doi: 10.7251/ZSAN2302177S **Review paper**

POSSIBILITIES OF USING WHEY PROTEIN AS SUPPLEMENTS IN THE NUTRITION OF ATHLETES

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Abstract: *Proteins are the building blocks of the human body and they are needed to build: muscles, blood, internal organs, bones and nails, tendons, skin, hormones, enzymes, neurotransmitters and more. However, in the nutrition of top athletes, although high-quality proteins play an important role in the overall energy balance, as a rule, they cannot be taken into the organism in sufficient quantity with standard nutrition. Whey proteins are considered the most valuable proteins in the sense that they contain the most branched chain amino acids (BCCA) and a well-balanced ratio between essential amino acids, which makes them essential supplements in the nutrition of top athletes and recreational athletes. The big difference in the biological quality of whey protein, compared to other proteins, comes from the high content of lysine and the thioamino acids cysteine and methionine. The ratio of these amino acids is important for the utilization of protein in the body, which is far greater in whey proteins than in casein, egg, beef, soy, peas, etc. The concentration of leucine, isoleucine and valine (Branched-chain amino acids) is necessary during strenuous muscular work of athletes because, unlike others, these essential amino acids are directly metabolized and used in tissue building during exercise and conditional training. On the other hand, glutamine is an irreplaceable supplement for top athletes because during intense and strenuous sports training, its stores (which are about 60% of all free amino acids) are quickly used up, which leads to a drop in immunity and physical exhaustion. The aim of the paper is to present an overview of previous research related to the importance of whey proteins in nutrition, with a special emphasis on their potential and application as supplements in the diet of top athletes.*

Key words: *whey proteins, nutritional value, supplements, athletes*

Introduction

A proper balanced nutrition implies a balance between what the organism spend and what is fed into it through food. How nutrition for athletes will look like, depends on several parameters, primarily on the type of sport a person plays, personal characteristics and goals he wants to achieve. The required energy for athletes is directly related to the level of basal metabolism, the possibility of using food and the intensity of movement. Through individual menus, athletes should adapt their nutrition to their training and they need to consume all seven groups of foods that are represented in the nutrition.

Carbohydrates are the only ones that have the ability to burn an oxidative and anaerobic way, which is why they are an irreplaceable energy source for athletes. It is

not desirable that there is more than 25% fat in the food, of the total energy needs, because with a significant increase in fat at the expense of carbohydrates and proteins, the work of the muscles decreases. Also, athletes need to take enough vitamins, have a proper schedule of meals and compensate for lost fluids.

Proteins are certainly irreplaceable ingredients in the nutrition of athletes. Their role is very important either for results or for regular functioning of the body. Proteins are building substances because they are a structural component of the body and they participate in building muscles (myoglobin), hormones, plasma proteins (albumin, globulin, fibrinogen), antibodies, vitamins (tryptophan, niacin), bones, teeth, etc. [1,2] . In addition, proteins ensure cell growth and renewal, maintain acid-base and osmotic balance, and as enzymes they catalyze many biological and chemical reactions [2]. The natural sources of proteins are almost all groceries of plant and animal origin and the only difference between them is their amino acid content. Amino acids can be essential or non-essential. Animal origin proteins are rich in all essential amino acids and they are considered to be the complete proteins with high biological value. On the other hand, foods of plant origin do not have a complete amino acid composition (except soy) and such foods provide only quantity, i.e. they satisfy nitrogen needs [1,2]. There are twenty one known amino acids in the nature out of which only eight are essentials: phenylalanin, tryptophan, leucine, isoleucine, valine, lysine, threonine and methionine, while histidine is essential for athletes and kids. These amino acids cannot be synthesized in the body and must be obtained through food. Whey proteins represent an ideal supplement to a properly balanced nutrition and should be included in the daily nutrition plan, especially for people who have an increased need for proteins, which certainly includes top athletes [3]. Compared with some other proteins that are processed in the liver, whey proteins are directly metabolized in the muscle tissue [4], which enables fast muscle recovery after training. On the other hand, although they are a good source of energy, whey proteins regulate body composition and it is not possible to get fat deposits from them. According to Morr [5], whey protein is a complex protein composed of smaller parts of β-lactoglobulin protein (50% of total whey proteins) and α-lactalbumin (20%). The following are immunoglobulins [6,1], glycomacropeptides, lactoferrin and other smaller peptides such as lactoperoxidase, lysozyme, etc. [6, 7]. Each of these small fractions has its own unique biological properties depending on the amino acid content. The positive influence of whey proteins was proved with the kids, adolescents, pregnant women [7], and a related decline in functional fitness [8].

The aim of this paper was to give a brief overview of the current knowledge about the potential properties of whey proteins and the possibility of their application as supplements in the nutrition of top athletes.

Biological value of whey protein

The biological value (BV) of a protein is the protein capability to be completely converted and synthesized into tissue protein, which depends on the content and arrangement of essential amino acids in it. The higher biological value of proteins the better is absorption of proteins which supply more amino acids for the body needs [9, 10]. The biological values of plant proteins are of lower values because they are in

deficit in some essential amino acids, and for that reason they are considered as incomplete proteins. Foods of animal origin (meat, milk, eggs) are high-quality or complete proteins with a biological value close to 100% [11]. The biological usability of whey proteins is much higher than in casein, beef and chicken meat, soy, peas, etc. (Table 1).

Table 1. The biological value of different proteins

Proteins	Biological Value (%)
Whey Protein	104
Whole Egg	100
Fish	83
Beef	80
Chicken	79
Casein	77
Soya	74

When the individual milk proteins are discussed, their actual digestibility is very similar, however, the biological value, as well as other indicators of nutritional value, are higher for whey proteins (100%) than for casein (77%). This is due to a more favorable composition of amino acids, thanks to the most valuable α-lactalbumin (104%), whose amino acid composition (lysine, leucine, threonine, tryptophan and cysteine) is close to the biological optimum [12]. As the cystine/methionine ratio increases, the utilization of protein in the body also increases [13]. Also, the high content of lysine (40% more in whey, than in milk) and thioamino acids (2.5 times more in whey) contributes to the higher biological value of whey proteins compared to milk proteins. The differences in the BV of various whey proteins depend on the method by which they are obtained. Cold process production is of the highest quality as proteins denatured at high temperatures, and their efficiency decreases which causes the decrease in biological value.

Today, it is known that the most valuable whey proteins are obtained by the cold process, ion exchange and microfiltration. Heat treatment of whey causes changes in the structure of amino acids, whereby a significant part of cysteine, lysine and arginine is destroyed, and the availability of other amino acids is also significantly reduced (e.g. lysine, proline, glutamic acid, threonine, alanine, glycine and serine). The amount of amino acids in g/100 g of whey protein is shown in Table 2.

The health value of whey protein

Due to their amino acid composition, whey proteins, in addition to their high nutritional value, also exhibit significant health properties, which is why some products based on whey proteins can be characterized as functional food. Certain whey proteins, such as immunoglobulins and other glycoproteins (lactoferrin, transferrin), and the enzymes lysozyme and lactoperoxidase protect the organism from

bacteria, viruses and infectious diseases, and they are used for the treatment of even the most serious diseases [14].

Table 2.Amino acid composition of whey protein (g/100g)

Amino Acid	Whey Protein	Amino Acid	Whey Protein
Alanine	3.5	Lysine	7.5
Arginine	2.3	Methionine	1.6
Aspartic Acid	8.4	Pheniylalanine	2.6
Cystine	1.7	Proline	6.6
Glutamic Acid	13.3	Serine	4.6
Glycine	1.4	Threonine	4.5
Histidine	1.6	Tryptophan	1.3
Isoleucine	4.6	Tyrozine	2.3
Leucine	8.8	Valine	4.4

It was established, that whey protein is effective in lowering blood sugar, increasing insulin levels and sensitivity to its effects [15]. In addition, whey is also a significant source of biologically active peptides, which mostly represent decomposition products of glycomacropeptides that exhibit favorable therapeutic effects [16]. It has been proven that the use of whey protein in nutrition, thanks also to lactoferrin [17] and lactalbumin [18], contributes to the prevention of certain forms of cancer. There are more and more studies that indicate the potential power of whey protein in the fight against HIV [19], in reducing the impact of stress, increasing serotonin in the brain, improving liver functions in people suffering from some forms of hepatitis, reducing blood pressure and preventing osteoporosis. One of the most significant effects that whey proteins cause in the organism is their ability to raise the level of cellular glutathione [20], considering that it is the most important water-soluble antioxidant found in the body. A reduced level of glutathione in the organism is closely related to a number of degenerative conditions and diseases (atherosclerosis, cataracts, cystic fibrosis, aging, Alzheimer's, Parkinson's and Crohn's disease, etc.). New possibilities of using whey protein in the treatment of obesity, goiter, kidney disease, etc. are also being investigated.

Protein requirements for athletes

Carbohydrates, fats and proteins are macronutrients that supply the organism with energy and they affect on various physiological functions of the organism [21]. Optimum energy intake is crucial for maintaining body mass, increasing muscle mass or maintaining good condition. Given that they are exposed to intense physical activities that cause physical and psychological stress, great metabolic changes occur in the athletes' bodies. Because of this, athletes have a higher energy consumption, which means that they need more food, but also food richer in nutrients [22]. The total daily energy needs of athletes who participating in team sports and endurance and strength sports are 10460-16736 kJ for women and 12552-25104 kJ for men.

The exact protein needs for each athlete are different and depend on age, gender, nutritional status, physiological state, and of the type, intensity and duration of their training. Contrary to belief, studies have shown that beginners compared to other athletes (exercisers) have a slightly higher need for protein, per kilogram of body weight (BW). When an individual begins to train, his needs increase due to an increase in protein decomposition and synthesis [23]. After about three weeks, the body adapts to exercise and becomes more efficient at conserving protein. The type of physical activity determines what type of protein will be built.

Strength training (with load) increases the amount of myofibrillar proteins (actin and myosin), i.e., it causes muscle hypertrophy and an increase in strength, and optimal protein intake is especially important in the early phase of strength training [24].

Dernling and DeShanti, [25] observed that athletes who participated in weight trainings and who used whey protein supplements (1.5 g/kg BW/day) for 11 weeks, show improvements in strength and lean body mass compared to groups supplemented with carbohydrates, creatine, or a combination of creatine and whey protein supplementation.

Endurance training results in an increase in oxidative capacity, i.e. muscle resistance to fatigue, through an increase in mitochondrial volume and density [26]. Just as in strength sports, exogenous essential amino acids are required to recover from muscle damage induced by endurance exercise [27]. Sports such as marathons and triathlons do not require a lot of muscle mass, but the long duration and high energy demands of those sports often exceed the immediate availability of carbohydrate and fat stores. A part of the energy in training must be compensated by the oxidation of proteins (amino acids).

According to the RDA (Recommended Dietary Allowances), healthy adults who do not engage in sports should consume 10-15% of their total energy intake, which corresponds to 0.8 gr/kg of BW. However, recent papers, which are based on better methodology, call for raising the recommendation to 1.0 g/kg of BW [28]. For recreational players, protein needs are 1.0-1.5 g/kg of BW, and for top athletes, on average, 1.2-1.8 g/kg [29]. Thus, the recommended intake of protein in strength sports is around 1.6g/kg of BW [24], while some studies show that the protein intake, required to maintain balance in endurance sports, ranges from 1.2 to 1.4 g/kg of BW [30]. Particularly expressed needs for proteins (over 2.0 g/kg of body mass) refer to athletes in aerobic endurance sports (long-distance athletes), weight lifters (strength athletes) and bodybuilders whose intense training produces a significant degree of muscle catabolism. However, the highest protein intake was recorded in hockey players, 2.2±0.5 g/kg/day. The lowest intake is in wrestlers of 0.96±0.6 g/kg/day. That's why complete addition (supplementation) of protein consumption is one of the most important pre-conditions for meeting all the needs of organism in building muscle tissue, and whey proteins have been considered to be one of the best sources of proteins for long time. Without regards to their importance, we should never over use the proteins. This could have a negative effect on health because the end product of decomposition is ammonia, which is toxic in large quantities.

The importance of whey protein for the resorption and building of muscle mass in athletes

Whey proteins have long been considered the "gold standard" and irreplaceable supplements in the nutrition of top athletes [3]. Due to the rich source of branched chain amino acids: leucine, isoleucine and valine (BCAA-branched chain amino acids), whey proteins are an ideal supplement for improving physical constitution and muscle mass by participating in the production, maintenance and repair of muscle tissue [31]. In a study on BCAA intake required to maximize muscle function, it was shown that at least 5 g (0.077 to 0.087 g/kg) is necessary of BCAA, before and after each exercise and a Leu:Ile:Val ratio should be 2:1:1 [32]. Namely, these amino acids, especially leucine, help in the regulation of protein metabolism by stimulating their synthesis and preventing protein decomposition, i.e. catabolism [33]. The amino acids just mentioned can be directly metabolized in the muscle tissue and the muscles primarily use them during exercise and conditioning training [34].

In fact, skeletal muscles take up BCAAs from the blood and they decompose them into glucose for energy. Therefore, BCAAs are unique among amino acids in their ability to provide a source of energy during endurance exercise [35].

Different forms of exercise affect the muscles in many different ways and stimulate the processes involved in building and decomposition of muscle tissue. Thus, endurance exercises and long-term aerobic activity exercises lead to catabolic processes that cause muscle protein degradation and reduce the rate of protein synthesis [36]. On the other hand, exercises that include loading are unique compared to other forms of exercise, because they affect the increase of protein synthesis and the building of muscle mass. However, even this form of exercise, due to excessive volume and poor nutrition and supplementation, can lead to muscle breakdown. Balancing the stress caused by training, through rest and nutrition, is very important for the positive direction of protein synthesis. Intake of protein in the form of supplements additionally helps with recovery, building and development of muscle tissue, and whey proteins have the highest biological value and absorption capacity [34]. Whey proteins are highly soluble, easily digested and absorbed in the body in high concentration. They belong to fast-dissolving proteins, because they provide significant nutrients to the muscles in a short time. These proteins are also a strong support for immunity because they raise the body's level of glutathione, which is a strong antioxidant. The antioxidant effect of glutathione is manifested thanks to the increased amount of cysteine in whey proteins, which is found in glutathione in the form of tripeptide amino acids: L-cysteine, L-glutamine and glycine. L-cysteine is the main source of free sulfhydryl group in glutathione and is a crucial factor in its synthesis [20]. Glutathione, in addition to increase immunity, is considered essential for preventing oxidative stress and improving the general state of the body. Strong trainings and exercises reduce the amount of glutathione, and whey proteins compensate for it [37]. In general, by using whey protein, the body is supplied with the best possible amino acid profile and low carbohydrate content, protein synthesis is accelerated, anabolic and anti-catabolic effects occur, recovery time from strenuous training is shortened, body mass is lost, etc. Athletes are recommended to use whey

proteins, as well as other supplements, selectively and sparingly under the mandatory supervision of a professional.

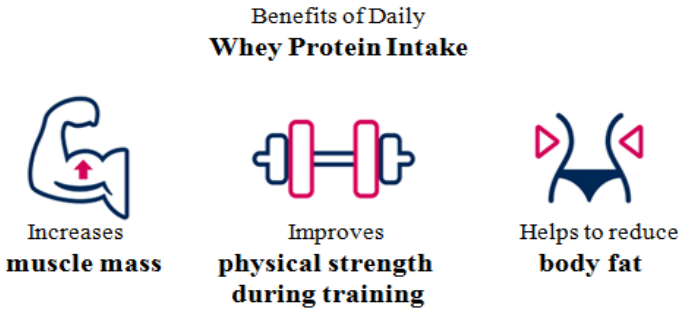

Figure 1. Benefits of daily intake of whey protein
https://www.agropur.com/us/news/Advantages-of-whey-protein-for-athletes

Dietary supplements based on whey protein in the nutrition of athletes

Whey proteins, which are widely used as supplements in the nutrition of top athletes, can be obtained on the market in several different forms, of which the most common are whey protein concentrates (WPC), whey protein isolates (WPI) and whey protein hydrolysates (WPH) [38] and more recently native whey protein (NWP) [39].

The main difference between the types of whey protein supplements is the speed of absorption (the hydrolyzate is absorbed the fastest, then the isolate and finally the concentrate), but in terms of the effect on muscle development and recovery, there is practically no difference between whey protein concentrate and isolate.

Whey protein concentrates (WPC) have a wide variation in protein content ranging from 35-80% [40,41], with reduced amounts of lactose and fat. Although the concentrate contains a lower percentage of protein compared to the isolate, the high-quality concentrate contains a variety of beneficial components that are rarely found in isolates. A good concentrate has a much higher proportion of growth factors, such as IGF-1, TGF-alpha and TGF-beta. It also contains several different bioactive lipids, such as linoleic acid, and often a higher level of immunoglobulins, lactoferrin and glutathion. Whey protein concentrates are a relatively cheap and easily available source of whey protein, which consists β-lactoglobulin (9.8%), α-lactalbumin (3.7%), serum albumin (1.2%), immunoglobulin (2.1 %), proteose and peptone (2.4 %) [42]. Different techniques are used for its production, such as ultrafiltration and diafiltration [42], reverse osmosis, electrodialysis, microfiltration, dialysis, techniques based on sedimentation, or complexation with different reagents, and physical and chromatographic separation techniques [43]. The composition, quality and functional characteristics vary depending on the quality and whey origin, preparation procedures, as well as conditions and storage [41]. The use of whey protein concentrate is recommended between meals and before bedtime when the main aim is to maintain a

high intake of amino acids in the blood for a long time. WPC can be used as a supplement before and after training, or as a protein snack between meals.

Whey protein isolates (WPI) are most often obtained using ion exchange chromatography [43] and microfiltration (Flow microfiltration procedure), and their main characteristic is the high content of protein, more than 90% [40]. Passing through an ion exchanger protein molecules are absorbed and deproteinized whey is isolated. With this procedure, isolates can be obtained that contain up to 95% protein [44], they do not contain impurities, but they do contain more BCAA and calcium.

Whey protein isolates are characterized by more favorable functional characteristics compared to concentrates, considering that they contain smaller amounts of salt, lactose, fat and ash. During the production of whey protein isolate, special attention should be paid to preventing the removal of biologically active components of whey. Because of relatively high cost of production, their application is still limited. This protein is practically without carbohydrates and fats, it is excellent for the pre-competition period where strict attention is paid to the intake of carbohydrates and fats.

It is used mainly by people who want to improve performance, but also by people who are on a diet without carbohydrates. The use of whey protein isolate is recommended immediately after waking up when the level of amino acids in the blood is low and after strenuous training when it is necessary to protect the body from catabolism and start anabolic processes.

Whey protein hydrolysates (WPH) have a protein content greater than 95% [40]. They are produced by enzymatic decomposition at a controlled temperature and pH value, from raw materials such as whey protein concentrates or lactalbumins. Proteolytic enzymes split the bonds between amino acids, resulting in peptides with different numbers of amino acids and free amino acids. Native whey protein (NWP) is a relatively new whey product obtained by filtration of unprocessed raw milk [39]. Native whey differs from typical WPC and WPI by not containing glycomacropeptides and maintaining a higher leucine content than conventional WPC or WPI [39].

The different types of whey protein and their uses are shown in Table 3.

Functional food with high whey protein content

Functional food is an integral part of the usual human nutrition and in its form is similar to conventional food. However, due to the content of bioactive compounds, functional food also has psychophysical benefits where, in addition to basic nutritional functions, it reduces the risk of chronic diseases [46]. Functional food certainly includes food with nutritional and health claims where health claims must be supported by scientific and clinical research [47]. Whey proteins as a functional food can be taken in different ways. What all products have in common is that they are an excellent source of protein and they contain few carbohydrates, but at the same time they are nutritionally balanced. In addition to supplying the necessary energy, the high amount of protein also helps in building muscle mass and protecting muscle tissue.

Table 3: Various types of whey protein and its uses (% w/w) [45]

Product	Protein Concentration	Lactose	Fat	Notes and Applications
Whey powder	11-14.5%	63-75%	1-1.5%	Produced by taking whey directly from cheese production, clarifying, pasteurizing and drying. Used in breads, bakery goods, snacks, dairy foods.
Whey protein concentrate (WPC)	25-89% (Most Commonly available as 80%)	4-52%	1-9% (as protein concentration increases, fat, lactose and mineral content decrease)	The most common and affordable form of whey protein. Used in protein beverages and bars, bakery goods, dairy, confectionary products, other nutritional food products.
Whey protein isolate (WPI)	90-95%	0.5-1%	0.5-1%	Used in protein supplementation products,protein beverages, protein bars, other nutritional food products.
Hydrolyzed whey protein concentrate	> 80% (hydrolysis used to cleave peptide bonds)	< 8%	< 10% (varies with protein concentration)	Used in sports nutrition products.
Hydrolyzed whey protein isolate	> 90%	0.5-1%	0.5-1%	Highly digestible form containing easy-to digest peptides that reduce risk of allergic reaction in susceptible individuals. Commonly used in infant formulas and sports nutrition products.

The classic way to take protein powder is to mix it with water or milk and make ready-made protein drinks for consumption after training or at any time of the day. They are also excellent in oatmeal or other porridges, in doughs for desserts full of protein, for various shakes, and they can be added to various smoothies or coffee. In addition, whey proteins can be added to commercial protein bars that represent a light and tasty protein snack that is appreciated by athletes.

However, functional foods with high content of whey protein, are usually dairy-based, such as yogurt, cottage cheese, and ricotta. These products, besides of whey protein content, which help muscle recovery during exercise, are also a good source of calcium and vitamins A and B12. On the other hand, they are low in fat and calories,

making them a good supplement to a healthy nutrition. The main carriers of the production process of fermented milk drinks, in addition to the raw material itself, are lactic acid bacteria (LAB) [46]. If whey proteins in the form of concentrates and isolates are added to such products, functional products with added value are obtained. This topic was researched by Stijepić et al.[48, 49, 50], Glušac et al. [51], Stokes et al. [52], Carbone and Pasiakos [53], Skrzypczak et al. [54], Gomaa et al. [55] and others. In the nutrition of athletes, special attention is paid to the importance of consuming probiotic yogurt, given that the increased intensity of training has a direct impact on the athlete's immune system, so the consumption of probiotics is especially useful in the winter months, when the exposure to respiratory infections is greater. Stijepić et al. [56, 57, 58] examined the possibility of producing yogurt enriched with probiotic cultures (*Lactobacillus* sp. and *Bifidobacterium* sp.) with the addition of isolates and concentrates of whey protein, cow and goat origin.

If honey is added to such a drink, it is additionally enriched with many different ingredients such as vitamins, minerals and substances with antioxidant activity (phytochemicals), which are not naturally present in whey (48, 49, 50, 51, 56, 57, 58).

Conclusion

By using whey protein, the organism is supplied with the best possible amino acid profile and low carbohydrate content. In athletes, protein synthesis is accelerated, anabolic and anti-catabolic effects occur, recovery time from strenuous training is shortened, health status is preserved and improved, body mass is lost, etc. Athletes are recommended to use whey proteins, as well as other supplements, selectively and sparingly under the mandatory supervision of a professional.

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MOGUĆNOSTI KORIŠTENJA PROTEINA SURUTKE KAO SUPLEMENATA U ISHRANI SPORTISTA

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Sažetak: *Proteini su gradivne jedinice ljudskog tijela te su potrebni za izgradnju: mišića, krvi, unutrašnjih organa, kostiju i noktiju, te tetiva, kože, hormona, enzima, neurotransmitera i drugo. Međutim, u ishrani vrhunskih sportista, iako kvalitetni proteini igraju važnu ulogu u ukupnom energetskom bilansu, oni se po*

pravilu standardnom ishranom ne mogu unijeti u dovoljnoj količini u organizam. Proteini surutke se smatraju najvrednijim proteinima u smislu da sadrže najviše aminokiselina razgranatog lanca (BCCA) te dobro izbalansiran odnos između esencijalnih aminokiselina, što ih čini osnovnim suplementima u ishrani vrhunskih sportista, ali i rekreativaca. Velika razlika u biološkom kvalitetu proteina surutke, u odnosu na druge proteine, potiče od visokog sadržaja lizina i tioaminokiselina cisteina i metionina. Odnos ovih aminokiselina bitan je za iskorištenje proteina u organizmu koji je u surutkinim proteinima daleko veći nego u kazeinu, jajetu, govođem mesu, soji, grašku i dr. Koncentracija leucina, izoleucina i valina (aminokiseline razgranatih lanaca) je neophodna pri napornom mišićnom radu sportista jer se, za razliku od drugih, ove esencijalne aminokiseline direktno metaboliziraju i koriste u izgradnji tkiva tokom vježbanja i kondicionih treninga. S druge strane, glutamin je nezamjenljiv suplement kod vrhunskih sportista jer se pri intenzivnim i napornim sportskim treninzima njegove zalihe (kojih je oko 60% od svih slobodnih aminokiselina) brzo troše, što dovodi do pada imuniteta i fizičke iscrpljenosti. Cilj rada je da se prikaže pregled dosadašnjih istraživanja vezanih za značaj surutkinih proteina u ishrani, sa posebnim akcentom na njihov potencijal i primjenu kao suplemenata u ishrani vrhunskih sportista.

Ključne riječi: *proteini surutke, nutritivna vrijednost, suplementi, sportisti*