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Effects of long term feeding diets differing in protein source and pre-slaughter starvation on biometry, qualitative traits and liver IGF-I expression in large rainbow trout

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ABSTRACT - The effects of feeding two complete extruded diets differing in protein source (fish meal-FM vs. vegetable proteins-VP) over 30 weeks and subsequent 30 days of starvation on biometry, fillet composition and liver IGF-I mRNA were studied in large rainbow trout. At the end of the feeding period, the dietary protein source little affected major biometry traits, dressing out yields and overall adiposity (P>0.05) but fish given the VP diet resulted in higher content of PUFA n-6 fatty acids in muscle (0.46 vs. 0.22 g/100g fillet, P<0.05). Regardless of the previous diet, 30 days of starvation resulted in increased carcass yield (P<0.05), in a slight reduction in adiposity (P>0.05) and of all fatty acids in fillet (P<0.05), except DHA. Liver IGF-I mRNA content was little affected by the test diet and starvation.

Key words: Rainbow trout, Vegetable proteins, Qualitative traits, Starvation.

Introduction - Replacing fish meal for protein-rich plant ingredients in complete diets for rainbow trout has been the focus of a number of studies which have shown very high inclusion rates, up to a nearly total dietary substitution, to be feasible with selected protein-rich vegetable materials in terms of growth performance. There is however evidence that some quality traits such as adiposity, dressing out yield and fatty acid composition of the edible portion could be affected to some extent in trout fed diets including substantial amounts of vegetable protein ingredients (Kaushik *et al*., 1995, Tulli *et al*., 2007). These changes have mostly been observed in juvenile or pen-size fish after relatively short culture periods, while quality traits in large-size rainbow trout subjected over a long time span to diets high in protein-rich plant materials, have been poorly investigated so far.

Fish undergo to a fasting period before slaughtering. Starvation has been suggested as a tool for improving dressing out yield, muscle proximate and fatty acid composition of the fillet (Einen *et al*., 1998; Grigorakis and Alexis, 2005) through metabolic mechanisms involving the somatotropic axis (Ayson *et al*., 2007; Pedroso *et al*., 2006).

The aim of this trial was to investigate the effects of 30 weeks of feeding diets differing in protein source and subsequent 30 days of starvation on biometric traits, fillet composition and liver expression of IGF-I in large rainbow trouts.

Material and methods - Two grossly isonitrogenous (7% DM) and isolipidic (19.6% DM) diets were prepared (table 1). The control diet (FM) with fish meal as the sole protein source was compared with a diet (VP) in which a mixture of pea protein concentrate and wheat gluten meal was used to replace 80 % of fish meal protein. Each diet was assigned to three groups of 18 triploid rainbow trouts each, according to a random design. Fish were kept in 500 litre fiberglass tanks in a flow-through system at 13.7±0.22°C, dissolved oxygen 8.17±0.8 mg/l, pH 7.49±0.21, and fed to satiety in two daily

meals over 30 weeks (first sampling time), then kept starved over 30 days (second sampling time). Eigtheen fish per dietary treatment (6 per groups) were sampled at each sampling time. Individual weight, morphometric traits, CF (condition factor), HSI (hepato somatic index), MF (mesenteric fat), DT (digestive tract), carcass and fillet yield were measured. Samples of fillet and liver were obtained from 6 specimens per dietary treatment at each sampling time to determine the fatty acid composition in muscles and to extract the hepatic RNA, respectively. The expression of liver IGF-I mRNA has been evaluated by a semiquantitative analysis of cDNA, using β-actin as house keeping gene. The amount of cDNA has been quantified using the Photo-capt Image Analizer (Vilber Lourmat). Data were analyzed by a two-way ANOVA (diet and sampling time).

Table 1. Composition and proximate analysis of the experimental diets.

		Diets				Diets	
		FM	VP			FM	VP
Composition	g/kg			Chemical analysis			
CPSP 72	"	50	50	Dry matter	%	94.2	92.4
Fish meal Chile	"	580	65	Protein	% DM	43.8	45.5
Pea protein conc.	"	-	210	Ether extract	"	18.9	19.8
Wheat gluten meal	"	-	210	Ash	"	11.2	4.4
Wheat meal	"	195	255	Starch	"	12.5	15
Fish oil	"	140	175				
Vit. and min. mix	"	35	35				

Results and conclusions - At the end of the feeding period, growth performance, feed efficiency (results not shown), major biometry traits and dressing-out yield (Table 2) were not affected by the dietary protein source (P>0.05) except for a slightly lower value of HSI and increased fillet yield in fish fed diet VP (P<0.05). After further thirty days of starvation, all biometry traits, particularly HSI and DT , were lowered and carcass yield increased (P<0.05) regardless of the past diet offered to trout. Fillet yield was significantly reduced in fish previously fed diet VP at the end of the fasting period, while no change due to starvation (P>0.05) occurred in those given diet FM.

Table 2. Morfometric traits of rainbow trouts at the end of the feeding time and at the end of starvation (n=18).

		Diet				
		FM		VP		SE
		End of feeding	End of starvation	End of feeding	End of starvation	
Weight	g	850.69	845.46	835.02	802.38	43.19
CF		1.19*	1.08*	1.24*	1.13*	0.036
HSI		1.10^{*A}	0.60^{*A}	0.92^{*B}	0.53^{*B}	0.040
DT	% BW	3.30*	1.93*	2.81*	2.15*	0.158
MF	% BW	4.07	3.94	4.24	4.07	0.434
Carcass yield	% BW	90.41*	92.70*	90.67*	92.14*	0.497
Fillet yield$	% BW	60.79^{B}	61.90^{B}	62.66^{A}	60.63^{C}	0.678

**within a diet P<0.05. $^{A, B,C}$ between diets P<0.05. $^{\$}$ interaction diet x sampling time P<0.05.*

As shown in table 3, at the end of the feeding period, the fillets obtained from fish given diets differing in protein source resulted similar in total lipid content and fatty acid composition (P>0.05) with the only exception of the n-6 PUFA fraction which level was almost doubled in muscle of fish fed diet VP (P<0.05). Starvation resulted in a similar slight decrease in the overall fat level in fillets irrespective of the past diet of trout. A similar pattern towards reduced levels was observed with all fatty acid categories (P<0.05) except DHA, of which the level tended to be preserved even after 30 days of fasting.

The results obtained in this study suggests that feeding diets high in vegetable protein ingredients for a long period had no major effects on growth performance, overall adiposity, dressing out yield in large trout. As in a previous study (de Francesco *et al*., 2004) long term feeding diets high in vegetable protein over a standard FM-based diet resulted in increased n-6 PUFAs content in muscle without affecting levels of n-3 PUFAs. Starvation has been confirmed to be a possible tool to improve the final product quality particularly in case of fish previously fed plant protein based diets being not detrimental in terms of changes in commercial yields while apparently ensuring a substantial decrease in n-6 fatty acids in the edible portion. However, more studies are needed to define the duration of the pre-slaugther fasting to optimise its potential beneficial effects on fillet yield and nutrient composition without adversely affect the animal welfare.

Table 3. Total lipid content and fatty acids composition of rainbow trout fillets at the end of the feeding time and at the end of starvation (n=6).

		Diets				
		FM		VP		SE
		End of feeding	End of starvation	End of feeding	End of starvation	
Total lipid	g/100g fillet	3.53	3. 21	4.02	3.38	0.319
Saturated	"	0.934*	0.710*	1.088*	0.672*	0.892
Monoun-saturated	"	0.978*	0.967*	1.224*	0.869*	0.211
n-3	"	0.796*	0.731*	0.883*	0.587*	0.153
n-6	"	0.217*^{A}	0.195*^{A}	0.456*^{B}	0.281*^{B}	0.063
EPA	"	0.130*	0.109*	0.139*	0.087*	0.031
DHA	"	0.471	0.458	0.503	0.362	0.100
n-3/n-6		3.661^{A}	3.749^{A}	1.945^{B}	2.915^{B}	0.173

**within a diet P<0.05. $^{A, B}$between diets P<0.05.*

In this trial the nutritional status or the protein source did not seem to affect the expression of liver IGF-I gene as reported by Cabillard *et al*. (2006) who didn't found a significant decrease of liver IGF-I mRNA in trout starved for 4 weeks. The role of the protein source on liver IGF-I gene expression in large rainbow trout remains unclear and needs deeper investigations.

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