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Note

Nitrogen and phosphorus utilisation in rainbow trout (*Oncorhynchus mykiss*) fed diets with or without fish meal

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Abstract –Dietary nitrogen and phosphorus utilisation were evaluated in rainbow trout, *Oncorhynchus mykiss*, fed fish meal and soy protein concentrate-based diets. Two short term trials were conducted with rainbow trout having initial mean weights of 24 and 156 g maintained at a water temperature of 17 ± 1 °C, during which triplicate groups of trout were fed the respective diets two times a day to apparent satiety by hand. In both trials, fish fed fish meal-based diet exhibited significantly higher growth. In small trout, feed/gain ratio and protein utilisation were slightly higher in groups fed the fish meal-based diet; however, in larger fish, there was no significant difference between treatments. Nitrogen retention and loss were not affected by dietary treatments, while phosphorus retention was higher in fish fed diet containing fish meal (30 and 17 %) than in fish fed diet containing soy protein concentrate (25 and 15 %) and greatly reduced in larger fish. Phosphorus losses were 10 and 13 g·kg gain^{-1} of fish fed the fish meal-based diet, while those for fish fed the diet without fish meal were 14 and 17 g. © Ifremer/Elsevier, Paris

Fish meal / soy protein concentrate / growth / losses of nitrogen and phosphorus / trout

1. INTRODUCTION

In current salmonid diets, fish meal supplies a large percentage of nitrogen and phosphorus to meet the requirements of fish and is also one of the most expensive single ingredients. Instability in world fish meal production, however, has driven many researchers to look for less expensive plant feedstuffs as alternative protein sources over the last two decades [4, 5, 13]. Although soybean meal is considered to be a suitable substitute for fish meal [9], growth of trout fed soybean-based diets was hitherto not promising. Recently, Kaushik et al. [7] reported that partial or total replacement (33 to 100 %) of fish meal with soy protein concentrate did not affect growth or feed utilisation in rainbow trout (80 g initial body weight). Sustainable fish farming relies on the reduction of pollution loads with an optimal production. In the present trials, we examined nitrogen and phosphorus losses through whole body retention of small (24 g body weight) and large (156 g body weight) trout fed fish meal-based and soy protein concentrate-based diets for 4 weeks.

2. MATERIALS AND METHODS

2.1. Diet preparation

Two isonitrogenous and isoenergetic diets containing either 32 % soy protein concentrate or 30 % Norwegian herring meal as a major protein source were formulated (*table I*). Reagent grade dicalcium phosphate was incorporated to the former diet at a level of 4 % to meet the phosphorus requirement [11]. Diets were dry pelleted into 2.5 and 4.0 mm diameter for small and bigger fish, respectively, using an experimental compression pellet machine. Ingredient composition of the diets is reported in *table I*. The essential amino acid composition of the diets based on our own

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Table I. Ingredient and chemical composition of the experimental diets[1].

Ingredient (as-fed basis, %)	Diet	
	SPC	FM
Norwegian herring meal	-	30.0
Soy protein concentrate[2]	32.0	-
Corn gluten meal	10.0	10.0
Fish oil	12.0	10.0
Extruded peas	25.0	33.0
Soybean meal	15.0	15.0
Vitamin premix[3]	1.0	1.0
Mineral premix[3]	1.0	1.0
$CaHPO_4\ 2H_2O$	4.0	-
Chemical composition (g or kJ/100 g DM):		
Experiment 1		
Crude protein	40.5	41.5
Crude lipid	13.6	13.9
Crude ash	8.6	8.3
Ca	2.9	3.0
P	1.6	1.3
Gross energy	2 109.0	2 141.0
Experiment 2		
Crude protein	41.3	41.7
Crude lipid	13.8	14.0
Crude ash	8.6	8.2
Ca	2.7	2.6
P	1.6	1.3
Gross energy	2 117.0	2 164.0
Essential amino acid composition (%) of the diets[4]		
Arginine	2.1	3.0 (1.5)
Histidine	0.8	2.3 (0.7)
Isoleucine	1.6	1.8 (0.9)
Leucine	3.0	3.4 (1.4)
Lysine	1.8	2.6 (1.8)
Methionine + Cystine	1.0	1.5 (1.0)
Phenylanine + Tyrosine	3.0	2.7 (1.8)
Threonine	1.2	1.4 (0.8)
Tryptophan	0.5	0.4 (0.2)
Valine	1.8	2.8 (1.2)

[1] SPC, soy protein concentrate-based diet; FM, fish meal-based diet.
[2] Provided by SOGIP, France.
[3] From Kaushik et al. [7].
[4] Data on essential amino acid requirements of rainbow trout [10] are presented within parentheses.

data along with data on the requirements for rainbow trout [10] are also reported in *table I*.

2.2. Feeding trial

Two growth trials were conducted in the Inra experimental fish farm at Donzacq (Landes, France) at a constant water temperature of 17 ± 1 °C for a period of 4 weeks each. Two size groups of rainbow trout (*Oncorhynchus mykiss*) having a mean initial body weight of either 24 g (Exp. 1) or 156 g (Exp. 2) were allotted in groups of 50 fish each into 6 circular (fiberglass reinforced plastics) and 6 rectangular (concrete) tanks, respectively. Triplicate groups were fed by hand twice a day to apparent satiety. Daily growth coefficients [6] were calculated as $100 \times [(Wf^{1/3} - Wi^{1/3})/\text{duration}]$. At the beginning and after the final weighing, 20 and 10 fish each (Exp. 1) and 10 and 5 fish each (Exp. 2) were withdrawn from each group for analyses. Nitrogen and phosphorus losses were calculated based on intakes and whole body nutrient gains.

2.3. Analytical methods

Chemical composition of the experimental diets and carcass was determined by the following procedures [1]: dry matter by drying in an oven at 110 °C for 24 h; crude protein (N × 6.25) by the Kjeldahl method after acid digestion; crude fat after ether extraction by the Soxhlet method; crude ash by incineration in a muffle furnace at 550 °C for 24 h, calcium by a wet ash method and titration with $KMnO_4$ and phosphorus by the vanado-molybdate method. Gross energy was determined using an adiabatic bomb calorimeter. Statistical analyses were performed by using analysis of variance and a multiple range test [3] at the 5 % probability level.

3. RESULTS AND DISCUSSION

In both feeding trials, fish provided with the fish meal-based diet showed greater voluntary feed intake than those fed the soy protein concentrate-based fish meal-free diet, which resulted in more weight gain ($P < 0.05$). In small trout, feed:gain and protein efficiency ratios were significantly improved in fish fed the former diet, while these values were not different ($P > 0.05$) between groups in larger sized trout (*table II*). Nitrogen intake per fish for 4 weeks ranged from 2.06 to 2.37 g in small fish and from 6.49 to 6.86 in larger ones. Chemical composition was similar between the two groups of each trial, although moisture content was significantly reduced with increase in lipid level compared to initial fish. Small trout fed diet containing soy protein concentrate gained 0.72 g nitrogen, which was lower than fish fed the other diet (0.82 g). This trend was also found in larger fish for which 2.11 and 1.97 g nitrogen were retained in fish fed soy protein concentrate- and fish meal-based diets, respectively. However, nitrogen retention efficiency was not different between the two groups ($P > 0.05$) in both trials. Nitrogen losses per kg weight gain varied little and were 49.4 to 51.4 g in small trout and 57.6 to 58.9 g in larger fish (*table III*).

There was significant difference in phosphorus intake per fish for 4 weeks between the two treatments, which ranged from 0.46 to 0.50 g and from 1.30 to 1.55 g in trials 1 and 2, respectively. However, the gain per fish remained constant at 0.13 g in small and 0.23 g in larger groups. On the other hand, phosphorus loss per kg weight gain was significantly higher in fish fed diet with soy protein concentrate (14.4 and 17.2 g) than in fish fed diet with fish meal (10.1 and 13.0 g) in two trials (*table IV*).

Table II. Growth and feed utilisation of rainbow trout fed two diets for 4 weeks[1].

Items	Exp. 1		Exp. 2	
	SPC	FM	SPC	FM
Wt gain (g/fish)	26.2 ± 0.86[b]	31.5 ± 0.45[a]	76.9 ± 1.83[b]	82.6 ± 0.61[a]
Feed intake (g DM/fish)	31.9 ± 0.67[b]	35.5 ± 0.37[a]	98.5 ± 0.70[b]	102.9 ± 0.47[a]
Feed:gain ratio	1.22 ± 0.02[a]	1.13 ± 0.00[b]	1.29 ± 0.03	1.25 ± 0.01[ns]
PER[2]	2.03 ± 0.03[b]	2.13 ± 0.01[a]	1.90 ± 0.04	1.93 ± 0.01[ns]
DGC[3]	3.00 ± 0.07[b]	3.44 ± 0.01[a]	2.84 ± 0.06[b]	3.03 ± 0.02[a]

[1] Initial weight of fish was 24 and 156 g for Exp. 1 and Exp. 2, respectively; values (means ± SE of triplicate groups) in the same row not sharing a common superscript letter are significantly different ($P < 0.05$); ns = non significant.
[2] Protein efficiency ratio = wet wt. gain/protein intake.
[3] Daily growth coefficient, % = $100 \times [(Wf^{1/3} - Wi^{1/3})/\text{duration}]$ [6].

Table III. Nitrogen utilisation of rainbow trout fed two diets for 4 weeks[1].

Items	Exp. 1		Exp. 2	
	SPC	FM	SPC	FM
Intake (g/fish)	2.06 ± 0.04[b]	2.37 ± 0.02[a]	6.49 ± 0.05[b]	6.86 ± 0.03[a]
Gain (g/fish)	0.72 ± 0.02[b]	0.82 ± 0.01[a]	1.97 ± 0.04[b]	2.11 ± 0.01[a]
Retention efficiency (%)	34.7 ± 0.42	34.4 ± 0.13[ns]	30.4 ± 0.57	30.6 ± 0.17[ns]
Losses:				
g/kg weight gain	51.4 ± 1.01	49.4 ± 0.29[ns]	58.9 ± 1.72	57.6 ± 0.54[ns]
g/kg feed intake	38.0 ± 0.81	37.9 ± 0.23[ns]	40.4 ± 1.01	42.2 ± 0.28[ns]

[1] Values (means × SE of triplicate groups) in the same row not sharing a common superscript letter are significantly different ($P < 0.05$); ns = non significant.

Table IV. Phosphorus utilisation of rainbow trout fed two diets for 4 weeks[1].

Items	Exp. 1		Exp. 2	
	SPC	FM	SPC	FM
Intake (g/fish)	0.50 ± 0.01[a]	0.46 ± 0.00b	1.55 ± 0.01[a]	1.30 ± 0.01[b]
Gain (g/fish)	0.13 ± 0.01	0.14 ± 0.00[ns]	0.23 ± 0.01	0.23 ± 0.00[ns]
Retention efficiency (%)	25.0 ± 0.33[b]	30.5 ± 0.14[a]	14.8 ± 0.54[b]	17.3 ± 0.23[a]
Losses:				
g/kg weight gain	14.4 ± 0.25[a]	10.1 ± 0.06[b]	17.2 ± 0.47[a]	13.0 ± 0.12[b]
g/kg feed intake	10.7 ± 0.11[a]	7.8 ± 0.04[b]	11.8 ± 0.24[a]	9.50 ± 0.08[b]

[1] Values (means × SE of triplicate groups) in the same row not sharing a common superscript letter are significantly different ($P < 0.05$); ns = non significant.

Irrespective of size, rainbow trout fed the diet with fish meal showed more feed intake and weight gain than the other group. Considering the earlier data with trout fed soy protein concentrate-based diet supplemented with 0.4 % methionine [7], decrease in weight gain of fish fed the diet with same protein source in the present experiment might be due to a possible marginal deficiency of sulphur amino acids in this diet (see *table I*). The lack of significant differences in feed:gain and protein efficiency ratios between the two groups in larger fish nevertheless suggests easier adaptation of the fish to the plant protein diet, even though the trial lasted only 4 weeks. Nitrogen gain in fish groups fed diet with fish meal was superior to that of the other groups owing to increase in its intake. However, the retention efficiency was not different ($P > 0.05$) between the two size groups. The results show the difference in nitrogen retention (34 vs. 30 %) between two different growing stages. Nitrogen loss, which did not vary much between the two groups in either size group, was somewhat higher in larger fish owing to an increase in its intake than in small ones.

While phosphorus gain was constant, its intake was significantly higher in fish fed diet with soy protein to which 4 % dicalcium phosphate was added. This resulted in a higher phosphorus loss in both trials. Generally, the availability of phytic phosphorus found in plant protein sources such as soybean meal is very low in fish [10]. Our data confirm that when fish are fed diets containing relatively high levels of plant sources, phosphorus requirement could be met by addition of an inorganic P source. While dietary phosphorus is essential for growth and metabolic purposes of fish [8], excessive supply might lead to environmental degradation, since it is a growth limiting factor for algal growth, and consequently eutrophication [2]. Because the two experimental diets in this experiment were adjusted to meet the P requirement [11], it seems that the higher P loss from the groups fed the soy protein containing diet would be due to an excess in total available P supplied mainly in an inorganic form. On the other hand, the fact that phosphorus retention of larger fish was two-fold lower than that of small fish in the two diet groups suggests that absolute P requirement of fish could be reduced with growing stage. Recently, Rodehutscord [12] reported that P requirement for growth and P gain in whole body of trout was 0.37 and 0.56 % of diet, respectively. The present results showed that a diet in which soy protein concentrate was the major protein source was not as efficient as that with fish meal in terms of growth of especially small fish, though dietary nitrogen retention was the same in each size of fish group regardless of protein sources. Further studies over longer periods taking into account possible beneficial effects of sulphur amino acid supplementation are warranted to develop practical diets devoid of fish meal.

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