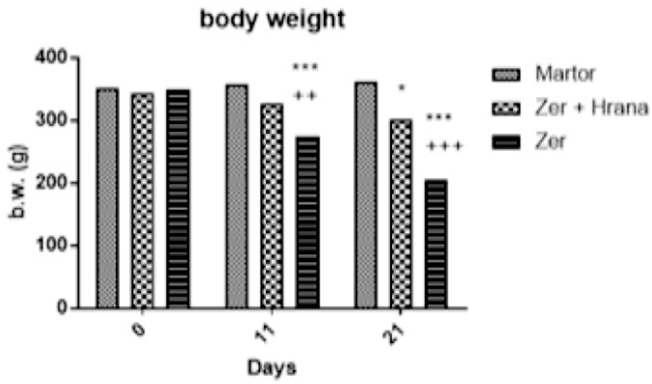

Fig.1. Graphical representation of weight values in the experimental groups *** p ≤ 0,001, * p ≤ 0.05

In group 2 (H + Z) there was a 10% decrease in baseline body weight, and in group 3 (Z), fed exclusively with Z there was observed a 42% decrease in baseline body weight at the last weighing. On day 21, there was a decrease in body mass in group 2 (302.6 ± 14.31) compared to the control group (345 ± 37.00) (p≤0.05) and group 3 decreased statistically significant (206.5 ± 21 13) compared to the control group (345 ± 37.00) (p≤0.001) and group 2 (302.6 ± 14.31) (Fig. 1).

Table 4

The values of number of leucocytes

	WBC (10^9/l)	LYM (10^9/l)	MID (10^9/l)	GRA (10^9/l)
Group 1	8.72 ± 1,7	7.02 ± 1	0,8 ± 0,06	0.6 ± 0,7
Group 2	5.90 ± 0,9	4,46 ± 0,7*	0,4 ± 0,07	0.8 ± 0,2
Group 3	5.20 ± 0,09	1.71 ± 0,4***	0,3 ± 0,1	3.1 ± 0,3

Values shown are mean ± standard deviation.
WBC - total number of cells (2.1-19.5 x 10^3), LYM number of lymphocytes (2-14.1 x10^3), MID-Mid average cell number (0-0.98 x10^3), GRA - the number of granulocytes (0.1-5.4 x10^3), *** p ≤ 0,001, * p ≤ 0.05.

No statistically significant differences were observed in total white blood cell evaluation (Table 4). However, the values of lymphocytes in the group fed exclusively with Z is significantly lower (1.71 ± 1.16 x 103) than the control group (7.02 ± 0.71 X 103), and the values of lymphocytes in group 2 are lower (4.46 ± 1.89 X 103) compared to control (7.02 ± 0.71 X 103).

No significant differences were observed in the red blood cell parameters, erythrocyte constants, being included within the physiological limits of this species (RBC 5.3-10 X 10^9, HTC 35-52%) Related by platelet constants, No changes were observed, the values were within the species limits (500-1370 x 10^3).

Related by carbohydrate metabolism parameters represented by serum glucose, the oscillations were recorded as follows: the amount of glucose in group 2 (H+Z) is significantly increased (241.7±20.53 mg/dl) compared to control group (95.8± 24.3 mg/dl) and group 3 (Z) (61±12.30 mg/dl);glucose value for group 3 (Z) (61±12.30 mg/dl) is significantly lower than the control group (95.8± mg/dl) and group 2 (Fig. 2).

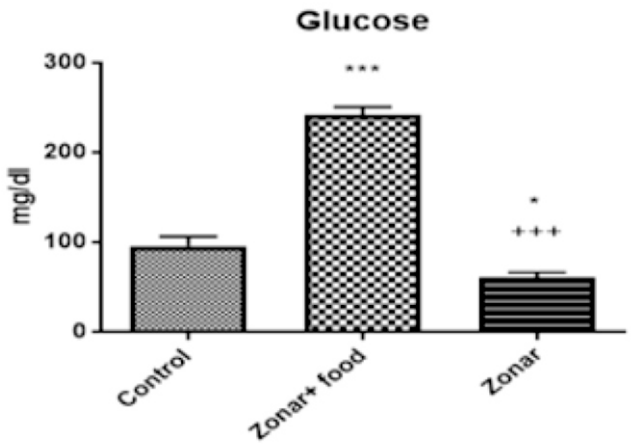

Fig. 2. Graphical representation of serum glucose concentration in the experimental groups, *** p ≤ 0,001, * p ≤ 0,05, +++ p≤0.001 in comparison to group 2

No statistically significant differences were detected with respect to the variation of the concentration of total proteins and serum albumin. Values are found in physiological limits in terms of total protein concentration (5.6-7.6 g/dl). A slight decrease is observed in group 3 regarding albumin concentration (3.3±0.14 g/dl) compared to other groups. The physiological limit for serum albumin is between 3.8-4.8 g/dl (Table 5).

The cholesterol value is significantly lower in group 3 (82.2±12.91) than the control group (104.4± 12.09) and group 2 (103±13.01).

Table 5

Protidic metabolism parameters

	Total proteins (g/dl)	Albumin (g/dl)
Group 1	7.28 ± 0.14	4 ± 0.27
Group 2	6.52 ± 0.23	4 ± 0.10
Group 3	5.8 ± 0.27	3.3 ± 0.14

Values shown are mean ± standard deviation

Also, the triglycerides value is highly significant lower in group 3 (90.48±10.02) than in control (143.8 ±31.03) and group 2 (172.24±2.25) (Fig. 3, 4).

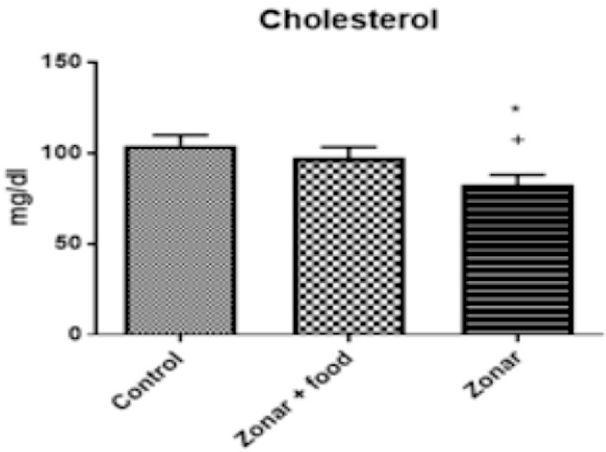

Fig. 3. Graphical representation of serum cholesterol concentration in the experimental groups, * p ≤ 0,05, + p≤0.05 in comparison to group 2 Normal values (40-130 mg/dl)

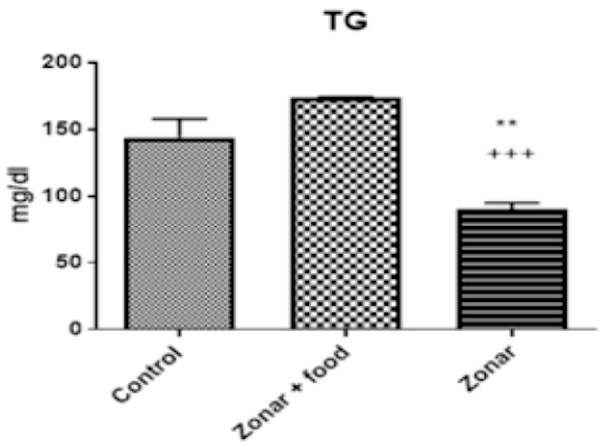

Fig. 4. Graphical representation of serum triglycerides concentration in the experimental groups, ** p ≤ 0,01,+++ p≤0.001 in comparison to group 2 Normal values (60-180 mg/dl).

The study showed a decrease in ALT in group 2

(58.76±5.98 IU/L) compared to the control group (81±3.16 IU/L) and regarding AST, there was a decrease in both experimental groups (group 2 141.62± 16.24 IU/L) (group 3 154.86±39.34 IU/L) compared to the control group (233.82±7.48 IU/L).

The concentrations of urea serum are significantly higher in group 2 (43.06±5.90 mg/dl) and 3 (42.66±11.53 mg/dl) compared to control group (17.96±1.40 mg/dl) (Table 6).

The value of serum creatinine concentration is not significantly changed. The value falls within physiological limits for the species (0.2-0.8 mg / dl) (Table 6).

Table 6

Renal and hepatic enzyme parameters

	Urea (mg/dl)	Creatinine (g/l)	ALT (U.I/l)	AST (U.I/l)
Group 1	17.96 ± 1.40	0.63 ± 0.06	81 ± 3.16	233.82 ± 7.48
Group 2	43.06 ± 5.90***	0.55 ± 0.02	58.76 ± 5.98*	141.62 ± 16.24***
Group 3	42.66 ± 11.53***	0.55 ± 0.02	77.78 ± 17.42	154.86 ± 39.34***

Values shown are mean ± standard deviation
Normal values Urea: 15-21 mg/dl; ALT: 16-89 U.I/l; AST: 192-269 U.I/l.
*** p ≤ 0,001, * p ≤ 0,05

The animals were accustomed relatively easily to the diet change. Significant variations were detected in the animal's body weight, particularly from the whey-fed. The animals lost about 42% of the initial body weight, which demonstrates the dietary effect in a relatively short period of time. We didn't find significant variations between experimental groups regarding blood analysis, which may be due to the relatively short time the research was conducted over. The study shows a slight lymphocytopenia without a change in total number of leukocytes and erythrocyte parameters which are within physiological limits of species. Biochemical determinations showed significant changes in the level of serum glucose. It is increased in group 2 fed with Z and standard chow and significantly lower in the group fed only with Z. Numerous studies have examined dairy foods for their potential prevention role in the development of insulin resistance, as well as their ability to increase sensitivity in type 2 diabetics [1, 10]. Dairy limits postprandial hyperglycemia (blood glucose levels rise after consuming a meal). When smaller increase of blood sugar exists, insulin needs to be produced are low. This is believed to be the result of actions of whey proteins that are unique in milk and milk-derived foods. Whey proteins contain essential amino acids that have been demonstrated to stimulate the pancreas to produce insulin [1, 13].

Whey-derived peptides and calcium can interfere with insulin's ability to move glucose from the bloodstream into the cells [13]. Insulin sensitivity is influenced by numerous factors, all interacting with each other. Increased glucose level for group 2 may be also linked to the consumption of standard food. Eating only whey for a limited period of time is attesting its hypoglycemic effect. Increased serum urea level is based on the protein diet, protein from whey and standard food composition. However, these values do not indicate kidney pathology, being accompanied by serum creatinine levels within physiological limits.

ALT liver enzyme level is low only for experimental group 2. This reduction does not indicate a hepatic pathology. Regarding AST there is a statistically significant decrease in both experimental groups. Aspartate aminotransferase (AST) is an enzyme that belongs to the class transaminase and it has the ability to catalyze the amino group and transfer keto group of ketoglutarate, forming oxaloacetic acid [5].

Unlike ALT, which is found primarily in the liver, AST is found in many tissues: myocardium, liver, skeletal muscle, kidney, pancreas, brain, spleen, making it [5], an indicator less specific for the liver function. The decline of enzyme levels can be attributed to the muscle mass loss with sharp weakening due to diet and caloric restriction.

Z consumption is cholesterol-lowering due to reduced amount of fat existing in its composition and its lipolytic effect of whey [9, 12].

CONCLUSIONS

1. Z consumption over 21 days had an effect on body weight in experimental groups, inducing its decrease significantly.

2. Regarding haematological parameters, there were not detected major changes in the values of leukocytes, erythrocytes and platelets.

3. Z consumption as exclusive source of food has a hypoglycemic effect.

4. Z consumption as a supplement to the diet as well as exclusive source of food has a lowering serum triglycerides effect and as exclusive source a cholesterol-lowering effect.

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