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ANALYSIS ON THE USE OF NEW INGREDIENTS IN TROUT FEED

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Abstract

The fodder for captive-bred trout contains ingredients such as fish oil, fishmeal, blood meal, peas, soybeans, wheat, oats, rapeseed, animal protein. The quality of the ingredients is the basis of healthy, mineralizing, protein and vitaminizing feeds. Excessive use of fishmeal in the market puts pressure on the fisheries sector, which is struggling to meet demand. Algae, crustaceans, aquatic plants, insects and seeds are ingredients that could replace fishmeal by helping the fishing industry as well as sustainability of the aquatic environment. In the process of feeding trout with new, experimental feed, research has highlighted the importance of several factors for the development and growth of fish. The level of protein in the feed directly affects the body weight of the trout and the replacement of fishmeal and fish oil with other types of flour and oils can lead to a change in the taste of trout meat. Feeding and control can be of particular importance in reducing losses and increasing environmental sustainability.

Key words: *environment, feed, fish, innovative, sustainability.*

INTRODUCTION

Due to its organoleptic qualities, trout are among the main species of fish consumed nationally and internationally. Aquaculture trout need fodder that reproduces the food from the natural environment as faithfully as possible from a nutritional point of view. Currently, when obtaining a feed for rainbow trout (*Oncorhynchus mykiss*), one of the main ingredients is fishmeal. Excessive use of fishmeal in fish farming and aquaculture as a protein base for rainbow trout feed and not only has led the European Union to focus on finding new sources of protein ingredients in fish feed in aquaculture.

By identifying new sources of ingredients used in trout feed, the aim is to eliminate all or part of the fishmeal from the feed currently used in intensive farming systems and at the same time protect the environment by reducing the consumption of fish meat for fishmeal. Removing fishmeal from feed and replacing it with other ingredients can reduce the final cost of feed, with an impact on the shelf price, and also reduce the negative impact on the environment.

Therefore, researchers have begun to use different ingredients, both of animal origin (insects, crustaceans, worms) and of vegetable origin (algae, different types of oils, cotton flour, rapeseed, brewer's yeast), to replace the fishmeal.

According to the European Commission (2021), the European Green Pact and the "Farm to Fork" Strategy emphasize the potential of marine food, obtained from aquaculture. This is considered a source of protein for food and feed with a low carbon footprint, which plays an important role in building a sustainable food system. In an effort to ensure a sustainable growth of the sector, global aquaculture is in a constant search for new solutions to reduce dependence on fishmeal and fish oils and to efficiently manage manure in animal and poultry facilities. Insects are a new source of sustainable, high-protein ingredients that can be use to feed farmed fish, especially rainbow trout (A European Green Deal - Farm to Fork strategy, 2019).

Aquaculture creates jobs in fisheries and related fields such as aquaponics, and the agro-tourism sector, thus helping the economic development of the countries involved. Directly or indirectly, aquaculture combats climate change and mitigates its impact by reducing pollution. Through a long-term strategic approach to aquaculture, it can become sustainable, profitable, and environmentally friendly.

MATERIALS AND METHODS

This paper aims to conduct a bibliographic review of the research on new sources of protein ingredients that can be used in feed intended for aquaculture fish.

The paper can be a basis for studies aimed at replacing conventional feed with alternative feed.

RESULTS AND DISCUSSIONS

The studied bibliographic literature shows that **fishmeal** is one of the classic ingredients used in the trout diet. Administered individually or as an integral part of certain mixtures, it has become, over time, the subject of numerous researches, in order to establish the possibility of replacing it, in certain proportions, with other types of feed. Thus, those of vegetable origin, but also those of animal origin were studied as alternative sources of protein ingredient.

Sources of plant origin

In a long-term comparative study (2 years), published in 2004 by Francesco et al., the diet based on fishmeal is analyzed in relation to that based on a mixture of vegetable protein sources supplemented with free amino acids. These studies were carried out by the Department of Animal Science at the University of Florence, Italy, in collaboration with the Fish Nutrition Laboratory of France. They showed significant changes to the detriment of rainbow trout fed with enriched mixtures, in terms of growth rate, organoleptic characteristics and the level of the chemical composition of the fillet in n-3 polyunsaturated fatty acids and n-3 / n-6 ratio. As regards replacing the rainbow trout diet based on fishmeal in variable proportions with 60 to 330 g/kg^{-1} of protein concentrate from **rapeseed**, the Canadian researchers at the University of Saskatchewan conclude in the same year 2004 that it does not compromise fish farming performance (Thiessen et al., 2004).

Experimental diets based on the partial or total replacement of fishmeal in rainbow trout feed were also the basis of a study published in 2006 by Chinese researchers at the Beijing Academy of Agricultural Sciences.

Cotton flour, extracted as a solvent and supplemented with lysine and methionine, replaced 0; 112.5; 225; 337.5; 450 g/kg^{-1} protein from fishmeal for 8 weeks. Luo et al. (2006) consider that, while the diet that completely excluded fishmeal adversely affects the growth rate of juvenile rainbow trout, there is no significant difference in growth or fish meat taste when replaced by up to 305 g/kg^{-1}.

The use of other types of flour has also been considered by many researchers. Kasiga & Brown (2019) replaced fishmeal with 50, 100 and 150 g of carinata flour (*Brassica carinata* - Ethiopian mustard), and studies have shown that there has been no change in whole body weight or visceral weight.

In order to reduce dependence on fish oils and to avoid problems related to contamination of aquaculture products (used for the production of fishmeal and fish oil) with persistent organic pollutants and heavy metals, the efforts of the farms have been directed towards reducing the percentage of fish oil in fish food, by partially replacing it with vegetable oils. This trend has emerged from a series of studies that have shown that a significant percentage of salmonid feed fish oil can be replaced without affecting the survival, growth and efficiency of feeding. However, vegetable oils have a different fatty acid profile than fish oils. When included in high levels in the diet, they influence the composition of meat fatty acids and reduce the concentration of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFA), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), well known for their beneficial cardiovascular and cognitive properties (Bélanger-Lamonde et. al., 2018).

Coconut oil has been the subject of research based on experimental diets, replacing herring oil and cod liver oil in rainbow trout food. The research was conducted in 2006, over a period of 231 days, by Italian researchers Ballestrazzi et al., using four diets with increasing coconut oil content, from 0 to 13%, but no significant changes were recorded in carcass features or meat composition.

Flaxseed and sunflower oil have been used since 2006 by Czech researchers in the diet of rainbow trout in the amount of 2% and 5%

individually or 5% mixture of both oils. Although this did not affect the firmness or juiciness of the meat, the major impact was on the intensity and oily taste of the fish (Drobna et al., 2006). Replacing fishmeal and fish oil with rapeseed protein concentrate and a mixture of rapeseed oil and flaxseed oil, led to a significant reduction ($P < 0.05$) in the residual concentration of PCDD/F and DL-PCB in diets and fish fillets according to studies by Sealey et al. (2011).

Algae and microalgae

Algae are a highly valued source of protein, essential amino acids (Fabregas & Herrera, 1985; Becker, 1994) and vitamins (Becker, 2004). They play an important role in human nutrition as well as in animal feed, such as broilers (Marton et al., 1968; Venkataraman et al., 1994) or pigs (Fevrier & Seve, 1976). In aquaculture, algae feeding experiments are related to their use as general sources of protein, due to the complete replacement of fishmeal or as additives in fish feed. The positive effect of the additive used lowers cholesterol and fat levels and improves lipid metabolism in fish. Due to the wide variety of biologically active agents found in spirulina species (*Arthrospira platensis*, *A. fusiformis* and *A. maxima*), they have been the subject of numerous investigations into their use in biotechnology and medical sciences. Spirulina is a rich source of the pigment C-phycocyanin C-PC (Richmond, 1986), with role as an antioxidant (Romay et al., 1998; Bhat & Madyastha, 2000; Pinero et al., 2001), as well as its anticancer properties (Dasgupta et al., 2001).

Different types of algae have been use to determine a stronger coloration of rainbow trout. Microalgae, *Chlorella vulgaris* was used in a study to compare the quantitative effect of the incorporation of food algal biomass (ALG) on rainbow trout muscle pigmentation with that obtained by feeding diets supplemented with a synthetic mixture of canthaxanthin and astaxanthin (MIX) equivalent with the amounts of carotenoids found in dried seaweed (Gouveia et al., 1997). The research was conducted over nine weeks by administering to two batches of fish. One batch was fed *Chlorella vulgaris* microalgae included in the diet, and the other batch was fed synthetic carotenoids (canthaxanthin and astaxanthin). At the end of the research, no significant differences were found between the weights of the batches compared to the total food consumption. The effect on the development of microalgae-fed fish was not as expected, compared to the results obtained in fish fed on a diet containing synthetic carotenoids.

Iranian researchers (Teimouri et al., 2013) following an experiment on 216 rainbow trout, which lasted 10 weeks, concluded that *Arthrospira platensis* could be introduced as an alternative, natural source of carotenoids instead of synthetic astaxanthin in rainbow trout diets. The inclusion of 7.5% *S. platensis* proved to be an adequate food level to ensure pigmentation, as there were no negative effects on fish growth.

Brewer's Yeast

Brewer's yeast is an ingredient, rich source of chromium, and B vitamins, used in the production of beer and bread. It is obtain from *Saccharomyces cerevisiae*, a one-celled fungus. Brewer's yeast can be use as a nutritional supplement in human and animal alimentation.

According Coroian et al. (2019), yeasts seem to provids new sources of protein in fish feed. It also acts as a probiotic. Dry brewer's yeast (1.5%) added to standard feed for rainbow trout has led to better-feed use and strengthened immunity.

Sources of animal origin

Insects and insect larvae are a new source of sustainable and high-protein ingredients that can be use in farmed fish feed, especially rainbow trout.

In 2007, in a 9-week study, American researchers St-Hilaire S. et al. investigated the effects of the partial replacement of fishmeal with diets containing 40% crude protein and 15% fat (67% of dietary protein was derived from fishmeal and all fat was derived from fish oil). Two of the test diets included the black soldier fly, *Hermetia illucens*, and a third test diet involved the use of housefly pupae, *Musca domestica*, which have 70% protein and 16% fat as a 25% substitute for the fishmeal component of the control diet. Studies have shown that with the rise in the price of fishmeal and fish oil, fly prepupae can be an economically advantageous food, but also sustainable for carnivorous fish diets. In areas where there is an aquaculture industry close to intensive farming units, fly larvae could be used to reduce animal waste and provide a good source of protein and fat for the aquaculture industry. Research in this direction has also been conducted by Sealey et al., in 2011, when four test diets were developed by replacing 25 and 50% of fishmeal with fly prepupae (BSF) or fly prepupae enriched with fish organs (EBSF). The dietary fat was adjusted to about 20% fat, using fish oil and poultry fat. In fish fed using these diets, there was no significant difference in the taste of meat compared to the one of the fish on control diet. There were significant differences in their growth however, the enriched diet offering visible effects clearly superior to the others. The effectiveness of insect meal was tested on the occasion of the study by Rema et. al., in 2019, which assessed the effect of incorporation levels of defatted yellow mealworm protein meal (*Tenebrio molitor*). It was concluded that yellow mealworm protein flour could effectively replace 100% fishmeal in the rainbow trout brood diet, with positive effects on its overall zootechnical performance and raising questions about the potential effects of the diet on immunity and the general health of fish.

To determine the potential of insects as a substitute for fishmeal in aquaculture fish feed, Barroso et al., 2014 examined 16 different species, 5 of them in different stages of development, from the orders *Coleoptera* (4), *Diptera* (7) and *Orthoptera* (5). The resulting conclusion was that insects have an amino acid profile similar to fishmeal, *Diptera* being the group most similar to fishmeal. In addition, insects have been shown to have higher ratios of omega 6 and monounsaturated fats, which is an advantage in growing rainbow trout. Researchers at the University of Turin (Belforti et al.) also investigated in 2015 the effects of including a defatted BSF in rainbow trout feed on performance, somatic indices and certain quality parameters. The studies were performed on 360 fish randomly divided into four batches that were fed diets with different percentages of TM: 0% (TM0), 25% (TM25) and 50% (TM50) as a feeding base. *Tenebrio molitor* larvae flour (TM) has been considered an innovative raw material and seems promising to be used as an alternative food to fishmeal in trout feed (Belforti et al., 2015).

Even more recent studies show that insect meal can be widely considered a promising raw material for aquatic food (Józefiak et al., 2019) and can be used even in the early stages of fish development (Lock et al., 2016), i.e., as a starter food. The main advantages of using insects are their easy production, rapid growth, short reproductive cycle of insects and high efficiency of feed conversion (Katayama et al., 2008). The use of insect meal in fish diets is an ecological method of obtaining a valuable source of protein. Furthermore, the use of combinations of different protein sources in aquatic food has been shown to have a positive effect on fish growth (Coyle et al., 2004; Mundheim et al., 2004; Lim & Yildirim-Aksoy, 2008; Sookying & Davis, 2011).

However, researchers such as Stadtlander et al., 2017, consider that further studies are needed on specific feeding strategies and the establishment of feed formulas. These conclusions were drawn following the use in the diet of rainbow trout of a content of 28% insect flour, mechanically defatted, prepared from black soldier fly larvae, *Hermetia illucens* (HIM).

Complementary studies are considered necessary to limit the negative effects on the lipid fraction of fillets and to avoid decreasing the efficiency of protein utilization in HIM-fed fish, which could lead to decreased production efficiency when applied over an entire production cycle and not only during the 7 weeks of their research. Moreover, insect food can also be considered a source of protein and a functional component of feed, which can positively affect the histomorphological structure of the gastrointestinal tract of fish and stimulate the expansion of beneficial bacterial populations in the intestine, according to Polish researchers (Józefiak et al., 2019).

Crustaceans

Chicks et al., conducted in 2021 a study of trout fed isoproteins (42%) and isolipids (24%) without fishmeal for 15 weeks, in which 10% of the protein in a mixture of plant ingredients was replaced with cyanobacteria and dried microalgae biomass (*Arthrospira platensis*, *Tisochrysis lutea* and *Tetraselmis suecica*) or Louisiana red swamp crayfish flour (*Procambarus clarkii*). The content of carotenoids and lipids in the fillets was determined. The coloring capacity of the rainbow trout fillet in fish fed with crayfish meal was also compared with that obtained from the use of commercially available synthetic astaxanthin. For the latter purpose, the test diets were additionally administered for 12 weeks.

Image analysis and colorimetric data indicated that trout fed cyanobacteria and microalgae showed an unwanted yellowish color compared to those fed Louisiana crayfish meal (*P. clarkii*) which showed a desired pink pigmentation.

Another study by Hoffmann et al. (2021) evaluated a combined feeding system, which included the addition of live food (*Artemia salina*) for 0; 3 or 7 days, the addition of insect flour (test diet) and the addition of fishmeal (control diet). Compared to the control diet (based on fishmeal) it was concluded that diets containing 20% larval insect flour can be used as an effective component of feed for juvenile fish.

The application of live feed (brine shrimp) did not significantly affect the growth results, although the type of insect meal had a crucial impact on the growth of the fish.

CONCLUSIONS

The data collected from the studied bibliography show that fishmeal is the main ingredient in trout feed, of paramount importance in its growth and development. However, researchers have tried to replace fishmeal with other ingredients. Some researchers have obtained significant results with vegetable protein diets with enriched mixtures, protein concentrates based on rapeseed or cottonseed meal, with positive results in most cases where fishmeal has been partially and not totally replaced.

The partial replacement of fish oil with vegetable oils does not significantly affect the growth and development of fish, but it does have a negative impact on polyunsaturated fatty acids in salmonid meat. The use of coconut oil did not change significantly the composition of the fish meat, but the flaxseed and sunflower oil adversely influenced the taste of the meat. Spirulina from algae and the protein they contain lowers cholesterol and fat in fish meat but also improves lipid metabolism, while increasing the color of rainbow trout fillets, and can be introduced into fish diets as a natural source of carotenoids to the detriment of synthetic astaxanthin. Insects and insect larvae have given good results in replacing fishmeal with different concentrations in the respective diets. Insect flour can be used in the early stages of growth and development of fish larvae with very good results, having the nutritional qualities as close as possible to fishmeal. Crustaceans (crayfish flour) played an important role in the pink pigmentation of trout, and the Artemia salina crustacean used as a live food in the diets of some trout did not significantly affect the growth results.

Algae, crustaceans, vegetable oils, seeds, various types of flour, insects and larvae are ingredients that could replace fishmeal, thus helping to develop aquaculture, increase sustainability, but also protect the environment.

As a result of the presented aspects that research is necesary in order to replace conventional feed with alternative feed, not only on an experimental scale, but also at high production level, in order to establish all the advantages and disadvantages that would result from this change in the types of raw materials in trout (fish) feed.

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