Balancing dietary lipid and cholesterol to increase fillet omega-3 deposition in rainbow trout fed a soy-based diet

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Background

Omega-3 fatty acids, particularly EPA (20:5n-3) and DHA (22:6n-3) have been identified as beneficial nutrients in human nutrition leading to improved heart and brain health (Calder, 2014). For this reason, farmed seafood must meet consumer needs and expectations. One-for-one substitutions of fish oil with plant oils currently is not sufficient to ensure healthful levels of EPA and DHA in farmed fish products.

Cholesterol appears to play a significant role in regulating FA metabolism by stimulating fatty acid β-oxidation and the conversion of ALA to DHA (Norambuena *et al.,* 2013). Even so, cholesterol use in optimizing fish feed formulations for maximizing n-3 LCPUFA biosynthesis has been limited and not previously reported for fish fed an all-plant based diet replacing fish oil with soy oil. This is important since cholesterol' metabolism may be affected by phytosterols in plant oils, and phytosterols are known to inhibit intestinal cholesterol absorption (Ostlund, 2004). While linseed oil is the richest source of ALA among common plant oils, soy oil has less than half the phytosterol content (300 mg/100 g; Verleyen *et al*., 2002) of linseed oil (700 mg/ 100 g; Schwartz *et al*., 2008).

The objective of the proposed research is to optimize dietary soy oil utilization by providing dietary sources of ALA and cholesterol to rainbow trout fed a fishmeal/fish oil free diet (low in LCPUFA's) and demonstrate increased conversion of ALA to DHA in the edible fillet.

Materials and Methods

- 12 diets (Table 1)
  - Fish meal / fish oil (FM/FO) and plant meal / fish oil (PM/FO) controls
  - Graded ratios of soybean oil (SO) / linseed oil LO), with or without added cholesterol (+C)
- Initial weight : 18.8 ± 0.3 g (mean ± SD)
  Donaldson X USDA-UI selected strain (Female)
- 25 fish / 145-L tank, triplicates
- Flow-through system, 15℃ spring water
- Apparent satiation (2 times / day) for 12 weeks
- RT qPCR will be used to determine the expression of the genes that are involved in fatty acids oxidation, desaturation, and elongation
- Dietary cholesterol supplementation was at a level of 1.43 mg/g of diet to mimic the amount of cholesterol in a fish oil-based diet.
- Data was subjected to one-way or multi-factorial ANOVA test. Treatment effects were considered significant at $P < .05$.

Results

- The survival rate (93.3% to 100%) and feed conversion ratio (1.02 to 1.09; Fig. 1) were similar among dietary treatments groups (P> .05).
- Compared with the fish fed other diets, the weight gain of fish fed diet FM/FO, PM/FO or L100+C was greatest (P< .05; Fig. 1).
- Different ratios of SO/LO oil did not affect trout weight gain; however, the addition of cholesterol to the LO100 diet (LO100+C) significantly increased fish weight gain (Fig. 1).
- The interaction between the two main factors (Cholesterol and SO/LO ratio) significantly impacted feed intake (Table 2, Fig. 2; P< .05); but had no significant effects on growth performance or feed utilization (P> .05).
- Preliminary FA analysis suggests that cholesterol plays a role in fatty acid bioconversion of 18:3n-3 to 20:5n:3, and 20:5n:3 to 22:6n:3 in LO100+C fed fish, compared to the LO100 (Table 3).

Table 1. Formulation and proximate composition of the experimental diets.

Ingredients (%)	Diets											
	FM/FO	PM/FO	SO100	SO100 +C	SO75 LO25	SO75 LO25 +C	SO50 LO50	SO50 LO50 +C	SO25 LO75	SO25 LO75 +C	LO100	LO100 +C
Fishmeal, sardine	30.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Poultry by-product meal	8.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blood meal, spray dried	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Corn protein concentrate	14.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	0.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soy protein concentrate	5.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Wheat gluten meal	5.97	11.60	11.60	11.60	11.60	11.60	11.60	11.60	11.60	11.60	11.60	11.60
Wheat flour	17.90	6.60	6.60	6.46	6.60	6.46	6.60	6.46	6.60	6.46	6.60	6.46
L-Lysine HCL	0.00	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
DL-methionine	0.00	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Threonine	0.00	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix 702	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Stay-C (vitamin C, 35%)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Trace mineral premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Dicalcium phosphate	0.00	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43
Fish oil	15.20	19.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Soybean oil	0.00	0.00	19.00	19.00	14.30	14.30	9.51	9.51	4.76	4.76	0.00	0.00
Linseed oil	0.00	0.00	0.00	0.00	4.76	4.76	9.51	9.51	14.30	14.30	19.00	19.00
Cholesterol	0.00	0.00	0.00	0.14	0.00	0.14	0.00	0.14	0.00	0.14	0.00	0.14
Nutrients (% as-fed basis)												
Dry matter (%)	92.2	92.5	92.7	92.9	92.1	92.5	92.7	91.8	92.9	91.7	94.3	92.1
Crude protein (%)	47.5	48.1	48.2	48.8	47.9	48.0	47.9	47.1	47.8	47.4	48.1	47.4
Crude fat (%)	21.5	21.3	21.2	21.2	21.4	21.5	21.3	21.3	21.3	21.3	21.3	21.6
Ash (%)	6.79	6.15	5.99	5.86	5.82	6.10	5.93	5.97	5.87	5.75	6.03	5.82
Gross energy (MJ/kg)	22.5	22.5	22.4	22.5	22.4	22.5	22.8	22.3	22.5	22.5	22.7	22.2
Cholesterol (mg/kg)	2040	1430	55	1290	89	1230	93	1220	106	1270	92	1150

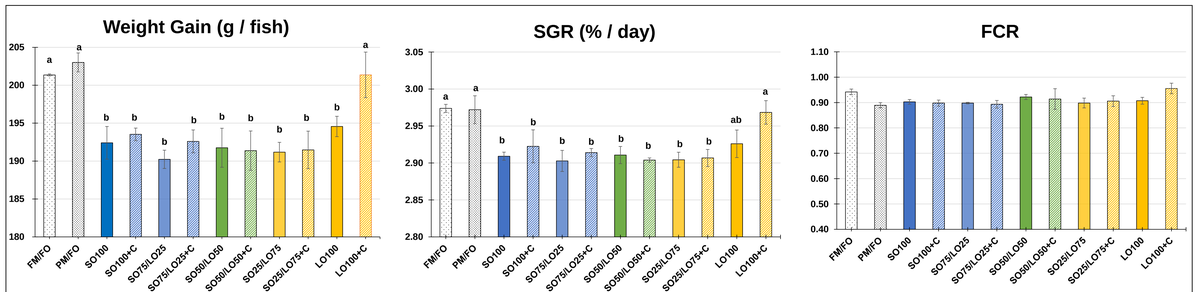

Figure 1. Weight gain (g/fish), SGR (%/day) and FCR of juvenile rainbow trout fed experimental diets for 12 weeks. Bars represent mean ± SE of triplicate samples.

Table 2. Growth performance and feed utilization of rainbow trout juveniles fed experimental diets for 12 weeks.

Diets	Initial weight (g / fish)	FBW (g / fish)	WG (g / fish)	SGR (% / day)	Survival (%)	FI (g DM / fish)	FCR
Means of main effects							
Cholesterol (mg/kg)							
0	18.8	211^{b}	192^{b}	2.91^{b}	98.4	174	0.91
1430	18.8	213^{a}	194^{a}	2.92^{a}	96.3	177	0.91
Soy oil / Linseed oil (%)							
100 / 0	18.8	211^{b}	193^{b}	2.91^{b}	96.0	174^{b}	0.90
75 /15	18.8	210^{b}	191^{b}	2.91^{b}	99.3	171^{b}	0.90
50 /50	18.8	210^{b}	192^{b}	2.91^{b}	97.3	176^{b}	0.92
25 / 75	18.6	210^{b}	191^{b}	2.91^{b}	96.7	173^{b}	0.90
0 / 100	18.8	217^{a}	198^{a}	2.95^{a}	97.3	185^{a}	0.93
Multi factors ANOVA (P Value)							
Cholesterol	0.794	0.046	0.033	0.024	0.111	0.573	0.367
Soy oil / Linseed oil	0.976	<.001	<.001	<.001	0.563	<.001	0.064
Cholesterol x Soy oil / Linseed oil	0.976	0.178	0.121	0.090	0.464	0.042	0.227

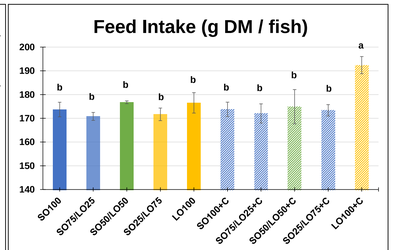

Figure 2. Feed intake (g DM/fish) of rainbow trout juveniles fed experimental diets for 12 weeks. Bars represent mean ± SE of triplicate samples. Two-way ANOVA indicated a significant Cholesterol X Soy/Linseed oil interaction ($P < .05$)

Table 3. Preliminary analysis of fatty acid compositions (% total fatty acids) in fillet of rainbow trout juvenile fed the experimental diets for 12 weeks. (n=1, 2)

Fatty acids (%)	Diets											
	FM/FO	PM/FO	SO100	SO100 +C	SO75 LO25	SO75 LO25 +C	SO50 LO50	SO50 LO50 +C	SO25 LO75	SO25 LO75 +C	LO100	LO100 +C
18:1n:9 (Oleic acid)	14.6	13.6	16.7	16.5	16.5	17.0	14.6	15.5	16.1	14.4	16.2	14.5
C18:2n:6 (Linoleic acid, LA)	4.66	4.65	29.5	33.6	27.4	29.9	21.2	21.6	17.5	16.4	12.0	10.8
C18:3n:3 (α-Linolenic acid, ALA)	0.64	0.74	3.19	3.71	8.33	9.09	13.1	13.1	19.4	18.3	24.9	22.5
C20:5n:3 (EPA)	5.34	5.11	0.68	0.76	1.21	1.15	1.70	1.74	1.90	2.10	2.23	2.79
C22:6n:3 (DHA)	12.8	13.3	3.90	3.54	4.78	4.12	5.75	5.41	5.86	6.18	5.08	6.72

Conclusions

- Both oil source and cholesterol affected final body weight, weight gain, and specific growth rate.
- Fish fed LO100+C diet showed significantly higher weight gain compared with the fish fed LO100, possibly because phytosterol inhibits intestinal cholesterol recycling and creates a conditional dietary requirement for cholesterol.
- Although, fish can synthesize cholesterol, *de novo* cholesterol biosynthesis and recycling is not enough for optimal growth relative to fish fed diets containing FO.
- Preliminary fatty acid analysis suggests that cholesterol improves fatty acid bioconversion (desaturation, elongation and peroxisomal chain shortening) when fish are provided with enough ALA, *via* linseed oil and not soy oil, in the diet.
- Cholesterol supplementation improves fatty acid bioconversion in edible fillets when fish are fed diets containing linseed oil and no animal feedstuffs.

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