

Effects of various lengths of starvation on body parameters and meat composition in intensively reared pikeperch (*Sander lucioperca* L.)

Varju-Katona M.^{1*}; Müller T.¹; Bokor Z.¹; Balogh K.²; Mézes M.²

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Abstract

The aim of this work was to evaluate the influence of various lengths of starving periods on weight changes of different body parts and on chemical composition of the pike perch fillets. Forty cultured, market size (average bodyweight: 732.2±129.8 g), pikeperch of mixed sex were divided into 5 groups (n=8 in each), where one group were sampled at the starting time as a farm control, two groups were continuously fed for 3 and 6 weeks before slaughter, and the experimental groups were withdrawn from feed for 3 and 6 weeks before slaughter. Slaughter took place without any stress caused by transport or waiting times. Body parameters such as total body weights and the weights of 10 differentiated body parts, namely the gonad, the alimentary canal, the liver, the abdominal fat, the vertebral with unmatched fins, the head with the double fins, the two side fillets and the skins of both side fillets were monitored during the experimental period in each group. All body parts were defined and compared as the percentages of the total body weights. The chemical compositions of the fillets were examined by Weende analysis, thus dry matter, crude protein, crude fat and crude ash were measured. Increasing the length of the starving period reduced the body weight, mainly due to losses in intraperitoneal fat, although decreased fillet weights were found, caused by the depression of the intramuscular fat.

Keywords: Body parts, Chemical composition, Pike perch, Starvation, Weende

1-Department of Aquaculture, Institute of Aquaculture and Environmental Safety, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő, Hungary

2-Department of Nutrition, Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő, Hungary

*Corresponding author's Email: varju.milan@gmail.com

Introduction

Percid fishes are one of the most appreciated fish species for costumers, due to the taste and quality of their flesh, with low fat (2.8-3.0%) and high protein (18-19%) levels (Jankowska *et al.*, 2003; Çelik *et al.*, 2005).

Recently, intensive culture technology of pikeperch (*Sander lucioperca*, Linnaeus, 1758) and other percid species generally use formulated pellet feeding (Molnár *et al.*, 2004; Zakeš, *et al.*, 2004; Steinfeldt, 2015; Steinfeldt *et al.*, 2015; Ljubobratović *et al.*, 2016). However, only little information is available on conditions and nutritional requirements in market size pikeperch, which is a limiting factor for the development of this species reared in aquaculture. Therefore the effects of the changes in nutrient content on the diet of fish up to market size should be determined more precisely.

Studies carried out with percid fish species indicated that low dietary lipid (120-180 g kg⁻¹) and high protein content (350-550 g kg⁻¹) seem to be beneficial for growth performance of percids (Brown *et al.*, 1996; Fiogbé *et al.*, 1996; Kestemont *et al.*, 2001; Xu and Kestemont, 2002). Furthermore, increasing lipid tissue incorporation and peritoneal deposition observed were caused by the differences in physiological needs of the species. Percid species store excess energy mostly in the viscera (Mathis *et al.*, 2003; Boujard *et al.*, 2004, Mairesse *et al.*, 2005). Fat deposition in the body cavity can result in lower slaughter yields (Jobling *et al.*, 1998; Jobling

2001), because the fat is removed when the fish is gutted during initial processing, and this can determine profitability. Over condition may also result in other physiological deficiencies such as reduced reproduction performance or spermatogenetic and oogenetic disorders. Therefore, temporary food deprivation technology may be an effective prevention strategy.

Unlike mammals or poultry, fish can generally tolerate long periods of starvation (Paul *et al.*, 1995; Collins and Anderson, 1997; Power *et al.*, 2000). Fasting or food deprivation is a normal phenomenon that many fish species may experience in their lifetime due to seasonal fluctuations of poor food availability and migration trips. Even under culture conditions several fish can undergo starving periods during stressful conditions, fluctuations of water quality or disease outbreaks (Barcellos *et al.*, 2010; Sridee and Boonanuntanasarn, 2012; Najafi *et al.*, 2015).

In the past decades several studies have monitored the physiological effects and metabolic responses of feed deprivation in fish (Ince and Thorpe, 1976; Kheyyali, 1990; Csengeri, 1996; Figueiredo-Garutti *et al.*, 2002; Falahatkar, 2012; Chatzifotis *et al.*, 2018).

The observations of starvation-caused total body mass losses (Table 1.) should be compared with each other carefully. Changes in body mass losses show wide variability in different fish species. They differ in regard to their overall energy requirements which are

dependent on factors like the initial body weights, body temperatures or evolutionary adaptation mechanisms, as well as on fish age and nutritional state (Navarro and Gutiérrez, 1995).

Furthermore species differ in how they save their fuel resources under food deprived periods (McCue, 2010).

Table 1: Rates of body mass losses among various starving fish species (modified by McCue 2010).

Fish species (Temperature)	Period (Day)	Mass loss (%)	Daily mass loss (% day ⁻¹)	Reference
Carp	56	24	0.4	Blasco <i>et al.</i> (1992)
Chinese catfish	28	14	0.5	Fu <i>et al.</i> (2011)
European perch (15C°)	13	12	0.9	Mehner and Wieser (1994)
European perch (20C°)	13	14	1,1	Mehner and Wieser (1994)
Meagre	60	27	0,4	Chatzifotis <i>et al.</i> (2018)
Nile tilapia	45	16	0.3	De Silva <i>et al.</i> (1997)
Porgy	28	12	0.4	Rueda <i>et al.</i> (1998)
Rainbow trout	120	23	0.2	Pottinger <i>et al.</i> (2003)
Salmon	42	2	0.1	Soengas <i>et al.</i> (1996)
Sea bass	60	20	0.3	Stirling (1976)
Striped bass	30	17	0.6	Small <i>et al.</i> (2002)
Walleye	42	25	0.6	Czesny <i>et al.</i> (2003)

Fish can adapt to feed restricted environments with different behavioural strategies, such as reducing their activity (Love, 1980; Hogendoorn, 1983; Ultsch, 1989) and migrating to environments of lower temperature (Sogard and Olla, 1996; van Dijk *et al.*, 2002) in order to save their metabolic energy. Many species can also adapt to starvation with physiological, cellular and molecular strategies to limit energy consumption (Land and Bernier, 1995). In situations, where food is readily available, animals support the energy themselves from continuous feeding to survive and reproduce. Therefore they are able to maintain a physiological state, where the animal's mass and energy inputs are balanced with its mass and energy requirements (Kleiber

1975). During starvation this balance between mass and energy flux becomes disrupted, thus several psychological changes occur in the animal body. In case of fish, the most observed and documented phenomenon is the change of total body mass. The reduction of body mass, effects on the condition factor, in order of the viscerosomatic- and the hepatosomatic-indexes (Hung *et al.*, 1997; Falahatkar, 2012).

Through starvation, the gastrointestinal tissues show more measurable changes in the first place by reducing their lipid contents (Ostaszewska *et al.*, 2006), than organs such as the liver and adipose stores undergo slower, but equally large reductions in mass (Zammit *et al.*, 1979). Other organs such as the heart,

brain, kidney and gonads may not undergo measurable reductions in relative mass during fasting or even increase in relative mass (Soengas *et al.*, 1996).

The three major physiological fuels are namely carbohydrates, lipids and proteins. Each has unique energy densities that ultimately influence net rates of body mass loss during starvation. Food deprivation is generally associated with the mobilisation of these energy reserves in order to maintain the homeostasis (Navarro and Gutiérrez, 1995; Gillis and Ballantyne, 1996; Vigliano *et al.*, 2002).

Several studies investigated the influences and metabolic responses of fasting in different fish species, and reported that in most species, glycogen is generally the first substrate used as an energy source, and glucose derived from glycogen is used to maintain glycaemia during the first stage of starvation. Furthermore, a depressed activity of glycogenolytic enzymes and lipogenesis were found, whereas the hydrolysis of triglycerides, and the fatty acids released, were mainly used as energy fuel through the corresponding oxidative pathways (Moon *et al.*, 1989; Sánchez-Muros *et al.*, 1998; Metón *et al.*, 2003; Guderley *et al.*, 2003).

Lipids are primarily stored in triglyceride form, in three different parts of the body, as intramuscular, intraperitoneal and subcutaneous fat. Through starvation the primary lipid depots - where free fatty acids are mobilised from - and the mass change ratio of these depots may widely differ

between species (Caruso *et al.*, 2012; Einen *et al.*, 1998; Falahatkar, 2012). In addition, the weight changes of fat depots, their fatty acid contents and the ratio of saturated and unsaturated fatty acids may be diverse between species (Csengeri, 1996; De Silva *et al.*, 1997; Zajic *et al.*, 2012).

In case of prolonged starvation, many scientists examined proteins as an energy resource, and the physiological mechanism, where the lipid-dominated catabolism switches to protein-dominated catabolism (Black and Love, 1986). It happens, when liver glycogen reserves are practically depleted, and the reducing lipid content of the body reaches a critical low level. Protein is mobilized mainly from skeletal muscle (Navarro and Gutiérrez, 1995; Echevarría *et al.*, 1997; Metón *et al.*, 2003), although in case of migrating species, protein catabolism may have an outstanding importance (Volkoff, 2011). Adequately nourished animals maintain a neutral protein balance, where the total rate of protein synthesis is equal to the protein degradation of the body. Therefore in case of long time starvation, starving animals eventually go into negative protein balance to minimize their net losses of protein (McCue, 2010).

Through starvation, alongside the weight losses of organs and tissues, their water content also changes. Although water provides no energetic value, while tissues and organs lose carbohydrate, lipid or protein, their relative water content increases (Woo and Cheung, 1980; Moon, 1983; Black and Love, 1986; Frick *et al.*, 2008).

Meanwhile, starvation caused relative growth of mineral content of the tissues was also found in several species (Stirling, 1976; Cook *et al.*, 2000; Czesny *et al.*, 2003).

Materials and methods

The experiments were carried out with two years old, market sized, intensively reared pikeperch (*Sander lucioperca*), at the fish farm of Győri “Előre” Fisheries Co-operative (Kisbajcs, Hungary). The fish used in the experiment, were hatched and reared in this same farm. Fish were stocked for starvation (n= 40) in the experimental space in 5 groups: an absolute control group, sampled at the starting time (day 0); two feeding groups served as controls: one sampled after 3 weeks, and the other after 6 weeks; and two starving groups: one sampled after 3 weeks and the other after 6 weeks of starvation. The initial body weights of fish groups are shown on Table 2. Fish were weighed at the start of the trial and at slaughtering. During the experimental period, fish were kept from any additional stress effects.

Even though there are specific pikeperch feeds in the market, a pelleted feed with similar nutrient content (Coppens Marico FOCUS, 6.0 mm) was chosen for the fish groups. This grower feed has 18.0 MJ kg⁻¹ DE, 49% crude protein, 10% crude fat, 0.8% crude fibre and 8.3% ash content. According to the manufacturer’s recommendation, this complete feed is suitable for carnivorous species, such as percid fish.

The initial group, which the experimental pike perch individuals were chosen from were fed the same feed for over 1 year, and it was used during the whole experimental period. Fish were fed daily at 0.5% of average initial body mass in each fed group.

The experiment lasted for 6 weeks, in 4 m³ cuboid tanks (400x100x100 cm), in continuous water-flow system (4-5 L min⁻¹). The groups were held separately. The tanks were filled up to 50% of total volume to avoid jump out losses. Water parameters were continuously monitored. Temperature was measured daily over the 42-day with laboratory thermometer (± 0.1 °C); dissolved oxygen and pH with HACH Portable Multi-Parameter Meter; total ammonia levels with photometric method by a HANNA HI 83203 Multiparameter Photometer.

Fish were held for 24 hours in low intensity red light, 80 Lux at the water surface, measured by a Voltcraft BL-10 Lux-Meter. The water temperature was 20 \pm 1 °C, dissolved oxygen concentration was 13 \pm 1.7 mg L⁻¹, the pH was between 7.2 and 7.5 and the average total ammonia level was 0.2 \pm 0.03 mg L⁻¹ during the whole experimental period.

Sampling and biochemical methods

Samples were taken at start (day 0 group), in the 3rd and 6th weeks of the experimental period from the fed and starvation groups as well. Slaughtering took place after live chilling, by rapidly decreasing fish body temperature in ice slurry (ice/water mixture 1:2). Weight measurements of the body parts were

taken right after slaughter, with a 0.01 g 1000g⁻¹ scale digital libra. The alimentary canal was measured without the intestinal contents. Post mortem muscle samples were taken from each pikeperch individual. Samples were kept in deep-frozen condition (-78 °C) until analysis.

Chemical compositions were made by proximate analysis (Weende method), with a quantitative method to determine different crude macronutrients in the samples. The analysis has the advantages of being simple, relatively quick, inexpensive and highly reproducible.

Before analysis, all meat samples were prepared by grinding. Determining dry matter was by drying the samples in an oven at 105 °C, until all the free water evaporated. The water content was the difference in weight of a sample before and after drying. The crude fat content was determined by the Soxhlet method, extracting the sample with n-hexane that removes the fats and other soluble substances. The crude protein fraction was calculated from the

nitrogen content of the sample, determined by the Kjeldahl procedure, by digesting in hot, concentrated sulphuric acid. Nitrogen was subsequently measured and expressed by multiplying the N figure by 6.25 as percentage. The ash content was determined by ignition of the sample at 550 °C. The residue represents the inorganic constituents of the sample.

Statistical evaluation of the data (unpaired t-test, one-way ANOVA with Tukey-Kramer Multiple Comparison post-hoc test) was done after calculating the means and standard deviations by GraphPad InStat for Windows version 3.05 (GraphPad Software, San Diego, California, USA).

Results

The body weight of fed animals showed significant increase up to the third and sixth week of the experimental period, while as an effect of starvation, significant decrease in the body weight was found during the same period (Table 2.)

Table 2: Body weight changes during the experiment (Letters indicate values that are significantly different ($p \leq 0.05$, independent sample t test) within the experimental groups, stars indicate values that are significantly different ($p \leq 0.05$, independent sample t test) between the experimental groups (3. week, 6. week).

	starting point (g)	3. week (g)	6. week (g)
0. time control (n=8)	702.8±96.1	-	-
feeding group 1. (n=8)	775±94.8 ^a	797.1±103.9 ^a	-
feeding group 2. (n=8)	738.5±87.3 ^a	-	812.7±88.2 ^a
starvation group 1. (n=8)	701.8±106.3 ^a	652.7±98.8 ^{a*}	-
starvation group 2. (n=8)	743.2±88 ^a	-	637.2±61 ^{b*}

In the first treated group, after a 21 day long starving period, 6.99 % of mass loss was measured, that is 0.33 % of *por diem* loss (% day⁻¹). In the second

treated group, after 42 days of fasting, 14.26 % of mass loss was measured, with 0.34% daily weight losses (% day⁻¹).

In the feeding groups, significant growth was measured in liver and abdominal fat, besides all the other body parts remained constant. However

in the experimental groups, the starvation had a diminishing effect on alimentary canal, liver, abdominal fat and filets too (Table 3).

Table 3: Changes of body parts in treated and non-treated groups (mean \pm SD, n=8).

Organ	Treatment	Initial	3rd week	6th week
		body %		
Gonad	Feeding group	0.24 \pm 0.23	0.29 \pm 0.20	0.23 \pm 0.19
	Starved group		0.39 \pm 0.22	0.31 \pm 0.23
Digestive tract	Feeding group	1.90 \pm 0.55	1.82 \pm 0.32	1.62 \pm 0.34
	Starved group		1.42 \pm 0.21	1.43 \pm 0.21
Liver	Feeding group	0.82 \pm 0.17	0.99 \pm 0.18	1.03 \pm 0.20
	Starved group		0.69 \pm 0.18	0.73 \pm 0.07
Abdominal fat	Feeding group	5.53 \pm 1.38	6.13 \pm 1.19	7.20 \pm 0.95
	Starved group		4.90 \pm 1.53	4.72 \pm 1.01
Vertebral with unmatched fins	Feeding group	18.66 \pm 0.98 ^a	18.23 \pm 1.58	17.80 \pm 1.53
	Starved group		21.13 \pm 1.42 ^b	19.59 \pm 0.90 ^a
Head with the double fins	Feeding group	24.39 \pm 0.99 ^a	23.55 \pm 1.07	23.08 \pm 0.71
	Starved group		25.95 \pm 1.29 ^a	26.39 \pm 1.69 ^b
Filets with skin	Feeding group	46.41 \pm 1.46	46.78 \pm 1.49	47.13 \pm 1.52
	Starved group		44.55 \pm 2.37	44.52 \pm 1.08
Filets without skin	Feeding group	39.84 \pm 1.40	40.18 \pm 1.56	40.90 \pm 1.67
	Starved group		37.29 \pm 2.79	37.46 \pm 1.20
Skin	Feeding group	6.57 \pm 0.19 ^a	6.60 \pm 0.35	6.23 \pm 0.32
	Starved group		7.26 \pm 0.55 ^b	7.06 \pm 0.44 ^a

^{ab} Different superscripts in the same column means significant difference at $p < 0.05$ level (one-way ANOVA with Tukey post-hoc test)

The proximate analysis showed that the measured chemical parameters of the fed fish meat remained at the initial levels, whereas in the fish samples from the starving groups, significantly

decreasing crude fat levels were found (Table 4). The depression of intramuscular fat causing the relative weight loss of the filets is shown in Table 3.

Table 4: Changes of body composition in treated and non-treated groups (mean \pm SD, n=8).

	initial	3 rd week	6 th week
	Feeding groups (%)		
Dry matter	22.68 \pm 0.91	22.67 \pm 0.30	22.68 \pm 0.25
Crude protein	21.46 \pm 0.71	21.47 \pm 0.38	21.49 \pm 0.15
Crude fat	3.94 \pm 0.43	3.99 \pm 0.28	4.07 \pm 0.59
Crude ash	1.26 \pm 0.08	1.25 \pm 0.06	1.25 \pm 0.05
Starving groups (%)			
Dry matter	22.68 \pm 0.91	22.56 \pm 0.22	22.55 \pm 0.22
Crude protein	21.46 \pm 0.71	21.45 \pm 0.30	21.29 \pm 0.49
Crude fat	3.94 \pm 0.43	3.27 \pm 0.63	3.17 \pm 0.13
Crude ash	1.26 \pm 0.08	1.26 \pm 0.11	1.31 \pm 0.04

No significant differences were found in measured groups at $P < 0.05$ level (one-way ANOVA with Tukey- post-hoc test).

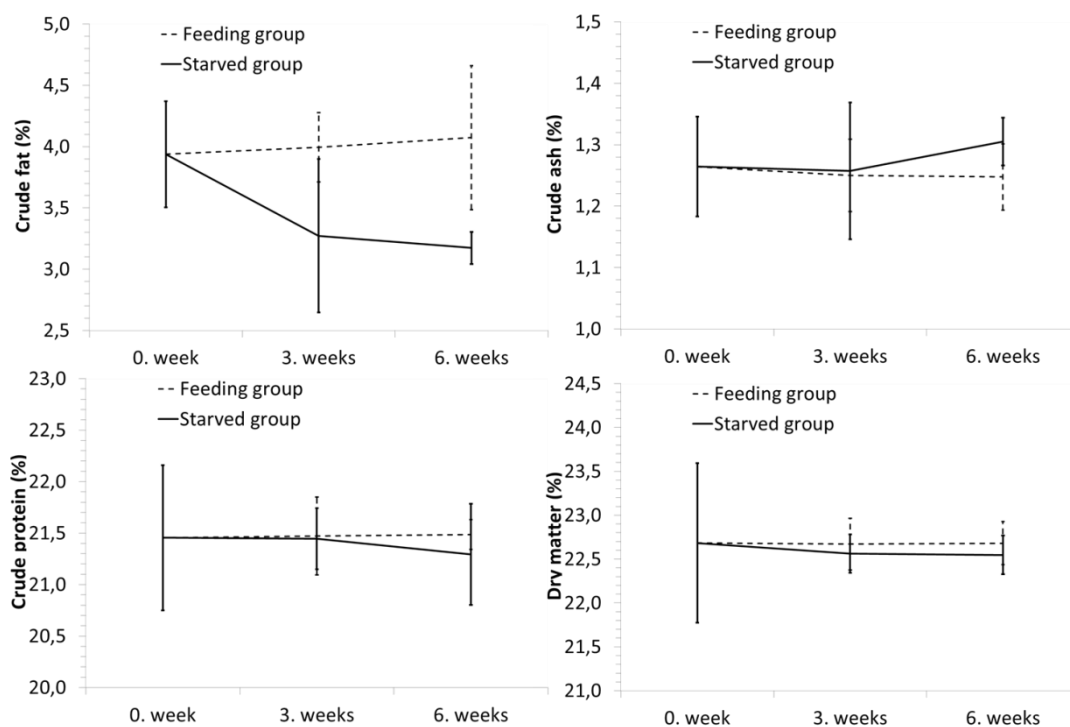


Figure 1: Changes of body composition parameters in the 3rd and 6th weeks (mean ± SD, n=8).

The reduction of crude fat showed notable difference in the starved group at $p=0.079$ (between week 0. and 6.), and among the fed and starved groups (6 week) at $p=0.07$.

Discussion

The varying lengths of starving periods had dramatic effects on bodyweight and on some meat composition parameters in pike perch. The reason for these changes is that due to starvation, glycogen and lipid reserves are mobilised and used as energy resources to maintain animals' homeostasis. Between different fish species, the changes in body mass losses show wide variability even between percid species. In our case we measured 7 % and 14 % of body weight losses from the initials after 21 and 42 days long fasting periods. This outcome was a lower level of weight loss, than was experienced in European perch (*Perca*

fluviatilis) in the same water temperature, or in walleye (*Sander vitreus*) after the same length of starvation but at a cooler temperature (Mehner and Wieser, 1994; Czesny *et al.*, 2003).

A radical decrease was found in the weights of the alimentary canal, the liver and the abdominal fat. These changes are in line with the findings of Ostaszewska *et al.* (2006), where histopathological changes were measured in the intestinal epithelium and liver in tench (*Tinca tinca*), and with the results of Zammit *et al.* (1979) where decreasing liver and adipose tissue weights were measured in European sea bass (*Dicentrarchus labrax*) after starvation.

In starved groups, a notable weight depression of the filets was also found, which suggests the mobilisation of energy reserves from meat in order to maintain homeostasis during the food

deprived period. These observations show connections with our other findings, where significantly depressed crude fat levels were measured in meat samples from starved fish. However these results are in line with the findings, that during prolonged starvation, when glucose and liver glycogen reserves are practically depleted, lipid reserves are used for energy, primarily mostly the saturated and monounsaturated fatty acids (Csengeri, 1996; Falahatkar, 2012; Zajic *et al.*, 2012).

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