

**THESES OF DOCTORAL (Ph.D.)  
DISSERTATION**

KAPOSVÁR UNIVERSITY  
FACULTY OF ANIMAL SCIENCE  
Department of Nature Protection

Head of the doctoral school:  
PROF. DR. MELINDA KOVÁCS  
Doctor of the Hungarian Academy of Sciences

Supervisor:  
DR. CSABA HANCZ  
CSC

INFLUENCE OF DIETARY LIPID SOURCES AND  
ALTERING FEED SELENIUM LEVELS ON THE FILLET  
COMPOSITION OF AFRICAN CATFISH AND NILE TILAPIA

Author:  
JANKA BIRÓ

KAPOSVÁR

2013.

# 1. BACKGROUND OF RESEARCH, OBJECTIVES

Intensive fish farms highly depend on fish meal and fish oil originating from capture fisheries. In 2006, 88.5 % of fish oil production was utilized by aquaculture sector (Tacon and Metian, 2008). Capture fisheries are not able to satisfy the demands of this rapidly increasing sector, thus conception of fish-from-fish is unsustainable. Significant research effort was focused into possible means of replacing fish oil with alternative lipid sources as vegetable oils.

African catfish (*Clarias gariepinus*) is the most important fish species in Hungarian intensive fish culture. African catfish gave 8.9 % of total fish production, and 93 % of intensively farmed fish production in 2010 (Jámborné and Bardócz, 2011). Nile tilapia (*Oreochromis niloticus*) is not so popular in Hungary as African catfish, but Tilapia (including all species) is the second most important group of farmed fish after carps, and the most widely grown of any farmed fish (FAO, 2005).

Fish consumption in Hungary is 4.2 kg/capita/year which is significantly lower than the EU average (22 kg/capita/year) (Failler, 2007). Level of fish consumption is a good indicator of a given country's nutritional standard. In Hungary the level of fish consumption has been hardly changed for years, thus functional food production could be advantageous in its recovery.

Fish is a healthy, easily digestible food which is exceptionally rich in unsaturated fatty acids. The lipid composition of farmed fish is more constant and less affected by seasonal variations than that of wild fish (Cahu *et al.*, 2004), as its flesh fatty acid profile directly reflects the fatty acid composition of the diet (Ng *et al.*, 2007). Fish flesh's fatty acid profile can be easily modified to contain larger proportions of eicosapentaenoic acid

(EPA) and docosahexaenoic acid (DHA) which are essential fatty acids for humans. Thus by modification of fillet fatty acid profile functional food can be produced.

Soil in Carpathian-basin belongs to the less well selenium supplemented regions. Thus increasing selenium content of the human diet is very important. Throughout nutrition, enriching fish flesh with selenium functional food also can be produced. Research on this field is breaking new ground since methods of extra quality fish production - especially for functional food -, have not been spread yet in the practice of Hungarian fish farming. Aims of my studies were the following:

1. To investigate the effects of different lipid sources as fish oil and two vegetable oils such as linseed oil and soybean oil on the body composition, with special emphasis on fillet's chemical composition, fillet fatty acid profile and on flesh quality of African catfish and Nile tilapia. In the case of Nile tilapia I also studied the effect of sex on the fatty acid profile.
2. To determine how increased amount of selenium can be incorporated in the fillet of the investigated species.
3. Additionally my objective was to study effects of soybean oil and selenium supplementations used for functional food production on survival, feed conversion ratio, weight gain, fillet fatty acid profile and fillet selenium content of both species.

## **2. MATERIALS AND METHODES**

Six experiments are presented in the thesis as three trials were carried out with both species. Experiments were conducted in the same recirculation system, 5<sup>th</sup> and 6<sup>th</sup> experiments were carried out at the same time. At the first two experiments African catfish and Nile tilapia were fed with diets containing fish, soybean or linseed oil supplementation. In the 3<sup>rd</sup> and 4<sup>th</sup> experiments fish were fed with diets supplemented with different selenium concentrations. In 5<sup>th</sup> and 6<sup>th</sup> experiments the best selenium and soybean oil supplementation – found in the previous trials - was used (Table 1).

Table 1: Experimental setup

Exp.	Involved species	Duration	Treatment	Number of treatments	Number of repeats	Investigated parameters
1	African catfish	6 week	12 % fish, linseed or soybean oil	3	2	production traits, chemical composition, fatty acid profile, fillet quality
2	Nile tilapia	6 week	11 % fish, linseed or soybean oil	3	3	production traits, chemical composition, fatty acid profile (fillet, liver, mesenteric fat), fillet quality, effect of sex on fatty acid profile
3	African catfish	6 week	0,5 mg kg <sup>-1</sup> , 2 mg kg <sup>-1</sup> , 4 mg kg <sup>-1</sup> Se supplementation + control	4	2	production traits, fillet selenium concentration
4	Nile tilapia	6 week	0,5 mg kg <sup>-1</sup> , 2 mg kg <sup>-1</sup> , 4 mg kg <sup>-1</sup> Se supplementation + control	4	3	production traits, chemical composition, fillet selenium concentration
5	African catfish	8 week	10 % soybean oil + 4 mg kg <sup>-1</sup> Se; control	2	3	production traits, chemical composition, fatty acid profile, fillet selenium concentration
6	Nile tilapia	8 week	10 % soybean oil + 2 mg kg <sup>-1</sup> Se; control	2	3	production traits, chemical composition, fatty acid profile, fillet selenium concentration

## **2.1. Origin and setting of experimental stocks**

Experiments were carried out in the Fish laboratory of Kaposvár University. In the studies market sized African catfish and Nile tilapia stocks were used. Experimental stocks were purchased from Szarvas-fish Ltd.

In the 1<sup>st</sup> and 2<sup>nd</sup> experiments fish were introduced into 15 tanks with a volume of 1 m<sup>3</sup> as a part of recirculation system with a total volume of 10 m<sup>3</sup>. 3<sup>rd</sup>-6<sup>th</sup> experiments were carried out in the renovated recirculation system consisting of 12 tanks with a volume of 500 l with a total volume of 20 m<sup>3</sup>. Water temperature was 28±0.5 °C (African catfish) 23.5 ± 1.0 °C and 27.9±1.1 °C (Nile tilapia) at the first two experiments, and 23.5±1.0 °C during the 3-6<sup>th</sup> trials

## **2.2. Experimental design**

### *2.2.1. Studies on the effect of fish, linseed or soybean oil supplementation on the production traits, fillet fatty acid profile and flesh quality traits of African catfish*

Feeding lasted for 42 days. 60-65 kg African catfish were stocked in each tank with a volume of 1 m<sup>3</sup>. Average initial body weight was 1026±121 g (n=374). The experimental feeds contained, besides the basic 6 % crude fat content 6 % added oil from different sources: fish oil, and two vegetable oils, soybean and linseed oil. All experimental diets were fed in 2 tanks. Fish oil supplemented feed was used as control. Experimental fish were sampled at the initial point and after 3 and 6 weeks in the study. Each time 5-5 fish from each dietary treatment were killed. Fillets' chemical composition, fatty acid profile, and production traits as growth, feed conversion ratio and survival

were determined. pH45 and pH24, flesh colour, dripping, cooking and thawing loss were also measured and evaluated.

*2.2.2. Studies on the effect of fish, linseed or soybean oil supplementation on the production traits, fillet fatty acid profile and flesh quality traits of Nile tilapia, furthermore the effect of different lipid sources and sex on the fillet fatty acid profile*

Feeding lasted for 42 days. 65 individuals of Nile tilapia with a total amount of 11-12 kg were stocked in each tank with a volume of 1 m<sup>3</sup>. Average initial body weight was 175.3±7.8 g (n=585). The experimental feeds contained, besides the basic 6 % crude fat content 5 % added oil from different sources: fish oil, and two vegetable oils, soybean and linseed oil. All experimental diets were fed in 3 tanks. Fish oil supplemented feed was used as control. Experimental fish were sampled at the initial point and after 3 and 6 weeks in the study. Each time 6-6 male Nile tilapia from each dietary treatment were killed. Fillet's chemical composition, fatty acid profile of fillet, liver and mesenteric fat, and production traits as growth, feed conversion ratio, fillet ratio and survival were determined. pH45 and pH24, flesh colour, dripping and cooking loss were also studied. To study the effect of sex on the fatty acid profile at the end of the experiment 4-4 (2 males, 2 females) Nile tilapia from each dietary treatment were killed and fillet and gonad samples were taken for determination of fatty acid profile.

### *2.2.3. Study on the influence of altering feed selenium levels on the fillet selenium content and production traits of African catfish*

Feeding lasted for 42 days. 40-45 kg African catfish were stocked in each tank with a volume of 1 m<sup>3</sup>. Average initial body weight was 1513±235 g (n=229). The three experimental diets were supplemented with 0.5; 2.0 and 4.0 mg kg<sup>-1</sup> selenium in form of selenoyeast. Commercial African catfish feed was used as control diet. All experimental diets were fed in 2 tanks. Experimental fish were sampled at the initial point and at the end of the study. Each time 5-5 fish from each dietary treatment were killed. Fillet selenium content and production traits as growth, feed conversation ratio, fillet ratio and survival were determined.

### *2.2.4. Study on the influence of altering feed selenium levels on the fillet selenium content and production traits of Nile tilapia*

Feeding lasted for 42 days. 30 individuals of Nile tilapia with a total amount of approximately 10 kg were stocked in each tank with a volume of 500 l. Average initial body weight was 335.5±29 g (n=360). The three experimental diets were supplemented with 0.5; 2.0 and 4.0 mg kg<sup>-1</sup> selenium in form of selenoyeast. Commercial African catfish feed was used as control diet. All experimental diets were fed in 3 tanks. Experimental fish were sampled at the initial point and at the end of the study. Each time 5-5 fish from each dietary treatment were killed. Chemical composition, fillet selenium content and production traits as growth, feed conversation ratio, fillet ratio and survival were determined.

*2.2.5. Study on the effect of soybean oil and selenium supplementations on the production traits, fillet selenium content and flesh quality of African catfish*

Feeding lasted for 56 days. 31 individuals of African catfish with a total amount of approximately 26 kg were stocked in each tank with a volume of 500 l. Average initial body weight was  $870\pm 160$  g (n=185). Diets were supplemented with 5 % soybean oil and  $4\text{ mg kg}^{-1}$  selenium in the form of selenoyeast. Commercial African catfish feed was used as control diet. Both experimental diets were fed in 3 tanks. Experimental fish were sampled at the initial point and at the end of the study. Each time 6-6 fish from each dietary treatment were killed. Chemical composition, fillet selenium content, fillet fatty acid profile and production traits as growth, feed conversion ratio and survival were determined.

*2.2.6. Study on the effect of soybean oil and selenium supplementations on the production traits, fillet selenium content and flesh quality of Nile tilapia*

Feeding lasted for 56 days. 35 individuals of Nile tilapia with a total amount of 12-14 kg were stocked in each tank with a volume of 500 l. Average initial body weight was  $393\pm 90$  g (n=210). Diets were supplemented with 5 % soybean oil and  $2\text{ mg kg}^{-1}$  selenium in the form of selenoyeast. Commercial Nile tilapia feed was used as control diet. Both experimental diets were fed in 3 tanks. Experimental fish were sampled at the initial point and at the end of the study. Each time 6-6 fish from each dietary treatment were killed. Chemical composition, fillet selenium content, fillet fatty acid profile and production traits as growth, feed conversion ratio and survival were determined.

### 2.3. Sampling, chemical analysis

Numbers of samples in the different experiments are presented in Table 2.

Table 2: Number of samples in the different experiments

	<b>0. week (pieces)</b>	<b>3. week (pieces/treatment)</b>	<b>6. week (pieces/treatment)</b>	<b>8. week (pieces/treatment)</b>
<b>Experiment 1</b>	5	5	5	-
<b>Experiment 2</b>	6	6	6 + 2 males, 2 females	-
<b>Experiment 3</b>	5	-	5	-
<b>Experiment 4</b>	5	-	5	-
<b>Experiment 5</b>	6	-	-	6
<b>Experiment 6</b>	6	-	-	6

Before sampling fish were killed after anaesthesia (with clove oil or Norcaicum; Egis, Budapest, Hungary). In the first two experiments fillet samples originated from the left dorsolateral fillet. In the 3-6<sup>th</sup> experiments fillet samples for selenium concentration determination also originated from the left dorsolateral fillet. In the 4-6<sup>th</sup> experiments fillet samples for chemical composition determination originated from the left dorsolateral fillet as well. In the 5<sup>th</sup> and 6<sup>th</sup> experiments fillet samples for determination of fatty acid profile originated from the right fillet. Mesenteric fat and liver were also sampled for fatty acid profile analysis in the 2<sup>nd</sup> experiment.

Chemical composition and selenium content of fillet were determined in the Analytical Laboratory of Kaposvár University. Flesh quality parameters were measured in the Laboratory of Animal Production Certification. Fatty acid profile was determined in the Research Institute for Animal Breeding and Nutrition.

## **2.4. Statistical analysis**

Statistical data were evaluated by SPSS for Windows 10.0 (1999). In the first and second experiments ANOVA (GLM, Univariate) with Tukey post-hoc test ( $P < 0.05$ ) was used to evaluate the effect of different treatments on fillet, liver and mesenteric fat, on fatty acid profile, on fillet fatty acid composition and on flesh quality parameters.

Growth, feed conversion ratio, survival, fillet's chemical composition, fillet fatty acid profile, selenium content and flesh quality data of the 3-6<sup>th</sup> experiments were evaluated by one-way ANOVA with Tukey post-hoc test.

### 3. RESULTS

#### 3.1. Effect of fish, linseed or soybean oil supplementation on the production traits, fillet fatty acid profile and flesh quality traits of African catfish

##### 3.1.1. Effect of the different oil supplementations on the production traits

Considering the production traits no significant difference was found between the different treatments. However the best daily weight gain, specific growth rate, feed conversion ratio and survival was achieved in the soybean oil (SO) supplemented group (Table 3). Feed conversion ratio was favourable in all treatments, ranging between 1.23-1.62 kg kg<sup>-1</sup>. Survival rate was also high in all groups. Low value of specific growth rate (0.7 %) can be explained by the lower growth capacity of market sized fish.

Table 3: Effect of different oil supplementations on the main production traits of African catfish at the 6<sup>th</sup> week (mean±SD)

Treatment (diet)	Daily weight gain (g day <sup>-1</sup> )	SGR. (% day <sup>-1</sup> )	Survival (%)	FCR. (kg kg <sup>-1</sup> )
Fish oil	9.34±6.32	0.64±0.28	88.4±2.65	1.62±0.04
Linseed oil	9.62±2.60	0.72±0.06	89.71±1.17	1.5±0.16
Soy-bean oil	10.99±4.38	0.79±0.16	91.69±1.34	1.23±0.07

### *3.1.2. Effect of the different oil supplementations on fillet chemical composition and on fillet flesh quality traits*

Neither fillet dry matter content, nor the crude fat and the crude protein content showed statistically significant alterations during the entire experiment.

Statistically proven effect of the dietary fat source was experienced only on two fillet flesh quality traits (pH45 and b\*), water holding capacity was not affected by it. Duration of dietary fatty acid supplementation had marked effect on most of the traits (pH45, pH24, L\*, dripping loss, thawing loss (Table 4). The mainly interesting finding on the fillet quality is the marked influence of treatment duration on the water holding capacity of fillet. At the 3<sup>rd</sup> week dripping loss was higher in linseed oil (LO) and fish oil (FO) groups, while cooking loss was higher in SO group, as compared to the data obtained at the end of the trial (6<sup>th</sup> week).

This may be a result of a longer-term adaptation, namely the one-step change to the LO and FO diets may have led to a progressive in vivo lipid peroxidation, which was later compensated by an adaptation of the antioxidant enzymes. The fact that the effect of treatment duration was more expressed on the fillet quality traits suggests that three weeks on a „finishing” diet may not always lead to the demanded quality alterations of the fillet.

Table 4: Flesh quality traits of African catfish fed with three different diets (mean±SD)

Treatment (diet)	Fish oil			Linseed oil			Soybean oil		
Treatment duration (week)	0	3	6	0	3	6	0	3	6
pH45	6.93±0.04 <sup>b</sup>	6.56±0.06 <sup>a</sup>	6.73±0.12 <sup>a4B</sup>	6.93±0.04 <sup>b</sup>	6.45±0.25 <sup>a</sup>	7.04±0.11 <sup>bB</sup>	6.93±0.04	6.73±0.08	6.87±0.18 <sup>4</sup>
pH24	6.01±0.01 <sup>b</sup>	5.88±0.04 <sup>a</sup>	5.95±0.09 <sup>ab</sup>	6.01±0.01 <sup>a</sup>	5.88±0.08 <sup>b</sup>	5.92±0.06 <sup>ab</sup>	6.01±0.01	5.90±0.10	5.89±0.05
L*	48.10±1.28 <sup>a</sup>	53.40±1.18 <sup>b</sup>	50.22±1.18 <sup>a</sup>	48.10±1.28	50.8±4.49	49.10±3.44	48.10±1.28	50.60±0.66	50.20±2.86
a*	-1.65±1.88	-1.90±0.91	-2.11±0.73	-1.65±1.88	-2.11±1.23	-3.19±0.44	-1.65±1.88	-2.06±1.18	-1.34±1.90
b*	4.41±1.75	7.21±0.37 <sup>A</sup>	4.55±0.86	4.41±1.75	5.23±0.67 <sup>A</sup>	4.46±1.34	4.41±1.75	5.42±1.13 <sup>B</sup>	5.15±0.10
Dripping loss	1.79±0.18 <sup>a</sup>	2.36±0.44 <sup>b</sup>	1.81±0.17 <sup>a</sup>	1.79±0.18 <sup>a</sup>	2.81±0.59 <sup>b</sup>	1.79±0.34 <sup>a</sup>	1.79±0.18	2.17±0.045	1.84±0.16
Cooking loss	7.78±1.51 <sup>a</sup>	11.61±1.57 <sup>b</sup>	11.71±0.95 <sup>b</sup>	7.78±1.51	11.10±3.36	11.80±3.73	7.78±1.51 <sup>a</sup>	11.70±1.89 <sup>b</sup>	8.59±0.80 <sup>a</sup>
Thawing loss	2.98±1.31 <sup>a</sup>	2.18±0.49 <sup>a</sup>	3.93±1.07 <sup>b</sup>	2.98±1.31 <sup>a</sup>	2.64±0.98 <sup>a</sup>	3.81±0.16 <sup>b</sup>	2.98±1.31	3.01±0.75	3.68±0.18

Different small superscripts represent significant differences within treatment, while different capital superscripts refer to significant differences between different treatments.

### *3.1.3. Effect of different oil supplementations and treatment duration on fillet fatty acid profile*

Both the dietary fat source and the duration of the feeding had marked effects on fillet fatty acids. The proportion of total n-3 fatty acids increased in the FO and SO groups, while in the LO group no statistically proven increment was shown. The proportion of  $\alpha$ -linolenic acid (C18:3 n3, ALA) increased in all groups, though the SO group reached lower proportions, as compared to the FO, in spite of the ca. two-fold higher ALA supplementation of the latter diet. Not surprisingly the one magnitude higher ALA supplementation caused significantly higher fillet ALA content in the LO group.

The fillet proportion of eicosapentaenoic acid (C20:5 n3, EPA) increased according to the dietary supplementation. Double amounts of dietary EPA (FO vs. SO) led to a nearly double tissue proportion of this fatty acid in the FO fillets. In contrast, identical dietary EPA supplementation in the LO and SO groups led ultimately to higher tissue EPA levels in the LO group, suggesting the role of endogenous fatty acid transformation, based on ALA as precursor. The docosapentaenoic (C22:5 n3, DPA) and docosahexaenoic acid (C22:6 n3, DHA) proportions in the fillet increased according to the graded dietary uptake of these acids.

Proportion of DPA and DHA in the FO and LO groups was significantly higher compared to the SO group. In the tissue proportion of DPA and DHA some slight effects of the dietary precursor (i.e. ALA) load might be supposed, since by totally identical dietary DPA, and as well EPA levels (SO and LO), the fillet in the LO treatment tended to contain significantly higher DPA and DHA proportions, however without statistical significance. In the FO group DPA and DHA proportion was significantly

higher at the 6<sup>th</sup> week compared to the initial and 3<sup>rd</sup> week's values. The substitution of fish oil with vegetable oils does not always lead to identically beneficial fatty acid profile of the catfish fillet. However, the dietary ensured proportions of EPA, DPA and DHA were higher in the FO diet, resulting ultimately in slightly higher proportions of these acids, most probably resulting from direct incorporation. The total n6 fatty acids showed either no change (SO, LO), or decreased, in parallel with the decreased dietary uptake (FO). The proportion of linoleic acid (C18:2 n6, LA) decreased only in the fillet samples of the FO group, according to the drastically decreased LA proportion of the diet. In contrast, arachidonic acid (C20:4 n6, AA) did not show marked proportional reduction in either of the treatments, albeit the SO and LO diets contained lower amounts of this acid, as compared to the basal diet. The markedly altered dietary n3 and n6 doses were effective in the reduction of the n6/n3 fatty acid ratio in the FO and LO groups.

### **3.2. Effect of fish, linseed or soybean oil supplementation on the production traits, fillet fatty acid profile and flesh quality traits of Nile tilapia, furthermore the effect of different lipid sources and sex on the fillet fatty acid profile**

#### *3.2.1. Effect of the different oil supplementations on the production traits*

Considering the production traits no significant difference was found between the different treatments. The low value of the specific growth rate (S.G.R. = 0.7 % day<sup>-1</sup>) can be explained by the occasional reproduction of fish (Table 5). Survival rate was satisfying in all groups.

Table 5: Effect of different oil supplementations on the main production traits of Nile tilapia at the 6<sup>th</sup> week (mean±SD)

Treatment (diet)	Daily weight gain (g day <sup>-1</sup> )	SGR (% day <sup>-1</sup> )	Survival (%)	FCR (kg kg <sup>-1</sup> )
Fish oil	1.6±0.1	0.77±0.03	92.3±0.9	1.83±0.06
Linseed oil	1.43±0.09	0.71±0.02	92.3±0.9	1.97±0.09
Soybean oil	1.36±0.07	0.66±0.03	90.8±0.9	2.06±0.15

### 3.2.2. Effect of the different oil supplementations on fillet chemical composition and on fillet flesh quality traits

Neither different lipid sources nor treatment duration affected the dry matter, crude fat, crude protein and ash content.

Redness (a\*) increased in all treatments. SO group had significantly lower L\* value, than FO group (Table 6). Dripping loss increased for the 3<sup>rd</sup> week in all treatments, however it was significant only in LO and SO groups. In the case of SO group it returned to the initial value for the 6<sup>th</sup> week, but remained high during the entire experiment in FO and LO groups. Similarly cooking loss was significantly higher in all treatments at the 3<sup>rd</sup> week than the initial value, but for the 6<sup>th</sup> week it returned to the original level.

Table 6: Flesh quality traits of Nile tilapia fed with three different diets (mean±SD)

Treatment (diet)	Fish oil			Linseed oil			Soybean oil		
Treatment duration (week)	0	3	6	0	3	6	0	3	6
pH45	6.63±0.12 <sup>a</sup>	6.74±0.20 <sup>a</sup>	6.63±0.14 <sup>a</sup>	6.63±0.12 <sup>a</sup>	6.61±0.14 <sup>a</sup>	6.61±0.21 <sup>a</sup>	6.63±0.12 <sup>a</sup>	6.66±0.18 <sup>a</sup>	6.61±0.13 <sup>a</sup>
pH24	6.23±0.14 <sup>a</sup>	6.11±0.09 <sup>b</sup>	6.01±0.08 <sup>b</sup>	6.23±0.14 <sup>a</sup>	6.11±0.04 <sup>b</sup>	6.04±0.13 <sup>b</sup>	6.23±0.14 <sup>a</sup>	6.20±0.10 <sup>a</sup>	6.02±0.07 <sup>b</sup>
L*	51.00±2.41 <sup>a</sup>	46.28±2.68 <sup>b</sup>	49.84±4.04 <sup>a</sup>	51.00±2.41 <sup>a</sup>	46.07±2.25 <sup>b</sup>	48.26±2.34 <sup>ab</sup>	51.00±2.41 <sup>a</sup>	47.49±2.21 <sup>b</sup>	47.08±1.29 <sup>b</sup>
a*	-3.74±0.39 <sup>a</sup>	-3.07±0.49 <sup>b</sup>	-2.77±0.68 <sup>b</sup>	-3.74±0.39 <sup>a</sup>	-2.76±0.57 <sup>b</sup>	-2.8±0.40 <sup>b</sup>	-3.74±0.39 <sup>a</sup>	-3.31±0.53 <sup>ab</sup>	-2.85±0.52 <sup>b</sup>
b*	1.71±0.91 <sup>a</sup>	2.03±1.03 <sup>a</sup>	1.21±1.28 <sup>a</sup>	1.71±0.91 <sup>a</sup>	2.37±1.59 <sup>c</sup>	0.59±1.05 <sup>a</sup>	1.71±0.91 <sup>a</sup>	1.89±1.02 <sup>a</sup>	0.72±0.89 <sup>b</sup>
Dripping loss	1.71±0.29 <sup>a</sup>	2.39±1.14 <sup>ab</sup>	3.30±1.44 <sup>b</sup>	1.71±0.29 <sup>a</sup>	2.22±0.54 <sup>bc</sup>	2.33±0.21 <sup>d</sup>	1.71±0.29 <sup>a</sup>	2.05±0.87 <sup>b</sup>	1.98±0.23 <sup>ab</sup>
Cooking loss	15.81±2.77 <sup>a</sup>	22.74±4.20 <sup>b</sup>	13.85±2.32 <sup>a</sup>	15.81±2.77 <sup>a</sup>	21.37±4.39 <sup>c</sup>	13.6±3.73 <sup>a</sup>	15.81±2.77 <sup>a</sup>	22.72±5.54 <sup>b</sup>	14.00±1.86 <sup>a</sup>

Same superscripts do not differ significantly.

### *3.2.3. Effect of different oil supplementations on fillet fatty acid profile*

In the fillet almost all of the fatty acid proportions were significantly affected by the different treatments. In the SO group the proportion of C18:0, C18:2n-6, C20:2n-6, C20:3n-3, C20:3n-6, C20:4n-6, C22:0, C24:0 increased, compared to the FO group. Similar changes were observed in the LO group where the proportion of C20:2n-6, C20:3n-3, C20:3n-6, C22:0, C24:0 and also C18:3n-3, increased but the difference in C18:0, C18:2n-6 and C20:4n-6 was not significant. The effect of vegetable oil complementation resulted decreasing proportion of C17:1n-7, C20:1n-9, C22:1n-9 in both vegetable oil groups (SO and LO). The proportion of C14:0, C22:5n-3 and C24:1n-9 decreased only in LO group. In the main fatty acid groups, the total n-6 PUFA increased significantly in both vegetable oil groups, the SO group exceeding the LO group. This resulted in a higher n-6 to n-3 ratio in the former, since in the LO and FO groups this ratio showed no significant differences in the fillet.

LO complementation was merely effective in increasing of the tissue ALA proportion. However, this increase failed to mirror the effect of the large C18:3n-3 provision by the LO diet, as further elongated and desaturated products (EPA, DPA, and DHA) were not affected by the precursor fatty acid feeding. The vegetable oil feeding (especially SO diet) led ultimately to a reduction of the fillet DPA proportion, and was found not to be effective in either enriching or maintaining the fillet EPA and DHA proportions. An increase was experienced for arachidonic acid (AA) in vegetable oil groups, which was the most expressed by the SO diet. LO feeding resulted a similar n-6 to n-3 ratio as in the FO group, indicating that by LO supplementation n-3 PUFA proportion corresponds to effect of FO on the fatty acid composition better than SO. This was however mostly

attributed to the direct increment of the ALA and not to its further elongated and desaturated metabolites.

#### *3.2.4. Effect of sex and different oil supplementations on fillet fatty acid profile*

The incidence of ALA in the fillet was higher in the males by all diets, however it was significant only in groups FO and LO, and the latter was higher than in fish fed other diets. Differences were found between the two sexes in the n-3 PUFA of FO and LO groups, resulting in a higher n-3/n-6 ratio in the males, but these were significant only in the FO group. In case of EPA, the males had generally higher values; however the difference was significant in the gonads of FO group. Fillet MUFA was affected by the fat source in the diet. LA, AA, and DHA and the main FA groups, n-3 PUFA, n-6 PUFA, the n-3/n-6 ratio and the DHA/EPA ratio were affected by both treatments (sex and diets). In case of n-3/n-6 ratio the interaction of the two treatments was also significant. Similarly to the fillet, females had a higher MUFA incidence in the gonads; however it was significant only in the FO group.

### **3.3. Influence of altering feed selenium levels on the fillet selenium content and production traits of African catfish**

#### *3.3.1. Effect of altering selenium supplementations on the production traits*

No tendencies were found in the production traits according to the different selenium level treatments. The treatments had no significant effect on the survival since losses did not occur during the experiment, supposing

that not either 4 mg kg<sup>-1</sup> supplementation caused toxicity (Table 7). S.G.R. of the 0.5 mg kg<sup>-1</sup> group was significantly lower compared to the control, but did not differ significantly from the other two treatments. Low value of specific growth rate can be explained by the lower growth capacity of market sized fish.

Table 7: Effect of different selenium supplementations on the main production traits of African catfish at the 6<sup>th</sup> week (mean±SD)

Treatment (diet)	Daily weight gain (g day <sup>-1</sup> )	SGR (%)	Survival (%)
Control	4.80±4.68 <sup>b</sup>	0.29±0.3 <sup>b</sup>	100
0.5 mg kg <sup>-1</sup>	2.32±2.82 <sup>a</sup>	0.14±0.17 <sup>a</sup>	100
2 mg kg <sup>-1</sup>	3.18±3.72 <sup>ab</sup>	0.18±0.19 <sup>ab</sup>	100
4 mg kg <sup>-1</sup>	4.07±3.27 <sup>ab</sup>	0.24±0.16 <sup>ab</sup>	100

Same superscripts do not differ significantly.

### 3.3.2. Effect of selenium supplementation on fillet selenium content

The selenium content of the African catfish fillet showed a moderate positive correlation with the feed selenium content, described well by linear regression. ( $Se_{\text{Fillet}}=5.62*Se_{\text{Feed}}+81,02$ ,  $r^2=0.45$ ,  $P=0.001$ ). Fillet selenium content was significantly higher (109 µg kg<sup>-1</sup>) in the 4 mg kg<sup>-1</sup> treatment, than in the others (Figure 1). In this group 1.5 fold higher increment was observed compared to the initial value, but in the two other groups significant increment also occurred.

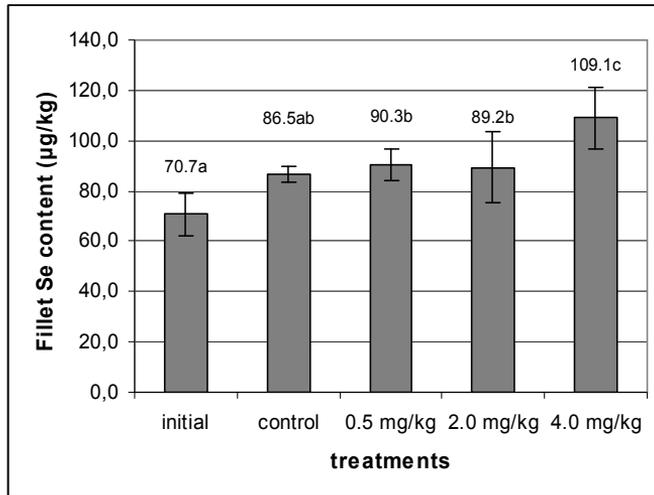


Figure 1: Selenium content in the fillet of African catfish

### 3.4. Influence of altering feed selenium levels on the fillet selenium content and production traits of Nile tilapia

#### 3.4.1. Effect of altering selenium supplementations on the production traits

Differences between groups were significant in the feed conversion ratio, where the 0.5 and 4 mg kg<sup>-1</sup> selenium supplemented groups showed better values than the control group (Table 8). Best feed consumption was achieved by 4 mg kg<sup>-1</sup> selenium supplemented group.

Table 8: Effect of different selenium supplementations on the main production traits of Nile tilapia at the 6<sup>th</sup> week (mean±SD)

Treatment (diet)	Feed consumption (kg)	FCR (kg kg <sup>-1</sup> )
Control	7.46±1.11	3.88±0.57 <sup>b</sup>
0.5 mg kg <sup>-1</sup>	7.71±0.93	2.58±0.30 <sup>a</sup>
2 mg kg <sup>-1</sup>	7.49±0.4	3.01±0.33 <sup>ab</sup>
4 mg kg <sup>-1</sup>	7.34±0.8	2.30±0.6 <sup>a</sup>

Same superscripts do not differ significantly.

### 3.4.2. Effect of selenium supplementation on fillet chemical composition and selenium content

In case of the body composition significant differences were found (Table 9). Lipid content increased significantly in all selenium supplemented groups as compared to the control, due to the high fat content of the diets.

Table 9: Fillet chemical composition of Nile tilapia fed with different selenium supplemented diets (mean±SD)

Treatment (diet)	Initial	Control	0.5 mg kg <sup>-1</sup>	2 mg kg <sup>-1</sup>	4 mg kg <sup>-1</sup>
Dry matter (%)	23.25±0.57	23.78±1.47	23.48±2.32	25.64±0.81	25.54±0.73
Crude protein (% D.M)	18.53±0.51	17.86±0.50	17.79±0.98	18.57±0.47	18.4±0.33
Crude fat (% D.M)	0.71±1.42 <sup>a</sup>	3.2±0.86 <sup>b</sup>	3.03±1.32 <sup>b</sup>	4.42±0.66 <sup>b</sup>	4.4±0.66 <sup>b</sup>

The feed supplementations produced significantly elevated selenium levels in the fillet. In the group with 2 mg kg<sup>-1</sup> supplementation the fillet selenium level was 1.6 times higher (127.80 µg kg<sup>-1</sup>) than the initial value (Figure 2). Selenium content of the fillet showed a moderate positive cubic relationship with the feed selenium content described by the ( $Se_{\text{Fillet}} = 75.6 +$

$46.8 \times (\text{Se}_{\text{Feed}}) - 12.3 \times (\text{Se}_{\text{Feed}})^2 + 0.72 \times (\text{Se}_{\text{Feed}})^3$  equation ( $r^2=0.65$ ,  $P=0.001$ ). According to my results a 100 g portion of Tilapia fillet fortified with the optimal selenium supplementation can cover approximately 25 % of the RDA of selenium.

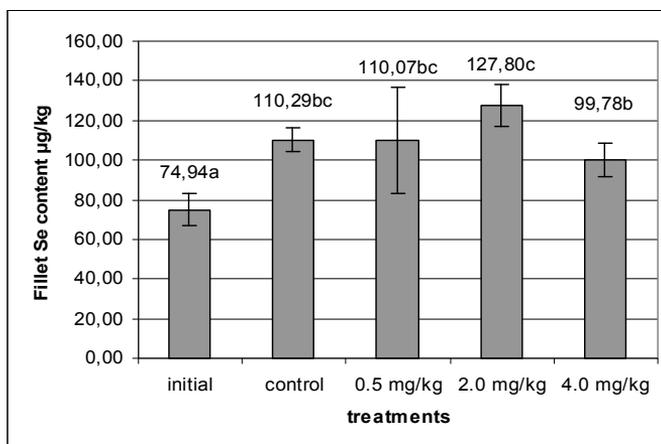


Figure 2: Selenium content in the fillet of Nile tilapia

### 3.5. Effect of soybean oil and selenium supplementations on the production traits, fillet selenium content and flesh quality of African catfish and Nile tilapia

#### 3.5.1. Effect of soybean oil and selenium supplementations on the production traits

Production traits of African catfish were not affected by the soybean oil + 4 mg kg<sup>-1</sup> selenium supplementation. No mortalities occurred during the entire experiment (Table 10). Low value of specific growth rate can be explained by the lower growth capacity of market sized fish.

Table 10: Effect of soybean oil and selenium supplementations on the main production traits of African catfish at the 8<sup>th</sup> week

(mean±SD)

Treatment (diet)	Daily weight gain (g day <sup>-1</sup> )	SGR (%)	Survival (%)	Feed consumption (kg)	FCR (kg kg <sup>-1</sup> )
Control	5.45±0.73	0.54±0.08	100	24.44±0.12	2.64±0.35
4 mg kg <sup>-1</sup> Se + SO	5.30±0.32	0.52±0.03	100	23.43±0.48	2.55±0.16

Production traits of Nile tilapia were not affected by different treatments. Daily weight gain and specific growth rate was rather poor, while survival was high (Table 11).

Table 11: Effect of soybean oil and selenium supplementations on the main production traits of Nile tilapia at the 8<sup>th</sup> week

(mean±SD)

Treatment (diet)	Daily weight gain (g day <sup>-1</sup> )	SGR (%)	Survival (%)	Feed consumption (kg)	FCR (kg kg <sup>-1</sup> )
Control	1.4±0.32	0.34±0.91	99.05±0.77	12.41±0.25	4.66±0.96
2 mg kg <sup>-1</sup> Se + SO	1.22±0.16	0.28±0.30	98.09±1.34	11.80±0.25	4.96±0.56

### *3.5.2. Chemical composition of African catfish and Nile tilapia fillet*

Fillet dry matter and crude ash content of African catfish fillet was significantly higher in treated and control groups compared to the initial value. There was no significant difference between control and treated groups. Fillet chemical composition of Nile tilapia was not affected by different treatments.

### *3.5.3. Effect of soybean oil and selenium supplementations on the fillet fatty acid profile of African catfish and Nile tilapia*

All of the FA proportions were significantly affected by the vegetable oil supplementations in the fillet. The proportion of LA, ALA and n-6 PUFA in the treated group increased but EPA decreased compared to the control group in the Tilapia, while only the proportion of ALA showed significant increment in the treated group of African catfish. However, comparisons to the initial value resulted, that C14:0, C16:1n-7, C18:1n-9, and MUFA decreased, while C20:4n-6 and PUFA increased in both control and treated groups in Tilapia. In case of LA, ALA, C20:3n-6 and IA value only the treated group showed significant increment compared to the initial value. Similar changes were observed in the African catfish where the proportion of C16:0, SFA, IA and IT value decreased but C18:3n-3, C20:3n-6, C20:5n-3, n-3 PUFA and total PUFA increased in both groups compared to the initial value. LA and n-6 PUFA increased but C18:1n-9 and MUFA decreased only in the treated group compared to the initial value. The differences between the two species were significant in the followings: the proportions of C14:0, C20:2n-6, C20:3n-6, C20:4n-6, C22:5n-3, C22:6n-3, n-6 PUFA, PUFA and

n-6 to n-3 ratio were higher but proportions of C18:1n-9, C18:3n-3, C20:5n-3 and MUFA were lower in Tilapia fillet.

In the case of Nile tilapia ALA proportion increased reflecting the ALA content of the diet. DHA content did not decrease significantly however fish oil containing control diet contained two-fold higher proportion of this fatty acid. EPA proportion decreased significantly. Fillet ARA content did not change, however control diet contained two-fold higher proportion than soybean oil and selenium supplemented diet. n-6/n-3 ratio increased due to soybean oil supplementation. ALA content of African catfish fillet increased by 28 %; while EPA and DHA levels have not changed. n-6/n-3 ratio in African catfish fillet showed more advantageous value than in Nile tilapia.

### 3.5.4. Effect of soybean oil and selenium supplementations on fillet chemical composition and fillet fatty acid profile

The selenium supplementation resulted significant increase in the fillet selenium content only in the African catfish, however selenium content of Nile tilapia also increased. The initial value and control value were not statistically different in the two species (ranging between 65.6 and 77.7  $\mu\text{g kg}^{-1}$ ), while supplementations resulted 76.6 and 106.0  $\mu\text{g kg}^{-1}$  selenium levels in the Tilapia and African catfish fillets, respectively.

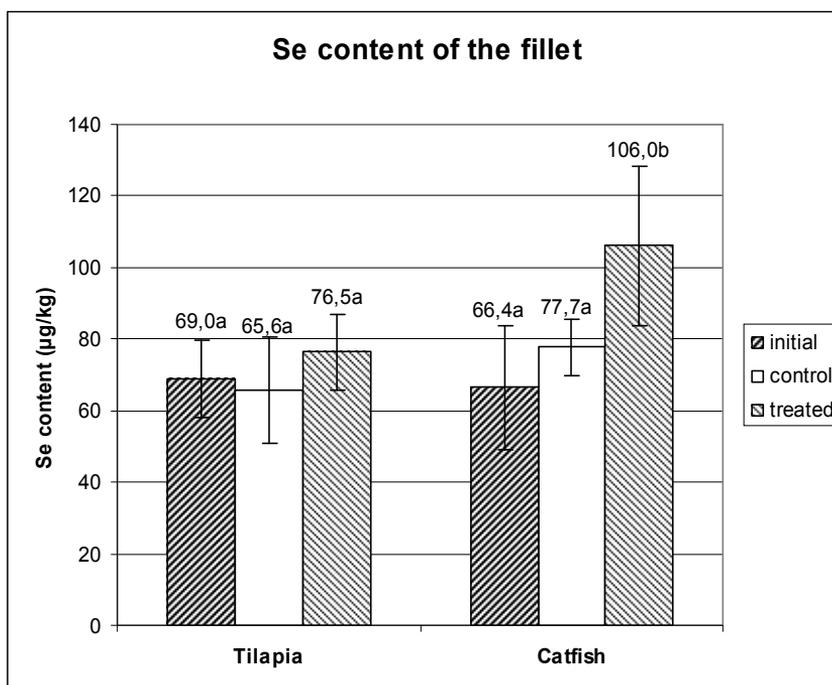


Figure 3: Fillet selenium content of African catfish and Nile tilapia fed with soybean oil and selenium supplemented diets

## 4. CONCLUSIONS

Production traits and fillet chemical compositions of the two species were not negatively affected by the vegetable oil supplementation. Both the dietary fat source and the duration of the feeding had marked effects on fillet fatty acids of both species. High n-3 fatty acid content of the diets decreased the water holding capacity of fillet, which was later compensated by adaptation of the antioxidant enzymes. In my opinion 6 weeks feeding of the finishing diet is needed to assure the desired fillet quality. Effect of linseed oil supplementation on the fillet fatty acid profile was more similar to the effect of fish oil supplementation, than those of soybean supplementation.

It was found that dietary vegetable oil fatty acids are effectively incorporated into tilapia's liver, fillet and mesenteric fat. The vegetable oil feeding (especially SO diet) led ultimately to a reduction of the fillet DPA proportion and was found not to be effective in either enriching or maintaining the fillet EPA and DHA proportions, as Nile tilapia has a limited capacity to synthesize EPA and DHA from dietary ALA precursor. LO feeding resulted similar n-6 to n-3 ratio as in the FO group, however it was mostly attributed to the direct increment of the ALA and not to its further elongated and desaturated metabolites. In summary, African catfish is more suitable candidate for functional food production than Nile tilapia. It could be explained with the different feeding habits of the species.

The maturation process resulted in marked differences between the two sexes of the Nile tilapia in terms of fatty acid composition of fish fillet. The more favourable fatty acid profile makes the male Tilapia more advantageous in the aquaculture production.

Production traits of African catfish were not affected by the high selenium content of the diet. However they were less prosperous in the 0.5

mg kg<sup>-1</sup> selenium supplemented group, which incorporation level is allowed by the Hungarian authorities. Supposedly the relatively high selenium content of the diet was not toxic for the fish, as no mortalities occurred during the experiments. The selenium content of the African catfish fillet showed a moderate positive correlation with the feed selenium content, described well by the following linear regression:  $Se(\text{fillet}) = 5.62 * Se(\text{feed}) + 81.02$ ; ( $r^2=0.45$ ). Production of functional food enriched with selenium could be achieved in 42 days of feeding a final diet containing 4.66 mg/kg organic selenium supplementation.

Feed conversion ratio, final weight and fillet yield was better in 0.5 and 4 mg kg<sup>-1</sup> selenium supplemented groups, but other production traits were not affected by different treatments. Fat content of diet was too high for Nile tilapia, fillet fat content increased in all treatments. Production of functional food enriched with selenium could be achieved in 42 days of feeding a final diet containing 2.47 mg/kg organic selenium supplementation. Selenium content of the Nile tilapia fillet showed a moderate positive relationship with the feed selenium content which could be approximated by the following equation of the third degree:  $(Se_{\text{Fillet}} = 75.6 + 46.8x(Se_{\text{Feed}}) - 12.3x(Se_{\text{Feed}})^2 + 0.72x(Se_{\text{Feed}})^3)$  ( $r^2=0.65$ ,  $P=0001$ ). According to my results a 100 g portion of Tilapia fillet fortified with the optimal selenium supplementation can cover approximately 25% of the RDA of selenium.

Soybean oil supplementation significantly affected the fatty acid profile of both species. Soybean supplementation of the diet was more suitable for the functional food production with African catfish than with Nile tilapia. Although level of ALA increased by 48 % in the Nile tilapia fillet, proportion of EPA and DHA - which are more important in terms of human diet – decreased. In the African catfish fillet proportion of ALA increased by 28 % and levels of EPA and DHA were not affected. Thus

production of ALA enriched African catfish fillet is possible. Selenium supplementation resulted in significant increase in the fillet selenium content only in the African catfish, but selenium content of Nile tilapia fillet also increased somewhat. Selenium supplementations resulted in 76.6 and 106.0  $\mu\text{g kg}^{-1}$  selenium levels in the Nile tilapia and African catfish fillets, respectively. Lower selenium content in Nile tilapia fillet could be explained by the increased oil content of the diet. Increased PUFA proportion of the fillet may be caused higher selenium requirement to prevent the oxidative stress, but further investigations are needed to its verification. According to my results African catfish is a more advantageous candidate in term of functional food production than Nile tilapia.

## 5. NEW SCIENTIFIC RESULTS

1. Production traits and fillet chemical compositions of African catfish and Nile tilapia are not negatively affected by the soybean oil and linseed oil supplementations. However only linseed oil supplementation results in similarly advantageous fatty acid profile in African catfish and Nile tilapia fillet as fish oil.
2. High n-3 fatty acid content of the diets decreases the water holding capacity of fillet, but it returns to the initial value for the 6<sup>th</sup> week. Six weeks feeding of the finishing diet is needed to assure the desired fillet quality.
3. The maturation process resulted in marked differences between the two sexes of the Nile tilapia in terms of fatty acid composition of fish fillet. The more favourable fatty acid profile and n-3/n-6 ratio, especially in FO group, makes the male Nile tilapia more advantageous in the aquaculture production.
4. Highest selenium content in African catfish fillet can be achieved ( $106-109 \mu\text{g kg}^{-1}$ ) by  $4 \text{ mg kg}^{-1}$  selenium supplementation of the diet. Selenium enriched functional food can be produced from African catfish.
5. Using traditional growing diets highest selenium content in Nile tilapia fillet can be achieved ( $127.8 \mu\text{g kg}^{-1}$ ) by  $2 \text{ mg kg}^{-1}$  selenium supplementation. Selenium content of the Nile tilapia fillet showed a moderate positive relationship with the feed selenium content which could be approximated by an equation of the third degree. However due to the increased soybean oil incorporation in the diet  $2 \text{ mg kg}^{-1}$  selenium supplementation is not enough to the increment of selenium content in the Nile tilapia fillet.
6. Selenium and  $\alpha$ -linolenic acid enriched functional food can be produced from African catfish. However Nile tilapia is a less advantageous candidate in terms of functional food production as soybean oil supplementation has a negative effect on fillet EPA and DHA proportion.

## 6. RECOMMENDATIONS

High n-3 fatty acid content of the diets decreases the water holding capacity of fillet, but it returns to the initial value for the 6<sup>th</sup> week. 6 weeks feeding of the finishing diet is needed to assure the desired fillet quality.

The more favourable fatty acid profile, n-3/n-6 ratio, and higher body weight makes the male Nile tilapia more advantageous in the aquaculture production. Use of homogenous male stock is recommended.

For functional food production from African catfish and Nile tilapia 4 mg kg<sup>-1</sup> and 2 mg kg<sup>-1</sup> selenium supplementation of the diet and 42 days of feeding is recommended, respectively.

Soybean oil and linseed oil supplementation is efficient in the increment of ALA proportion of African catfish fillet, thus vegetable oil lipid source could be recommended for functional food production and for substitution of fish oil in fish diets.

Increased PUFA proportion of the fillet may be caused higher selenium requirement to prevent the oxidative stress. I recommend further investigations to verify my findings.

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

### Articles in foreign languages:

**Biró, J.**, Hancz, Cs., Szabó, A., Molnár, T. (2009): Effect of sex on the fillet quality of Nile tilapia fed varying lipid sources. In: Italian Journal of Animal Science Volume 8 - Supplement 3, 225-227. p.

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

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