EFFECT OF FISH FEEDS WITH DIFFERENT FATTY ACID CONTENTS ON STRESS RESPONSE OF COMMON CARP (PRELIMINARY RESULTS)

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UTICAJ HRANE SA RAZLIČITIM UDELOM MASNIH KISELINA NA STRESNE REAKCIJE KOD ŠARANA (PRELIMINARNI REZULTATI)

Abstrakt

Riblje brašno i riblje ulje su važne komponente riblje hrane koja se koristi u akvakulturi. Oni sadrže nezasićene masne kiseline koje su važne kako u ishrani riba tako i u ishrani ljudi. Međutim, izvori ribljeg brašna i ulja su ograničeni. Cilj AQUAMAX projekta, koga finansira EU, je da se riblje brašno i ulje zamene ili dopune sa biljnim uljima. Tim za imunologiju u Institutu za ribarstvo, akvakulturu i irigaciju ispitivao je efekat dve različite hrane za ribe (hrana dostupna na tržištu i hrana sa dodatkom Camelina ulja) na pojavu stresa izazvanog ograničenim prostorom kod šarana. Na bazi rezultata ovog istraživanja može se zaključiti da primenjena ishrana nema uticaja na toleranciju šarana na stres izazvan ograničenim prostorom.

Ključne reči: *šaran, stres, otpornost, Camelina ulje, riblja hrana*

INTRODUCTION

In these days, the world's fish consumption increases by approx. 1.5% per year (D e l g a d o et al., 2002/1). According to the prognoses, the world's fish production will reach 130 million metric tons until 2020, and 40% of this quantity will be provided by aquaculture, instead of the current rate of 35% (D e l g a d o et al., 2002/1). In 2020, the share of the European Union in annual fish production will be 12.6 million metric tons (F a i l l e r and L e c r i v a i n, 2003; F a i l l e r, 2005). The demand of fish feeds will increase because of the increasing significance of aquaculture. One of the most important ingredients of commercially available fish feeds is fish meal (and/or fish oil). Its production is more and more expensive due to the decreasing opportunities of natural-water fishery (D e l g a d o et al., 2002/2). Because of this, there is a growing interest in using vegetable oils, which enable the replacement of fish oil used in fish feeds.

Similarly to fish oils, vegetable oils are rich in unsaturated fatty acids, of which the ω-3 and ω-6 fatty acids are essential in human nutrition (S i n c l a i r et al., 2002). For example, docosahexaenic acid (DHA), eicosapentaenic acid (EPA) and linolenic acid (ALA) belong to this group. They have an important role in embryonic development, (L a u r i t z e n et al., 2001; D u n s t a n et al., 2008), regulating the blood pressure (T e r e s et al., 2008), normal function of the eye and brain (S i m o p o u l o s, 2008; J o h n s o n and S c h a e f e r, 2006). These unsaturated fatty acids are essential nutrients for fish as well (L e e et al., 1967, W a t a n a b e et al., 1974; S t e f f e n s, 1997).

The ω-3 and ω-6 fatty acids have an important role in the function of the immune system (C a l d e r, 2007). EPA and DHA are the precursors of docosatriens, which reduce the effect of inflammation and restore the pre-inflammation state (S e r h a n et al., 2004; S e r h a n, 2005). In addition, poly-unsaturated fatty acids help to reduce the effects of various stress factors (M a r t i n s et al., 2007; T r e n z a d o et al., 2008).

Since 2006, Research Institute for Fisheries, Aquaculture and Irrigation (HAKI, Szarvas, Hungary) has been participating in the EU-funded “AQUAMAX” project, which has an aim of replacing the decreasing fish meal and fish oil sources with oil- and protein-containing feed components that can be produced on a sustainable way. Fish nutritional, toxicology, analytical and immunological experiments are being carried out in the framework of the project. An *in vivo* experiment was implemented by the research group of immunology in HAKI. In this experiment the effect of two different fish feeds (commercially available silurus feed and feed supplemented with Camelina (*Camelina sativa*) oil) on the confinement stress tolerance of common carp was investigated by measuring lysozyme, glucose and cortisol levels of blood plasma at the beginning of the experiment and 21 days later. Results of this study are reported in this article.

MATERIALS AND METHODS

Two feeds with high protein content and different oil supplements were fed to juvenile Common carp at two stocking densities. The experiment was carried out in the recirculation fish rearing system of HAKI, Szarvas, Hungary. During the experiment, fish were kept in fibreglass tanks with a constant water flow-through of 7 litres/minutes. The water volume was 200 litres for “normal” and 100 litres for “crowded” stocking densities (14 kg/m^3 and 54 kg/m^3, respectively). Temperature and pH were constant during the experiment (22°C, pH 8.5). Dissolved oxygen content was changing between 80% and 90%. Experimental fish were allocated into the following four groups:

Group 1: Fish fed on experimental feed in normal stocking density (“Experimental normal”)

Group 2: Fish fed on experimental feed in high stocking density (“Experimental crowded”)

Group 3: Fish fed on control feed in normal stocking density (“Control normal”)

Group 4: Fish fed on control feed in high stocking density (“Control crowded”)

The experimental feed was supplemented with Camelina oil. A commercially available silurus feed was used as control feed. Some composition data of feeds are presented in Table 1. The daily feed rations were 2% of the total body mass in each tank. Feeding was performed during daylight periods using belt feeders. The experiment was implemented with three parallels (tanks) of each group; therefore altogether 12 tanks were used. There were four fish in tanks belonging to "normal" groups and seven fish in tanks belonging to "crowded" groups. At the beginning of the experiment, the average individual body mass of the fish was 843±42g in group 1, 708±68g in group 2, 630±184g in group 3 and 815±13g in group 4.

Table 1. Composition of fish feeds used in the experiment. +: the exact composition is unknown

Ingredient	Control feed	Experimental feed
Camelina oil (%)	-	5.8
Fish oil (%)	+	+
Calculated composition		
Dry matter (%)	89.00	90.5
Crude protein (%)	42.00	35. 0
Lysin (%)	+	2.10
Methionin (%)	+	1.00
Methionin + cystein (%)	+	1.30
Crude fibre (%)	+	2.34
Crude fats (%)	+	9.8
Energy content (MJ/kg)	+	13.48

At the beginning of the experiment (first sampling) and 21 days later (second sampling), 1 ml of blood was taken from three fish per tank (12 fish per group). Syringes and needles used for sampling were treated with heparin to avoid blood clotting. Samples were taken into 1.5 ml microcentrifuge tubes and blood plasma was isolated by centrifugation (1400G, 15 min). Blood plasma samples were stored at -20°C before the measurements.

Plasma lysozyme activity was measured using the method of S a n k a r a n and G u r n a n i (1972). Glucose concentration of blood plasma was measured by the GOD-POD-PAP method using a Fluitest GLU diagnostic reagent kit (Biocon, Germany). Cortisol level of blood plasma was determined by radioimmunoassay using a ^{125}I-RIA diagnostic reagent kit (Institute of Isotopes Co. Ltd., Hungary).

For lysozyme activity, each blood plasma sample was measured with three parallels and their average was used for the analysis. Glucose and cortisol levels were measured once for each sample. Differences between the results of each experimental group was evaluated by one way analysis of variance (ANOVA) at a significance level of $p<0.05$. Experimental results are presented in tables. SigmaStat statistical software (SPSS, Inc.) was used for statistical evaluation of experimental data.

RESULTS AND DISCUSSION

At the beginning of the experiment, plasma lysozyme activity was significantly higher in the "control crowded" group than in the "control normal" group. Twenty-one days later the lysozyme activity in the “control crowded” group was still significantly higher than in the “control normal” group. There was no significant difference of lysozyme activities in the two groups fed on experimental (Camelina oil containing) feed (Table 2.). There was no significant difference of the two other parameters, plasma glucose and cortisol level among the experimental groups at the beginning of the experiment or 21 days later (Tables 3. and 4., respectively).

Table 2. Lysozyme activity (µg/ml) of the blood plasma at the beginning of the experiment (1st sampling) and 21 days later (2nd sampling). *: significant difference compared to the relevant control (p<0.05)

	Experimental, crowded	Experimental, normal	Control, crowded	Control, normal
1st sampling	2.409±0.226	2.131±0.159	3.237±0.359*	2.094±0.137
2nd sampling	3.200±0.314	3.245±0.490	5.435±0.561*	3.648±0.370

Table 3. Glucose level (mmol/l) of the blood plasma at the beginning of the experiment (1st sampling) and 21 days later (2nd sampling)

	Experimental, crowded	Experimental, normal	Control, crowded	Control, normal
1st sampling	3.606±0.193	3.952±0.365	4.100±0.280	3.571±0.350
2nd sampling	4.625±0.459	4.939±0.584	4.767±0.544	5.443±0.407

Table 4. Cortisol level (ng/ml) of the blood plasma at the beginning of the experiment (1st sampling) and 21 days later (2nd sampling)

	Experimental, crowded	Experimental, normal	Control, crowded	Control, normal
1st sampling	180.3±104.8	202.3±52.6	373.6±110.3	358.6±86.5
2nd sampling	139.4±39.5	181.7±62.1	350.9±99.3	388.3±75.8

In a previous experiment only slight differences in cortisol and no differences in glucose levels were found in rainbow trout held under crowded conditions (100 kg/m^3) and fed on diets with different levels of vitamins E and C and highly unsaturated fatty acids (HUFA) as compared to normal crowding density (20 kg/m^3) (T r e n z a d o et al., 2008). No significant differences in plasma glucose levels were found in Nile tilapia fed on high protein diets and held at low and high stocking densities (Abdel-Tawwab et al., 2005).

CONCLUSION

From our observations it seems possible that tolerance of carp to crowding stress does not depend on the applied diet composition.

Acknowledgements:

Our research was financed by the EU-funded AQUAMAX project (Sixth Framework, contract number: FOOD-CT-2006-16249). Renata Relic participated in research work in the frame of the EU-funded ROSA project (Coordination and Support Actions, project number: REGPOT-2007-3).

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