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Poultry By-Product Meals as Partial Fish Meal Replacement Yielded Higher Somatic Growth in Alsatian Charr (*Salvelinus alpinus X fontinalis*) than Pork or Vegetable-Based Fish Meals

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Abstract

Freshwater salmonids gain increasing popularity as diet fish and are an important dietary source of omega-3 polyunsaturated fatty acids (PUFA) for humans. The aim of this study was to investigate effects of alternative feeds from domestic sources on fish growth and lipid composition of the sparctic Alsatian charr (*Salvelinus alpinus X fontinalis*), commonly used in freshwater aquaculture in Europe. In this fish feeding experiment, we used a control feed (32% fish meal; feed F1) and experimental diets containing generally only half the amount of fish meal, which was replaced in equal parts by poultry (F2), two different pork by-product meals (F3 and F4), plant-based feeds (F5), or by a feed containing 25% fish and 15% poultry meal (F6) that had a similar caloric value compared to the other feeds. Six hundred charrs of similar initial weight (ca. 90 g) were randomly distributed into 12 tanks (50/tank) of 1.4 m^3, supplied with subalpine spring water, and fed one of the feeds. Fish biomass development was the highest in fish fed diet F6, followed by F1, F2, and F5. Pork by-product meals as partial replacement of fish meal resulted on average in a 25% lower biomass gain compared to charr feeding on poultry by-product meal (F6). The use of poultry or pork by-product meals as partial fish meal replacements did not significantly change the total lipids or fatty acids retained in these fish. This study shows that these alternative feeds, with similar lipid sources, had no significant effect on the fatty acid composition in Alsatian charr, however, poultry by-product meal as partial replacement of marine fish meal clearly enhanced charr biomass by 15% relative to conventional fish meals.

Keywords

Alternative Protein Sources, Feed Formulation, Salmonids, Muscle Lipids, Fillet Composition

1. Introduction

The global production of freshwater fish, crustaceans, and molluscs increased two-fold within 10 years from 21.8 billion t (in 2001) to 41.7 billion t (in 2010) [1]. Among freshwater salmonids, farming of rainbow trout (*Oncorhynchus mykiss*) keeps also augmenting from 0.5 to 0.7 billion tons (2001-2010) [1]. Such rapidly increasing freshwater fish production requires equally rapid scientific development of sustainable and economical nutritious diets. It is crucial that such alternative diets contain all essential amino acids, fatty acids, vitamins, and minerals to satisfy the fish's physiological requirements because dietary lack thereof may compromise somatic fish growth and health and consequently may also affect healthy food supply for humans.

A promising approach to reduce the increasingly more expensive fish meal (FM) in pellet feeds for fishes and also crustaceans are to fully or at least partially replace FM with plant sources [2] [3] [4] or other animals sources, such as poultry by-products [5] [6]. Although recent research suggests that plant sources as full or partial FM replacement yield similar fish growth as conventional FM feeds [3] [7], there is experimental evidence that marine and freshwater salmonids fed diets with vegetable oils contain less omega-3 (n-3) long-chain polyunsaturated fatty acids (PUFA), in particular docosahexaenoic acids (DHA; 22:6n-3) [8] [9]. Contrary to amino acids, fish can store and thus accumulate dietary fatty acids. Moreover, in addition to proteins, lipids and their fatty acids support various physiological processes, including somatic growth [10] [11] [12], reproduction, behavior and vision, as well as immune response of fishes [13]. It is thus a scientific challenge to find dietary substitutions that result in similar growth and fatty acid retention in fishes and other consumers (e.g., crustaceans [5]) than conventional FM feeds.

The use of regional animal by-products for freshwater aquaculture is of economic and ecological importance, especially for countries without access to the sea. Feeding studies using animal by-products as partial replacement of FM are thus far mostly conducted on marine fish (but see, e.g., [6]). For example, poultry by-products as partial replacement (30%) of FM in salmon diets resulted in reduced growth of Chinook salmon (*Oncorhynchus tshawytscha*), which may have been due to reduced palatability [14]. Using pork in fish diets as partial replacement of marine FM, no significant growth difference in Coho salmon (*Oncorhynchus kisutch*) was reported [15], suggesting that such animal by-product meals may also be valuable for other salmonids. Thus far, it is not known how terrestrial animal by-products or vegetable feeds as partial replace-

ment of FM affect somatic growth and, from a human nutrition perspective, highly precious n-3 PUFA in freshwater salmonids.

We conducted a feeding study to examine the effect of partial replacement of dietary FM with poultry or pork by-products, or partial replacement with vegetable feeds on the growth rate and tissue fatty acid profiles in the consumer-sized sparctic charr *Salvelinus alpines X fontinalis*, commonly known as Alsatian charr, used in freshwater aquaculture particularly in Europe [16]. We tested the null-hypothesis that there is no difference in fish growth rates or tissue fatty acid profiles in charr feeding on the different diets over their entire growth cycle. This test underlies the assumption that these dietary substitutions in fish feeds can fully replace commonly used FM resulting in equal fish growth rates. Alternatively, if fish growth is enhanced by certain fish feeds it is expected that PUFA contents in muscle tissues decrease relative to more slowly growing charr (effect of growth dilution).

2. Materials and Methods

2.1. Fish, Husbandry, and Experimental Diets

The Alsatian charr was descended from female brook trout (*S. fontinalis*) x male Arctic charr (*S. alpinus*) cross. Fish (15 - 20 g body weight at the beginning of the feeding experiments; n = 600) were held for 180 days at the aquarium facilities at the Wasser Cluster Research Centre Lunz, Austria, from June until November 2014. The experiment was conducted in a flow-through system containing twelve 1000-L rectangular tanks with a continuous supply of gravel filtered spring water (ca. 25 L min^{-1}). Wastewater was drained using a sinkhole covered by a 5 mm mesh screen. Fish were subjected to natural photoperiod (latitude = 47.8604˚N), delivered by artificial fluorescent lighting and adjusted weekly. A total of 600 juvenile Alsatian charr were randomly distributed as 50 fish of mixed sexes per tank. Feeds were supplied to two replicate tanks per dietary treatment, thus it was not possible to statistically test for tank effects. However, we collected enough fish to conduct analysis of variance (see below) and test for differences among treatments.

Dissolved oxygen, pH and water temperature were recorded daily. Throughout this long-term feeding experiment, fish were exposed to natural variability of water temperature (9˚C to 12˚C; mean = 10.5˚C), dissolved oxygen (7 to 11 mg L^{-1}) and slightly above neutral pH values (~8), typical for water in the limestone Alps.

Six isocaloric fish feeds were formulated (Garant™, Austria) to provide sufficient lipid and protein to meet somatic requirements for salmonids [17]. Fish in duplicate tanks were fed 1 of the 6 different diets that contained 32.5% FM (F1,control) or only 17.5% FM (F2, poultry; F3, Pork SP; F4, Pork D; F5, vegetable feeds) or 25% FM (F6, Poultry Plus)as partial FM replacements (**Table 1(a)**). All feeds contained wheat, marine fish and rapeseed oil, wheat flower, blood meal, rapeseed cake, and wheat gluten (**Table 1(a)**). Diets were dispensed daily

Table 1. (a) Feed components (in %) with decreasing contents in fish meal and increase in animal by-product meals and vegetable feed, and relative mean contents (in %) of saturated, mono-unsaturated, and omega-3 and -6 polyunsaturated fatty acids; (b) proximate composition of experimental diets (g/100 g diet).

(a)

	Feed 1 Control	Feed 2 Poultry	Feed 3 Pork SP	Feed 4 Pork D	Feed 5 Vegetable	Feed 6 Poultry plus
Marine fish meal	32.5	17.5	17.5	17.5	17.5	25
Poultry by-product meal	-	15	-	-	-	15
Pork by-product meal SP	-	-	17	-	-	-
Pork by-product meal D	-	-	-	17	-	-
Soy grain GMO-free	-	-	-	-	13	-
Sunflower protein concentrate	17	17	17	17	20	12.5
Wheat	10	10	8.4	9.3	4.5	13
Fish oil	9	9	9	9	9	9
Rapeseed oil	8	8	8	8	8	8
Wheat flower	7.5	7.5	7.5	7.5	5.4	7.5
Blood meal	6.5	6.5	6.5	6.5	6.5	6.5
Rapeseed cake	5	5	5	5	5	3
Wheat gluten	3.0	3.0	3.0	3.0	6.9	-
Monocalciumphosphat	-	-	-	-	1.1	-
Lysine-HCl	-	-	-	-	0.09	-
Premix	0.6	0.6	0.6	0.6	0.6	0.6
Saturated fatty acids	15.8	16.2	17.7	19.2	15.4	16
Mono-unsaturated fatty acids	52.2	51.5	51	50	51.9	52.2
Omega-3 polyunsaturated fatty acids	13.9	12.6	12.8	12	13	12.8
Omega-6 polyunsaturated fatty acids	17.6	19.3	18.8	18.5	19.5	18.6

(b)

	Feed 1 Control	Feed 2 Poultry	Feed 3 PorkSP	Feed 4 PorkD	Feed 5 Vegetable	Feed 6 Poultry plus
Total proteins	42.9 ± 0.9	43.2 ± 1.1	43.4 ± 1.7	43.3 ± 1.5	42.8 ± 2.0	43.5 ± 1.9
Total lipids	20.1 ± 0.2	20.5 ± 0.1	20.9 ± 0.1	21.5 ± 0.2	19.1 ± 0.2	18.9 ± 0.1
Ash	10.4 ± 1.0	9.3 ± 0.8	9.3 ± 1.1	10.3 ± 1.1	10.7 ± 0.7	9.8 ± 1.3
Moisture	5.9 ± 0.4	7.7 ± 0.8	7.9 ± 0.2	7.3 ± 0.4	8.7 ± 1.1	7.6 ± 0.4

into the tank by a clockwork belt feeder (Dryden Aqua Ltd) over a 12 hrs feeding period. The daily feed ration exceeded the recommended feeding rate for salmonids for the prevailing water temperature.

2.2. Sampling Procedure

During the entire feeding experiment, every 2 wks one third of the fish in each

tank was randomly selected, weighed (g) and measured (cm) for the assessment of specific growth rates and biomass. After the biomass assessments, all fish were returned to their respective tanks. The specific growth rate (SGR, % body weight day^{-1}) was calculated as

$$\left[\left(\ln W_1 - \ln W_0\right)/t\right]\times 100,$$

where W_0 and W_1 are weights in grams per fish at the start and at the end of the feeding period, respectively, and t is the time of feeding in days. At the end of the feeding trial (after 180 days), twelve fish were selected at random from each treatment to determine lipid contents and fatty acid composition in the dorsal muscle tissue. Fish were rendered unconscious (blow on the head) and then killed by cardiac incision following the Federal Act on the Protection of Animals, Austria (http://www.ris.bka.gv.at), then a sub-sample of muscle was dissected and stored in plastic vials (8 mL). Muscle samples were obtained by cutting a fillet from above the lateral line as a border between the dorsal and ventral tissue. No skin or bone was included in the sample. All tissue samples were stored at −80˚C and subsequently freeze dried before analysis.

2.3. Proximate Analysis

The gross nutrient composition of the 6experimental diets was determined as below (**Table 1(b)**). Moisture was determined by drying to constant weight in an oven at 110˚C for 24 h [18]. Sample weight was recorded before drying and after removal from the oven. This process was repeated at 1 h intervals until the weight change was <5 mg. Total protein content in experimental diets was determined by modified Bradford assay [19] and total lipids by solvent extraction and gravimetric determination [20]. Ash content was determined by placing pre-weighed diets in a muffle furnace at 550˚C for 8 h or until white ash was obtained [18] that was subsequently weighed.

2.4. Lipid Extraction and Fatty acid Analysis

Total lipids from homogenized, freeze-dried dorsal muscle samples (25 - 35 mg) were analyzed as in Heissenberger *et al.* [20]. In brief, samples were sonicated and vortexed (4X) in a chloroform-methanol (2:1) mixture. Organic layers were removed and transferred into solvent-rinsed vials. For gravimetrical determination of total lipid contents (*i.e.*, mg lipids g dry $weight^{-1}$), subsamples (100 µL) of the extracts (duplicates) were evaporated and weighed. Fatty acids were derivatized to obtain fatty acid methyl esters (FAME) using toluene and sulphuric acid-methanol solution (1% v/v, 16 h at 50˚C). In contrast to Heissenberger *et al.* [20], hexane without butylated hydroxytoluene (BHT) was used for each washing step after methylation to avoid BHT-related peak interference in chromatograms (data not shown). FAME were analyzed using a gas chromatograph (TRACE GC THERMO, Detector: FID 260˚C, Carrier gas: H_2: 40 mL/min, N_2: 45 mL/min, air: 450 mL/min, temperature ramp: 140˚C (5 min) − 4˚C/min − 240˚C (20 min) = 50 min) equipped with a temperature-programmable injector

and an autosampler. A Supelco™ SP-2560 column (100 m, 25 mm i.d., 0.2 µm film thickness) was used for FAME separation. Excalibur 1.4™ (Thermo Electron Corporation) was used for calculation and, if necessary, manual resetting of the chromatograms. Fatty acid concentrations were calculated using calibration curves based on known standard concentrations.

2.5. Data Analysis

Principle components analysis (PCA) was used to reduce the number of individual FA into a single FA composition score and to analyse the difference between dietary and tissue FA compositions. Significant differences in somatic growth, total lipids and fatty acid contents among dietary treatments were determined by one-way ANOVA with subsequent Tukey's HSD tests to examine between treatment effects. Data identified as non-homogeneous, using variance test, were subjected to log transformation before applying the statistical tests. SPSS 15.0 was used for data analysis. Fatty acid retention ratios were determined as the quotient of FA in fish muscle tissues (mg FA per unit biomass) and FA in the respective diet. We define retention as the ability of fish to regulate and control ingested FA. To evaluate the effect of somatic growth differences on PUFA contents in the obtained biomassper fish after 180 d (end of this feeding experiment; $PUFA_{fish}$), we calculated $PUFA_{fish} = (PUFA_{mt}/5) \times BM_{180}$, where $PUFA_{mt}$ was the PUFA content in muscle tissues, "5" was the measured conversion factor from freeze-dried (dry weight; dw) to wet tissue weight, and BM_{180} the measured biomass (wet weight; ww) of fish in all diet treatments after 180 d of feeding. Finally, we calculated the variance of PUFA (n-3 and n-6) retention as a measure of the dispersion of the PUFA around the mean.

3. Results

3.1. Diet Composition

All feeds contained similar contents of total proteins (~43%), total lipids (~20%), total ash (~10%), crude fiber (1.8% - 2.7%), and moisture (~6% - 9%; **Table 1(b)**). As partial FM replacement in feeds F2-F6, F2 and F6 contained 15% poultry by-product meals, F3 and F417% pork by-product meals, and F5 13% soy grain GMO-free. All feeds contained wheat, marine fish and rapeseed oil, wheat flower, blood meal, rapeseed cake, and wheat gluten (**Table 1(a)**). The total lipid concentrations ranged from 125 - 189 mg g dry weight^{-1} (mean values) and were not significantly different among the 6 feeds ($p = 0.31$; **Table 2**). The fatty acid contents (mg per unit biomass) for total saturated (SAFA), mono-(MUFA), and PUFA were similar in all feeds (**Table 2**).

3.2. Fish Biomass and Specific Growth Rate

There was an overall statistically significant difference in fish biomass among these six feeding treatments in this experiment ($F_{(5, 78)} = 4.0$, $p = 0.003$), with fish feeding on F6 being significantly larger than those feeding on F2 ($p = 0.004$), F3

Table 2. Contents (mg per g dry weight; n = 6 per treatment) of total lipids, fatty acids (>0.5 mg per g dry weight), and grouped in omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFA) in fish feeds and fish (Alsatian charr; *Salvelinus alpinus X fontinalis*). F1 = Control, F2 = Poultry, F3 = Pork SP, F4 = Pork D, F5 = Vegetable, F6 = Poultry plus.

	Feed 1 Control	Feed 2 Poultry	Feed 3 Pork SP	Feed 4 Pork D	Feed 5 Vegetable	Feed 6 Poultry plus
Total lipids						
Feeds[a]	201 ± 19	205 ± 9	209 ± 9	215 ± 15	191 ± 18	189 ± 10
Fish[b]	66 ± 17	82 ± 33	109 ± 33	95 ± 31	75 ± 24	89 ± 14
Fatty acids Feeds						
14:0	3.9 ± 0.7	3.2 ± 0.2	3.4 ± 0.2	3.3 ± 0.1	3.3 ± 0.0	3.0 ± 0.2
16:0	16.5 ± 3.2	17.1 ± 1.2	19.0 ± 1.4	18.6 ± 0.2	15.7 ± 0.4	14.8 ± 0.1
16:1n-7	4.2 ± 0.8	4.0 ± 0.3	3.9 ± 0.3	3.6 ± 0.0	3.4 ± 0.3	3.5 ± 0.1
18:0	4.3 ± 0.8	4.6 ± 0.3	6.2 ± 0.5	6.8 ± 0.1	3.0 ± 4.2	3.9 ± 0.1
18:1n-9	74.0 ± 5.4	74.8 ± 5.0	79.7 ± 5.8	71.3 ± 0.2	71.6 ± 6.0	65.6 ± 9.7
18:1n-7	5.3 ± 1.1	2.4 ± 3.4	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 3.2	4.5 ± 0.5
18:2n-6	28.7 ± 2.1	31.1 ± 1.4	31.4 ± 2.4	28.3 ± 2.0	29.7 ± 2.5	26.7 ± 2.9
20:1n-9	4.5 ± 0.9	4.2 ± 0.3	4.4 ± 0.4	3.9 ± 0.0	4.2 ± 0.4	3.8 ± 0.6
18:3n-3	9.6 ± 1.9	9.4 ± 0.4	9.3 ± 0.8	8.5 ± 0.0	9.2 ± 0.8	8.3 ± 1.4
18:4n-3	0.9 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1
20:2n-6	1.0 ± 0.2	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	0.9 ± 0.1
20:4n-6	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
20:4n-3	0.8 ± 0.2	0.8 ± 0.1	0.8 ± 0.11	0.7 ± 0.0	0.8 ± 0.1	0.7 ± 0.1
20:5n-3	4.8 ± 1.0	3.8 ± 0.3	3.8 ± 0.3	3.4 ± 0.1	3.6 ± 0.3	3.6 ± 0.1
22:5n-3	1.5 ± 0.4	1.4 ± 0.1	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.1	1.2 ± 0.1
22:6n-3	5.7 ± 1.3	4.7 ± 0.4	4.7 ± 0.5	4.2 ± 0.1	4.5 ± 0.4	4.3 ± 0.3
Fatty acids Fish						
14:0	0.6 ± 0.1	0.8 ± 0.5	1.2 ± 0.4	0.9 ± 0.4	0.7 ± 0.4	1.0 ±0.2
16:0	6.4 ± 0.7	8.0 ± 2.9	10.0 ± 3.0	8.5 ± 2.1	7.2 ± 2.0	7.0 ±1.6
16:1n-7	1.0 ± 0.2	1.6 ± 1.1	2.2 ± 0.8	1.4 ± 0.6	1.0 ± 0.6	1.8 ±0.5
18:0	1.6 ± 0.4	1.8 ± 0.8	2.4 ± 1.9	2.1 ± 0.7	1.6 ± 0.5	1.9 ±0.3
18:1n-9	14.3 ± 3.9	21.5 ± 3.8	34.2 ± 5.7	25.6 ± 5.0	20.2 ± 3.9	24.8 ±5.1
18:1n-7	1.3 ± 0.4	0.9 ± 0.9	0.0 ± 0.0	1.6 ± 1.1	1.6 ± 0.8	1.9 ± 0.4
18:2n-6	5.7 ± 1.6	8.3 ± 1.9	13.1 ± 3.5	9.7 ± 4.9	8.0 ± 3.1	9.5 ± 2.1
18:3n-6	0.2 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.1
20:1n-9	1.1 ± 0.4	1.5 ± 0.8	2.6 ± 1.0	1.8 ± 0.9	1.5 ± 0.9	1.7 ± 0.4
18:3n-3	1.5 ± 0.4	2.0 ± 0.2	2.9 ± 1.1	2.3 ± 1.1	2.0 ± 0.8	2.3 ± 0.5
18:4n-3	0.3 ± 0.1	0.5 ± 0.1	0.7 ± 0.3	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
20:2n-6	0.5 ± 0.2	0.6 ± 0.1	1.0 ± 0.3	0.7 ± 0.3	0.6 ± 0.3	0.7 ± 0.1
20:4n-6	0.7 ± 0.2	0.7 ± 0.3	0.9 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1

Continued

20:4n-3	0.3 ± 0.0	0.3 ± 0.1	0.5 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
20:5n-3	2.1 ± 0.4	2.1 ± 0.3	2.5 ± 0.4	2.2 ± 0.5	2.1 ± 0.3	2.3 ± 0.2
22:5n-3	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	0.7 ± 0.1
22:6n-3	9.1 ± 1.3	9.5 ± 1.8	11.6 ± 3.9	9.8 ± 1.2	10.2 ± 2.3	10.0 ± 1.2
n-3 PUFA						
Feeds[c]	23 ± 5	21 ± 2	21 ± 2	19 ± 1	21 ± 2	19 ± 2
Fish[d]	14 ± 2	15 ± 4	19 ± 6	16 ± 3	16 ± 4	16 ± 2
n-6 PUFA						
Feeds[e]	30 ± 7	32 ± 2	33 ± 3	30 ± 0	31 ± 3	28 ± 3
Fish[f]	7 ± 2	10 ± 6	15 ± 7	12 ± 6	10 ± 5	11 ± 3
n-3/n-6						
Fish[g]	2	1.8	1.3	1.6	2	1.5

OW-ANOVA: [a]$F = 1.29$. $p = 0.31$; [b]$F = 1.91$. $p = 0.12$; [c]$F = 1.56$. $p = 0.21$; [d]$F = 1.94$. $p = 0.12$; [e]$F = 1.29$. $p = 0.31$; [f]$F = 1.79$. $p = 0.15$; [g] mean n-3/n-6 values.

($p = 0.031$), and F4 ($p = 0.014$). At the end of the feeding experiment, fish feeding on F6 enhanced their biomass by 15% relative to F1 and had the higher mean biomass (264 mg wet weight). However, fish feeding on feeds with pork by-product meals (F3 and F4) resulted on average in a 25% lower biomass gain (205 and 210 mg wet weight, respectively; Figure 1) compared to fish feeding on F6.

Somatic growth rates among the different feeding treatments were not affected by water temperatures that ranged from 9˚C - 12˚C during this experiment ($p > 0.2$ for all diet treatments). Fish feeding on F6 had the highest growth rate (0.6 ± 0.08), while F1, F2, and F5 fish had lower growth rates (0.54 ± 0.13, 0.54 ± 0.08, and 0.53 ± 0.16, respectively), and the lowest was detected in fish feeding on feeds containing poultry by-products (F3: 0.46 ± 0.14 and F4: 0.46 ± 0.08). The overall feed conversion ratios (FCR), determined as dry feed weight per total wet weight gain for the entire feeding period, were lower in the F1 (FCR = 1.08), F6 (FCR = 1.09) and F2 (FCR = 1.12) than in F3 (FCR = 1.28), F5 (FCR = 1.29), and F4 (FCR = 1.30).

3.3. Total Lipids and PUFA Composition in Fish

Total lipid concentrations in dorsal fish muscle tissues (n = 6 per treatment; mean values ranged from 66 - 109 mg g dry weight^{-1}) did not significantly differ among these 6 feeding treatments ($p = 0.12$; Table 2). Similarly, there was no statistically significant difference observed in n-3 ($p = 0.12$) or n-6 ($p = 0.15$) PUFA in fish (Table 2). We tested also for differences in concentrations of the individual n-3 PUFA alpha-linolenic acid (ALA; 18:3n-3), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3) because freshwater salmonids are an important dietary supply of long-chain n-3 PUFA to humans that are otherwise well provided by marine fish ([21] [22]); there was no significant

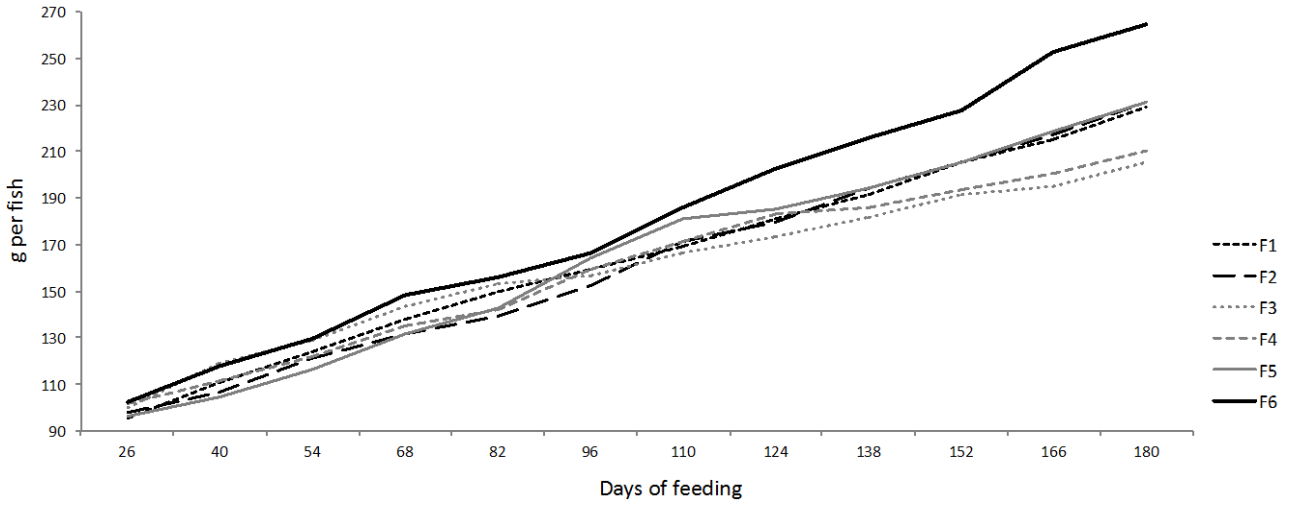

Figure 1. Average biomass (in g) of Alsatian charr (*Salvelinus alpinus X fontinalis*) fed diets containing 32.5% fish meal (F1; control diet), 17.5% fish meal and 15% poultry by-product meal (F2), 17.5% fish meal and 17% pork by-product meals (F3 and F4), 17.5% fish meal and 13% soy grain feeds (F5), and 25% fish meal and 15% poultry by-product meal (F6).

difference in dorsal muscle tissues of Alsatian charr in ALA ($F = 1.39$; $p = 0.25$), EPA ($F = 1.06$; $p = 0.4$), or DHA concentrations ($F = 0.97$; $p = 0.45$) among these 6 different feeding treatments. Fish in these treatments contained on average 29% - 34% PUFA per total lipid content and retained on average 1.2 - 1.3X more PUFA per total lipids than their diets; there was no significant difference in PUFA retention (normalized to total lipids; $p = 0.43$). However, relative to its dietary supply, DHA was less strongly retained in dorsal muscle tissues of F1 fish (only 1.6X more DHA) than in all other fish that were feeding on meals partially replaced by other animal or vegetable by-products (2 - 2.5X more DHA than their dietary DHA supply). Similarly, the retention of the omega-6 long-chain PUFA arachidonic acid (ARA; 20:4n-6) in dorsal muscle tissues was lower in F1 fish (1.3X) than in other fish (1.4 - 1.8X; **Table 3**).

The first and second principle components (PC1 and PC2) together of the PCA explained 66% of the variability in eigenvalues (PC1: 40.5%, PC2: 25.5%). The proportions of n-3 PUFA and SAFA were positively correlated, whereas the proportions of the long-chain SAFA 20:0, 22:0, and 24:0, used as fatty acid markers of terrestrial sources, were negatively correlated to the PC1 scores (**Figure 2**). Omega-6 PUFA were negatively correlated to the PC2. Thus, feeds were clearly separated by terrestrial fatty acids, whereas fish were strongly associated with n-3 PUFA.

Omega-3 PUFA contents per total individual fish weight at the end of the feeding experiment (after 180 days) showed lowest n-3 PUFA contents (ww) in F1-fish (640 ± 90 mg per fish; ww) and highest in F6-fish (866 ± 99 mg per fish), however, without any significant differences among these 6 treatments (OW-ANOVA; $F = 1.7$, $p = 0.16$; **Figure 3(a)**). Omega-6 PUFA contents in fish were also lowest in F1-fish (322 ± 85 mg per fish) and highest in F3-fish (845 ± 308 mg per fish), but not significantly different among these treatments

Table 3. Retention ratios of polyunsaturated fatty acids (PUFA) contents (mg g dry weight^{-1}; calculated as ratios between PUFA contents in fish and feeds) in Alsatian charr (*S. fontinalis X alpinus*) fed on 6 different feeds: F1 = Control, F2 = Poultry, F3 = Pork SP, F4 = Pork D, F5 = Vegetable, F6 = Poultry plus.

	Feed 1 Control	Feed 2 Poultry	Feed 3 Pork SP	Feed 4 Pork D	Feed 5 Vegetable	Feed 6 Poultry plus
18:2n-6	0.2	0.3	0.4	0.3	0.3	0.4
18:3n-6	1.8	3.5	5	4	3.2	3.8
20:4n-6	1.3	1.4	1.7	1.5	1.8	1.7
18:3n-3	0.2	0.2	0.3	0.3	0.2	0.3
18:4n-3	0.4	0.7	0.9	0.8	0.7	0.8
20:4n-3	0.3	0.4	0.6	0.5	0.4	0.5
20:5n-3	0.4	0.6	0.7	0.6	0.6	0.6
22:6n-3	1.6	2	2.5	2.4	2.3	2.3

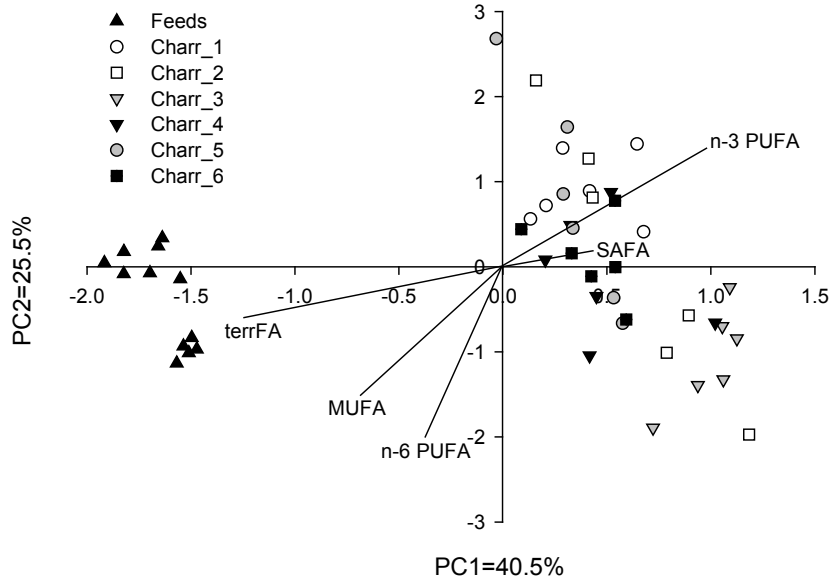

Figure 2. Principle components analysis of fatty acids in experimental feeds and dorsal muscle tissues in Alsatian charr (*Salvelinus alpinus X fontinalis*) supplied with 6 different feeds: 32.5% fish meal (F1; control diet), 17.5% fish meal and 15% poultry by-product meal (F2), 17.5% fish meal and 17% pork by-product meals (F3 and F4), 17.5% fish meal and 13% soy grain feeds (F5), and 25% fish meal and 15% poultry by-product meal (F6).

(OW-ANOVA; $F = 1.5$, $p = 0.22$; Figure 3(b)). Finally, the variance of retained n-6 PUFA (55181) in all fish was almost twice as large as that of n-3 PUFA (30685).

4. Discussion

This long-term feeding experiment shows that poultry by-products as partial FM replacement (~25% less FM) caused the highest weight gain in Alsatian charr (F6) during 180 days of feeding. The final weight gain in Alsatian charr was very

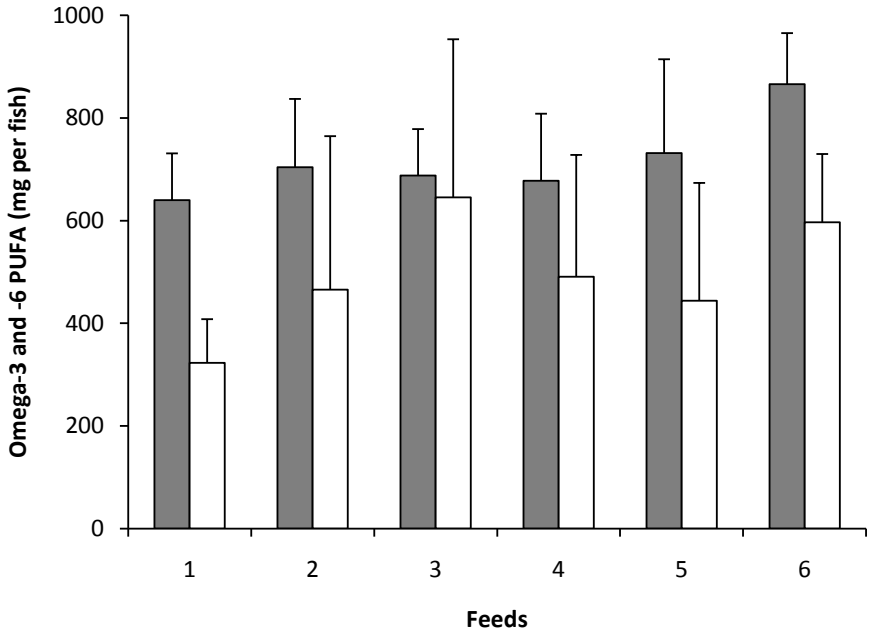

Figure 3. Contents (mg; mean ± standard deviation) of omega-3 (grey bars) and omega-6 (white bars) polyunsaturated fatty acids (PUFA) per total weight of individual fish at the end of the feeding experiment (after 180 days) in 6 feeding treatments: 32.5% fish meal (F1; control diet), 17.5% fish meal and 15% poultry by-product meal (F2), 17.5% fish meal and 17% pork by-product meals (F3 and F4), 17.5% fish meal and 13% soy grain feeds (F5), and 25% fish meal and 15% poultry by-product meal (F6).

similar when feeding on conventional FM feed (F1; containing 32.5% FM), feeds containing poultry by-products (F2) or vegetable diets (F5) as partial FM replacement (~50% less FM). However, pork by-products as partial FM replacement in feeds F3 and F4 (~50% less FM) resulted in the lowest fish weight gain, indicating that animal by-products in isocaloric fish feeds can still cause differences in somatic growth of this freshwater salmonid. At the same time, such diet composition induced differences in fish weight did not significantly affect the retention of fatty acid contents in muscle tissues.

The largest weight gain in F6 coincides with the highest dietary contribution of animal meals in this feed (40%), compared to 32.5% FM in F1, 34.5% animal meals in F2, F3, and F4, or only 17.5% FM in F5. All of these feeds were isocaloric and differed only in their ingredient composition. It is likely that F6 with the highest animal meal contribution and slightly higher amounts of wheat in the feed caused higher weight gain in F6-fish. Bureau *et al.* [23] reported similar digestibility of animal protein ingredients in feeds for rainbow trout and Dosanjh *et al.* [15] found no biomass differences in marine salmon that were fed on pork by-product meals. This is in contrast to our finding that charr grew less efficiently when feeding on feeds containing pork by-product meals (F3 and F4). It is likely that pork by-product meals may have been less digestible for charr than F1 and the poultry by-product feeds as indicated by their higher FCR. Lower digestibility of pork than poultry by-product meals was recently reported in a feeding study on Pacific white shrimp (*Litopenaeus vannamei*) [24]. It is also

possible that other unidentified ingredients in pork by-product meals caused lower weight gain because charr feeding on F5 (vegetable feed by-products) had similar FCR as F3 and F4, yet higher weight gain. These results indicate, however, that the higher contribution of animal meals in F3 and F4 (34.5%) compared to F5 (with only 17.5% animal meal) did not account for the higher weight gain measured in F5 charr. Furthermore, the similar weight gain in fish feeding on F1, F2, or F5 demonstrates that this freshwater salmonid can be supplied with 50% lessmarine FM in their diet and that poultry by-products or vegetable feeds as partial FM replacement result in very similar final fish biomass. Thus, 50% reduction of FM and proportional replacement by poultry by-products or vegetable feeds can fully replace commonly used FM in feeds for Alsatian charr.

Contrary to our hypothesis that charr with highest somatic growth will result in lowest PUFA contents in muscle tissues (effect of growth dilution), there was no significant difference in charr PUFA contents among these treatments. This suggests that independent of weight gain the dietary PUFA supply was not limiting PUFA retention in Alsatian charr. It can be consequently speculated that, in addition to FM replacements, slight reductions of dietary PUFA supply may have little effects on PUFA retention in this sparctic charr. It is, however, clear that full replacement of fish oil with vegetable oil may also reduce fish-derived long-chain n-3 PUFA content in charr or other aquaculture-raised freshwater salmonids [25], although fish weight gain may not be equally compromised [7]. Thus, further research should aim at identifying by how much dietary fish oil can be replaced with other oil sources in a combined effort to reduce both FM and highly valuable fish oil in aquaculture feed production for freshwater salmonids and other freshwater fish.

Principle components analysis revealed that charr of all feeding treatments were strongly associated with n-3 PUFA, independent of diet ingredients and somatic growth differences. The most highly retained n-3 PUFA was DHA in fish of all feeding treatments, whereas all other identified n-3 PUFA contents were lower than in their dietary supply, indicating that DHA is the most required n-3 PUFA in Alsatian charr, as is the case of freshwater fish in general [26]. However, F1-fish retain on average only 1.6 times more DHA relative to its dietary supply, whereas it was 2.4 to 2.5 times more in F4 and F3-fish, respectively. Similarly, the n-6 PUFA gamma-linolenic acid (18:3n-6) and ARA were effectively retained in fish of all treatment, but the lowest retention in F1-fish and highest in F3 and F4-fish suggests that PUFA retention efficiency in Alsatian charris affected by the feed composition and not directly regulated by dietary fatty acid supply. Finally, the higher variance in n-6 than n-3 PUFA retention in fish among these different feeding treatments suggests that, independent of these diet compositions, Alsatian charr retained n-3 PUFA more consistently than n-6 PUFA.

5. Conclusion

In conclusion, this long-term feeding trial of 180 days furthers our knowledge

that reducing marine fish meal by 50% and using poultry by-product meal and vegetable feeds, both used as partial replacements of marine fish meal, results in similar weight gains of this sparctic charr. When marine fish meal was only reduced by 25% and substituted by poultry by-product meal, the fish biomass was clearly enhanced by 15% relative to conventional fish meal feeds. However, it became evident that pork by-product meal as partial replacement yielded lower biomass gain than the conventional marine fish meal. The use of poultry or pork by-product or vegetable meals as partial fish meal replacements did not significantly change the total lipids or fatty acid contents in these fish, suggesting that these alternative feeds, yet with similar lipid sources, have very little effect on the fatty acid composition in this freshwater salmonid. Further studies are warranted to assess the effect of increasing use of animal by-product and vegetable meals as well as lower fish oil contribution to fish feeds on weight gain and fatty acid composition in freshwater salmonids that are increasingly raised and eaten by humans worldwide [1].

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