NUTRITIVE VALUE OF RAPESEED PROTEIN CONCENTRATE FOR RAINBOW TROUT (Oncorhynchus mykiss)

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INTRODUCTION

Owing to the cyclical nature of fish meal prices, variability in the quality of fish meal protein, extensive use of fish meal protein to furnish most of the high protein needs of cultured salmonids and the present high cost of food (40 to 50% of fish farm operating costs), it is essential to identify and develop suitable economical alternative protein sources. Previous research on rainbow trout (Yurkowski et al. 1978) and juvenile chinook salmon (Higgs et al. 1982) has shown that rapeseed protein concentrate (RPC) may comprise > 24% of the dietary protein by partial replacement of fish meal. However, neither of the foregoing studies established how much RPC could be substituted for fish meal without compromising performance. Moreover, no attempt was made in these studies to assess whether the nutritive value of RPC could be improved by dietary zinc supplementation or by removal of its phytic acid content (5.3 - 7.5%). Phytic acid, the hexaphosphate of myoinositol, is strongly negatively charged at all pH values normally encountered in food. Indeed, this compound has strong affinity for proteins at low pH and for cations such as zinc at intestinal pH. Consequently, high dietary levels of phytic acid may depress growth, feed efficiency, protein availability and zinc bioavailability in salmonids (Higgs et al. 1988). Further, replacement of fish meal by plant protein may be accompanied by reduction in food intake. Hence, Yurkowski et al. (1978) noted significant depression of appetite in trout when fish meal was replaced totally by RPC.

This study was undertaken to (1) assess whether the nutritive value of RPC for rainbow trout could be improved by dietary zinc supplementation and, (2) evaluate the merits of three sources of RPC (undephytinized, untreated control; undephytinized, solvent treated control; dephytinized) as partial or total replacements of steam-dried whole herring meal in a dry basal diet for rainbow trout. The appetite enhancer, Finnstim, was included in all diets at 1.5% in the second experiment in an attempt to overcome depression of food intake as the dietary fish meal content was replaced progressively by each of the RPC sources.

MATERIALS AND METHODS

Experiment 1

Seven dry diets (basal diet based upon the Abernathy 19-2 formulation) of equivalent protein and lipid content (48.2 and 13.6% of dry matter, respectively) were prepared. Undephytinized Bronowski RPC replaced either 0, 30 or 60% of the protein provided by steam dried whole herring meal (HM) and supplemental zinc was either 75, 150, or 225 (60% RPC level only) ppm (Table 1). All diets were cold pelleted (California pellet mill) and subsequently they were crumbled and sieved to suit fish size and stored at -20°C.

TABLE 1. Diet treatments given to rainbow trout in experiment 1.

Diet	Major protein sources[1] (g/kg as fed)		Other[2]	Supplemental Zn (ppm)
	HM	RPC		
1	480.0	0	520.0	75
2	480.0	0	520.0	150
3	278.0	260.0	462.0	75
4	278.0	260.0	462.0	150
5	96.0	525.0	379.0	75
6	96.0	525.0	379.0	150
7	96.0	525.0	379.0	225

[1] HM = steam-dried whole herring meal; RPC = Bronowski rapeseed protein concentrate.

[2] The common dietary components (g/kg) were as follows: blood meal, 80; choline chloride (70%), 5; mineral premix, 1.0; vitamin supplement, 15; permapell, 19; and dried whey, 100. The levels of α-cellulose, wheat middlings and herring oil, were respectively, 71, 139 and 90 (diets 1 and 2), 18, 139 and 85 (diets 3 and 4) and 20, 57 and 82 (diets 5, 6 and 7).

The seven diet treatments were each assigned randomly to duplicate groups of 50 trout. Each group was held in a 150 L isopropylene tank supplied with running (4 L/min), filtered fresh water from Lake Washington. Water temperatures ranged from 11 - 15°C and a natural photoperiod was provided during the study. The daily ration was initially 5% of body weight. But this was reduced to 3% of body weight as the fish grew and water temperature declined. Rations were adjusted following lot weighing of the groups on day 0, 14, 23 and 38 of the 53-day study. The fish in each group were weighed individually on day 53.

Data for body weight gain and feed efficiency (body weight gain (g) ÷ food intake (g)) were analyzed by one-way ANOVA and the SAS REGWF multiple range test.

Experiment 2

Ten dry diets (basal diet based upon University of Guelph trout formulations) of equivalent protein, lipid and estimated metabolizable energy content (43%, 18% and 3.9 kcal/g on a dry weight basis, respectively) were prepared. Each of the three RPC sources

comprised about 19.2, 38.9 and 58.9% of dietary protein by replacement of 33.3, 66.6 and 100% of HM protein (Table 2).

Table 2. Diet treatments given to rainbow trout in experiment 2.

Diet	Major Protein Sources[1]				
	HM	RPC			Other[2]
		Undephy. Control	Dephy. (g/kg dry diet)	Undephy. Sol. Control	
1	320	-	-	-	680
2	213.3	121.1	-	-	665.6
3	106.7	242.2	-	-	651.1
4	-	363.3	-	-	636.7
5	213.3	-	130.1	-	656.6
6	106.7	-	260.2	-	633.1
7	-	-	390.3	-	609.7
8	213.3	-	-	126.5	660.2
9	106.7	-	-	253.0	640.3
10	-	-	-	379.5	620.5

(1) HM = steam-dried whole herring meal; RPC = Bronowski rapeseed protein concentrate; undephy. control = undephytinized control; dephy. = dephytinized; undephy. sol. control = undephytinized solvent control.

(2) common dietary components (g/kg dry diet) were as follows: poultry-by-product meal, 70; blood meal, 40; corn gluten meal, 65; dried whey, 60; vitamin/mineral supplement, 50; choline chloride (60%), 5; ascorbic acid, 2; permapell, 9.92; Finnstim, 15; dextrin, 15. The variable dietary constituents were: wheat middlings, herring oil; DL-methionine, L-lysine, ethoxyquin, $CaHPO_4$, and $CaCO_3$.

Mean dietary concentrations (g/kg dry matter) of Ca, Cu, Fe, K, Mn, Na, P and Zn were respectively, 17.4, 0.019, 0.364, 7.33, 0.100, 5.64, 15.6 and 0.237. Dietary Mg levels ranged from 2.57 - 2.78 (diets 1, 2, 5 and 8), 3.27 - 3.37 (diets 3, 6 and 9), and 4.15 - 4.33 (diets 4, 7 and 10). All diets had $\geq$ 0.7% inorganic P and $\geq$ 0.06% Mg from animal and inorganic sources. Dietary glucosinolate levels ranged from 156 - 467 μmoles/kg dry diet (diets 2-4), 65 - 196 (diets 5-7) and 48 - 144 (diets 8-10).

Each of the 10 test diets was fed to duplicate groups of 74 - 77 trout (initial mean weight 4.2 - 4.4 g) held in 10.0 - 10.3°C well water on a natural photoperiod for 84 days. Water flow rate into each 800-L fiberglass tank averaged 7.5 l/min. Dissolved oxygen content varied between 9.9 and 10.6 mg/l. All groups were fed their prescribed diet by hand to satiation three times daily. Records of daily food intake and mortality were maintained.

Random samples of 60 fish were removed from each tank and they were individually weighed at 21-day intervals. Four samples of 9 fish each were analyzed for initial whole body proximate composition. Ten fish were removed randomly from each replicate on day 84 for determination of final whole body proximate and mineral composition (five pools of 2 fish each per replicate). Three fish were sampled from each replicate on day

85 for histopathological examination of the liver, kidney, thyroid and alimentary tract. Specific growth rates (GR; % wet wt/day) were derived from the covariate slopes as follows: SGR = (e^{slope} -1) x 100. Food intake was calculated for each 21-day interval by dividing the mean daily dry food intake per fish x 100 by the geometric mean wet weight of the fish. Feed efficiency (FE) was calculated as wet weight gain (g) ÷ dry food (g). Percent protein deposited (PPD) and gross energy conversion efficiency (GECE) were calculated respectively as protein or energy gain x 100 ÷ protein or gross energy intake. Data for appetite, FE, PPD, GECE and whole body Zn concentration (dry basis) were analyzed by one-way ANOVA with replicate nested in diet and replicate treated as random and Newman-Keuls test with P = 0.05, where appropriate. Data for GR were analyzed by a one factor Analysis of Covariance of the natural log of wet weights with a test for equality of slopes, and Scheffe's test with P = 0.05.

RESULTS

Experiment 1

Rainbow trout growth, appetite and FE were not compromised when 30% of the herring meal protein in diet 1 (basal diet) was replaced by protein from RPC. Additional, replacement of herring meal protein (60%) by RPC did, however, adversely affect growth and appetite. Because of the latter response, it was not possible to accurately calculate FE for groups fed diets 5, 6, and 7. Dietary zinc supplementation did not significantly influence trout performance regardless of the dietary RPC level (Table 3).

Table 3. Initial weights (IBW), body weight gains and feed efficiencies (FE; weight gain (g) ÷ food intake (g)) of rainbow trout in relation to diet treatment.

Diet	% Replace HM protein	Dietary RPC (g/kg)	Suppl. Zn (ppm)	IBW (g/fish)	Wt. gain (g/fish)	FE[(1)] (g/g)
1	0	0	75	2.39	4.43[a]	0.82[a]
2	0	0	150	2.39	4.88[a]	0.84[a]
3	30	260	75	2.38	4.17[a]	0.76[a]
4	30	260	150	2.44	4.08[a]	0.77[a]
5	60	525	75	2.66	0.98[b]	-
6	60	525	150	2.66	1.42[b]	-
7	60	525	225	2.04	1.42[b]	-

[(1)] FE was not calculated for fish fed diets 5 - 7 due to poor fish appetite (over feeding).

Experiment 2

Rainbow trout growth rates, appetite (data not shown), FE, PPD and GFCE, mortality (< 3% of initial number in each group) and health (no signs of pathology) were not compromised when either the undephytinized control or the dephytinized RPC sources were substituted for 66% of the HM protein in the basal diet (diet 1; Table 4).

Table 4. Influence of diet treatment on rainbow trout growth rate (GR), feed efficiency (FE), percent protein deposited (PPD) and gross energy conversion efficiency (GECE) during the study. Refer to Material and Methods section for additional information.

Diet	% Replace[1] HMPRT	RPC[1] Source	GR[2] (%/d)	FE[3] (g/g)	PPD[3] (%)	GECE[3] (%)
1	0	-	$2.39^{d,e,f}$	$0.61^{d,e}$	19.7^{d}	21.8^{c}
2	33.3	undeph. contrl.	2.51^{f}	0.69^{f}	23.1^{f}	27.5^{d}
3	66.6	"	$2.15^{c,d}$	$0.56^{c,d}$	19.1^{d}	22.3^{c}
4	100	"	$1.83^{a,b}$	0.41^{b}	13.4^{b}	15.8^{b}
5	33.3	dephy.	$2.28^{d,e,f}$	$0.60^{d,e}$	$20.6^{d,e}$	21.3^{c}
6	66.6	"	$2.19^{c,d,e}$	$0.55^{c,d}$	19.2^{d}	21.9^{c}
7	100	"	$1.94^{b,c}$	0.43^{b}	14.2^{b}	16.2^{b}
8	33.3	undephy. sol. contrl.	$2.44^{e,f}$	$0.66^{e,f}$	$22.3^{e,f}$	25.4^{d}
9	66.6	"	$2.13^{c,d}$	0.50^{c}	16.5^{c}	20.1^{c}
10	100	"	1.63^{a}	0.33^{a}	11.0^{a}	12.5^{a}

[1] % replacement of herring meal protein; RPC = rapeseed protein concentrate.
[2] Analysis of Covariance indicated $P < 0.001$.
[3] One-way ANOVA indicated $P < 0.001$ (FE, PPD and GECE).

Total replacement of HM protein with each of the RPC sources did not depress trout appetite relative to fish fed diet 1. Indeed, fish appetite was positively correlated with the dietary level of each RPC source. By contrast, GR, FE, PPD and GECE were reduced significantly (Table 4).

The procedure used to dephytinize RPC significantly reduced the quality of the RPC protein. For instance, groups ingesting the diets in which undephytinized solvent-treated RPC replaced 66.6% or all of the HM protein generally exhibited poorer performance than corresponding controls receiving diets with undephytinized RPC (Table 4). The benefits of phytate removal on the nutritive value of RPC were consequently less than anticipated (compare performance of groups fed diets 4, 7 and 10).

Dietary treatment had little influence on final whole body proximate compositions and terminal whole body mineral levels (i.e., Ca, P, Mg, Cu, Fe, Na, Mn and K) except zinc. In this regard, zinc was noted to be significantly depressed in fish fed diet 10 (data not shown).

Thyroid follicle epithelial cell heights (TFEH) in trout on day 84 were directly related to the dietary level of each RPC source. Significant elevation of TFEH was observed only in groups fed diets 4 and 10 (data not shown).

DISCUSSION

Collectively, our findings suggest that undephytinized and dephytinized RPC may comprise about 38% of the dietary protein (fish meal only 11% of diet in exp. 2) for rainbow trout without adversely affecting their performance. The foregoing estimate for

the acceptable level of RPC in trout diets is markedly higher than those obtained for canola and rapeseed meal ($\leq$ 21% of dietary protein; Higgs et al. 1990). This is probably because RPC contains lower levels of glucosinolates (antithyroid compounds), fiber, nitrogen-free extract (carbohydrate) and phenolic compounds than in canola meal (Jones, 1979; Higgs et al. 1990). Also, RPC has more digestible (available) protein and energy for rainbow trout than in canola meal (Forster and Hardy, 1990, unpubl. data).

In contrast to the situation in mammals (Jones, 1979), dietary zinc supplementation above 75 ppm (basal diet had 204 ppm zinc in experiment 1) did not reinstate normal growth and food intake in trout fed diets with low or high levels of RPC (phytic acid). Further, phytate removal from RPC generally did not enhance trout performance contrary to what would be expected from the findings of other studies on salmonids (reviewed by Higgs et al. 1988). This was in part due to the dephytinization procedure employed in this study. Also, care was exercised in experiment 2 to ensure that all diets had not only equivalent, but also adequate levels of minerals (especially phosphorus, magnesium and zinc) to satisfy the known dietary mineral needs of trout.

Trout in experiment 1 had poor appetite when the dietary RPC level was high. By contrast, fish in experiment 2 did not show any depression of appetite even when all of the fish meal protein was replaced by RPC. It is probable, therefore, that Finnstim was efficacious in maintaining trout appetite regardless of diet treatment.

Complete replacement of HM protein in rainbow trout diets by RPC may be possible if, 1) the methodology for phytate removal is improved and 2) the available levels and balance of dietary protein and energy and of the essential amino acids are optimized.

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