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Review article

PROTEIN SOURCES AND THEIR SIGNIFICANCE IN CARP (*Cyprinus carpio* L.) NUTRITION

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Abstract: Common carp (*Cyprinus carpio*) is one of the most widely cultured fish species in the world. It is predominantly cultivated in the Balkans in the semi-intensive system that is based on the utilization of natural food from the fish pond and supplemental feed (cereals, pelleted and extruded feed). Currently, an intensified type of semi-intensive production is starting to be more present in Serbia, where cereals, as the most common supplemental feed, are replaced by concentrated feed that fill in the lack of proteins from carps' natural food in periods of its decreased production. The nutritional requirements for growth, reproduction and normal physiological functions of fish are similar to other animals, but generally fish need more proteins in their diet. Due to this, the efficiency in the use and utilization of proteins is more significant for fish than for other animals. The selection of supplemental feed in semi-intensive system of fish culture depends on the natural potential of the fish pond, culturing period, fish category, price and quality of feed.

Key words: common carp, protein sources, semi-intensive system.

Introduction

Fish production in Serbia is mostly based on carp (warmwater) and trout (coldwater) fish farms. Between 13,500 and 14,000 ha of land is used as fish farms, consisting of 99.9% of carp and 0.01% of trout farms (Marković et al., 2009). In aquaculture there are three types of production systems depending on the type of fish feed, stocking density and application of ichthyological and agrotechnical measures: extensive, semi-intensive and intensive. The extensive system is scarcely present at carp fish farms and represents only 1 to 2% of the total fish production. During the production cycle, fish are not fed with supplemental feed, and depend mainly on the natural production of the fish pond, available plankton, benthos and water plants. This type of fish production is characterized by low stocking density, low production expenses and low yield.

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Semi-intensitve production system is the dominant type of carp production in the world (Tacon, 1993). According to Marković et al. (2009) more than 80% of produced fish in Serbia are produced in this type of system, with common carp being the main species representing 80% of the total fish produced in polyculture. It is based on the utilization of natural food from the fish pond as a high quality protein component of the diet (Marković et al., 2003). The production of natural food is increased by the application of agrotechnical measures as: drying out the fish ponds during the winter, soil treatment, fertilization etc. For fulfilling the energy resources, in the traditional semi-intensive system, fish are supplied with carbohydrate feed in the form of grains (corn, wheat, barley, etc.) (Marković et al., 2002).

The intensive system of carp production in Serbia is rarely present, usually as culturing carp fish fry. It concerns high stocking density on a relatively small production surface where fish are fed only supplemental compound feed.

In intensive and, during recent years, in semi-intensive production in Serbia, feed expenses present 40 to 60% of the total production cost. Having in mind the economical and environmental aspects, it is important to use fish feed that has low conversion rate, high growth rate and that provides good health, high quality of fish meat and has a low impact on the environment (Jahan et al., 2003).

Nutrient requirements for growth, reproduction and normal physiological functions are similar to other animals, but fish have much higher requirements in proteins, so feed mixtures with 25 to 45% of raw proteins are mainly used (Davies and Gouveia, 2010). Given that the general aim is a global increase of aquaculture production, there is a need for introduction of different components in feed mixtures and their maximal utilization (Tacon, 2005; Kraugerud, 2008).

Different animal and plant components are used in the production of fish feed. From the nutritional aspect, the components of animal origin (mainly fish meal) have the best nutritional potential but are the most expensive. Ingredients of plant origin, as soy meal, have a reasonable price and are continuously available on the market (Storebakken et al., 2000).

For some species and categories of fish, appropriately prepared meals are the most important precondition for good growth, thus, during making formulations, except providing enough proteins, it is very important to ensure a specific level and share of certain amino acids (Watanabe, 2002; Ruohonen and Kettunen, 2004). Optimally balanced proteins in feed for animals should meet the requirements and provide enough amino acids, that are neither in surplus nor in shortage.

In terrestrial animals isoleucine, leucine and valine can have an antagonistic effect when their ratios are not adequately balanced (D'Mello, 2003). However, antagonism of amino acids in fish, specially their effects on growth rate and utilization of nutrients, is not completely understood and currently only some inconsistencies are reported (Yamamoto et al., 2004).

For fish and other animals, the feed with a balanced or optimal share of amino acids in mixtures is of great significance for the maximal protein utilization (Green and Hardy, 2002). There are two conditions that should be fulfilled in order to have a good balance of amino acids in the feed: optimal ratio of essential amino acids (EAA) and optimal ratio of essential and nonessential amino acids (NEAA).

Protein sources

In the semi-intensive system of culturing, the nutritional requirements of carp are fulfilled from two sources: natural food and supplemental feed.

Natural food for carp in fish ponds is mainly represented by zooplankton (*Rotifers*, *Cladocerans* and *Copepods*) and zoobenthos (mostly *Chironomids* and *Oligochaeta*). Natural food is a valuable source of proteins, free amino acids and oligopeptides, fat and fatty acids and vitamins that are essential substances for growth and development of fish (Kirbia et al., 1997). The production of natural food is increased by applying agrotechnical measures such as drying out of fish ponds during winter, soil treatment and fertilization (Marković et al., 2000, 2005). However, during the season of rearing carp, there is a distinctive seasonality in the natural food production. In our climatic region, the maximal production of zooplankton and zoobenthos biomass is in spring, but during midsummer, when the water temperatures are optimal for carp culturing, a significant decrease of natural food occurs (Dulić, 2007; Dulić et al., 2009).

Except as a protein source for all fish categories, natural food, especially zooplankton, is used as a feed for larvae of fish and crustaceans in semi-intensive and intensive systems of culturing. Out of an array of potential zooplankton organisms, currently mostly in use are two species of Rotifers, *Brachionus plicatilis* and *B. calyciflorus*, and naupliar larvae of species *Artemia salina* (*Branchiopoda*, crustaceans). These organisms are chosen primarily for their dimensions (*Brachionus* 50 to 200 µm, *Artemia* 400 to 550 µm) making them an ideal meal for larvae of different fish species. Culturing of these organisms is relatively easy (Lavens and Sorgeloos, 1996; Moretti et al., 1999). Nevertheless, a lot of research has been done showing that these organisms by their nutritive characteristics and specially by promoting a visual stimulus in fish larvae, due to their constant locomotion, are decisively better than an artificial feed (Rønnestad et al., 1999; Kolkovski, 2001). The negative side of live feed, particularly for marine fish, is its high prices that can occasionally be as high as 79% of the total production cost. During the last 20 years, this has initiated a lot of investigation on new alternatives for live feed, mostly as microparticulate diets (MPD) (Callan et al., 2003). Even though a lot of work is done in this area, still an ideal MPD that gives the same results in fish growth rate as live feed has not been yet formulated (Lazo et al., 2000).

The selection of supplemental feed in semi-intensive system of production depends on the nutritional requirements of fish species, availability, price and quality of feed (Tacon, 1993).

In the production of aquafeed different components of animal and plant origin are used. Concerning the nutritive aspect, components of animal origin, especially marine, have the best nutritional potential. These feeds have been used for decades with no alternatives since they provide essential amino acids. They have proteins of high biological value. They are of different origin and are mostly dairy by-products, by-products of slaughterhouses and fish processing. They are considered as the most expensive feed, therefore should be effectively used. Lately, their use has been reduced due to an effective balancing of meals and introduction of synthetic amino acids.

Fish meal is considered to be the best source of proteins in feed for fish, due to its high content of high quality proteins, essential amino acids, high content of phospholipids and essential fatty acids, high level of digestibility and palatability (Barlow, 2003). The limiting factors in applying fish meal in the production of aquafeed are high prices, limited resources and microbiological quality (Du and Niu, 2003). Data from FAO (2006) show that the total world production of fish meal in the last two decades was around 6 to 7 million tons and for fish oil 1.2 to 1.4 million tons. From the total production of fish meal, 46% is used in aquafeed production, and from the total production of fish oil, 81 % is used in the fish feed industry. In the year of 2004 around 19.5 million tons of aquafeed was produced, with feed for Cyprinids' comprising 45% (Tacon, 2005). The protein content in fish meal varies from 57 to 77%, and due to good quality the digestibility of these proteins ranges from 92 to 95%. It is rich in essential amino acids, especially in lysine, cystine, methionine and tryiptophan, representing a very valuable supplement to meals based on cereal grains, especially corn. Fish meal has a high content of mineral matter (10-22%) of high nutritional value, since they have 3-6% of calcium, 1.5-3% of phosphorus and a number of desirable microelements, as manganese, iron and iodine. They are a good source of vitamin B complex, especially holin, vitamin B12 and riboflavin and have an increased nutritional value due to the presence of growth factor, known as animal protein factor (APE).

Krill is a group of marine crustaceans that have a high potential as a valuable ingredient for the production of commercial feed, not only for fish. The most used species in aquaculture currently is an Antarctic species *Euphausia superba* that showed an excellent performance test in salmon, trout and other coldwater species. Krill meal is nutritionally well balanced with a high protein content and excellent profile of amino acids that are necessary for coldwater fish species. Krill is a good feed attractant, has an excellent palatability and is a natural source of carotenoid pigments, minerals, essential fatty acids as well as a component that increases survival rates of fish larvae. It is a good source of phospholipids and HUFA, as eicosapentnoic acid (C20:5n-3) and dokosaheksenoic acid. (C22:6n-3). A high level of fluorine (around 1500 ppm) and copper (around 75 ppm) can partially limit its use. Studies on salmon showed that at least 30% of krill meal can be used in feed without deposition of fluorine in tissues (Albrektsen, 2007).

Rendered (recycled) by-products of terrestrial animals have had an application in the production of feed for aquaculture for many years. These by-products include lipids-industrial fat (suet), edible beef suet, fat, yellow fat, animal protein meal-meat and bone meal (MBM), meat meal, hydrolyzed feather meal, poultry by-product meal, blood meal and specialized mixtures as well as other products-liver meal, lung meal, egg powder. Rendered products are a cheap source of good quality proteins, a good source of lysine, sulfur amino acids, histidine, arginine, phosphorus (El-Sayed, 1998). Animal by-products are very palatable for many fish species (Otubusin et al., 2009). Their application was limited in the '70 and '80 due to the studies that indicated low digestibility in fish, but new technologies of processing of rendered by-products showed a significant increase of digestibility providing an increase in their use in fish feed production (Cho et al., 1982; Gill, 2000).

Feed of plant origin has a big potential for a wide application in fish feed industry: they are a cheaper source of high quality proteins compared to proteins from animal sources, there is a global bioavailability and they are relatively easily renewed. On the other hand, plant feeds have the limited application due to several reasons: the lack of some essential amino acids (lysine, trypsin, sulfur amino acids, methionine and cystine), the presence of antinutritional factors and palatability, etc. (Fransis et al., 2001).

The substitution of fish meal by plant proteins in fish feed was and still is a topic in many studies in the area of fish nutrition (Viola et al., 1982; Pongmaneerat et al., 1993; Uran et al., 2008). However fish meal and plant protein sources are very different in the amount of proteins, structure of amino acids, energy availability and amount of mineral matter.

Soy meal is the most important feed of plant origin in the fish feed industry. It is a source of biologically valuable proteins that are very similar in the structure of amino acids to proteins of animal origin (Watanabe, 2002). The nutritional value of soy is determined by its amino acid composition and content of antinutritional factors that depends on the soy cultivar, cultivation conditions, soil etc. (Vucelić-Radović et al., 2005). The most used soy products in production of feed for aquaculture are oil-free soy meal and soy protein concentrate. According to Gatalin (2002), other products such as high fat soy meal, mechanically extracted soy cake, soy flour extracted from solvent are used depending on the technology of production. Soy flour lacks in lysine, methionine and cystine which can be overcome by the addition of synthetic amino acids or by a compatible combinations with other feeds. It is also deficient in the content of vitamin B complex.

As well as in most of other oilseeds, soybean consists of a number of toxic, stimulative and inhibitory substances, as allergens, goitrogens and anticoagulation factors. Antinutritional factors are a group of 'resistant substances' present in plants, but undesirable and toxic to animals. Depending on the effect, Francis et al. (2001) divided antrinutritional factors into: those which have an effect on the utilization of proteins and digestion (protease inhibitors, tannins), on the utilization of minerals (fitates, gossypol pigments,), antivitamins and other substances (saponins, alkaloids, photosensitive agents). Saponins are a big problem since their presence in water has a significant toxic effect on fish because it damages the respiratory epithelium of gills (Francis et al., 2001). In addition, they cause damage to intestinal epithelium by building complexes with enzymes and thus preventing the digestion and absorption of amino acids.

The most heat-labile anti-nutritional factor is trypsin inhibitor that inhibits the action of trypsin and himotripsine reducing protein digestibility and growth rate of rainbow trout (Olli and Krogdahl, 1994), carp (Viola et al., 1992), and channel catfish (Fagbenro and Davies, 2001).

The study on different levels of protein digestibility of soybean meal in feed for carp of 5.4 to 33.6% indicated a high degree of digestibility, 82 to 94.5%, indicating that the protein digestibility of soy flour does not affect the protein level (Watanabe, 2002). The best results in growing carp are achieved when up to 56% fish meal is replaced by soybean products (Pongmaneerat et al., 1993). Larger share of soybean meal in combination with a small amount of fish meal and a larger quantity of corn in the mixture, results in lower growth rates of fish (Stanković et al., 2009). Replacement of fish meal to a greater extent (78, 89 and 100%) reduces growth and feed utilization.

Single-cell proteins are produced from unicellular organisms. They can be produced using non-photosynthetic and photosynthetic organisms. In recent years there has been a considerable global interest in using microbial fermentation for the production of proteins. Unicellular organisms as yeast and bacteria have a fast growth and can double their biomass in industrial fermentors in just 3 to 5 hours (Ratledge, 2004). According to Skrede et al. (1998), the flour produced from bacterial biomass is an ingredient with the amino acid composition closest to fish meal. Nutritive value of proteins from the flour of bacteria was confirmed by many researchers examining trout and other cultivated animals (Storebakken et al., 1999). Some studies suggest that the amino acids of unicellular proteins can be suboptimal because of their unbalanced ratio that has resulted in lower growth in tilapia (Davies and Wareham, 1988). It was found that the single-cell proteins are deficient in amino acids that contain sulfur, and perhaps in isoleucine. Although the amino acid profile of unicellular proteins is more balanced than the profile of cereal grains, they are certainly inferior to protein supplements. This can often be eliminated when adding methionine. Murray and Marchant

(1986) found that the addition of methionine to single-cell proteins increases the effect of feed utilization in rainbow trout. Storebakken et al. (2004) reported that the replacement of fish meal by single-cell protein up to 50% in trout diet does not result in reduced growth or the expression of deficiency of any essential amino acids in the mixture.

Benefits and disadvantages of alternative protein sources

Aquaculture is highly dependent on the production of fish meal and fish oil, because of their use in fish feed, particularly for carnivorous species, and to a lesser extent in omnivorous species (Watanabe, 2002; Pike, 2005). However, the limiting factors for application of fish meal in the production of aquafeed are: limited resources due to a drastic reduction in populations of fish that are used for fishmeal production (Deutsch et al., 2007); increased needs, due to aquaculture annual growth of about 9%; and price increase due to reduced world production of fish meal (Kristofersson and Ierson, 2006). All this has resulted in the replacement of fish meal and in finding alternative sources of proteins.

Cereals used in an unprocessed form in fish feed have a small input as building blocks and serve mainly as a source of energy for fish. Such management of nutrition in semi-intensive farming systems provides on average 1000 kg/ha yield of carp. The possibility of a slightly better utilization of cereals, but not providing a large change in the growth rate and fulfillment in nutritional requirements, is obtained by processing-extrusion of cereals. Bekrić et al. (1997) reported that the extrusion can be applied to a large number of agricultural products: cereals (corn, barley, oats, sorghum, wheat, rice and triticale), oil crops (soybean, sunflower, canola, cotton seed and peanuts) and coarse grain legumes (beans, peas, vetch, and lupine). Starch gelatinization is considered to be the most important result of cereal extrusion, where the crystal structure of the starch transforms into a gelatinous mass (Gonzalez et al., 2005). Therefore, this procedure is used in processing of corn and other grains. In the process of extrusion of starch macromolecules, amylase and amylopectin, ‘tear’ to molecules of lower weight, and as a result nutrients in the diet are more efficiently utilized (Perez et al., 2007).

Knowledge about the benefits of concentrated feed is slowly leading to substitution of grains by pelleted and then extruded diets (Stanković et al., 2010). In recent years, in Serbia, half of the carp produced was fed with concentrated extruded feed. This type of carp diet provides the possibility of increasing stocking density. Yields may be from 1,500 to over 3,000kg/ha (Marković et al., 2007, 2009). According to Barrows et al. (2007), extrusion, in addition to improving the nutritional content of the mixture and digestibility in animals, has a significant impact on the conservation and compactness of food in the aquatic environment, and therefore the preservation of the aquatic environment.

Soy products are by their amino acid composition the most similar to proteins of animal origin, which is why many studies are based mainly on this plant source. Watanabe (2002) suggests that the proteins of soy products are very digestible for carp, regardless of the level of protein in food, but the presence of anti-nutritional factors and the lack of lysine, methionine and cystine require additional investments in terms of adding synthetic amino acids and heat treatment. According to many researchers, partial replacement of fish meal by soybean products is possible with the addition of synthetic amino acids.

Waste from terrestrial animals would be an excellent source of protein for fish, but the organization for food safety has concluded that the obstacles for the use of rendered by-products of animals is in the spreading of BSE disease. Such feed stuff can be vectors of influent agents such as BSE and TSEs so in August of 2001, the EU banned the use of these products (including fish meal due to forgery) for the production of animal feed, with the exception of the use of fishmeal in the production of food for dogs, chicken and fish (FIN, 2004).

Single-cell proteins are proteins of the future. Besides quick and easy production (Ratledge, 2004), they have the most similar nutritive value of protein structure and amino acid composition to fish meal (Skrede et al., 1998; Storebakken et al., 1999). Shortcomings of single-cell proteins are: low palatability and digestibility, nucleic acid content, presence of toxins, protein quality and economic feasibility.

Conclusion

Problems with fish meal in the world market led many manufacturers of fish feed to look for alternative sources of protein. This paper gives an overview of some alternative sources with their benefits and disadvantages. Soy is still generally used as an alternative source of protein because it is the most studied component, but the current research is directed towards investigating unicellular proteins and krill as components with better potential.

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IZVORI PROTEINA I NJIHOV ZNAČAJ U ISHRANI ŠARANA (*Cyprinus carpio* L.)

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R e z i m e

Šaran (*Cyprinus carpio*) kao jedna od najrasprostranjenijih gajenih vrsta riba u svetu, na prostorima Balkana dominantno se gaji u poluintenzivnom sistemu. Intenziviranjem poluintenzivnog sistema i saznanjima o prednosti koncentrovanim hranama, žitarice se polako potiskuju peletiranim i ekstrudiranim smešama. Takođe, u cilju globalnog povećanja proizvodnje, javljaju se potrebe za unošenje raznolikih komponenti u smeše za ishranu riba i njihove maksimalne iskoristivosti. Akvakultura je visoko zavisna od proizvodnje ribljeg brašna i ribljeg ulja zbog velike primene u industriji hrane za ribe. Ograničavajući faktori primene ribljeg brašna su: ograničenost resursa, porast potreba i porast cene. To je navelo na potrebu da se riblje brašno zameni alternativnim izvorima proteina kao što su: proizvodi od soje koji su najsličniji proteinima animalnog porekla, otpaci terestičnih životinja kao jeftiniji izvori kvalitetnih proteina, kril koji ima veliki potencijal u proizvodnji hrane, kao i jednoćelijski organizmi sa velikom brzinom rasta.

Ključne reči: šaran, izvori proteina, poluintenzivni sistem.

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