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Influence of various fat levels on meat quality in rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*)

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Abstract. Rainbow trout (*Oncorhynchus mykiss*) is one of the widely distributed species of fresh water worldwide and in Europe, while brook trout (*Salvelinus fontinalis*) presents very good meat quality and is very much preferred by consumers both as fresh fish in cleaned and packed conditions. Besides, brook trout have very high flesh quality with excellent pigmentation, texture and composition. Meat quality is primarily given by nutrition. Although nutritional aspects of those two species were fairly well studied, an evolving process of new insights into trout (but many other farm species) nutrition constitute a continuous process. In regard with the main nutritional needs of trout, the most expensive are primarily sources of proteins and fats. Fish origins for proteins and fats have been demonstrated to give best results, but they are becoming more and more limited. Here we tested traditional cheese as a partial substitute for fat in trout nutrition, in a mixture with classic fodder and in a small familial farm. Cheese has increased the fat level with 2.5% of growth ratio of rainbow trout and brook trout. That has conducted to better average daily gain and live weight ($P<0.05$ for rainbow trout, and $P<0.01$ for brook trout respectively) for cheese mixture. Meat quality of plot fed with higher fat level has a higher dry matter content and significant more fat content ($P<0.001$ within rainbow trout plots and $P<0.01$ for brook trout). In conclusion, a higher fat content of 2.5% has beneficial effects of trout meat, considering that this fat is high in benefic fatty acids for human consumption.

Key Words: fish nutrition, feed substitute, alternative fodder, organic feeding supplement.

Introduction. Meat quality is a dynamic concept evolving in parallel with actual insights into human nutrition. Although meat chemical composition is primarily influenced by feeding and little variation occurs in the main nutrients percentages within the same subspecies, breed or variety, the concept of quality is an evolving concept. Fish meat represents an increasingly demanded product worldwide (FAO 2014), having real health benefits (FAO 2010) and far more variability as market offers than red meat. That is simply because of huge variety of species from aquaculture and relatively easy to grow. Within fresh water species, rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) constantly increase their livestock due to high species plasticity and farm breeding suitability. *O. mykiss* is one of the widely distributed species of fresh water in Europe (FAO 2014). *S. fontinalis* has lower livestock and implicitly smaller impact on aquaculture. But there are advantages in regard with tradition and consumer preferences. In a consumer acceptance study on these two species, brook trout reached consumers preferences in a survey by selling the fish in cleaned and packed conditions (Köse et al 2001).

If the main trout livestock is sold as fresh meat, various methods of preservation add commercial value to this meat. Smoked, salted, dried are only a few preserving methods and variation within process of production are questions of tradition, labeling, consumer preferences or various other factors. Trout meat presents a growing interest for consumers for its biological value, cheap price and relatively easy to produce.

Fresh fish quality most commonly refers to aesthetic characteristics and its degree of freshness. When longer term of preserving is needed, still aesthetic characteristics is one of the main consumer's criteria to buy. Chemical and nutritional values of meat are not mandatory to be presented than for packaged products. But these parameters highly contribute to conservation and overall quality of fish meat.

Both rainbow trout and brook trout demands high protein and fat levels in their diets for a proper growth and health. Although several studies have focused on those species nutrition (proteins – Kim 1997; Jobling et al 2010; Amin 2013; Amin et al 2014; fat and fatty acids – Austreng et al 1980; Pieper & Pfeffer 1980; Ng et al 2010), meat quality and consumer preferences are an evolving and dynamic domain.

Romania has a long tradition in trout production mainly because a large network of fresh water suitable for species farming. On the other hand, those two species have demonstrated to better survive during the cold season in this specific temperate climate with cold and long winters. The species ability to survive cold winters has been also proven for North American continent also in temperate climate and in pond conditions (Coyle & Tidwell 2008).

Given that meat chemical composition is primarily influenced by nutrition, the use of alimentary products or residues rich in proteins and fats from traditional farms is sometimes a practice in rural parts of Romania. Cheese could be such an example. Here we investigate the rainbow trout and brook trout meat quality fed with classic fodder and cheese supplementation.

Material and methods

Biological material. 100 individuals from rainbow trout *O. mykiss* and 100 *S. fontinalis* were fed for a period of 360 days with two levels of protein and energy in a traditional farm from Suceava County, Romania.

Water quality. The fish farm is located in Broşteni village from Suceava County, at an altitude of 750 m, on Neagra creek that flows into the river Bistriţa. The water source is from a tributary of Neagra creek, with optimal characteristics for trout breeding. Temperature (T °C), pH and dissolved oxygen (DO) were monthly recorded with portable multi-parameter HANNA HI 9828. Temperature variation ranged between 2.8 and 13.2°C (average of 6.83°C for entire 2014 year), dissolved oxygen level ranged between 8.3 and 10.15 mg O_2/L (average of 9.13 mgO_2/L) and water pH ranged between 6.7-7.2 (average of 7.04).

Diet and chemical composition of the fodder. One plot (plot 1 from both *O. mykiss* and *S. fontinalis*) received a classic granulated fodder with various levels of protein and energy, depending of stage of growing (Table 1). The second plot received a mixture of the same fodder + cow cheese in percent of 10% from estimated daily intake. Estimated daily intake was calculated as percent of total biomass (Anderson & Newman 1996).

Table 1

Chemical composition of the fodder fed to *Oncorhynchus mykiss* and *Salvelinus fontinalis*

Specification	*Chemical composition of the fodder*				
	Starter	*Grower*			
		Plot 1	*Plot 2*	*Plot 1*	*Plot 2*
	2-3 mm	*3 mm*		*4.5 mm*	
Crude protein (%)	56	45	44.5	43	42.7
Crude fat (%)	18	20	22.6	22	24.4
WSC (%)	8.9	18	16.9	18	16.9
Fiber (%)	0.7	2	1.8	2	1.8
Ash (%)	9.5	7	6.9	7	6.9

Starter composition: fish meal, wheat, fish oil, yeast, minerals and vitamins.
Grower composition: plot 1 - classic fodder: fish meal, wheat, fish oil, hydrolyzed proteins, rapeseed, soy, blood meal, minerals and vitamins; plot 2 – mixture of classic fodder as for plot 1 (90%) with cow cheese (10%).

Chemical analyses of the mixture were made using Kjeldahl method for crude proteins (automatic analyzer Gerhardt Turbotherm with VapodestDistiler); Soxhlet method was used for crude fats based (automatic analyzer VelpScientificaSer 148 Solvent Extractor); crude fibers were determined by automatic analyzer Dosi-Fiber. Dry matter and water content were determined with classical methods of 105°C drying in oven, while ash content has been determined in a calciner.

Statistical analyses*.* Statistical analyses were performed with StatSoft Statistica version 10 (http://www.statsoft.com). For equal or unequal variances assuming preliminary F-test were computed, for each comparison between variables and for statistical differences a T-test was performed.

Growth parameters*.* Average daily gain (ADG) was calculated as difference between final and initial live weight, all divided to number of experimental days (360 days).

Meat chemical analyses. Meat chemical analyses followed the same methods as for fodder and used the same equipments.

Results and Discussion. *O. mykiss* showed a higher ADG (ADG = 0.438 g for classic fodder and 0.446 for mixture) than *S. fontinalis* (ADG = 0.401 g for classic fodder and 0.411 for mixture), which is expected given that *O. mykiss*, has normally higher growing performances than his relative (Kocaman et al 2006). Within the same species, fishes best capitalized mixture of classic fodder with cow cheese (Table 2). That was observed for both species ($P<0.05$ for *O. mykiss*, $P<0.01$ for *S. fontinalis* respectively for live weight comparison).

Slaughter percentage has been improved in plot fed with cheese mixture. Although the final weight is at lower limit for marketable purpose, positive variation occurred for cheese mixture fed individuals. This variation would probably become higher with the age. In aquaculture large number of individuals finally contributes to the total amount of fresh meat when higher slaughter percent is recorded.

Chemical composition of the meat did not reveal much variation as absolute values. But variance has been significant for fat content between the two plots fed with various fat levels ($P<0.001$ for fat content within *O. mykiss* plots and $P<0.01$ for *S. fontinalis* for the same comparison). Given that *S. fontinalis* meat has normally higher protein, lipid and dry matter content in both whole-body and fillet (Nistor et al 2014a), our results reconfirm this. Higher values for all parameters were shown in plots fed with cheese mixture, most probably due to higher fat content of the fodder. Fats are known to generate higher energy for growth from all nutrients, twice as higher than proteins and carbohydrates and they are more efficiently used as energy for fish (De Silva & Anderson 1995). In salmons and trout even higher amounts of fats (especially oils) are needed (FAO 2010 - species profiles).

Plot 1 received a classic fodder well equilibrated for protein and energy (here fat level is critical), while plot 2 ensured on average of 2.2% higher fat content for entire growing period. Fat supplementation was done by cheese, a natural product with good quality of proteins and fats, but not as good as fish oil or fish meal. The main fat energy was given by classic fodder, here cheese has been just a supplement from own farm production, which maintained protein level of the mixture and added energy. Benefits of this fat supplementation conducted to a more fat meat ($P<0.001$ in *O. mykiss* and $P<0.05$ in *S. fontinalis*). That fat content of the meat, although little as absolute value, could be high in quality. It has been shown that even at high variation of total fat content, the variation of the main fatty acids proportion in meat is relatively low in rainbow trout, but higher total fat content leaded to more fatty acids (Timberg et al 2011).

Table 2

Variation of live weight, anatomical proportions and tissues composition of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) fed with two fat levels

Specification	*Oncorhynchus mykiss*				*Salvelinus fontinalis*			
	Classic fodder		*Mixture of classic fodder with cheese*		*Classic fodder*		*Mixture of classic fodder with cheese*	
	X±sx	*%*	*X±sx*	*%*	*X±sx*	*%*	*X±sx*	*%*
Live weight (g)	157.68±1.60	-	160.42±2.33	-	144.58±1.77	-	147.96±0.92	-
Head (g)	17.22±0.77	10.92	16.13±0.37	10.05	15.72±0.31	10.87	14.26±0.25	9.64
Flippers (g)	3.98±0.12	2.52	4.07±0.36	2.54	3.93±0.13	2.72	3.65±0.17	2.47
Intestines (g)	22.59±0.38	14.33	21.28±0.71	13.27	17.72±0.33	12.26	17.28±0.19	11.68
Skin + scales (g)	9.98±0.42	6.33	10.84±0.22	6.76	9.68±0.25	6.70	10.48±0.49	7.08
Bones (g)	10.26±0.28	6.51	10.84±0.50	6.76	10.57±0.25	7.31	10.14±0.38	6.85
Meat (g)	73.1±0.45	46.36	76.30±0.19	47.56	71.07±0.85	49.16	74.72±1.11	50.50

Fish oil is the main fat in salmon's diet and the highest amount of oil goes into feed for carnivorous fish such as salmon and trout to ensure an end product rich in omega-3 fatty acids (EPA and DHA). The profile industry claims that around 50 percent of omega-3 fatty acids, from either fish oil or fishmeal, consumed by the fish are finally found in meat (FAO 2014). Scientific studies reconfirm those retention proportions of EPA and DHA in salmon of 30–75 percent depending on the level of fish oil in feed (NIFES 2013).

Comparing the two species, rainbow trout has better responded to higher fat content of the fodder. A slightly fattest meat improves its organoleptic characteristics, thus consumers attraction too. In spite of dietetic demands of the consumers for meat in general, a certain percent of fat is more than necessary for a proper cooking. *S. fontinalis* meat has shown to have a more consistent texture than *O. mykiss*, also lower losses at frying but also at frying preceded by flouring (Nistor et al 2014b). A fattest meat is thus more affordable for the consumer's preferences.

Regarding the diet costs, permanent efforts are invested to decrease the amount of rich ingredients in fish food. A success example is Norway, where nutritional demands of *Salmo salar* ratio were optimized during past 25 years. In 1990, 3.8 kg of marine protein was used to produce 1 kg of salmon protein, while in 2013 the amount had fallen to 0.7. The corresponding marine oil dependency ratio was 2.8 in 1990, whereas in 2013 the amount was 0.5 kg of marine oil origin to produce 1 kg of fat (Ytrestøyl et al 2015).

Cheese scrap was used to feed *O. mykiss* and significant results ($P<0.05$) were recorded for weight gain and feed efficiency in larger trouts (Hertrampf & Piedad-Pascual 2001). In Romania traditional cheese has a low price on the market but is a valuable source of good quality proteins and fats. Trout feeding supplementation is thus a convenient way to use it, in low amount within the mixture with classic fodder. Meat quality improvement was significant for fat level and dry matter content, but probably for cooking characteristics too. That could increase the meat demands from the consumers, more so because the fishes are raised in traditional farm system.

Table 3

Chemical composition of meat from *Oncorhynchus mykiss* and *Salvelinus fontinalis* fed with two fat levels

Specification	*Oncorhynchus mykiss*			*Salvelinus fontinalis*		
	Classic fodder (CF)	*Mixture of classic fodder with cheese (MF)*	*P values*	*Classic fodder (CF)*	*Mixture of classic fodder with cheese (MF)*	*P values*
	X±sx	*X±sx*	*CF vs MF*	*X±sx*	*X±sx*	*CF vs MF*
Water (%)	74.77±0.92	74.1±0.69	0.26004	73.32±0.76	72.81±0.88	0.2130
DM (%)	25.23±0.08	25.9±0.24	0.18723	26.68±0.18	27.19±0.40	0.4038
Fat (%)	3.18±0.41	3.47±0.34	0.00024	4.12±0.30	4.31±0.27	0.0109
Protein (%)	20.78±0.74	21.18±0.81	0.4999	21.23±0.28	21.68±0.31	0.4709
Minerals (%)	1.27±0.06	1.25±0.09	0.2211	1.33±0.04	1.2±0.07	0.000004

*** = P<0.001; ** = P<0.01; * = P<0.05; ns = P>0.05.

Conclusions. *O. mykiss* and *S. fontinalis* are ones of the widely bred species of trout in Romania, which has very good ecological conditions and quality of the water for species exploitation. In spite of tradition, breeding conditions and nutrition are generally inconsistent with the last scientific inputs. Many small familial farms were founded in the past decade as an additional source of incomes in agriculture. When there is an excess of agricultural products and can be given in fish feed, the farmers do that. When cheese was given in mixture with a classic fodder (90% classic fodder and 10% cheese) in trout nutrition and increased the fat percent with 2.5% for growing period, both *O. mykiss* and *S. fontinalis* showed better performance. Higher ADG, slaughter percentage and fat content of the meat were the most obvious features. Besides higher final live weight, fat content of the meat presented significant variation ($P<0.001$ for fat content within rainbow trout plots and $P<0.01$ for brook trout for the same comparison). That could increase the meat demands from the consumers, because better cooking properties of the meat and more so because the fishes are raised in traditional farm system, with organic feeding supplementation.

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