THESES OF DOCTORAL (PhD) DISSERTATION

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INVESTIGATIONS ON ENVIRONMENTAL FRIENDLY INTENSIVE POND CULTURE PRODUCTION OF EUROPEAN CATFISH

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1. BACKGROUND OF RESEARCH, OBJECTIVES

The contribution of fish to world's animal protein supply is significant, topping out at 17%. Aquaculture production is continuously increasing since the 1950s. The share of aquaculture products in total food fish consumption was 53 percent (171 million tons) in 2016 (FAO, 2018).

Market demands products of good quality, which are available throughout the whole year. These needs can only be achieved by application of intensive technologies. Due to the high operation costs of intensive rearing systems, only producing of high market value fishes such as the carnivorous species is viable in them. Profitability of Hungarian aquaculture also can be increased by raising the current 3-4 % share of carnivorous species.

The European catfish (*Silurus glanis* L. 1758) is the largest predatory fish in Europe. It has white, boneless and tasty flesh, which meets the demands of the market. It grows rapidly; furthermore, it has especially good feed conversation ratio compared to other predatory fish species. It adapts well to commercial fish feeds, different rearing technologies and handling. Hungarian catfish production has a great potential in term of genetics and technology development as well.

Global demand for animal proteins is continuously increasing, thus to meet them aquaculture production is growing in parallel, especially in intensive pond rearing systems (Zhang et al., 2020). Fish utilize only part of the applied feed, while unused nutrients remain and accumulate in the water body and sediment of ponds (Sun and Boyd, 2013). Nutrients are accumulating mainly in the sediment (Gross et al., 2000; Zhang et al., 2016).

Due to the overfishing of seas and oceans production of fishmeal is undulating since 1994, but overall, it shows a declining trend. While in 1983 price of fishmeal was 400 $ per ton, in 2017 it approached 1600 $. Due to the constantly increasing prices and decreasing production research was focused

on alternative protein sources such as soybean meal, rapeseed meal, cottonseed meal, animal by-products etc. Ratio of fishmeal was successfully decreased in the diet of several fish species e.g. in the case of channel catfish (*Ictalurus punctatus*) (Twibell and Wilson, 2004) and rainbow trout (*Oncorhynchus mykiss*) (Gibson Gaylord et al., 2007).

The main aims of my studies were as the following:

1. To investigate the effects of the different protein sources of the diets such as the animal originated proteins (fish meal and meat meal) and plant-based proteins (corn and wheat) on the production traits of European catfish and on the chemical composition of water and sediment.
2. To evaluate the effect of different rearing technologies (monoculture, intensive-extensive pond system) on the production traits, water quality and chemical composition of the sediment.
3. To assess the effect of different treatments as mechanical mixing, aeration, biological and chemical treatments on chemical composition of the water and the sediment.
4. To study the opportunities of fishmeal substitution with soybean meal and processed animal protein, and their effect on production traits, growth and protein metabolism related gene expression in the liver.

2. MATERIALS AND METHODS

Four experiments are presented in the thesis. During the 1st trial European catfish was fed with different plant and animal origin protein containing diets. In the 2nd experiment effects of two different rearing technologies were evaluated. During the 3rd trial effects of different pond treating technologies as control (Ko), mechanical mixing (Ke), sodium percarbonate (Na), bacterium product (B), sodium percarbonate + bacterium product (NaB), aeration (L), aeration + sodium percarbonate (LNa), aeration + bacterium product (LB), aeration + sodium percarbonate + bacterium product (LNaB) were investigated. During the 4th trial catfish was fed with different levels of soybean meal and processed animal protein containing diets and their effects were evaluated. The main characteristics of the experiments are summarized in Table 1.

Table 1: Experimental setups

Experiment	Spices	Duration	Treatments	Aim of trials	Number of treatments	Number of replications	Examined parameters
1	European catfish	21. 06-25.09. 2013. (97 days)	Diets of 6 different protein sources (fishmeal, meat meal, corn, wheat, wheat + xylanase, wheat + beta-glucanase)	To investigate the effects of the different protein sources of the diets on the production traits and on the chemical composition of water and sediment.	6	3	production traits and chemical composition of water and sediment
2	European catfish, common carp	06.05-06.10. 2014 (154 days)	monoculture, intensive-extensive system	To evaluate the effect of different rearing technologies (monoculture, intensive-extensive pond system) on the production traits, water quality and chemical composition of the sediment.	2	4	production traits and chemical composition of water and sediment
3	-	15.05.-10.07 2013 (57 days)	Different pond treating technologies (control, mechanical mixing, Sodium percarbonate, Bacterium product, Sodium percarbonate + Bacterium product, aeration, aeration + Sodium percarbonate, aeration + Bacterium product, aeration + Sodium percarbonate + Bacterium product)	To determine the effect of different treatments as mechanical mixing, aeration, biological and chemical treatments on chemical composition of water and the sediment.	9	3	chemical composition of water and sediment
4	European catfish	06.05-24.07 2014 (80 days)	replacing fishmeal with soybean meal and processed animal protein (30, 60, 100 %) + control	To study the opportunities of fishmeal substitution with soybean meal and processed animal protein, and their effect on production traits, growth and protein metabolism related gene expression in the liver.	7	3	production traits and expression of genes

2.1. Origin and setting of experimental stocks

One and two years old European catfish stocks were used in the studies. For the 1st trial experimental stock was purchased from Attala Hal Ltd. In the 2nd and 4th experiments two years old stocks were originated from the stock of the previous year. In the 2nd experiment two years old common carp stock was originated from NAIK HAKI.

At arrival of fish the temperature of transporting and rearing water was equalized. Fish were quarantined in tanks of NAIK HAKI's recirculation system. Until the beginning of the experiments, fish were fed with commercial fish feed produced by Halták Ltd. Fish were acclimatized for 2 weeks to the new rearing conditions.

2.2. Experimental design

2.2.1. Effect of different protein sources of the diet on the production traits of European catfish and on the chemical composition of water and sediment

The feeding experiment lasted for 97 days. 144 catfish were stocked in 18 limnocorals, 8-8 one in each. Average initial body weight was 72.7±1.3 g. Fish were individually measured at the beginning and at the end of the experiment. At the 5th and 9th weeks of the experiment 50 % of the stock was measured.

The 6 experimental feeds contained 34,5-35,6 % protein of different source. All experimental diets were fed in 3 randomly chosen limnocorals. Composition of fed diets are shown in Appendix 1.

On the 1st, 14th, 28th, 42th, 56th, 70th, 84th and 95th days of the experiment water samples were taken from each limnocoral. Samples were taken from the entire water column. Sediment samples were taken from each limnocoral on

the 1st and 95th days of the experiment after filling and before draining the pond. Samples were taken from the upper 10 cm of the sediment.

2.2.2. Effect of different rearing technologies (monoculture, intensive-extensive pond system) on the production traits, water quality and chemical composition of the sediment

Feeding lasted for 154 days. Fish were stocked into 4-4 ponds of two different size (350 m^2 700 m^2). In monoculture treatment, only catfish were stocked into the ponds. In the case of intensive-extensive system, catfish were stocked into 2-2 cages. Size of the cages were as follows: 3x3x2 m and 3x6x2 m. Two years old common carp were stocked in the pond. Average initial body weight of catfish was 485.68±3.43 g (n=2480). Due to the huge number of fish, they were measured in groups (20 individuals/bucket). Average initial body weight of common carp was 348.9±2.5 g (n=432). From the beginning of the experiment 20 % of the whole stock was measured biweekly with probe harvesting.

Fish were fed with Aller Bronze (6 mm) fish feed during the entire experiment. Composition of fed diets are shown in Appendix 2.

On the 1st, 14th, 28th, 42th, 56th, 70th, 84th, 98th, 112th, 126th, 140th and 153th days of the experiment water samples were taken from each pond. Samples were taken from the entire water column. Sediment samples were taken at 9 points of each pond on the 1st and 153th days of the experiment after filling and before draining the pond. Samples were taken from the upper 10 cm of the sediment.

2.2.3. Effect of different treatments on chemical composition of water and sediment

27 pieces of 5 liter-sized jars were used during the 57 days long trial. 600 g sediment with high dry matter content were placed into each bottle. Sediment samples were originated from the Bikazugi Holt-Körös, which is the receiving water of NAIK HAKI's effluent water. Jars were filled up with 3.3 litre pond water. Sediment of the jars. Sediment of the jars were sampled at the beginning (1st day, 150 g) and at the end (57th day, 150 g) of the experiment. 100 ml water samples were taken weekly (9 times altogether). Jars were refilled with 100 ml distilled water after sampling. Oxygen content, pH, and conductivity was measured in the jars 3 times weekly.

2.2.4. Substitution of fishmeal with soybean meal and processed animal protein in European catfish diets

The feeding experiment lasted for 80 days. 75 two years old catfish were stocked into 3-3 cages/treatment (size 3x3x3 m). Cages were placed into a pond of 1700 m^2. Average initial body weight of catfish was 350.94±5.24 g. Fish were individually measured at the beginning and at the end of the experiment and in the meantime biweekly.

The 7 experimental feeds contained 435 g/kg crude protein. Control diet contained 49 % fishmeal as protein source. Diets were fed in randomly chosen cages. Composition of fed diets are shown in Appendix 3.

2.3. Sampling of fish and chemical analysis

At the beginning of the fourth experiment, nine fish from the start population and three fish from each cage at the end of experiment were randomly collected for analyses of whole-body proximate composition. In addition, another four fish from each cage were anaesthetized, killed with a blow to the head and then dissected to collect the targeted organ (liver) at the end of experiment. Four liver samples were taken from the same area of liver on each fish. Liver samples from two fishes were immediately frozen in liquid nitrogen and stored in at −80°C until analyses of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Similarly, liver samples from another two fishes were immediately stored in an RNA later at −80°C until RNA extraction for gene expression analysis.

In the first and fourth experiment the proximate composition of the feed and fish was analyzed by standard methods of AOAC (1995). Dry matter was determined gravimetrically after drying at 105 °C for 4 h. Total nitrogen was determined by Kjeldahl method using a digestion block (KJELDATHERM, Gerhardt, Germany) and distillation method (VAPODEST 30, Gerhardt, Germany), and crude protein calculated as $N \times 6.25$. Fat was determined by Soxhlet method using a semi-automatic system (SOXTHERM 2000, Gerhardt, Germany) and diethyl ether (boiling point, 40–60 °C) as a solvent, and ash content was estimated after combustion at 550 °C for 4 h. Crude fiber content was determined in fat extracted feed sample by digestion with sulphuric acid (0.51 mole/l) and potassium hydroxide (0.89 mole/l) in a GERHARDT Crude Fibre apparatus (Gerhardt, Germany). Total carbohydrate was calculated by differences as, 1000 − (crude protein + fat + ash + fiber). The gross energy of experimental diets was calculated as described by Halver (1976). The amino acid contents in diets were analyzed by the accredited laboratory of the

Hungarian Food Chain Safety Office following the ISO 13903:2005 Official Method.

The chemical analysis of water and sediment samples were examined in the National Agricultural Research and Innovation Centre Research, Institute of Irrigation and Water Management Laboratory.

2.4. Gene expression

2.4.1. RNA extraction

Total RNA was isolated from liver samples using a Promega RNA Isolation Kit (Cat No. Z3100, USA) according to the manufacturer's instructions. The quantity of the RNA was assessed using a Nano-Drop spectrophotometer (NANODROP 2000, Thermo Scientific, Wilmington, DE, USA). The integrity (quality) was checked by denaturing gel electrophoresis (1% agarose gel) and the purity by measuring the OD260/OD280 absorption ratio (>1.95).

2.4.2. cDNA synthesis

cDNA was generated from 1 μg of total RNA using the Omniscript Reverse Transcriptase cDNA synthesis kit (Qiagen, Germany) for reverse transcriptase polymerase chain reaction (RT-PCR) following the manufacturer's protocol. The product of the first strand cDNA synthesis was stored at −80°C until the quantitative RT-PCR (qRT-PCR) runs.

2.4.3. Real-time quantitative RT-PCR

Highly purified salt-free "OliGold" primers (Eurogentec, Seraing, Belgium) for the quantification of the target genes were designed using the LightCycler probe design software, version 1.0 (Roche Diagnostics, Vilvoorde, Belgium). qPCR analyses were performed on an Mx3000P QPCR System (Agilent Technologies, Belgium).

2.4.4. Protein metabolism enzymes

SIGMA-ALDRICH® Alanine Aminotransferase (ALT) Activity assay kit (Catalog # MAK052, SIGMA-ALDRICH, USA) and Aspartate Aminotransferase (AST) Activity assay kit (Catalog # MAK055, SIGMA-ALDRICH, USA) were used to determine ALT and AST activity in liver of fish.

2.5. Statistical analysis

Statistical data were evaluated by SPSS for Windows 22.0 (2013). Normal distribution of variables was checked by Kolmogorov-Smirnov test. Equality of variances was assessed with Levene-test.

In the first experiment, one-way ANOVA with Tukey post-hoc test ($P<0.05$) was used to evaluate the effect of different treatments on the production traits and on the chemical composition of water and sediment

In the second experiment, two-sample t-test was used to evaluate the effect of different treatments on the production traits and on the chemical composition of water and sediment.

In the third experiment variables were not normally distributed, thus effect of treatments was evaluated with the non-parametric Kruskal-Wallis test.

In the fourth experiment, ANOVA was used to evaluate the effect of different treatments on the production traits, whole body composition and growth-related gene expression. Duncan's new multiple range test was used to compare sets of means.

In the 1^{st}, 2^{nd}, and 4^{th} experiments no individual marking was used, thus limnocoral, cage and pond averages were counted in the case of production traits, water and sediment chemical composition.

3. RESULTS

3.1. Effect of different protein sources in diets on production traits of European catfish and on water and sediment quality

3.1.1. Results of examination of production traits

By the end of the trial mean body weights of HU and HA groups differed significantly ($p<0.05$) from the B, BA and BB groups (Table 2). However, the final mean weight of K group did not differ significantly from any of the other treatments. Weight gain of B and K differed significantly ($p<0.05$) from HA, but not from HU group. For the total experimental period, SGR ranged from 1.43 (BB) to 1.73 (HA). Considering this parameter, the enzyme-treated diets showed significantly poorer achievements than the HU and HA diets. The animal protein containing feeds did not differ significantly from each other. The feed conversion of HU group did not differ from the HA group significantly. Regarding PER, there were significant differences ($p<0.05$) between FM group and all the animal protein free groups.

Table 2: Growth performance of catfish (mean±SE)

Treatments	Initial weight (g)	Final weight (g)	Body mass gain (%)	SGR[7] (%/nap)	FCR[8] (g/g)	PER[9] (g/g)
HA[1]	73.6±0.5	381.5±1.5^{a}	418.3±20.9^{a}	1.73±0.04^{a}	1.13±0.06^{a}	2.57±0.08^{a}
HU[2]	71.6±1.1	353.1±1.1^{a}	391.7±34.8^{ab}	1.67±0.07^{a}	1.29±0.02^{ab}	2.17±0.02^{ab}
K[3]	73.2±1.0	332.2±1.3^{ab}	348.9±22.5^{abc}	1.58±0.05^{ab}	1.45±0.06^{ab}	1.99±0.06^{b}
B[4]	72.1±1.4	305.8±1.1^{b}	330.1±18.9^{bc}	1.53±0.04^{ab}	1.53±0.11^{b}	1.85±0.07^{b}
BA[5]	73.6±1.5	297.5±1.0^{b}	303.1±25.6^{c}	1.47±0.06^{b}	1.55±0.12^{b}	1.84±0.10^{b}
BB[6]	71.4±1.4	285.4±0.9^{b}	290.6±25.8^{c}	1.43±0.06^{b}	1.61±0.11^{b}	1.79±0.13^{b}

[1] fishmeal; [2] meat meal; [3] corn; [4] wheat; [5] wheat + xylanase; [6] wheat + beta glucanase; [7] specific growth rate; [8] feed conversation ratio; [9] protein efficiency rate

3.1.2. Results of examination of physical and chemical parameters of water

Dissolved oxygen (DO) levels were 6.67±0.34 mg/l in the treatments. pH was relatively stable during the experiment (7.86±0.05). The maximum values of water samples did not exceed the limit values determined by Hungarian regulations 28/2004. (XII.25.) KvVM order Appendix 2. The chemical composition of water samples is shown in Table 3. There were no statistically significant differences between the treatments. The values of ammonium nitrogen (NH_4-N) changed periodically during the experiment, which corresponded to the changes of amount of feed used. The nitrate-nitrogen (NO_3-N) values changed similarly to NH_4-N values in the study, more or less in contrast to that, which can be explained by the bacterial conversion of NH_4-N to nitrate. The concentration of total nitrogen (TN) changed similarly in all treatments during the culture period. The change of the TN concentration of the water bodies may have been correspond to the nitrogen accumulation of the sediment. It was found a continuous decrease in orthophosphate (PO_4-P) values, which can also be explained by the interactions between the sediment and the water body – the orthophosphate was transformed into a periodical formed organic phosphorus, which entered the sediment layer by sedimentation, either temporarily or permanently.

Table 3: Chemical composition of water samples (mean±SE)

	HA[1]	HU[2]	K[3]	B[4]	BA[5]	BB[6]
NH_4-N[7] (mg/l)	0.23±0.2	0.21±0.11	0.17±0.06	0.22±0.01	0.15±0.09	0.17±0.14
NO_3-N[8] (mg/l)	0.04±0.03	0.04±0.03	0.04±0.04	0.06±0.06	0.05±0.04	0.07±0.12
NO_2-N[9] (mg/l)	0.02±0.02	0.03±0.02	0.03±0.02	0.05±0.04	0.02±0.01	0.03±0.02
TN[10] (mg/l)	1.0±0.7	0.9±0.5	1.0±0.7	0.9±0.6	0.8±0.5	0.8±0.5
PO_4-P[11] (mg/l)	0.06±0.03	0.07±0.04	0.07±0.03	0.08±0.05	0.08±0.04	0.06±0.03
TP[12] (mg/l)	0.17±0.07	0.16±0.06	0.18±0.08	0.17±0.08	0.18±0.08	0.14±0.06
TSS[13] (mg/l)	18.8±16.1	13.1±11.5	14.6±10.9	6.9±7.9	10.1±9.6	12.8±15.4
Chl-a[14] (mg/l)	42.8±50.6	15.3±23.02	41.2±57.9	13.1±17.8	26.2±51.6	18.8±23.9

[1] fishmeal; [2] meat meal; [3] corn; [4] wheat; [5] wheat + xylanase; [6] wheat + beta-glucanase; [7] ammonium-N; [8] nitrate-N; [9] nitrite-N; [10] total N; [11] orthophosphate-P; [12] total P; [13] total suspended solids; [14] chlorophyll-a

3.1.3. Results of examination of sediment parameters

The values of dry matter, Kjeldahl-N and phosphate are shown in Table 4. There was no significant difference between the treatments in the case of dry matter. Slight differences were observed, which can be explained by the different digestibility of the feeds. The content of Kjeldhl-N changed differently between the groups. These values are increased in the treatment K and B, and are decreased in case of the other groups. The difference between the initial and final values of the HA and HU treatments can be explained by the better digestibility of the feeds. In the case of BA and BB treatments, the decrease might be caused by the enzyme supplementation used. The content of phosphate in dry matter in all treatment. There were no significant differences between groups. The apparent decrease in the nitrogen and phosphorus content of the sediment contradicts the fact that significant amount of N and P were introduced into the limnocorals with feed during the experiment. The explanation for this phenomenon may be the fish population and the aeration.

The nutrients in the upper layer of sediment became easily mobilizable and partly transferred to the water column.

Table 4: Dry matter, Kjeldahl-N and phosphate content of sediment the beginning and end of the trial (mean±SE)

Treatments		Dry matter (m/m%)	Kjeldahl-N (mg/kg d.m.)	Phosphate (mg/kg d.m.)
HA[1]	1. nap	46.4±2.4	1490±230.6	2776.7±263.9
	95. nap	52.7±4.4	1456.7±225	608.7±81
HU[2]	1. nap	55±1.2	1021.3±84.3	2060±87.2
	95. nap	56.7±3.3	913.7±83.4	517±72.7
K[3]	1. nap	47.6±1.6	1300±34.6	2643.3±102.1
	95. nap	57.6±3	1346.7±281.5	579.7±41.1
B[4]	1. nap	54.8±3.6	1138.3±211.7	2006.7±222.8
	95. nap	67.3±2.3	1293.3±135	748±135.5
BA[5]	1. nap	63±6	920.3±324.7	1880±197
	95. nap	64.6±4.6	825±222.8	499.3±125.6
BB[6]	1. nap	51.1±3.5	1320±255.3	2303.3±250.1
	95. nap	56.5±7.4	1099.7±416.3	539±58.6

[1] fishmeal; [2] meat meal; [3] corn; [4] wheat; [5] wheat +xylanase; [6] wheat + beta-glucanase

3.2. Effect of different rearing technologies (monoculture and extensive-intensive combined system) on production traits of European catfish and on water and sediment quality

3.2.1. Results of examination of production traits

Growth parameters of experimental stock are shown in Table 5. There was no statistical difference between the groups in the case of initial and final body weight. At the end of the experiment cage reared fish were approximately 100 g bigger compared to monoculture however both groups quadrupled their initial body weight (M: 3.92x; I-E: 4.13x). Specific growth rate (SGR) was rather low (0.9±0.1 %) in both groups than values expected in a predatory species. These results can be explained with the higher feed wastage of cage-

reared stocks, and in the case of monoculture with the number of feedings. Final body weight of common carp stocked into the pond were 7 times (6.8x) bigger than the initial body weight. However, results of production traits are not as good as it could be expected in the case of European catfish (FCR: 1 g/g; SGR: 2 %/day), but production of common carp can compensate that economically.

Table 5: Growth performance of catfish and common carp (mean±SE)

Treatments	Initial weight (g)	Final weight (g)	Weight gain (g)	Body mass gain (%)	SGR[3] (%/day)	FCR[4] (g/g)
M[1]	485.2±4.6	1903.3±238.3	1418.1±238.8	292.3±49.6	0.9±0.1	1.4±0.4
I-E[2]	485.5±2.7	2002.9±170.3	1517.5±167.6	312.6±32.8	0.9±0.1	1.4±0.4
Common carp	333.5±3.7	2266.9±87.6	1933.4±86.5	579.7±25.1		

[1] monoculture; [2] intensive-extensive system; [3] special growth rate, [4] feed convertion ratio

3.2.2. Results of examination of physical and chemical parameters of water

During the experiment, dissolved oxygen (DO) level of the ponds was 7.16±2.17 mg/l in the I-E treatment and 7.73±2.23 mg/l in the M treatment. Average pH values were 8.0±0.3 (I-E) and 8.09±0.36 (M). Data of water samples are shown in Table 6. Among tge eight examined parameters, four resulted in significant difference. Nitrate nitrogen (NO_3-N), orthophosphate phosphorus (PO_4-P), total suspended solid (TSS) and chlorophyll-a (Chl-a) differed significantly between the treatments. TSS was two times higher in the intensive-extensive treatment compared to monoculture. This significant difference can be explained with the bioturbing of common carp placed outside of the cages. Chl-a values of monoculture treatment were two time higher compared to I-E treatment. This difference also can be explained with the bioturbing of common carp, which lead to the increased turbidity of water. This caused decreased light penetration and latter lead to decreased production of

phytoplankton. TSS values exceed the limit values determined by Hungarian regulations (28/2004. (XII.25.) KvVM order Appendix 2).

Table 6: Chemical composition of water samples (mean±SE)

	Ammonium-N (mg/l)	Nitrate-N (mg/l)	Nitrite-N (mg/l)	Total N (mg/l)
M[1]	0.948±0.79	0.356±0.43[a]	0.142±0.19	4.565±3.11
I-E[2]	0.781±0.54	1.004±0.79[b]	0.188±0.12	3.491±1.75
	Orthophosphate-P (mg/l)	Total P (mg/l)	Total suspended solids (mg/l)	Chlorophyll-a (mg/l)
M	0.181±0.12[a]	0.537±0.42	60.839±42.92[a]	220.855±256.78[a]
I-E	0.072±0.02[b]	0.329±0.16	138.689±87.74[b]	110.067±120.37[b]

[1] monoculture; [2] intensive-extensive system

Figure 1, 2 and 3 show the changes of total nitrogen, total inorganic nitrogen, and total organic nitrogen content. In the I-E treatment changes of N containing parameters were consistent. Controversially, in the second half of the experiment in M treatment values of total nitrogen and total organic nitrogen differed largely from values of I-E group. Figure 3 shows that organic nitrogen content of M group started to increase to a great extent after the 5th sampling. It corresponds with temporal changes of Chl-a content which shows similar pattern. Decreased total nitrogen content of water body in I-E treatment can be explained with the filter effect of the extensive component, where significant amount of nitrogen is accumulating via the weight gain of the common carp. This also means that the nutrient transformation efficiency of the combined system together with the additional fish production yield exceeds that of monoculture ponds.

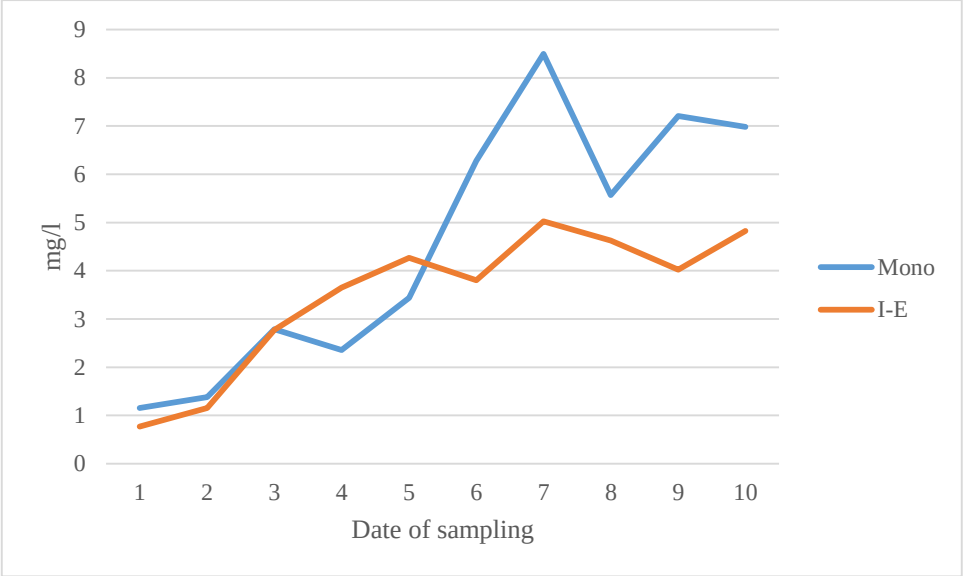

Figure 1: Total nitrogen values of the water samples

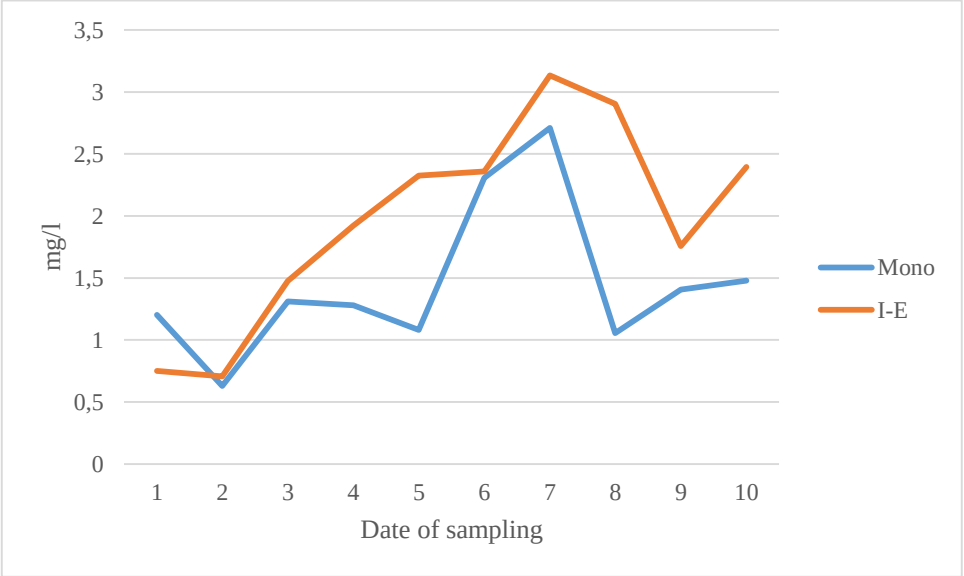

Figure 2: Total inorganic values of the water samples

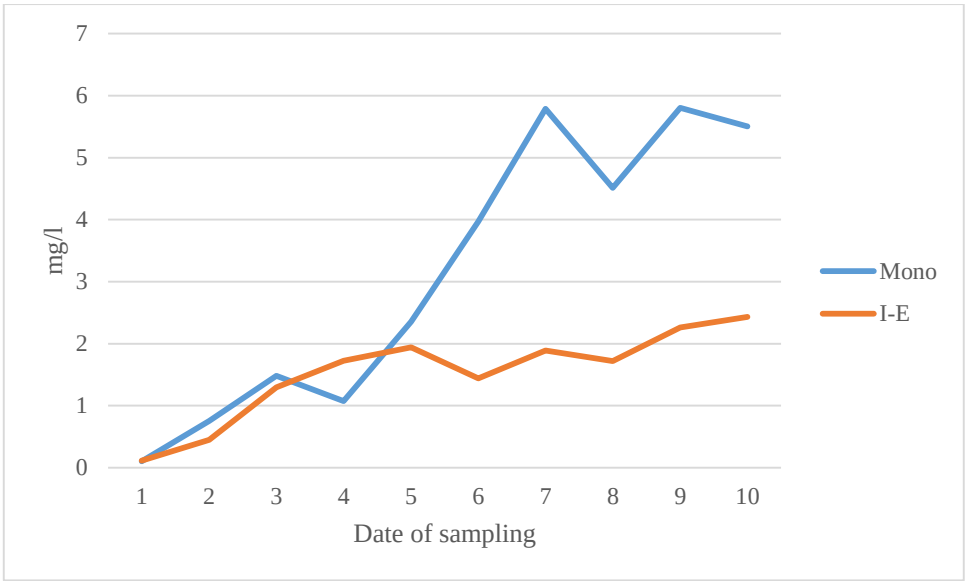

Figure 3: Total organic nitrogen values of the water samples

Figure 4 shows the changes of orthophosphate phosphorus through out the experiment. Similarly, to the nitrogenous parameters notable differences could be observed between the values of the two groups in the second half of the experiment. This phenomenon can be explained with the nutrient removing ability of the extensive part of the combined system. Stocking common carp in the extensive part led to increased organic matter (including organic particulate phosphorus) removal rate.

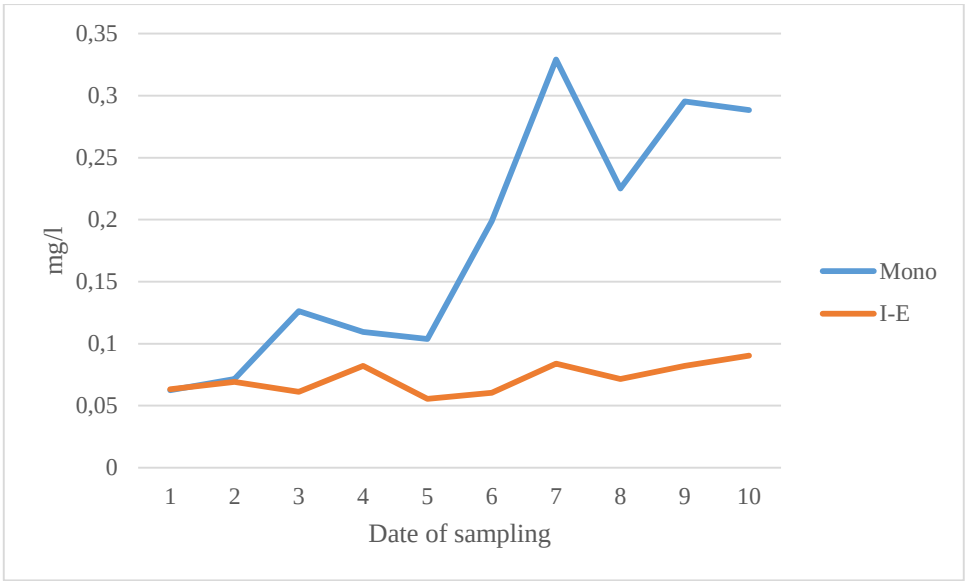

Figure 4: Orthophosphate values of the water samples

3.2.3. Results of examination of sediment parameters

Dry matter content of samples decreased by the end of the experiment in both treatments (Table 7). The values of the two other examined parameters (KN and P) – similarly to water samples TSS and Chl-a values – changed adversely between the groups. This also suggests that, nutrient transformation efficiency of combined system exceeds that of monoculture. Extensive pond removes significant amount of organic N and P compounds, and accumulates them in additional common carp production.

Table 7: Dry matter, Kjeldahl-N and phosphate content of sediment the beginning and end of the trial (mean±SE)

		Dry matter (m/m %)	Kjeldahl-N (mg/kg d.m.)	Phosphate (mg/kg d.m.)
M[1]	Initial	61.5±11.3	1630.8±1515.9	1133.6±751.8
	Final	43.3±10.6	2901.5±1661.8	1778.8±1654.1
I-E[2]	Initial	69.2±6.4	1622.5±1826.5	2001.5±2023.4
	Final	60.4±7.7	1200.8±859.0	1299.9±1318.4

[1] monoculture; [2] intensive-extensive system

3.3. Effect of different pond treatment methods on water and sediment chemical traits

3.3.1. Results of examination of chemical parameters of water

Dissolved oxygen content of the different groups during the experiment are shown in Figure 5. Curves of the certain treatments can be divided in three different groups. First group consists from the treatments where aeration was applied. In the middle control and sodium percarbonate groups can be seen separately. Aeration and the lack of heterotroph microbial activity-increasing treatments resulted in moderate oxygen level. Curves of mechanical mixing, bacterium product and bacterium product + sodium percarbonate treatments can be found at the bottom of the figure. Low DO level is caused - besides the lack of aeration – by the higher oxygen demand of heterotroph activity which increased due to mechanical mixing and bacterium product supplementation.

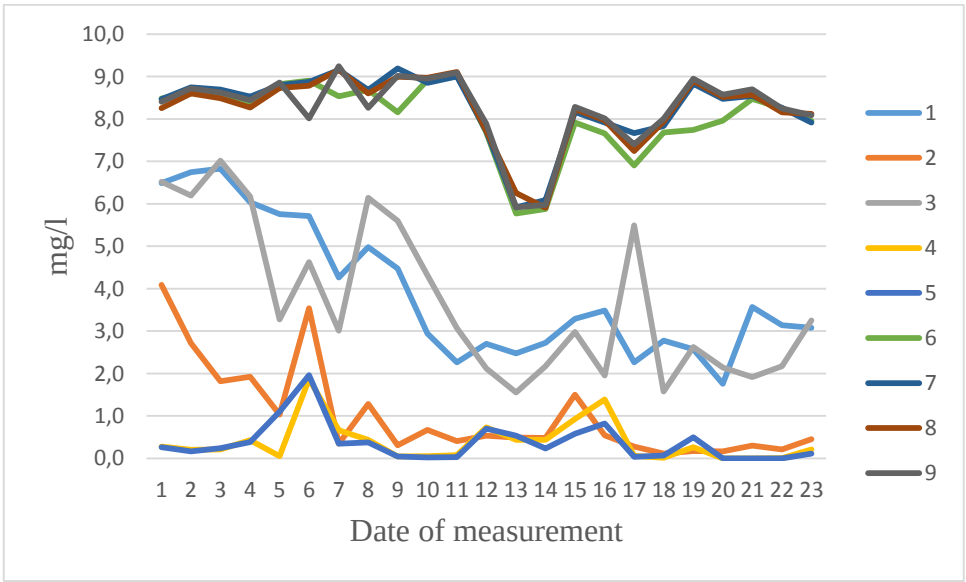

Figure 5: Time evolution of dissolved oxygen content of the treatments
[1] control; [2] mechanical mixing; [3] sodium percarbonate; [4] bacterium product; [5] sodium percarbonate + bacterium product; [6] aeration; [7] aeration + sodium percarbonate; [8] aeration + bacterium product; [9] aeration + sodium percarbonate + bacterium product

Results of water sample analyses are shown in Table 8. Ammonium nitrate content were significantly different in B and NaB groups compared to the other groups. Values belonging to LB and LNaB treatments were also higher compared to the other five treatments. Similar tendencies were observed in the case of total inorganic nitrogen, total organic nitrogen, total nitrogen and PO_4-P. This indicates that bacterium product supplementation significantly helped the degradation of sediments' dry matter content, which resulted in elevated inorganic N and P content of the water body. Bacterium supplementation combined with aeration lead to increased ammonium oxidation, and parallel to the decreased ammonium N concentration, nitrate and nitrate content increased.

Table 8: Chemical composition of water samples (mean±SE)

Treatments	Ammonium-N	Nitrate-N	Nitrite-N	Total Inorganic N	Total Organic N	Total N	Orthophosphate-P	Total P	Total Suspended Solids
	mg/dm^3	mg/dm^3	mg/dm^3	mg/dm^3	mg/dm^3	mg/dm^3	mg/dm^3	mg/dm^3	mg/dm^3
Ko[1]	0.15±0.05	1.1±0.3	0.1±0.2	1.4±0.4	0.5±0.2	1.9±0.5	0.05±0.04	0.06±0.05	7.5±7.9
Ke[2]	0.48±0.16	0.6±0.2	0.2±0.3	1.2±0.3	0.7±0.3	1.9±0.4	0.08±0.05	0.14±0.08	67.7±47.3
Na[3]	0.41±0.22	0.9±0.5	0.2±0.2	1.5±0.3	0.4±0.2	1.9±0.3	0.08±0.08	0.12±0.11	6.2±5.9
B[4]	22.80±4.18	0.1±0.1	0.2±0.3	23.02±4	4.2±2.7	27.2±3.7	1.48±0.58	2.00±0.65	25.2±24.2
NaB[5]	22.59±3.28	0.1±0.3	0.4±0.7	23.1±2.7	3.9±2.3	27.1±3.2	1.71±0.62	2.17±0.64	23.3±21
L[6]	0.27±0.24	1.6±0.8	0.03±0.02	1.9±0.9	0.6±0.4	2.5±0.9	0.09±0.03	0.11±0.03	13.2±13.5
LNa[7]	0.19±0.06	1.6±0.9	0.2±0.3	2±0.9	0.6±0.3	2.6±0.9	0.09±0.03	0.13±0.07	43.6±65.8
LB[8]	3.02±5.44	10.2±5.2	2±3.8	15.2±5.3	3.6±3.5	18.8±6.3	0.23±0.15	0.52±0.30	66.7±87.6
LNaB[9]	2.16±3.31	8.8±4.8	2.4±4.5	13.4±4.4	3.4±2.6	16.8±5.7	0.29±0.14	0.54±0.16	58.2±90.1

[1] control; [2] mechanical mixing; [3] sodium percarbonate; [4] bacterium product; [5] sodium percarbonate + bacterium product; [6] aeration; [7] aeration + sodium percarbonate; [8] aeration + bacterium product; [9] aeration + sodium percarbonate + bacterium product

3.3.2. Results of examination of sediment parameters

Initial and final values of sediment samples are shown in Table 9. Dry matter content of sediment samples decreased in every groups comparing them to the initial values. Increasing KN content of sediment samples were observed, in the B group it was two times higher. Considering B group, elevated KN amount is related with increased TAN content and heterotroph biomass. Phosphorus content as well as dry matter content decreased in all treatments.

Table 9: Dry matter, Kjeldahl-N and phosphate content of sediment the beginning and end of the trial (mean±SE)

	Dry matter	Kjeldahl-N	P[10]
	m/m%	mg/kg d.m.	mg/kg d.m.
Initial sample	52.2	1450	1480
Ko[1]	46±1.4	2033.3±308.9	1030±20
Ke[2]	43.78±3.2	3710±246.4	1060±26.5
Na[3]	45.9±1.1	3203.3±279.3	1026.7±20.8
B[4]	44.4±1.8	4233.3±1333.2	1050±36.1
NaB[5]	43.9±1.3	2953.3±1295.1	1083.3±51.3
L[6]	43.8±1.6	3606.7±424.4	1030±10
LNa[7]	42.9±1.1	3460±492.4	1026.7±15.3
LB[8]	43.5±2.6	2923.3±447.4	1076.7±50.3
LNaB[9]	40.9±4	2890±85.4	1110±50

[1] control; [2] mechanical mixing; [3] sodium percarbonate; [4] bacterium product; [5] sodium percarbonate + bacterium product; [6] aeration; [7] aeration + sodium percarbonate; [8] aeration + bacterium product; [9] aeration + sodium percarbonate + bacterium product; [10] phosphate

3.4. Effect of fish meal replacement by different ratio of soybean meal and processed animal protein on the growth response and liver gene expression of European catfish

3.4.1. Proximate composition of whole body

The effect of different inclusion levels of SM and PAP on the body composition of European catfish is presented in Table 10. Whole-body

composition analysis showed that replacement of dietary FM with either SM or PAP significantly increased ($p<0.05$) whole-body moisture content and decreased ($p<0.05$) whole-body crude protein content. Replacement of dietary FM with SM significantly ($p<0.05$) decreased whole-body crude lipid up to 60% replacement level. No significant differences were found in the whole-body ash content among different diet treatments.

Table 10: Whole body chemical composition (g/kg) (mean±SE)

Treatments	Moisture	Crude protein	Crude fat	Ash
FM (control)[1]	729.3±0.1^{c}	157.8±1.5^{a}	53.4±3.6^{b}	23.7±0.4
SM30[2]	774.2±1.5^{a}	143.9±1.1^{a}	40.2±1.7^{c}	20.4±0.1
SM60[3]	782.7±1.8^{a}	142.5±5.1^{a}	32.9±2.0^{c}	23.1±0.4
SM100[4]	752.8±1.7^{b}	137.6±5.2^{b}	72.6±9.1^{a}	19.6±0.8
PAP30[5]	749.6±0.6^{b}	141.7±3.4^{a}	75.5±0.3^{a}	20.6±0.8
PAP60[6]	769.6±0.6^{ab}	147.7±3.2^{a}	55.8±0.5^{b}	22.1±0.3
PAP100[7]	764.3±0.7^{ab}	137.0±0.8^{b}	63.5±0.9^{ab}	20.9±0.2

[1] fishmeal; [2] soybean meal 30%; [3] soybean meal 60%; [4] soybean meal 100%; [5] processed animal protein 30%; [6] processed animal protein 60%; [7] processed animal protein 100%

3.4.2. Growth performance and nutrient utilization

The dietary SM or PAP level significantly ($p<0.05$) affected the growth performance in the present study. There were no significant differences in body mass gain (%) and MGR among fish fed the diets with 30 and 60% replacement of FM from either SM or PAP (Table 11). When either SM or PAP replaced 100% FM, FCR and PER were lower ($p<0.05$) and higher ($p<0.05$), respectively, compared with the other groups. As the replacement level was increased, the PPV decreased ($p<0.05$) and lowest PPV was found in 100% substitution level.

Table 11: Growth performance of European catfish fed practical diets with different levels of substitution of FM by SM or PAP (mean ± SE)

Treatments	Initial weight (g)	Final weight (g)	Weight gain (g)	Body mass gain (%)	MGR[8]	FCR (g/g)[9]	PER (g/g)[10]	PPV (%)[11]
FM (control)[1]	351.03±0.09	935.12±7.10^{a}	584.08±7.10^{a}	166.39±2.02^{a}	6.50±0.10^{a}	1.31±0.04^{b}	1.78±0.05^{a}	30.02±1.88^{a}
SM30[2]	350.88±0.08	899.83±10.88^{a}	548.93±10.93^{a}	156.44±3.14^{a}	5.98±0.15^{a}	1.32±0.10^{b}	1.77±0.11^{a}	25.99±2.46^{a}
SM60[3]	351.31±0.05	903.68±18.61^{a}	552.37±18.61^{a}	157.23±8.14^{a}	6.04±0.21^{a}	1.32±0.11^{b}	1.75±0.12^{a}	25.20±2.31^{a}
SM100[4]	351.24±0.09	757.07±9.65^{c}	405.83±9.66^{c}	115.54±2.75^{c}	4.03±0.12^{c}	1.77±0.07^{a}	1.32±0.10^{b}	17.88±1.41^{b}
PAP30[5]	350.97±0.11	882.64±4.17^{a}	531.66±4.17^{a}	151.48±1.19^{a}	5.73±0.06^{a}	1.36±0.05^{b}	1.72±0.06^{a}	24.55±1.35^{a}
PAP60[6]	350.82±0.09	862.71±15.26^{ab}	511.89±15.69^{ab}	145.91±7.29^{ab}	5.46±0.16^{ab}	1.39±0.09^{b}	1.61±0.14^{a}	24.64±2.19^{a}
PAP100[7]	351.03±0.13	749.37±13.29^{c}	398.33±13.06^{c}	113.47±3.64^{c}	3.94±0.16^{c}	1.86±0.11^{a}	1.19±0.08^{b}	16.04±0.85^{b}

[1] fishmeal; [2] soybean meal 30%; [3] soybean meal 60%; [4] soybean meal 100%; [5] processed animal protein 30%; [6] processed animal protein 60%; [7] processed animal protein 100%; [8] metabolic growth rate; [9] feed conversion ratio; [10] protein efficiency ratio; [11] protein productive value

The dietary amino acid analysis showed that the essential amino acid (ΣEAA) decreased with increasing SM or PAP, which was positively correlated with growth response. Substitution of 100% dietary FM with SM or PAP results in a significant decrease the sum of EAA in the diet. The dietary lysine and threonine content decreased with the increasing dietary SM or PAP, whereas the dietary methionine level was decreased only due to inclusion of SM in place of FM.

3.4.3. Expression of growth-related genes

Liver IGF-I mRNA expression level was significantly downregulated in fish fed with SM_{100} and PAP_{100} compared to the control (FM) and to the other experimental groups (Figure 6a). Significant reduction in GHR mRNA level was also seen for fish fed the diets with SM_{100} and PAP_{100} with 3.87-and 3.44-fold lower expression than in the control (Figure 6b). The drops in the GHR transcript level in these two groups (SM_{100} and PAP_{100}) were also significantly lower than other dietary treatments. The expression pattern of the GH gene among dietary treatments was almost opposite to the IGF and GHR (Figure 6c). Contrary to IGF and GHR, the mRNA transcript level of GH in fish group subjected to SM_{100} and PAP_{100} elevated significantly and prevailed the control by 2.12-and 2.03-fold, respectively.

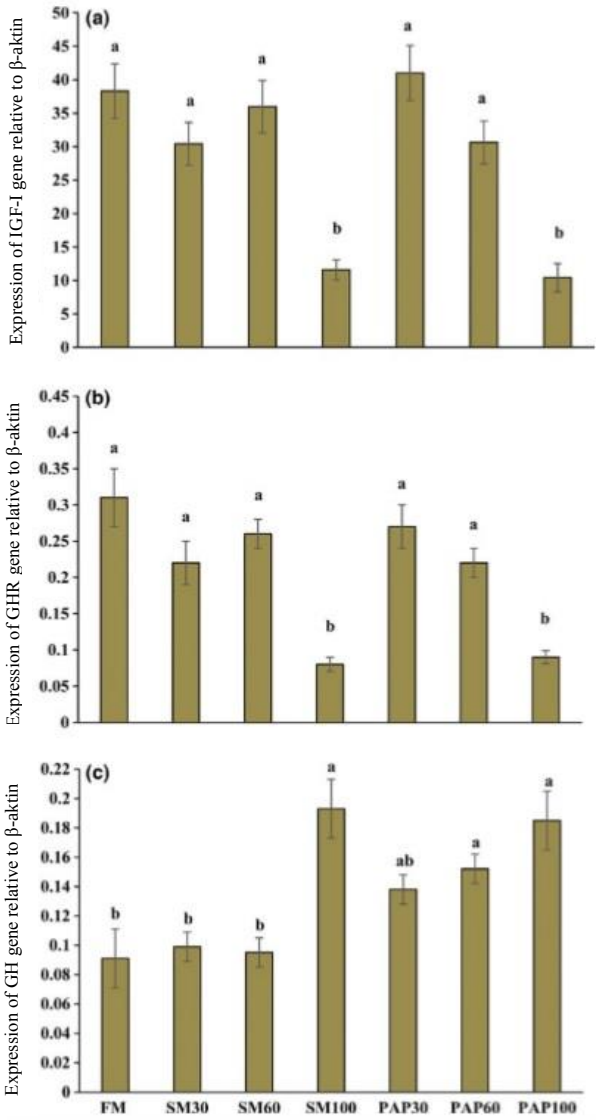

Figure 6: Expression of IGF-I (a), GHR (b) és GH (c) genes relative to β-aktin

3.4.4. Expression of protein metabolic enzyme genes

Under the different experimental conditions, mRNA expression level of ALT and AST followed the same pattern as the IGF-I expression profile; the level dropped significantly in SM_{100} and PAP_{100} relative to all treatments including the control (Figure 7a).

3.4.5. Protein metabolism enzyme activity

In general, ALT and AST activity responses (Figure 7b.) paralleled mRNA expression data quite well for these two enzymes. The activities of ALT and AST in liver decreased with increasing dietary SM or PAP level. When the substitution level was 60% or higher, activities of ALT and AST in liver were lower ($p<0.05$) than in the control group.

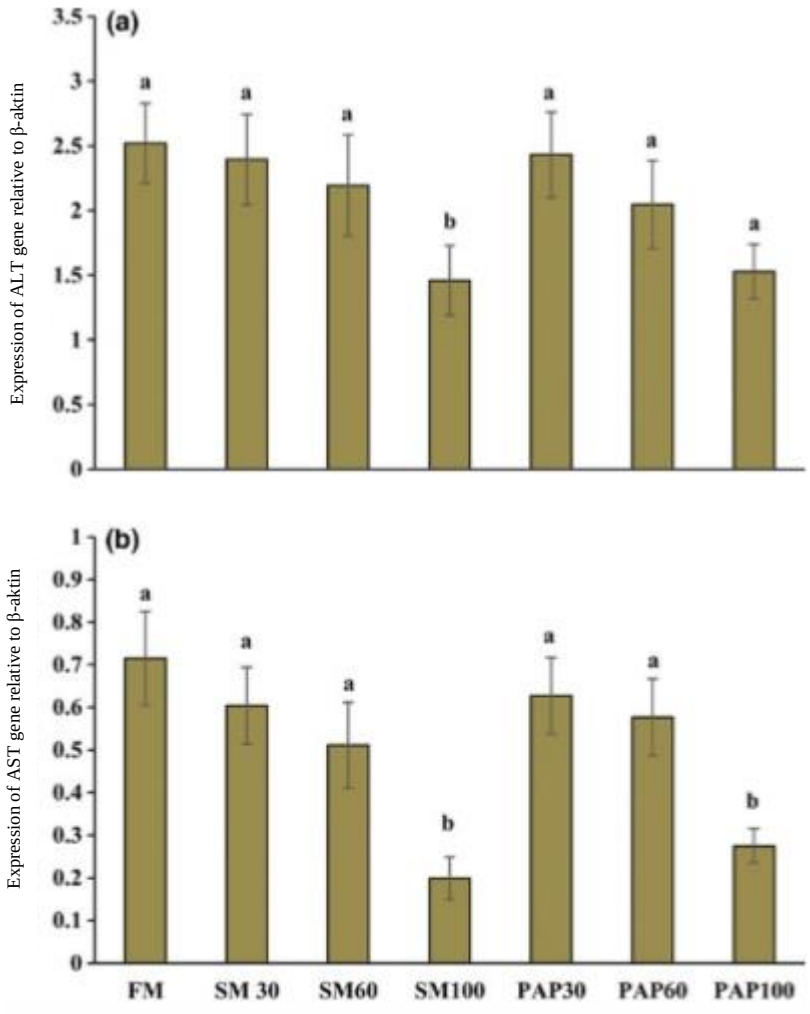

Figure 7: Expression of ALT (a) and AST (b) genes relative to β-aktin.

4. CONCLUSIONS

In the first experiment different protein sources of the diet significantly affected the production traits of European catfish. Diets containing exclusively plant protein resulted in significantly lower weight gain, SGR, FCR and PER compared to the other groups. In contrast, there were no significant differences between the groups fed either fishmeal or meat meal containing diets in terms of weight gain, SGR, FCR and PER. These results demonstrated that soybean meal with corn and meat meal is an acceptable alternative feed component in a European catfish diet. There were no statistical differences between the treatments in terms of the chemical composition of water samples. The maximum values of the water chemistry parameters did not exceed the limit values determined by Hungarian regulations (28/2004. (XII.25.) KvVM order Appendix 2). The dry matter content of sediment samples elevated in all groups, due to the increasing amount of feed.

In term of the production traits there were no significant differences between the different rearing technologies (M; I-E). Both feed conversion ratio and specific growth rate can be improved in treatment M by means of optimal feeding frequency and choosing the appropriate length of feeding. In I-E treatment, changes in stocking density can moderate the feed wastage of cage-reared fish. In I-E group common carp stocked into the ponds can be raised without supplementary feeding. At the end of the breeding season common carp as “by-product” can give an extra source of income. TSS values exceed the limit values determined by Hungarian regulations (28/2004. (XII.25.) KvVM order Appendix 2) in both groups.

In those groups of pond treatment methods where no aeration was used DO levels were lower than 3 mg/l, or 1 mg/l, which might be lethal for most of the farmed fish. Reason of this low values could be the lack of the algae population which occurs in natural water bodies. Different treatments caused

significant differences in water parameters. Outstanding TAN values were observed in B and NaB groups due to the bacterium product supplementation. High NO_3-N levels were found in LB and LNaB groups due to chemical processes of nitrification. PO_4-P values were also the highest in the above-mentioned 4 groups due to the bacterial activity. Dry matter and phosphorus content of sediment samples decreased due to the benthic organisms. In contrast, KN values increased in all treatments, which can be related to the elevated TAN content and heterotroph biomass.

Dietary SM or PAP levels significantly affected the growth response of European catfish. With increasing dietary SM or PAP, growth and nutrient utilization significantly decreased. In particular, when replacing FM more than 60%, the body mass gain, feed efficiency and protein efficiency were significantly lowered than those of control and other groups. These results indicate that 60% of FM could be replaced by either SM or PAP without significantly reducing growth and nutrient utilization. The dietary amino acid analysis showed that the ΣEAA decreased with increasing SM or PAP, which was positively correlated with growth response. Substitution of 100% dietary FM results in a significant decrease the sum of EAA in the diet. This suggests that the reduced growth was related to essential amino acids deficiencies, especially lysine and threonine at more than 60% substitution level. Another possible reason for depressed growth and nutrient utilization could be high undigested fiber in SM_{100} and PAP_{100} diets. The results presented herein indicate that the expression of the GH–liver axis was affected by dietary treatment. Besides dietary EAA imbalance discussed earlier explicitly for SM100 and PAP100, the cessation in growth rate in these two treatments is also attributed to the decline in the binding capacity of GH to the liver GH receptors concomitant with the decrease in GHR mRNA level. mRNA expression levels and activities of ALT and AST enzymes clearly reduced with

increase in dietary SM or PAP level and remarkably the lowest activities were found in the fish fed the diet substituted with 100% FM from SM or PAP.

5. NEW SCIENTIFIC RESULTS

1. Production traits of European catfish are not affected negatively by the substitution of fishmeal with meat meal. Substituting fishmeal with corn protein results in similar production triats as achieved with meat meal. 60 % of the fishmeal content of European catfish diets is substitutable with soybean meal or processed animal protein without the impairment of production traits.

2. The investigated rearing technologies such as monoculture and intensive-extensive system not affect the production traits of European catfish. Stocking density of 2850 kg/ha has no negative effect on water and sediment quality. However, there is no difference in the production traits between the technologies, extra income can be achieved in I-E system by the common carp produced. Nutrient transformation efficiency of the combined system together with the additional fish production yield exceeds that of monoculture ponds.

3. Bacterium product supplementation together with aeration significantly promote the degradation processes of the sediment's organic matter content. Kjeldahl-N content increases in all treatments, especially in case of bacterium supplementation. Sodium percarbonate supplementation per se or combined with aeration has no significant effect on water quality parameters.

4. The dietary lysine and threonine content decreases with the increasing dietary SM or PAP in feeds, whereas the dietary methionine level decreases only due to inclusion of SM in place of FM. Substituting 60 % of fishmeal with soybean meal results in better crude fat content compared to the control, while in case of processed animal protein, it is similar to that.

5. Substitution of 100% dietary FM with SM or PAP results in significantly lower gene expression level of IGF-I and GHR in the liver, while transcript level of GH elevates. ALT and AST mRNS expression levels also decrease significantly. The activities of ALT and AST in liver decrease with increasing dietary SM or PAP level. When the substitution level is 60% or higher, activities of ALT and AST in liver are lower than in the control group.

6. RECOMMENDATIONS

Exclusively plant protein containing diets resulted in significantly lower weight gain, SGR, FCR and PER compared to the other groups. In contrast, there were no significant differences between the groups fed either fishmeal or meat meal containing diets in term of weight gain, SGR, FCR and PER. According to the results, meat meal is a recommendable alternative of fishmeal in European catfish diets.

Comparing different rearing technologies combined system resulted lower total nitrogen content in the water body due to the filter effect of the extensive component. Extensive part of the system accumulates significant amount of organic Nitrogen in common carp's additional weight gain. This also means that the nutrient transformation efficiency of the combined system together with the additional fish production yield exceeds that of monoculture ponds. Application of combined systems in European catfish rearing is recommended due to better water quality and additional common carp yield.

Comparing different pond treating methods it can be concluded that bacterium product supplementation together with aeration significantly promote the degradation processes of sediment's organic matter content, thus aeration of ponds is recommended especially in combination with bacterium product.

SM and PAP level of the diets significantly affects the growth of European catfish. Substituting more than 60 % of fishmeal with SM or PAP leads to the impairment of weight gain, FCR and PER, thus use of higher ratios of these alternative ingredients in catfish feeds is not recommended.

7. SCIENTIFIC PAPERS AND LECTURES ON THE SUBJECT OF THE DISSERTATION

Articles in foreign languages:

Havasi, M., Kumar, S., **Nagy, Z.,** Beliczky, G., Nagy, S., Bercsényi, M., Gál, D. (2015): Effects of feeding regime on growth feed conversion and size variation of *Silurus glanis*. Croatian Journal of Fisheries/RIBARTSVO, 73(4):142-147 pp.

Havasi, M., Kumar, S., **Nagy, Z.,** Pál, L., Beliczky, G., Bercsényi, M., Gál, D. (2015): Effect of total fish meal replacement with vegetal protein alone or combined with rendered animal protein on growth performance and tissue composition of European catfish (*Silurus glanis*). Israeli Journal of Aquaculture-BAMIDGEH 67, Paper: 1236, 8 p.

Kumar, S., J. Sándor, Zs., **Nagy, Z.,** Fazekas, Gy., Havasi, M., Sinha, A.K., de Boeck, G., Gál, D. (2017): Potential of processed animal protein versus soybean meal to replace fish meal in practical diets for European catfish (*Silurus glanis*): growth response and liver gene expression. Aquaculture Nutrition, 23(5):1179-1189 pp.

Nagy, Z., Gál, D., Hancz, Cs. (2017): Effects of different European catfish feeds on production parameters and water quality in limnocorrals. Acta Agraria Kaposváriensis, 21(1):15-27 pp.

Full conference papers in proceedings in foreign languages:

Havasi, M., Kumar, S., **Nagy, Z.,** Beliczky, G., Bercsényi, M., Gál, D. (2015): Preliminary study on replacement of fishmeal with rendered animal protein in the feeds of *Silurus glanis*. XXXIX. Halászati Tudományos Tanácskozás, HAKI. Szarvas, 2015. május 20-21., AQUAREDPOT,

Kumar, S., J. Sándor, Zs., Fazekas, Gy., **Nagy, Z.,** Havasi, M., Gál, D. (2015): Potential of using processed animal protein (PAP) ingredients to replace fish meal in practical diets for European catfish (*Silurus glanis*). XXXIX. Halászati Tudományos Tanácskozás, HAKI. Szarvas, 2015. május 20-21., AQUAREDPOT

Abstract conference papers in proceedings in foreign languages and in Hungarian:

Nagy, Z., Gál, D. (2013): Tápos nevelés hatása a vízminőségre különböző népesítési sűrűségek alkalmazása esetén pontynál és szürke harcsánál. XXXVII. Halászati Tudományos Tanácskozás, HAKI. Szarvas, 2013. május 22-23., 38 pp.

Nagy, Z., Hancz, Cs., Havasi, M., Pál, L., Gál, D. (2014): A halliszt kiváltás lehetőségeinek vizsgálata szürke harcsa (*Silurus glanis*) tavi nevelése során. XXXVIII. Halászati Tudományos Tanácskozás, HAKI. Szarvas, 2014. május 28-29., 42-43 pp.

Nagy, Z., Hancz, Cs., Havasi, M., Pál, L., Gál, D. (2014): Replacement of fishmeal in feeds developed for European catfish (*Silurus glanis*). Aquaculture Europe 14, 405 pp.

Nagy, Z., Gál, D., Havasi, M., Hancz, Cs. (2015): Különböző intenzív harcsanevelési technológiák összehasonlító vizsgálata. XXXIX. Halászati Tudományos Tanácskozás, HAKI. Szarvas, 2015. május 20-21., 39-40 pp.

Nagy, Z., Gál, D., Havasi, M., Hancz, Cs. (2015): Comparative study of two different intensive pond rearing technologies of European catfish (*Silurus glanis*). Aquaculture Europe 15, 318 pp.

Nagy, Z., Gál, D., Hancz, Cs. (2016): Effect of stocking density on growth performance and water quality of European catfish (*Silurus glanis*). Aquaculture Europe 16, 366 pp.

8. ENCLOSURES

Table 1: Ingredients and proximate composition of the diets used Experiment 1.

	HA[1]	AP[2]	SC[3]	B[4]	SW-A[5]	SW-B[6]
Proximate composition, %						
Dry matter	89.40	90.70	88.90	89.50	89.50	89.50
Crude protein	34.55	35.60	34.65	35.50	35.50	35.50
Crude fat	5.05	5.15	4.39	4.49	4.49	4.49
Crude fibre	0.44	1.52	3.62	3.19	3.19	3.19
Total carbohydrate	42.26	42.53	40.64	40.62	40.62	40.62
Ash	7.10	5.90	5.60	5.70	5.70	5.70
Components, %						
Soybean meal	30.30	25.05	40.00	49.74	49.74	49.74
Fishmeal	19.00	0.00	0.00	0.00	0.00	0.00
Meat meal	0.0	17.00	0.00	0.00	0.00	0.00
Corn	26.50	27.14	16.48	0.00	0.00	0.00
Corn gluten	12.20	18.80	19.10	15.44	15.44	15.44
Wheat	10.00	10.0	10.00	30.00	30.00	30.00
Canola meal	0.00	0.00	10.00	0.00	0.00	0.00
Mono-calcium phosphate	0.00	0.00	1.30	1.30	1.30	1.30
Sunflower oil	1.50	0.87	2.20	2.60	2.60	2.60
DL-methionine	0.00	0.10	0.00	0.07	0.07	0.07
L-lysine	0.00	0.54	0.42	0.35	0.35	0.35
Vitamin and mineral premix*	0.50	0.50	0.50	0.50	0.50	0.50

[1] fishmeal; [2] meat meal; [3] corn; [4] wheat; [5] wheat + xylanase; [6] wheat + beta-glucanase

* Vitamin A: 400 000 IU/kg, Vitamin D_3: 200 000 IU/kg, Vitamin E: 6000 mg/kg, Vitamin K_3: 918 mg/kg, Vitamin B_1: 500 mg/kg, Vitamin B_2: 1200 mg/kg, Vitamin B_6: 1000 mg/kg, pantothenic acid: 3000 mg/kg, folic acid: 500 mg/kg, Vitamin C: 10 000 mg/kg, Ca: 22.8 g/100g, Fe: 6000 mg/kg, Zn: 40 324 mg/kg, Mn: 5022 mg/kg, Cu: 1000 mg/kg, Se: 22.5 mg/kg, I: 496 mg/kg, antioxidant: 2000 mg/kg

Table 2: Components and chemical composition of the diets used in Experiment 2.

Crude protein (%)	Crude fat (%)	Ash (%)	Crude fibre (%)	P (%)	Gross energy (MJ)	Digestible energy (MJ)
45	15	6.5	3.2	1.1	21.2	17.6

* Components: feather meal, fishmeal, haemoglobin, poultry meal, poultry oil, rapseed, rapseed oil, soya, sunflower protein conc., triticale, vitamins, minerals, wheat

Table 3: Ingredients and chemical composition of the diets used in Experiment 4.

	Treatments						
	FM (control)[1]	**SM_{30}[2]**	**SM_{60}[3]**	**SM_{100}[4]**	**PAP_{30}[5]**	**PAP_{60}[6]**	**PAP_{100}[7]**
Ingredient (g/kg)							
Fishmeal	490	350	200	0	350	200	0
Extr. soya	0	200	300	400	0	0	0
PAP-55	0	0	0	0	150	300	450
W. wheat	280	200	180	160	280	270	285
Corn	58	68	48	33	60	62	43
Corn gluten	0	0	80	200	0	20	80
Blood meal	50	50	50	50	50	50	50
Linseed oil	40	40	40	40	28	16	10
Fish oil	5	15	25	40	5	5	5
Yeast, f.g.	50	50	50	50	50	50	50
Vit-Min mix*	20	20	20	20	20	20	20
Lignin phosphate	7	7	7	7	7	7	7
Chemical composition (g/kg d.m.)							
Dry matter	922.5	913.1	909.8	903.5	924.9	918.6	901.2
Crude protein	427.0	428.2	433.4	428.7	427.9	448.4	451.5
Crude fat	110.1	110.8	107.0	110.9	98.1	87.1	80.9
Crude fibre	17.7	25.3	27.5	29.5	23.6	27.5	31.5
Ash	135.0	113.7	91.3	51.5	130.4	121.4	105.2
Total carbohydrate	232.7	235.1	250.6	282.9	244.9	234.2	232.1
Gross energy (MJ/kg)	16.49	16.73	16.99	17.71	16.23	16.08	16.15

[1] fishmeal; [2] soybean meal 30%; [3] soybean meal 60%; [4] soybean meal 100%; [5] processed animal protein 30%; [6] processed animal protein 60%; [7] processed animal protein 100%

* Vit-Min mix (Cargill Takarmány Zrt.) (quantity/kg): vitamin A: 1,000,000 IU; vitamin D_3: 80,000 IU; vitamin E:5000 mg; vitamin K_3: 334 mg; vitamin B_6: 200 mg; vitamin C: 11,300 mg; Ca: 114 g; P: 78 g; Na: 1 g; Fe: 670 mg; Zn: 1070 mg; Mn: 160 mg; Cu ($CuSO_4$*$5H_2O$): 200 mg; Se: 20 mg; lysine: 70 g; methionine: 198 g