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THE INFLUENCE OF SALVIA HISPANICA AS A SUBSTITUTION OF FISH MEAL ON THE GROWTH PERFORMANCE AND ECONOMIC PARAMETERS OF COMMON CARP (*CYPRINUS CARPIO L.*), RAISED IN NET CAGES

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Abstract

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The purpose of the study was to determine the effect of the addition of chia as a substitution of fish meal in extruded feed on the growth intensity and economic efficiency of common carp, grown in net cages. The fish were kept in net cages, each of them with an effective water volume of 1 m^3, which are part of a fish farm "Dani", situated in the Studen Kladenets dam. Five hundred and forty common carps, were divided into three groups – control (A), experimental B and C, each of them with three replicates, with a mean initial live weight, respectively 53.5±1.31 g, 53.4±1.33 g and 53.4±1.24 g ($p>0.05$). The feed was extruded pellets with a size 3.5 mm. The feed content for fish from experimental groups was as follows: for the control group (A) - 12% fish meal, for the group B - 6% fish meal and 12% chia, and that for the group C without fish meal, with a content of 25% chia. The duration of the trial period was 60 days. To monitor the impact of the addition of chia as a substitution of fish meal to extruded pellets on the growth intensity of common carp (*Cyprinus carpio L.*), control catches were performed at a 20-day interval. The live body weight (BW, g) in the control catches was established, with the carps of each group being weighed together and so the average weight of one fish calculated. At the end of the experiment, fish growth (g), their survival rate (%) and feed conversion ratio (K) were calculated. In the middle of the experimental period, there is a tendency for a higher live body weight in the fish of control group A, where extruded pellets contained 12% fish meal, compared to the values of this indicator of individuals in the groups B and C, fed with granules with content of 12% and 25% chia. The same trend remained at the end of the experiment, with the live weight, being highest in the common carp from the control group (A) - 152.89 ± 18.57 g, followed by that of fish from the experimental group B - 144.25 ± 14.77 g and the one of individuals from C group - 138.66 ± 14.59 g and the differences are statistically proven ($p<0.03$, $p<0.001$). Calculated economic conversion ratio (ECR) was 2.10 for the fish from the control variant A and it was accordingly with 59% and 229% lower, compared with the value of the same parameter, calculated for the individuals from experimental group B and C.

Key words: common carp; *Cyprinus carpio*; pelleted feed; replacement of fish meal; chia; growth performance; Feed Conversion Ratio (FCR); economic efficiency

Introduction

In the development of aquaculture, along with the improvement of the technological parameters of applied production systems for the cultivation of different species, the basic requirement will be for the nutritional ingredients applied in optimized quantities in the feed mixes to provide intensive growth, with minimal negative impact on the environment.

The production of various types of hydrobionts needs considerable amounts of fish meal, which is a major component in the extruded feed required for the cultivation of delicate

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species (Hardy, 1999). The total production of fishmeal in recent years is almost constant and is about 7 million tons (New and Wijkstrom, 2002). This is the result of over-exploitation of water basins and reduction of fish catches. Due to the fact that fish meal needs are constantly increasing as a result of the development of aquaculture, there is also an increase in its international market price (Hardy and Tacon, 2002). According to the EU requirements, fishmeal can only be used in sustainable aquaculture if it is certified and produced from waste products in the food industry. This is the reason for an increase in the price of this feed additive for the feed industry and alternative protein sources are therefore sought to replace fishmeal or a part of it in extruded feeds for biological production by the hydrobionts (Twibell and Brown, 2000; Cheng and Hardy, 2002; Cheng et al., 2002; Muzinic et al., 2004; Craig and McLean, 2005). Most alternative proteins are of vegetable origin and are inferior to fishmeal and can`t fully satisfy the nutritional requirements of the cultivated species and some of them also contain fish antimatter factors.

Feed with fish meal as a major ingredient is in shortage on the international markets. As mentioned, this is a reason to search for alternative protein sources for the feed industry in aquaculture (Maina et al., 2003). According to various authors, the fish meal substitutes can be protein rich oil seeds and waste products in the processing of cereal feeds including soy bean, rapeseed, corn gluten, wheat gluten and, to a lesser extent, ground peas and lupine (Abery et al., 2002; Pereira and Oliva-Teles, 2002; Grisdale-Helland et al., 2002a, 2002b; Abdelghany, 2003; Bautista- Teruel et al., 2003; Cremer et al., 2003). In this area other authors have conducted experiments with various hydrobionts subject to aquaculture (Guenco et al., 1985; Matsushita et al., 1998; Farhangi and Carter, 2001; Takagi et al., 2001; Grisdale-Helland et al., 2002a; Lee et al., 2002; Penaflorida, 2002; Du and Niu, 2003; Hari and Kurup, 2003; Jahan et al., 2003; Peres et al., 2003; Refstie and Tiekstra, 2003; Richter et al., 2003; Singh et al., 2003).

The opinion of most authors is that alternatives to fishmeal in the fish feed industry will increasingly be sought and used in the future, and that this will be at the center of the development of organic production. The case of replacing fish oil in granules with oil of plant and animal origin is particularly problematic, and this is particularly important for predatory fish and small fish (Tacon and Barg, 1998; Bharadwaj et al., 2002; Martino et al., 2003; Mente et al., 2003). For fish and other aquatic organisms the replacement have been successful when using soybean, rape, and fats from terrestrial animals, which is, of course, consistent with the cultivated species and age categories (Rinchard et al., 2002);

Many of the potential sources of protein for hydrobionts have a low nutritional value due to their low digestibility or the potential for resorption of amino acids. In recent years there has been a tendency to increase the volume of production from organic aquaculture and the projections are that it will increase by about 20% in the next few years (Dimitri and Greene, 2002; Harvey, 2003). The reason for this is the increasing demand for healthier and dietary foods (Mansfield, 2004).

The chia (*Salvia hispanica* L.) is an annual plant of the family *Lamiaceae*, which can be cultivated in a desert and semi-desert climate. Studies show that the seeds of these plants are characterized by high protein content and natural antioxidants, as well as a high content of unsaturated fatty acids, and more than 60% of their content are alpha-linoleic acid (omega-3 fatty acid). All this shows that the inclusion of these plants in the food for both humans and animals could benefit their health (Olivos-Lugo et al., 2010; Sandoval-Oliveros and Paredes-López, 2012).

So far, chia has been tested as a component in the feeding of lambs (Urrutia et al., 2015) and rabbits (Rodríguez-Abello et al., 2016). Studies on the use of this component in feed for fish and other hydrobionts are lacking.

The purpose of the study was to determine the effect of addition of chia as a substitution of fish meal in extruded feed on the growth intensity and economic efficiency of common carp, grown in net cages.

Material and methods

The fish are cultivated in cages with an effective volume of 1 m^3, which are part of a fish farm "Dani", situated in the Studen Kladenets dam. Five hundred and forty common carps were divided into three groups (control A, experimental B and experimental C) of 60, each with three replicates (A_1, A_2, A_3, B_1, B_2, B_3, C_1, C_2, C_3) and respectively with an average initial live weight 53.5±1.31 g, 53.4±1.33 g and 53.4±1.24 g. The feed was extruded pellets with size of 3.5 mm. The chia content as a substitution of fish meal for carps from different groups was as follows: for the control group (A) -12% fish meal, without chia, for the experimental group B - 6% fish meal and 12% chia, and that for experimental group C - without fish meal, with a content of 25% chia. Nutrients in the feed of the three groups are presented in Table 1. The daily ration that fish received from the three replicates of the control and trial groups was up to 3% of their live weight. The duration of the trial period was 60 days.

Hydrochemical Analysis

During the experimental period, the water temperature (^oC) in the net cages was measured daily.

Table 1
Nutrients in pelleted feed for common carp (*Cyprinus carpio L.*)

№	Ingradients	Groups		
		A	B	C
1	Wheat, %	20.84	15	10
2	Corn, %	12	10.59	5
3	Soybean meal, %	24	28	32
4	Fish meal, %	12	6	0
5	Dicalcium phosphate, %	5.7	6	6
6	Salt, %	0.5	0.5	0.5
7	Lysine - 98%	0.15	0	0
8	Methionine, %	0.1	0	0
9	Flavor	0.01	0.01	0.01
10	Premix carp, %	0.4	0.4	0.4
11	Sunflower meal, %	19	19	19
12	Kempbind, %	0.3	0.3	0.3
13	Lime stone, %	1	1.2	1.79
14	Chia, %	0	12	25
15	Sunflower oil, %	4	1	0
	Nutritional value			
16	Protein, %	27.02	27.02	26.99
17	Kcal/kg	3000	2995	3117
18	Lysine, %	1.69	1.89	2.28
19	Methionine + cystine, %	1.01	1.51	2.15
20	Calcium, %	2.40	2.40	2.42
21	Phosphorus, %	1.80	1.81	1.80

* 1 kg feed contains: vitamin A – 10000 IE; vitamin D_3 – 1500 IE; vitamin – E – 200 mg; vitamin K – 3 mg; thiamin – 10 mg; riboflavin– 15 mg; pyridoxin– 8 mg; vitamin B_{12} – 0.02 mg; nicotinic acid– 40 mg; folic acid– 3 mg; biotin– 0.3 mg.
** 1 kg feed contains: Fe – 145 mg; Mn – 67 mg; Cu – 16 mg; Zn – 68 mg; I – 1.5 mg; Co – 0.5 mg; Se – 0.6 mg

The remaining hydrochemical parameters were examined in an accredited laboratory "Alimenti" in the village of Tsaratsovo, Plovdiv region at the beginning and at the end of the experiment with the following methods:

- Nitrogen nitrite – BDS EN ISO 10304-1:2009
- Nitric nitrogen, $mg.l^{-1}$ – BDS EN ISO 10304-1:2009
- Dissolved oxygen, $mg.l^{-1}$ – BDS EN 25813:2004
- pH – BDS EN 3424:1981
- Electrical conductivity, $\mu S.cm^{-1}$ – BDS EN 27888:2002

Intensity of fish growth

At the beginning and at the end of the experiment carps were individually weighed (g) and their total body length (cm) was measured. To monitor the impact of the addition of chia to extruded pellets as a substitution of a fish meal on the growth intensity of common carp (*Cyprinus carpio* L.), control catches were performed at a 20-day interval. The live body weight (BW, g) in the control catches was established, with the carp of each group being weighed together and the average weight of one fish calculated. At the end of the trial, fish growth (g), their survival rate (%) and feed conversion ratio (K) were calculated.

Economic analysis

To analyze the cost-effectiveness of the addition of chia as a substitution of a fish meal in feed for common carp (*Cyprinus carpio L*) cultivated in cages, feed conversion ratio, fish growth and survival data were used. A comparison was made between these values of the carps from the different experimental variants and the cost (BGN) for granulated feed for this species cultivated in cages was determined. The prime cost (BGN) per 1 kg gain of fish grown in cages is determined.

The economic conversion ratio (ECR) was determined using the following equation (Piedecausa et al., 2007):

ECR = Cost of Diet x Feed Conversion Ratio (FCR)

Statistical evaluation of the data was done by STATISTICA 6.0 Software (StatSoft Inc., 2002).

Results

Hydrochemical analysis

During the experimental period hydrochemical parameters in the cage were optimal for carp growing. Water temperature, dissolved oxygen, pH, nitrate, nitrite and water conductivity were regularly monitored.

The temperature values during the carp trial to determine the influence of chia as a substitution of fish meal in the diet are presented on Table 2. It was from 23.50°C to 26.50°C in the water of the net cages, where fish from different experimental variants were grown during the trial period, which is optimal for this species. The amount of dissolved oxygen in the water during the experimental period was within the range adopted for the cultivation of common carp and it varies from 7.45 $mg.l^{-1}$ to 8.60 $mg.l^{-1}$ (Table 2). The pH values of water in the cage base range from 7.10 to 7.30, which is optimal for carp growing (Table 2). The amount of nitrate nitrogen during the experimental period ranged from 0.37 $mg.l^{-1}$ to 0.31 $mg.l^{-1}$, which is below the maximum allowable value set in Regulation No. 4 of October 20, 2000. The nitrite nitro-

gen content in the water is given in Table 2. It was less than 0.04 mg.l^{-1}, which is below the maximum admissible value set in Regulation No. 4 of 20.10.2000. The water conductivity during the carp experiment is presented in Table 2 and it ranged from 219.00 µS.cm^{-1} to 224.00 µS.cm^{-1}, which is optimal for the cultivated species.

Common carp growth

The mean values of body live weight of carp during the beginning of the trial from the three replications of control group A and experimental groups B and C were 53.5 ± 1.31 g, 53.4 ± 1.33 g and 53.4 ± 1.24 g, respectively, with differences not statistically proven (p>0.05) (Table 3). In the middle of the experimental period, there is a tendency for a higher live weight in fish of group (A), where carp feed has 12% fish meal compared to the values of this indicator in individuals in the groups fed with extruded pellets with content of chia 12% and 25% (Table 3). The same trend is maintained at the end of the experiment, with the live weight being highest in the carp from the control group (A) - 152.89 ± 18.57 g, followed by that of the experimental group B - 144.25 ± 14.77 g, and it was lower in the group C - 138.66 ± 14.59 g. The differences are statistically proven (p<0.03, p<0.001).

The survival rate of the carp from different experimental variants during the trial is presented on (Table 3). The values of this index in individuals from the repetitions of the trial groups B and C, fed with extruded pellets, containing chia as a substitution of fish meal and fish in the control group were 93.9%, 93.9% and 92.8%, respectively.

At the end of the experiment, the average individual weight gain of fish from control group (A), was 99.67 ± 18.41 g and it was higher than the values of this indicator of fish from experimental groups B and C by 5.9% and 10.2% respectively, the differences being proven (p <0.03, p <0.001) (Table 3).

During the experimental period the carp was fed five times per day. An analysis of daily fed pellets was performed on repetitions in the control and experimental groups. The feed conversion ratio in the control group is 1.93, in the experimental group B with the addition of 12% chia as a substitution of a fish meal it was 2.11, and in the individuals of the

Table 2
Water chemical parameters in net cages during the experiment with common carp

Parameter	n	Min.	Max.	Regulation №4
Temperature, ºC	60	24.00	26.50	22.0-28.0
Dissolved oxygen, mg.l^{-1}	2	7.45	8.60	> 6
pH	2	7.10	7.30	6.5-8.5
Nitrate nitrogen, mg.l^{-1}	2	0.31	0.37	< 2.0
Nitrogen nitrite, mg.l^{-1}	2	<0.04	<0.04	< 0.05
Electricalconductivity, µS.cm^{-1}	2	219.00	224.00	-

Table 3
Fish production parameters

Parameter	n	A	B	C
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Total body length at the beginning, cm	180	14.93±0.41	14.92±0.43	15.0±0.39
Total body length at the end, cm	180	21.20±1.48	20.85±1.48	20.68±1.48
Initial body weight, g	180	53.5±1.31	53.4±1.33	53.4±1.24
Final body weight, g	180	152.8±18.57	144.25±14.77	138.66±14.59
Survival rate, %	180	92.8	93.9	93.9
Average individual weight gain, g	180	99.67±18.41	91.01±14.80	85.39±14.40`
FCR		1.93	2.11	2.25

experimental group C, where the fish meal was substituted with 25% chia - 2.25 (Table 3). The differences are statistically proven between the values of this indicator in the fish from the control and experimental groups ($p<0.05$).

Economic analysis

The cost of extruded pellets, without VAT for the carp from the control group (A) with a fish meal content of 12% is 1090 BGN per ton, for the fish in the experimental group (B), which has 6% fishmeal and 12% chia price of 1580 BGN per ton and of the feed for fish from group C - without fishmeal, with a content of 25% chia of which is 2135 BGN. The calculated economic efficiency coefficient for control group (A) is 2.10, which is lower compared to the value of the same parameter for carps from experimental groups B and C by 59% and 229%, respectively.

Discussion

Analysis of the hydrochemical data obtained (water temperature, dissolved oxygen, pH and electrical conductivity) during the test period indicates that they are in the optimal range for growing common carp. The same applies to the maximum permissible nitrate and nitrite concentrations in water. For carp farms, these values should be up to 2 mg/L and up to 0.05 mg/L, respectively, significantly higher than those maintained in water during the experimental period (Regulation No 4 of 20.10. 2000). The maintenance of these optimal water values in all experimental variants is due to the fact that the carp from these groups is cultivated with optimized technological and technological parameters of the production technology.

The influence of chia as a substitution of fish meal in extruded pellets on the survival rate for carp cultivated on a cage was analyzed. The data reported at the end of the trial show that this indicator in the experimental group fed with 12% and 25% chia were 93.9%. The survival rate for fish in the control group was close to these values - 92.8% (Table 3). This is due to optimal hydrochemical values in net cages during the trial period as a result from applied optimized technological parameters - stocking density, daily ration, and multiplicity of meals.

The analysis of the final live weight of the carp shows that it is 152.8 ± 18.57 g in the control group and this is higher with 6% and 10.6%, respectively than the values of this indicator of individuals from the experimental variants B and C, the differences are reliable ($P <0.001$) (Table 3).

At the end of the experimental period, the analysis of feed consumption data shows that the feed conversion ratio in carp fed with granules with a fish meal content of 12% (control group A) is 1.93 and it is 10% lower than that of fish in the experimental group, fed with 12% chia as a substitution of fish meal and 17% less in the subjects of the experimental group C (Table 3). Better utilization of extruded pellets, containing 12% fish meal reflects positively the growth of fish from control group grown in net cages. At the beginning of the trial, live weight of the carp from different experimental variants was similar ($P>0.05$). At the middle of the experimental period there was a tendency for a higher live weight in fish from the control group compared to this indicator in the fish from the experimental groups B and C, where the chia was added as a partially or fully substitution of fish meal in extruded pellets.

The obtained data for the growth and FCR of carps in current experiment were in the agreement with the results obtained by Hossain and Jansey (1989) in which study the carps fed with the control feed (containing fishmeal) showed significant growth potential compared to those obtained in the experimental groups of fish fed with mustard flour, flax and sesame seeds. The same authors also found significant differences in growth and feed digestibility in the experimental carps by comparing different levels of protein substitution from the above-mentioned seeds. This also corresponds to the results obtained from us - significant differences in the growth and FCR in the fish fed with feed contained 12 and 25% chia in the favour of carps fed with the feed contained lower level of chia (12%).

The reason for lower growth and poorer nutritional utilization in fish when the fish meal was replaced with chia could be result from the factors which affected the growth and FCR of the carps in other conducted studies where fish meal was replaced by compositions of plant origin. These factors are: anti-nutritional factors, the lack of some micronutrients (Turchini et al., 2009; Pratoomyot et al., 2010) as well as the ap-

Table 4
Economic efficiency of the addition of chia in extruded pellets for common carp (*Cyprinus carpio L.*)

Parameters	A	B	C
Price, BGN/kg (without VAT)	1.09	1.58	2.14
ECR	2.10	3.33	4.8

pearance of aggregates between the fibers and other nutrients and the decrease of digestibility of feed because of the presence of enzyme inhibitors (Pradhan et al., 2014).

The analysis of economic data shows that the carps from the control group which showed the lowest feed conversion ratio have the best economic efficiency ratio as well.

Conclusion

The addition of 12% and 25% of chia as a substitution of a fish meal in extruded pellets for feeding of a common carp, grown in net cages influences the investigated indicators as follows:

- reduces the growth rate of fish from the experimental groups B and C compared to those in the individuals from control group (A) by 6% and 10%, respectively;
- increases the feed conversion ratio for carp from experimental groups B and C by 10% and 17%, respectively, compared to those of the fish from control group;
- does not affect the survival rate of fish from all experimental variants;
- increases the cost of growing carp in experimental groups B and C in comparison with the values of this parameter for fish in the control group.

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