THE BIOLOGICAL EFFECTS OF THE BOVINE MILK EXTRACT

EFECTELE BIOLOGICE ALE EXTRACTULUI DIN LAPTE DE BOVINE

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ABSTRACT | REZUMAT

The extract obtained from the bovine milk (whey) is a natural product that has in its composition numerous bioactive compounds with curative potential, confirmed by the literature.

The milk extract consists of about 93% water and 6.5% dry matter, from which the most important are lactose, proteins, numerous vitamins, micro/macro minerals, and fats in small quantity. The biochemical components offer to the extract a high degree of digestibility, mainly due to the content of branched chain amino acids.

Among the most studied physiological effects of the milk extract is the antioxidant effect developed at the cellular, tissue and systemic level, mainly by stimulating the formation of the glutathione. The high content of milk extract in lactoferrin, a glycoprotein involved in the metabolism of iron, gives it numerous other beneficial physiological effects, such as anti-inflammatory, antimicrobial, antiviral and antifungal activity.

The purpose of this paper is to present the biological effects of the whey obtained from the bovine milk in different categories of chronic degenerative diseases, such as obesity, diabetes mellitus, cardiovascular diseases, hepatopathies, cancer, respectively in tissue regeneration following some mechanical or other injuries. Investigation of the beneficial physiological effects of the whey can be performed also by using some experimental models of disease performed on the laboratory animals (e.g.: mice, rats, Guinea pigs).

Keywords: diabetes, cancer, obesity, extract from bovine milk (whey)

Extractul obţinut din lapte de vacă (lactoser) este un produs natural care are în compoziţia lui numeroase substanţe bioactive cu potenţial curativ confirmat de literatura de specialitate.

În componenţa lactoserului intră aproximativ 93% apă şi 6,5 % substanţă uscată, dintre care mai importante sunt lactoza, proteinele, diferite vitamine, micro/macrominerale şi grăsimi în cantitate relativ mică. Componentele biochimice oferă lactoserului un grad mare de digestibilitate, datorat în principal conţinutului în aminoacizi cu catenă ramificată.

Printre efectele fiziologice mai studiate se află rolul antioxidant puternic dezvoltat la nivel celular şi tisular, în principal prin stimularea formării de glutation. Conţinutul ridicat în lactoferină, o glicoproteină cu rol în metabolismul fierului, conferă acestui extract obţinut din lapte de vacă multiple alte efecte fiziologice benefice, cum ar fi acţiunea antiinflamatoare, antimicrobiană, antivirală şi antifungică.

Scopul lucrării îl constituie prezentarea efectelor biologice ale lactoserului obţinut din lapte de vacă, în diverse categorii de boli degenerative cronice, cum sunt obezitatea, diabetul zaharat, bolile cardiovasculare, hepatopatiile, neoplaziile, respectiv în regenerarea tisulară care urmează unor injurii mecanice sau de altă natură.

Investigarea efectelor fiziologice benefice ale lactoserului poate fi realizată şi cu ajutorul unor modele experimentale de boală realizate pe animale de laborator (ex: şoareci, şobolani, porci de Guinea).

Cuvinte cheie: diabet, cancer, obezitate, extract din lapte de vacă (lactoser)

The progressive loss of the metabolic control has common characteristics in diabetes, obesity, cardiovascular disease (CDV) and aging. This is usually manifested through decreased insulin sensitivity, increased lipid anabolism, pro-inflammatory environment and hypertension (18). Excess visceral adipose tissue deposition, lipid infiltration of liver, muscle and other organs, sarcopenia are the pathophysiological factors that may lead to metabolic destruction.

Sarcobesity and sarcopaenic diabetes are the first characteristics that show the degenerative response of the muscle mass (18, 21). The consumption of dairy products is associated with decreased prevalence of metabolic diseases. The European Region Dietary Guide from 2010 recommends the intake of dairy products as part of a healthy diet. The consumption of milk and dairy products is associated with a decrease of metabolic syndrome (MetS) by affecting directly the risk factors mentioned above (21, 23). Whey is a by-product of bovine milk. The composition and proper-

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ties of whey depend on processing technology. Liquid whey consists of about 93% water and contains 50% of milk constituents, of which lactose is majority. Lactose from whey is an important source of energy, but also has various effects, such as the stimulation of the peristaltic digestive tract, increasing the absorption of calcium, phosphorus and magnesium (11). The whey also contains proteins, minerals (calcium, phosphorus, zinc) and vitamins. Because of very low fat level, the whey is considered a dietary pro-duct (11, 15).

Table 1

Residues of aminoacids in bovine lactoferrin

(Hall et al., 2003)

Aminoacid	Lactoferrin	Aminoacid	Lactoferrin
Alanine	67	Glutamic Acid	40
Proline	30	Phenylalanine	27
Arginine	39	Methionine	4
Lysine	54	Leucine	65
Asparagine	29	Glycine	48
Valine	47	Tyrosine	22
Tryptophan	13	Aspartic acid	36
Cysteine	34	Histidine	9
Threonine	36	Total number of residues	689
Isoleucine	15	Pierce A, Colavizza D, Benaissa M, et. al., 1991, Molecular cloning and sequence analysis of bovine lactoferrin, Eur J Biochem; 196:177-184.	
Serine	45		
Glutamine	29		

Food industry has an advantage consecutive processing this product by various technologies: sweet drinks, fermented beverages, alcoholic drinks or protein extracts making. However, whey proteins are those that rank whey as a food product with medicinal properties (11). Whey proteins comprise about 20% of total milk proteins. These are not coagulated by acid and are resistant to the action of chymosin (16). Because these proteins are resistant to gastric digestion, they contribute to a wide variety of physiological activities in the gut lumen, blood stream and other tissues (16). The whey of cows have received a particularly attention because it is a human food and agronomic product. Its utilities are known in the food industry as whey powder/milk powder, raw material for alcoholic beverages and protein concentrates (16, 18). During industrial processing, the whey is usually heated and the heat treatment can have a negative impact on the nutritional value of the whey in terms of amino acids bioavailability and peptide function.

This is the main reason that leads to making of diets containing unprocessed whey and research of its potential curative biological effects (21).

The whey proteins are classified as high biological quality proteins due to the physiological requirements of amino acids in human diet.

In comparison to another protein sources, the whey has a higher amount of branched chained amino acids (BCAAs). The most important BCAAs are leucine, isoleucine, and valine. Particularly, leucine has a key role in metabolism of proteins (protein synthesis) and tissue growth and regeneration. Because the whey proteins contain the best combination of all amino acids, these outperform the biological value of proteins from meat, vegetable protein (15).

The whey proteins consist of betalactoglobulin, alfalactalbumin and immunoglobulins. It also contains glycoproteins (e.g., lactoferrin and transferrin) and enzymes (e.g., lactoperoxidase and lysozyme), which significantly increase immune function, together with immunoglobulins (15). Lactoferrin is a glycoprotein that has the ability to bind iron. It is found in large quantity in human milk (2.2 to 5.8 g/l), and in bovine milk reaches concentrations of 0.2-1.5%. Lactoferrin

Table 2

Components found in whey protein

(Marshall, 2004)

Whey components	% proteins	Benefits
Beta-Lactoglobulin	50-55 %	Source of essential and branched chained amino acids
Alfa-Lactalbumin	20-25 %	Primary protein found in human breast milk Source of essential and branched chained amino acids
Immunoglobulins	10-15 %	Primary proteins found in colostrums Immune modulating benefits
Lactoferrin	1-2 %	Antioxidant Antibacterial, antifungal, antiviral Promotes growth of beneficial bacteria Naturally occurs in breast milk, tears, saliva, bile, blood and mucus
Lactoperoxidase	0,50 %	Inhibits growth of bacteria
Bovine Serum Albumin		Source of essential amino acids Large chain of peptides
Glycomacropeptide	10-15 %	Source of essential amino acids Lacks the aromatic amino acids: phenylalanine, tryptophan and tyrosine

plays an antibacterial, antifungal, and antioxidant role within the cells. The amino acids that make up lactoferrin have a key role in its action. Residues of amino acids in bovine lactoferrin are 689 in comparison to 691 residues in human lactoferrin (Table 1, 2).

The biological effects of the extract obtained from bovine milk

The antioxidant activity of the whey is one of its most important properties. Glutathione (GSH), an intracellular antioxidant consisting of cysteine, glutamic acid and glycine, prevents the formation of oxygen reactive species and cellular injuries. It has been shown that older cells contain 20% less glutathione than younger cells, therefore glutathione has a direct role in cell aging processes. It also represents a safe cysteine donor for replenishment during immune deficiency states. The whey proteins are considered the most active components (3, 4), which form peptide and exert a ligand role for the receptors from gut lumen, other target organs and tissues, after their absorption into the blood. The synthesis of new cellular proteins is achieved through the interaction of these peptides of food origin. Short-chain peptides appear in the blood by intercellular junctions, while the long-chain peptides need specific transporters (22). The peptides from whey have an immunostimulatory, anti-inflammatory, antihypertensive and dietetic role (22, 23).

The biological effects of the extract obtained from bovine milk to the function of immune system

Immunoglobulins from whey are composed of gamma globulins (Ig G, Ig A), which represent 10-15% of the total protein (5). They have an immunoprotective effect by stimulating of T lymphocytes of various types production and maturation. A study shows (7) that an amount of 0.3 mg/ml of immunoglobulins of the type Ig G gives a significant increase of specific immunity of the organism. Bovine milk contains 0.6-0.9 µg/ml Ig G immunoglobulin, which is taken up by human consumption of milk and dairy products (7, 15). Immunoglobulins in milk provide specific antibodies for different bacterial or viral diseases in humans, such as human rotavirus, *E. coli*, *S. enteritis* and *S. typhimurium* (2, 5). Also, beta-lacto globulin represents half of the total proteins found in bovine milk. This, in addition to the rich content in essential branched chain amino acids, contains a specific protein (crpb I/II, crabps) capable of binding retinoic acid, with direct effect on stimulating lymphocyte response (7). Lactoferrin (bLF) has ability to activate NK cells and neutrophils and increase the cytotoxicity of macrophages. The recent studies demonstrate that orally administrated lactoferrin in animals has beneficial effects like: enhancement of Th1 cytokine response in splenocytes and lymph node cells, increasing leukocyte number in blood and lymphoid tissues (13). These effects were further studied on viral infections in human and animals. Lactoferrin is capable of binding with a certain virus and inhibit its development. Studies show that these effects are encountered in human infections with Rotavirus, Norovirus, feline Calicivirus, murine Norovirus, Herpes viruses (HSV 1, HSV 2) and common Adenoviruses that cause common colds (14). Due to its biological properties of inhibiting a wide range of viruses, scientists continue their research on the chronic hepatitis C also, although lactoferrin seems to have a gastro-intestinal and systemic tropism (13).

Modulation of the antiinflammatory response

The milk contains more than 50 growth factors and hormones, but their concentration is lower than that of the immunoglobulin and lactoferrin. Growth factors found in milk are consisted of chains of proteins or steroid hormones. Characterized growth factors from milk extract are: betacellulin, tumour growth factor (TGF-beta), fibroblast growth factor (FGF), platelet growth factor (PDGF) and insulin growth factor (IGF1, IGF2) (10). It should be noted that these factors are synthesized in the digestive tract. Their role is crucial for the health of the digestive tract through the development of intestinal mucus. Their physiological role is to neutralize inflammatory cytokines and develop an anti-inflammatory and protective effect of gastric and intestinal epithelium. Chronic inflammation is related to the continuous oxidative stress (12). Several studies show that systemic inflammation is a key factor in the development of the metabolic syndrome, particularly in diabetes and cardiovascular diseases. Consumption of a whey based diet showed a significant decrease of oxidative stress, pro-inflammatory markers (TNF-alfa, IL-6) and an increase of the anti-inflammatory adiponectin. Also, these effects are enhanced regarding inflammatory stress associated with overweight and obesity (16).

Biological effects on the cardiovascular system

The extract from bovine milk has antihypertensive effects due to its very rich content in some bio-

active peptides derived from the beta-globulin and alpha-lactalbumin. These peptides are represented by alpha-lactorfin, beta-lactorfin and albutensin, which have the ability to stimulate the contraction of the smooth muscle, mainly because of their opioid effect. The mechanism of action consists of blocking the conversion of angiotensin I to angiotensin II. The conversion blockade is realized by inhibition of angiotensin-converting enzyme (ACE), which is the key enzyme in the regulation of blood pressure (19). Also, calcium is thought to play a role in the regulation of blood pressure. There are some studies which highlights that high intake of calcium and peptides from whey is associated with maintenance of blood pressure (19). Beta-globulin inhibits the absorption of cholesterol by changing its solubility in the small intestine. The reduction of cholesterol level decreases the risk of obesity or cardiovascular diseases (21).

Modulating the tissue and muscle regenerative response

Skeletal muscle mass is determined by the balance between the synthesis of new proteins and degradation (catabolism) of the existing proteins.

A lower rate in the protein synthesis is a key factor in the aging sarcopenia (6, 7). Regular whey protein supplementation in individuals with poor muscle mass promotes skeletal muscle accretion and enhanced function (15). A study from the beginning of 1990's found that the whole body protein synthesis was increased postprandial with 68%, after the ingestion of whey protein. Also, another study performed on the older men demonstrated that the consumption of 20 g of whey protein lead to postprandial muscle accretion after 6 hours (7). This effect was attributed to the high content in leucine of the whey protein. A study from the University of Colorado (7) suggests that whey protein has a direct role in strengthening and growth of the bone by stimulating the production of collagen. After tissue injury, an increase in the intake of dietary protein and water helps in the synthesis and repair of the connective tissues involved in the healing process. The study claims that a protein deficiency during injury delays healing and lengthens the inflammatory phase. Also, collagen is a glycoprotein formed within the human body, which forms the basis of all fibrous connective tissues. Collagen provides strength, elasticity and permeability to the blood vessels (7). Proline and lysine are the main amino acids contained in the whey protein, which helps cells to build collagen. Other studies show that whey proteins are capable to stimulate proliferation and differentiation of osteoblastic cells and reduce bone resorption (6, 7). In vivo studies (6) showed that whey protein has the ability to increase the femoral bone strength, as well as biochemical markers released from osteoblasts formation in ovariectomized rats. Clinical trials in humans suggest similar biological effects (6).

The biological consequences of whey intake in obesity

Dairy products consumption is inversely associated with the adiposity (18). Studies on diet induced obesity in experimental mice models demonstrated that animals fed with low calorie diet with whey protein supplementation for 50 days had a significant result concerning weight loss. Mice that consumed alfa-lactalbumin had a significant weight loss with the reduction of visceral fat during the caloric restriction.

Authors suggest that whey protein may reduce obesity *via* improvement of leptin sensibility, and beta-lactoglobulin may capture hydrophobic molecules, decreasing the absorbtion of fat by intestinal cells (18). Whey-based diet has an anti-adipose, anti-obesity effect. Whey protein is known for its anabolic effect on skeletal muscle, due to the high content of branched chain amino acids with greater effect on protein synthesis. This stimulates the growth of fatty acid synthesis in the muscles while decreases the fatty acids in the liver. Together with Ca^{++}, whey components have lipolitic effect on the whole body.

In dyslipidemia, the whey protein acts to decrease the total triglycerides in the blood and liver (10). In a controlled trail, supplementation with 56 g/day of whey protein concentrate for 6 months resulted significantly lower body weight, fat mass and waist circumference in overweight or obese individuals (10). Satiety is a key factor in the regulation of food intake. Whey protein and specific amino acids are involved in the control of gastric and intestinal motility and pancreatic secretion, which induce satiety. Whey proteins stimulate cholecystokinine and glucagon-like peptide (10). These are hormones secreted by the cells of the small intestine that has a function in satiety control. Authors observed a decrease in appetite, food intake after the consumption of 50 g of whey protein. This suggests a potential application of whey based food in appetite control in obese individuals (18).

The biological effects of whey in Diabetes mellitus

Insulin is an anabolic hormone that increases

the muscle protein synthesis. Insulin is sensitive to the composition and concentration of amino acids in the plasma, so the ingestion of whey leads to an increase in blood insulin level (20, 21). The proteins in the extract are capable of reducing serum glucose in healthy patients and to increase glucose tolerance in diabetic and obese patients. Consumption of milk protein has a demonstrated postprandial hypoglycemic effect (8). Stimulation of insulin secretion has a beneficial effect on the whole metabolism of carbohydrate, fat and proteins. Thus insulin acts directly in the synthesis of glycogen, triglycerides and intracellular proteins. In the same time, it contributes to the reduction of hyperglycemia and body prolonged exposure to high levels of glucose in the blood, which generates free radicals, resulting in micro and macro-vascular lesions. In individuals with type 2 diabetes, it acts by inhibiting the enzyme peptidyl transferase, the enzyme involved in the control of blood glucose levels (8, 20, 23). The use of protein extracts also has the capacity to stimulate B-cell activation and T cell suppression mechanism which inhibits the expression of mRNA of TNF- α (8). This mechanism is a key factor in the destruction of pancreatic ß-cells by inhibition of apoptotic Fas receptor production, which prevents cell death. In the same time, this mechanism restores pancreatic ß-cell morphological features, as a result of increased clearance of glucose. The final result leads to islet recovery and their ability to produce insulin (20, 21). Recent studies have shown the role of whey protein supplementation in glycemia control by stimulation of endogen hormones, increasing insulin release. In another study (21), the authors describe oral administration of whey protein and glucose by nasogastric tube in mice, in comparison to administration of glucose only. The result showed an increase in insulin levels three times greater and insulin tolerance four times greater with the consumption of whey protein. In clinical trials, a hypoglycemic effect of whey protein was noticed eight hours after consuming a meal that contained 45 g of protein in patients with type 2 diabetes (21).

The biological effects of whey in cancer

The dietary and antioxidant effect of whey stimulates cellular immune mechanisms in the fight against the malignant cells (1, 2). The anticancer and antitumor potential is given mainly by the glutathione (GSH) high level, which has the ability to increase cellular defense, stimulates increase of lymphocytes and Natural Killer cells number and detoxifies the body from carcinogens (2,3,4). The fact that GSH depletion can be deleterious for the malignant cells and, potentially, enhances the effectiveness of chemotherapy and/or ionizing radiations, is also known. The value of GSH depletion in sensitizing tumor cells to ionizing radiation was first demonstrated in human lymphoid cell lines. Achievement of the selective tumor GSH depletion under *in vivo* conditions is a pharmacological challenge (18). In addition, the mitochondrial GSH oxidation favors opening of the mitochondrial permeability transition pore complex, facilitating in this way the releasing of death-related molecular signals. GSH is also involved in regulating other types of cancer cell death, including the necrosis and autophagy (18).

Identification of the mechanisms controlling GSH homeostasis in malignant cells allowed elucidating a potential, GSH-depletion-based, strategy to improve the efficacy of the cancer therapy. A research in colon cancer has demonstrated the ability of the whey protein to significantly reduce the tumor growth. Whey protein acts as a cysteine delivery system, capable of inhibiting tumor growth. An additional source of cysteine can restore Natural killer cells activity.

Also, sulfur-containing antioxidants, such as GSH, N-acetylcysteine selectively induce p53-dependent apoptosis in altered malignant cells (2, 3, 4, 5). Lactoferrin is able to inhibit the metastatic spread of the primary tumor in mice. Bovine lactalbumin, which represents 10-15% of the total protein in the extract, inhibits the growth of breast cancer cells *in vitro.*

Incidence of tumors and metastasis is directly influenced by the immune system function of the body. Six amino acids in the composition of proteins identified in the extract (Table 3) are designed to improve the immune function, increase the synthesis of collagen and stimulate antitumor defense.

Numerous scientific experiments were conducted in order to establish the effects of whey protein supplementation in clinical trials in individuals with various cancer types (12). The types of tumors studied consisted of uterine carcinoma, metastasis of renal carcinoma, prostate cancer and metastasis of prostatic carcinoma. All human subjects have had significantly clinical improvements in terms of tumor reduction, metastasis decrease, improved the appetite and energy level. In some cases, the metastatic lesions disappeared in 2 months after administration of over 20 g of whey protein concentrates and cytology exams were absent of neoplastic cells (2).

The anticancer activity of bovine lactoferrin has been demonstrated in experimental lung, bladder, tongue, colon, and liver carcinogenesis on rats, possi-

bly by suppression of the phase I enzymes, such as cytochrome P450 1A2 (CYP1 A2) (13).

Also, in another experiment *bLF* did not show any toxicity, so *bLF* offers promise as a potential chemo-preventive agent for oral cancer.

In other studies conducted on different animal experimental models of carcinogenesis *bLF* increases the production of NK cells, CD8+, CD4+ şi ILFγ (13). It also has a role in the regulation of cytokine production (13). Lactoferrin increases the active form of IL-18, which has an anti-angiogenic effect.

Now, *bLF* is used as an ingredient in yogurt, chewing gums, infant formulas and cosmetics (13).

Table 3

The biological function of the amino acids from whey (Balch Phylis, 2006)

Aminoacids	Biological functions
Arginin	Role in muscle metabolism; - Immune stimulation; - Maintain a proper balance of nitrogen in the body, the role of excretion / elimination when in excess thereof; - Role in healing damaged tissues.
Glicine	- Prevents muscle degeneration, by increasing the synthesis of creatine; - Has a role in post lesion healing / tissue regeneration.
Lizine	- Role in the absorption of calcium in bone; - Role in bone growth and development; - Stimulates the production of antibodies, enzymes and hormones.
Fenilalanin	- Source for the synthesis of tyrosine; - Inhibits appetite; Reduce painful sensitivity; - Antidepressants and anti-obesity effects.
Treonina	- Role in maintaining homeostasis proteins; - Stimulates synthesis of collagen and elastin - Helps the liver to function Lipotropics
Prolina	- Stimulates the immune system - Role in the production of collagen - Contributes to the healing of cartilage and muscles.

CONCLUSIONS

• The bovine whey is composed by bioactive substances that can improve the functions of the immune system, the main effect being immunostimulatory, due to a high level of immunoglobulin, beta-lactoglobulin and alpha-lactalbumin. These proteins play a role in B-cell synthesis.

• The whey proteins have an anti-inflammatory effect by inhibiting TNF α and interleukins.

• Lactoferrin is an antimicrobial substance, having also antiviral and antifungal activity.

• The whey proteins, by immunostimulating ability, can be used both in the treatment of diabetes and the prevention of its complications.

• The glutathione is a very strong antioxidant, that has a healing effect in several diseases, being the main component able to exert anti-proliferative and anti-metastatic effect.

• The whey could be a functional food that can be included in many types of diets.

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