Influence of Phytogenic Additives on Growth Parameters and Meat Biochemistry in *Cyprinus carpio*

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Abstract
Over time, antibiotics have been used in aquaculture to control fish diseases. Improper and sometimes unjustified use of them has raised concerns about their effectiveness and the possibility of resistant bacterial strains development. One of the attempts to maintain a good health status of fish was to introduce botanical or phytogenic additives into the feeding diets. Phytogenic compounds are natural bioactive compounds, with beneficial effects on technological performance by stimulating growth, feed consumption and food recovery. The aim of this experiment was to determine how the growth parameters and composition of carp (*Cyprinus carpio*) meat are influenced by the introduction of phytogenic compounds in the diet, such as licorice, echinacea and thyme. The inclusion of phytogenic additives in feeding diets has ensured a higher growth performance in the experimental groups, compared to the diet without the addition of phytogenic additives and a feed conversion ratio (FCR) with better values in the experimental groups (1.87 in the group fed with licorice addition, 2.03 in the group with added echinacea, respectively 2.31 for the group with added thyme), compared to the control group, where a value of 2.40 was obtained. Phytogenic additives supplemented in feeding diets cause an accumulation of protein and lipids in carp meat, which increase the fish nutritional value.

Keywords: biochemistry, *Cyprinus carpio*, growth parameters, phytogenic additives

1. Introduction

The fisheries and aquaculture sector has expanded significantly in recent decades, with total production, trade and consumption reaching an all-time high in 2018 [1]. The intensification of aquaculture has led to a number of challenges for farmers, due to the emergence of the disease among aquatic animals. For years, antibiotics have been used to control pathogens in fish and even as growth promoters. Due to growing concerns about the safety and health of humans and animals, much research has been done to find alternatives to chemicals that can be used both prophylactically and to stimulate growth.

An alternative ingredient for chemicals, which ensure the good health of the fish material, which increase production, but also provide the nutrients needed by the fish [2], are botanical or phytogenic additives.

Phytogenic compounds are plant bioactive compounds that have positive effects on health and technological performance by stimulating growth, feed consumption and food utilization [3]. These plant-derived ingredients are able to provide all the nutrients to the fish, ensuring a high-quality end product, safe for human consumption and the environment [4]. Speaking of "phytogenic compounds" we refer to the leaves, flowers, roots, seeds of the plants used but also to

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their extracts, in the form of essential oils or tinctures [5].

Phytogenic compounds have mainly antioxidant, antimicrobial, antiparasitic properties, stimulating food consumption, by increasing the enzymatic activity and can be considered growth promoters [6].

The beneficial properties of phytogenic compounds result from their bioactive molecules. Carvacrol and thymol, from thyme and oregano, have antimicrobial activity against pathogens; allicin from garlic has antibacterial action; glycyrrhizin from licorice has strong antibacterial, antiviral and anti-inflammatory effects; flavonoids from echinacea have an antioxidant effect, helping to combat oxidative stress. Many studies have shown the positive effect of phytogenic additives in animal feed. The health of fish has improved when supplemented with fennel or garlic, probably due to the presence of bioactive compounds in these plants [7, 8]. Beneficial effects have been reported in other animals (pigs, birds) that have been fed diets that have incorporated various phytogenic compounds [5, 9, 10].

The purpose of this experiment was to establish the influence of diets with the addition of phytogenic compounds from licorice, echinacea and thyme on the growth parameters and the composition of the meat of the carp species (*Cyprinus carpio*).

2. Materials and methods

The authors of this paper have complied with the legislation in force on the care and use of animals in experiments [11, 12]. The biological material involved in this study was anesthetized before any intervention, by soaking in a solution of clove oil (0.025 ml per 1 liter of water).

A number of 185 specimens of carp (*Cyprinus carpio*) one year old and an average weight of 40.5±0.31 g, obtained in the Research and Development Farm Brateş, Galați were divided into 4 experimental groups. The experimental treatments were performed in glass aquariums, with dimensions of 100 x 40 x 40 cm, with a water volume of 100 liters, in each aquarium being 45 specimens. From the initial number of fish, 5 specimens were kept for biochemical determinations. The fish were acclimatized for a period of 7 days. Water quality was monitored during the experiment. Dissolved oxygen, temperature and pH were measured daily using the HQ40d - Hach Lange portable analyzer with two measuring probes. Nitrites, nitrates and ammonium concentrations were determined weekly with the Hach DR 1900 portable spectrophotometer, using LCK - Hach Lange kits. The water was conditioned with external filters of the Tetra Ex 1200 Plus type, the elimination of dejections was done by siphoning, and the removed water was replaced with fresh one.

The basic feed was 34% protein and 9% fat. Each experimental diet was performed by adding a phytogenic compound to the basic feed, in a concentration of 0.5%, as follows:

- Control diet (AM) - without phytogenic compounds
- Diet 1 (A1) - with the addition of 5 g of licorice (*Glycyrrhiza glabra*) to 1 kg of basic feed
- Diet 2 (A2) - with an addition of 5 g echinacea (*Echinacea purpurea)* to 1 kg of basic feed
- Diet 3 (A3) - with the addition of 5 g of thyme (*Thymus serpyllum*) to 1 kg of basic feed

After weighing the phytogenic compounds, their extracts were obtained in water, which were then mixed with a 2% gelatin solution and sprayed over the feed. The feed with phytogenic compounds, thus obtained, was allowed to dry at room temperature and distributed to the fish, 2 times a day (8.00 and 16.00), for 52 days, the feed ratio being 2.5% of biomass.

Growth performance was determined by the following indicators:

Weight gain (WG)=Final weight-Initial weight; Bf, Bi=final and initial biomass of lot, respectively.

Individual weight gain (IWG)=(Wf-Wi)/N, where: Wf, Wi=final and initial average mass of the lot; N=number of specimens

Daily growth rate=(Wf-Wi)/t [g/day], where: t=number of days

Specific growth rate (SGR)

SGR=(lnWf - lnWi)*100/t [%/day]

Feed conversion ratio (FCR)=feed intake (g)/weight gain (g)

The biochemical composition of the meat (crude protein, moisture, fat content, ash), were calculated according to standard methods [13].

The total protein substances were determined by the Kjeldahl method, according to STAS 6514-75,

using a Gerhardt type system. By multiplying the value of the nitrogen content by 6.25, a coefficient characteristic of fish meat, the crude protein content was determined.

Moisture was determined according to STAS 6508-73 by heating in oven at 130°C for one hour to constant mass.

The content of lipids was determined by the Soxhlet method, using the VELP type extraction system, according to STAS 6512-73.

The ash content was determined by calcining the sample at 600°C and weighing the remaining residue.

The data obtained were statistically processed using Microsoft Excel for Windows. The statistical differences between the variables were analyzed using the ANOVA One Way test and the t test. The differences were considered significant at values of $p<0.05$.

3. Results and discussion

During the experiment, the mean values for dissolved oxygen and water temperature did not show statistical differences between the experimental groups ($p>0.05$). In contrast, pH indicated statistically significant differences ($p<0.05$) between lots.

The mean values for temperature, dissolved oxygen and pH were 20±1.16°C, 8.62±0.29 mg/l and 8.35±0.12 upH, respectively. The average concentrations of nitrates, nitrates and ammonium ions were 0.78±0.25 mg/l, 2.16±0.68 mg/l and 0.17±0.33 mg/l, respectively.

In general, the fish in the experimental groups, in which phytogenic compounds were added, showed an increased growth performance compared to the control group.

The effect of dietary supplementation with bioactive compounds on carp body weight is shown in Figure 1. All results were presented as mean±standard deviation.

In 2018, Hassan et al. obtained, by supplementing the diet of Nile tilapia with various phytogenic compounds, an increase of the individual average mass by 16% in the case of turmeric, by 18% in the administration of rosemary and by 14% in the case of thyme [14].

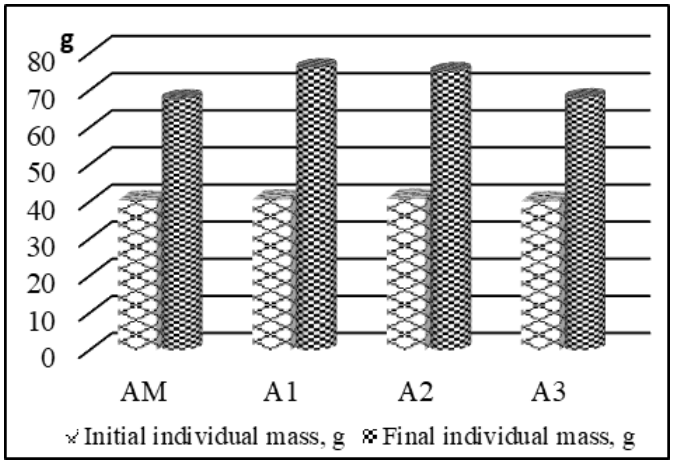

Figure 1. Evolution of the individual body mass of carp fed on diets with phytogenic compounds

Weight gain, the specific growth rate and the FCR are shown in Figure 2. Weight gain recorded at the end of the experiment was higher in group A1, 1.59 kg, compared to 1.46 kg in A2, 1.25 kg in A3 and 1.16 kg in the control group (AM).

Regarding the daily growth rate, it was found that the administration of thyme in the feed led to an increase of about 2% compared to the control. The lot fed with the addition of echinacea, A2, recorded a daily growth rate of 24% higher than in the control, the highest daily growth rate being recorded in the case of the group fed with the addition of licorice, by about 28% higher.

The specific growth rate, SGR, an important technological indicator, recorded the best value in the case of the group fed with licorice (1.23% per day), followed by the group in which echinacea was administered (1.15% per day), 1.03% per day for the group fed with thyme and 0.97% per day for the control group.

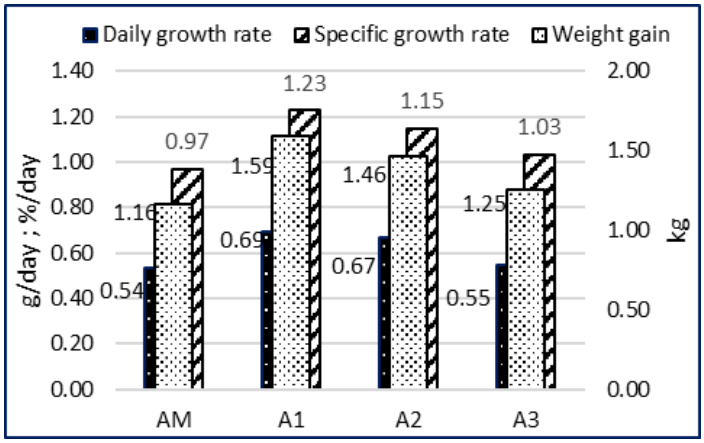

Figure 2. Daily growth rate, specific growth rate and weight gain in the three experimental lots compared to the control group

Results with positive effects on growth performance and food recovery were also obtained in the case of Nile tilapia, when administered in feed of encapsulated phytogenic compounds, in a concentration of 0.5 g/kg [15].

Regarding the feed conversion ratio (FCR), it was observed that the best use of the feed was made in the group fed with the addition of licorice, where an FCR value of 1.87 was obtained, followed by group A2 (2, 03) and A3 (2.31). The data presented show that the addition of sweet wood, echinacea and thyme have beneficial effects on the use of feed (Figure3).

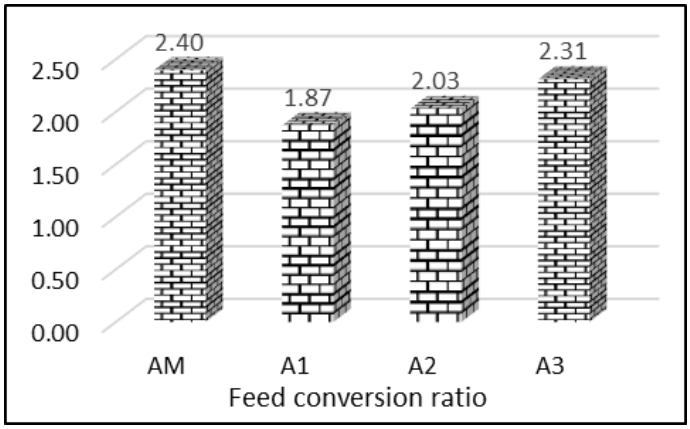

Figure 3. Evolution of the feed conversion ratio in the experimental groups

Similarly, Velichkova et. Al. (2019) [16] observed an improvement in food recovery by supplementing the food with 1% sweet flag (*Acorus calamus L*), by 19% compared to the control.

In order to correctly assess the nutritional value of the meat of the fish involved in this experiment, as well as the way in which the food was used, the biochemical composition of the meat was determined, presented in Table 1 as the mean±standard deviation.

The values obtained highlight the variation of the biochemical composition of the meat at the end of the experiment, depending on the diets used in the experimental groups, compared to the control group. Biochemical analysis of the meat indicated significant differences between the control group and the lots in which phytogenic compounds were administered, in terms of moisture content ($p<0.05$) and lipid content ($p<0.05$). the differences were insignificant in terms of protein content ($p=0.108$) and ash content ($p=0.727$). The protein content increased compared to the time of onset in all experimental groups, with the largest increase in the variant with the addition of echinacea (17.34%).

Table 1. Biochemical composition of carp at the end of the experiment

		Moisture %	Proteins %	Lipids %	Ash %
Initial		77.58±0.11	16.84±0.04	3.23±0.06	2.13±0.03
Final	AM	75.71±0.15	17.29±0.01	4.34±0.02	1.93±0.05
	A1	75.35±0.04	17.33±0.03	4.72±0.03	1.92±0.03
	A2	75.29±0.07	17.34±0.03	4.69±0.02	1.96±0.03
	A3	75.35±0.06	17.32±0.03	4.67±0.06	1.92±0.04

The lipid content of meat showed significant differences at the end of the experiment between the control group and the 3 experimental groups ($p<0.05$). The highest lipid content was recorded in the group fed with licorice (4.72 g%), and the lowest in the control group (4.34).

The results of this study are consistent with those of Hashem et al. (2017), who in the experiment of feeding the Nile tilapia for 83 days with different concentrations of moringa (*Moringa oleifera Lam.*) Obtained an increase in the protein and lipid content in fish meat, compared to control [17].

Also, Ahmadifar et al. (2011) [18], found a significantly higher lipid content in the meat of rainbow trout (*Oncorhynchus mykiss*) fed fodder supplemented with oregano extract.

Free water content is a valuable chemical indicator, as it indirectly reflects the proportion of dry matter that concentrates all nutrient-carrying substances. Humidity values decreased from 75.71% (AM) to 75.35% (A1 and A3) and to 75.29 (A2).

The results of this study are in contradiction with the results of other authors who found an increase in water content with the addition of 1g of oregano in the diet of carp raised in the recirculating system [19].

4. Conclusions

In conclusion, research has shown the effectiveness of phytogenic compounds in carp food in terms of growth performance. The best results on individual and total growth rate, daily growth rate and feed conversion factor (FCR), were recorded in the group that was given licorice, followed by the one in which echinacea was administered.

The addition of phytogenic compounds in the carp diet improves the protein and lipid content in carp meat, while decreasing the moisture content.

There are many studies that have shown the positive effect of supplemented phytogenic compounds in aquatic animal feed, so their use in aquaculture can be recommended in order to stimulate growth. However, research in this area needs to be continued.

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