Implementation of rendered fats in aquaculture feeds: maximizing ability to tailor nutritional value of cultured finfish while minimizing reliance on marine resources

Final Report

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Introduction

With domestic seafood consumption at record highs and many commercial food-grade fisheries in decline, the contribution of aquaculture products to the American diet continues to grow. Aquaculture's demand for fish oil continues to increase despite record-high pricing for this commodity. The aquaculture industry must reduce its consumption of marine-derived lipid or risk future exhaustion of this resource. In general, dietary FO can be partially or completely replaced with alternative lipids without affecting growth performance. Unfortunately, fish oil replacement significantly alters the FA composition of the resultant product and results in a corresponding decline in fillet highly unsaturated fatty acid (HUFA) content. Although cultured seafood produced using alternative lipid sources is attractive from sustainability and production cost standpoints, these products are also substantially less valuable in terms of providing beneficial HUFA to human consumers.

The inherent conflict between production of fillets with the greatest nutritional value (fish oil-based feeds) and the lowest feed costs (alternative lipid-based feeds) may be overcome through the use of 'finishing' feeds to augment fillet HUFA content of fish raised on alternative feeds prior to harvest. The fatty acid (FA) composition of fish tissues is plastic, and will change in response to altered dietary composition. By feeding high-HUFA content, finishing feeds at the end of the production cycle, fillet HUFA content can be restored immediately prior to harvest. Based on the various estimates of selective metabolism of FA in fishes, saturates (SFA) appear to be preferentially catabolized, whereas polyunsaturates, including HUFA and less beneficial, shorter-chain polyunsaturates (PUFA), are selectively deposited in tissues. Thus, to maximize fillet deposition of beneficial HUFA and reduce competition with less-beneficial PUFA, saturated grow-out feeds with little PUFA content would be indicated. We have confirmed the efficacy of this strategy in sunshine bass and Nile tilapia using coconut oil as a source of SFA. The purpose of the current project is to evaluate various rendered fats as more cost-effective sources of SFA to be used in grow-out feeds for rainbow trout.

Objectives

- Assess the relative suitabilities of beef tallow, pork lard, poultry fat, and yellow grease as partial substitutes for fish oil in rainbow trout grow-out feeds in terms of production performance and responsiveness of fillet tissue to FA profile restoration during finishing

- Employing the ideal rendered lipid identified in Objective #1, determine the relationship between fish oil substitution level and duration of finishing period in order to maximize utilization of the ideal rendered lipid throughout the production cycle of rainbow trout.

Industry Summary

Objective 1 was designed as a screening study to determine which of the rendered fats evaluated held the most promise for use in the context of finishing strategies for rainbow trout. Each of the rendered fats assessed yielded equivalent growth and growth efficiency. Given that no differences in production performance were observed among the fats, we selected beef tallow based on 1) the minimal impact this fat had on fillet composition in Objective 1, and 2) the more favorable price and widespread availability of this fat source relative to the other rendered fats we evaluated.

In Objective 2, greater levels of fish oil sparing (50-100% fish oil replacement) with beef tallow were evaluated in combination with finishing periods of different durations. The feeding trial for Objective 2 revealed no significant differences in growth performance among the treatments: All fish reached a marketable size (~1.25 lbs.) by the time of harvest, and weight gain (1000-1200% gain over the course of the trial) and feed conversion ratio (FCR; 1.4-1.7) were good, regardless of whether the fish were fed diets containing fish oil, beef tallow, or blends of these lipids exclusively or in combination with a finishing feed. Tissue analysis revealed that fillets with equivalent levels of beneficial HUFA may be produced by feeding rainbow trout beef tallow-based feeds according to one of several schemes. For example, 75% of dietary fish oil can be replaced with beef tallow in the grow-out feed if a 4-week finishing period is used. Alternatively, beef tallow can be used to completely replace fish oil in rainbow trout feeds if a 12-week finishing period is used to restore HUFA levels. Taken together, these results indicate that beef tallow may be used rather extensively in rainbow trout culture without concern for reduced production performance or the loss of fillet nutritional value. Lay publications describing these results are planned in order to encourage the aquafeed industry to consider greater use of rendered fats, particularly beef tallow, in rainbow trout feeds.

Manuscript sections for Objective #1

Please see attached publication, Trushenski et al. 2011a. An additional, indirectly related publication prepared during the course of our project, Trushenski and Lochmann 2009, is also provided.

Manuscript sections for Objective #2

Please see below. This manuscript is in preparation, and will likely be submitted to the North American Journal of Aquaculture when complete. A lay summary is also planned for publication in Global Aquaculture Advocate or a similar industry publication.

Maintaining Production Performance of Rainbow Trout *Oncorhynchus mykiss* While Reducing Reliance on Fish Oil-Based Feeds

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Abstract

Static landings and increasing cost of fish oil have led culturists to investigate alternative lipids and feeding strategies in order to reduce production costs and pressure on reduction fisheries. Rendered fats, like beef tallow, may prove to be suitable alternatives that could maintain production performance and allow tissue fatty acid profile to be conserved or restored utilizing fish oil-based feeds. Accordingly, we evaluated fish oil sparing with beef tallow (50, 75 or 100% replacement of added fish oil) in feeds for rainbow trout fed for a total of 31 weeks in combination with 4, 8, or 12 weeks of finishing with a fish oil-based feed. Fish oil replacement and finishing duration had no significant effect on production performance: survival – 91.5 ± 1.3%; final weight – 587.7 ± 13.1 g; weight gain – 1151 ± 29%; feed conversion ratio – 1.49 ± 0.03; specific growth rate – 1.15 ± 0.01 %BW/day; and feed intake – 2.19 ± 0.02 %BW/day. Tissue analysis revealed that fillets with equivalent levels of beneficial long-chain polyunsaturated fatty acids (LC-PUFA) may be produced by feeding rainbow trout beef tallow-based feeds according to one of several schemes. For example, 75% of dietary fish oil can be replaced with beef tallow in the grow-out feed if a 4 to 8-week finishing period is used. Alternatively, beef tallow can be used to completely replace fish oil in rainbow trout feeds if a 12-week

finishing period is used to restore LC-PUFA levels. Taken together, these results indicate that beef tallow may be used rather extensively in rainbow trout culture without concern for reduced production performance or the loss of fillet nutritional value, thereby reducing costs and reliance on marine-based feeds.

Introduction

Terrestrial rendered fats are inexpensive, widely available, and depending on the type of fat, rich in SFA (Trushenski and Lochmann 2009). Originally thought to be less valuable in aquafeeds than medium-chain polyunsaturated fatty acid (MC-PUFA, 18 carbon atoms, ≥ 2 double bonds) rich plant-derived lipids, rendered fats may actually be more advantageous in aquafeeds in terms of cost, production performance, and long-chain polyunsaturated fatty acid (LC-PUFA) retention. Previous research has demonstrated that, among rendered fats, beef tallow is an excellent lipid source for use in rainbow trout feeds (Trushenski et al. 2011). Although beef tallow-based feeds apparently minimize the lost of LC-PUFA from rainbow trout fillets, some modification is likely to occur if these feeds are fed throughout the production cycle. Accordingly, we evaluated the growth and fillet fatty acid composition of rainbow trout raised to a marketable size on feeds containing beef tallow or blends of fish oil and beef tallow in combination with a fish-oil based finishing feed.

Methods and Materials

Feed Preparation and Analyses

A practical rainbow trout feed (Table 1), including 91 g/kg fish oil (100 FO, Virginia Gold™, Omega Protein, Inc., Houston, Texas), served as the control dietary formulation and finishing feed in the present work. Experimental grow-out feeds were derived from the basal formulation by replacing 50 (50/50 Diet), 75 (75/25 Diet), or 100% (100/0 Diet) of the fish oil with beef tallow (Darling International, Inc., Irving, Texas). All feedstuffs were incorporated using a cutter-mixer (Model CM450, Hobart Corporation, Troy, Ohio), pelleted using a food grinder, dried at 100°C using a forced-air food dehydrator (Harvest Saver R-5A, Commercial Dehydrator Systems Inc., Eugene, Oregon), and stored frozen (-20°C) throughout the duration of the study. Triplicate feed samples were analyzed to confirm proximate composition (Table 1). Briefly, samples were lyophilized (Freezone 6, Labconco Corporation, Kansas City, Missouri) to determine moisture content, and then pulverized. Protein (LECO® FP-528, LECO Corporation, St Joseph, Michigan) and ash (Muffle furnace, 600°C for 3 hours) content were determined for each pulverized sample, and lipid content was determined gravimetrically following chloroform/methanol extraction (Folch et al. 1957). Total lipids extracted from the feeds were analyzed for fatty acid composition (Table 2) according to the procedures described by Lane et al. (2006).

Experimental Design and Feeding Trial

Ten feeding regimens were developed to address the influences of fish oil sparing with beef tallow and finishing on the production performance and fillet composition of rainbow trout (Figure 1). Experimental regimens represented feeding the grow-out feeds described above (50/50 Diet, 75/25 Diet, or 100/0 Diet) in combination with 4, 8, or 12 weeks of finishing with the control 100 FO feed. The control regimen represented feeding the 100 FO feed throughout the entire feeding trial. A recirculation system comprised of thirty, 190-L tanks and associated biological and mechanical filtration units was stocked with juvenile rainbow trout (47.0 ± 0.2g, mean ± SE). Each tank was randomly assigned to a feeding treatment regimen (3 tanks/treatment, $N = 3$), and all fish were fed daily to apparent satiation.

Throughout the trial, water quality parameters were maintained within ranges suitable for rainbow trout culture in freshwater and photoperiod was maintained on a 24:0 light/dark cycle. All culture and husbandry methods, as well as euthanasia and sample collection procedures described below, were conducted under the direction and approval of the Southern Illinois University Institutional Animal Care and Use Committee, protocol #08-042.

Harvest, Sample Collection, and Growth Performance

Prior to initiation of the finishing period, two fish were sampled from each tank for determination of pre-finishing fillet fatty acid composition; baseline fillet samples were collected following the same procedures described below for harvest sample collection. After completion of the feeding trial, feed was withheld for 24 h prior to harvest. All surviving fish within each tank were harvested, sedated by immersion in an ice water bath, and then counted and group-weighed by tank. After weighing, all fish were euthanized by rapid disruption of the posterior cranium/spinal cord. A subsample of fish from each tank were individually weighed and dissected to remove the viscera for determination of hepatosomatic and viscerosomatic indices (see *Calculations*). Shank (boneless, skinless, belly-flap removed) fillets were also harvested from these fish, weighed to determine dress-out (see *Calculations*). Subsamples of shank fillets were individually packaged and stored frozen (-80°C) prior to proximate and fatty acid analyses; remaining fillets were pooled by treatment regimen and stored frozen (-20°C) for use in the taste test. Survival, weight gain, feed conversion ratio (FCR), specific growth rate (SGR), and feed intake were also calculated for each tank (see *Calculations*). Shank fillets were lyophilized, pulverized, and extracted in the same manner as feed samples to determine total lipid content. Fillet lipid samples were similarly analyzed for fatty acid composition according to the procedures described above. Standard metrics of growth performance were calculated based on Trushenski et al. (2011) for all treatment regimens:

Results and Discussion

Fish oil replacement and finishing duration had no significant effect on production performance: survival – 91.5 ± 1.3%; final weight – 587.7 ± 13.1 g; weight gain – 1151 ± 29%; feed conversion ratio – 1.49 ± 0.03; specific growth rate – 1.15 ± 0.01 %BW/day; feed intake – 2.19 ± 0.02 %BW/day. Tissue analysis revealed that fillets with equivalent levels of beneficial LC-PUFA may be produced by feeding rainbow trout beef tallow-based feeds according to one of several schemes (Table 3). For example, 75% of dietary fish oil can be replaced with beef tallow in the grow-out feed if a 4-week finishing period is used. Alternatively, beef tallow can be used to completely replace fish oil in rainbow trout feeds without impacting fillet beneficial fatty acid profile if a 12-week finishing period is used to restore LC-PUFA levels.

Conclusions

Our results suggest that beef tallow can be used rather extensively in rainbow trout feeds without concern regarding loss of production performance. Although feeding beef tallow-based diets does alter the fatty acid profile of rainbow trout fillets, these effects are minimized by the relatively high levels of saturated fatty acids and monounsaturated fatty acids associated with this lipid. Furthermore, any effects on fillet composition can be corrected through the use of fish oil-based finishing feeds. Taken together, our results suggest an expanded role for rendered fats, particularly beef tallow, in rainbow trout feeds as an economically and strategically valuable alternative to fish oil.

References

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Table 1. Feed formulations. All values listed as g/kg.

Ingredient	100 FO	50/50 Diet	75/25 Diet	100/0 Diet
Corn Gluten Meal [a]	250.00	250.00	250.00	250.00
Menhaden FM [b]	200.00	200.00	200.00	200.00
Soybean Meal [c]	200.00	200.00	200.00	200.00
Blood Meal [d]	50.00	50.00	50.00	50.00
Wheat Bran [c]	149.80	149.80	149.80	149.80
Fish Oil (FO) [b]	91.00	45.50	22.75	---
Beef Tallow (BT) [e]	---	45.50	68.25	91.00
Carboxymethyl Cellulose	20.00	20.00	20.00	20.00
Dicalcium Phosphate	15.00	15.00	15.00	15.00
Sodium Phosphate	15.00	15.00	15.00	15.00
Choline Chloride	6.00	6.00	6.00	6.00
Vitamin Premix [f,h]	1.20	1.20	1.20	1.20
Stay-C (vitamin C, 35%) [g]	1.00	1.00	1.00	1.00
Mineral Premix [f,i]	1.00	1.00	1.00	1.00
Proximate composition [j]				
Dry matter	939 ± 6	923 ± 6	926 ± 6	931 ± 6
Lipid	138 ± 2	142 ± 2	147 ± 2	138 ± 2
Protein	477 ± 2	472 ± 2	478 ± 2	475 ± 2
Ash	87 ± 2	90 ± 2	94 ± 2	95 ± 2

[a] 60% protein, Grain Processing Corporation, Muscatine, Iowa
[b] Omega Protein Inc., Houston, Texas
[c] 47% protein soybean meal; Seimer Enterprises, Teutopolis, Illinois
[d] Darling International, Irving, Texas
[e] Beef Tallow – Darling International, Irving, Texas
[f] Purina Test Diet, Richmond, Indiana
[g] Argent Laboratories, Redmond, Washington
[h] Formulated to contain 25.000% L-ascorbyl-2-polyphosphate, 13.160% vitamin K, 12.500% inositol, 12.500% nicotinic acid, 7.500% riboflavin, 6.250% calcium pantothenate, 2.500% pyridoxine hydrochloride, 1.250% thiamine mononitrate, 1.000% vitamin A palmitate, 0.500% cyanocobalamin, 0.450% folic acid, 0.125% biotin, and 0.010% cholecalciferol in a cellulose base
[i] Formulated to contain 24.897% zinc oxide, 14.933% ferrous sulfate, 3.470% manganese oxide, 0.967% cupric carbonate, 0.262% potassium iodide, 0.060% sodium selenate, and 0.030% cobalt carbonate in a cellulose base
[j] Proximate composition listed in g/kg on dry matter basis

Table 2: Fatty acid composition of experimental feeds. All values listed as g/100 g fatty acid methyl esters. Values represent least-square means; PSE < 0.01 reported as 0.00.

Fatty acid(s)[f]	100 FO	50/50 Diet	75/25 Diet	100/0 Diet	PSE
14:0	7.48	5.34	4.50	3.51	0.01
15:0	0.63	0.57	0.55	0.52	0.00
16:0	18.71	21.54	23.72	25.38	0.01
17:0	0.53	0.84	1.01	1.19	0.00
18:0	3.47	10.11	13.67	17.20	0.02
20:0	0.27	0.25	0.25	0.24	0.00
Total SFA[a]	31.09	38.63	43.69	48.03	0.04
14:1	0.25	0.17	0.25	0.32	0.00
16:1(n-7)	9.47	6.25	4.91	3.37	0.01
18:1(n-7)	2.92	3.24	1.49	1.11	0.01
18:1(n-9)	9.03	19.65	24.06	29.03	0.02
20:1(n-9)	0.86	0.54	0.40	0.25	0.00
Total MUFA[b]	22.52	29.85	31.11	34.08	0.02
16:2(n-4)	1.11	0.62	0.40	0.16	0.00
16:3(n-4)	1.27	0.68	0.42	0.16	0.00
18:3(n-4)	0.37	0.25	0.20	0.15	0.01
18:2(n-6)	9.19	9.81	10.41	10.63	0.06
18:3(n-6)	0.36	0.27	0.22	0.18	0.00
20:2(n-6)	0.22	0.15	0.13	0.10	0.00
20:3(n-6)	0.23	0.15	0.14	0.11	0.01
20:4(n-6)	1.00	0.63	0.47	0.29	0.00
Total n-6	11.00	11.01	11.36	11.21	0.05
18:3(n-3)	1.72	1.32	1.17	0.98	0.01
18:4(n-3)	2.67	1.50	0.97	0.41	0.00
20:3(n-3)	0.19	0.11	0.00	0.00	0.00
20:4(n-3)	1.41	0.80	0.52	0.22	0.00
20:5(n-3)	12.93	7.24	4.69	1.94	0.01
22:5(n-3)	2.25	1.29	0.88	0.39	0.01
22:6(n-3)	11.47	6.70	4.60	2.29	0.03
Total n-3	32.64	18.96	12.83	6.22	0.04
Total PUFA[c]	46.38	32.52	25.21	17.89	0.04
Total LC-PUFA[d]	29.48	16.92	11.30	5.19	0.05
Total MC-PUFA[e]	14.31	13.14	12.97	12.34	0.06
n-3:n-6	2.97	1.72	1.13	0.55	0.01

[a] Saturated fatty acids (SFAs) – sum of all fatty acids without double bonds
[b] Monounsaturated fatty acids (MUFAs) – sum of all fatty acids with a single double bond
[c] Polyunsaturated fatty acids (PUFAs) – sum of all fatty acids with ≥ 2 double bonds
[d] Long-chain polyunsaturated fatty acids (LC-PUFAs) – sum of all fatty acids with chain length of ≥ 20 carbon atoms and double bonds ≥ 3
[e] Medium-chain polyunsaturated fatty acids (MC-PUFAs) – sum of all PUFA with chain length of 18 carbon atoms
[f] Number to the left of colon indicates number of carbon atoms in the fatty acid chain, the number to the right of the colon indicates the number of double bonds, number after the hyphen indicates the position of the first double bond from the methyl end

Table 3. Overall fillet fatty acid composition at harvest. Means with common letter labels are not significantly different (P >0.05).

Fatty acid(s)	100 FO	50/50 Diet: 12 week finish	50/50 Diet: 8 week finish	50/50 Diet: 4 week finish	75/25 Diet: 12 week finish	75/25 Diet: 8 week finish	75/25 Diet: 4 week finish	100/O Diet: 12 week finish	100/O Diet: 8 week finish	100/O Diet: 4 week finish	SE mean	*P*-value for 1-way ANOVA
Total SFAs	34.6	33.7	33.3	34.8	34.6	34.1	33.8	34.0	33.7	34.5	0.5	0.3286
Total MUFAs	29.3 d	28.6 d	30.8 cd	33.0 bcd	35.0 bc	34.6 bc	35.0 bc	31.4 bcd	36.2 ab	40.8 a	1.0	<0.0001
n-6	10.5	10.8	10.6	10.4	10.7	10.6	11.4	10.5	11.2	11.3	0.2	0.1135
20:5(n-3)	5.4 a	5.4 a	4.5 ab	3.8 bc	3.7 bc	3.8 bc	3.3c	4.5 ab	3.5 bc	2.1 d	0.2	<0.0001
22:6(n-3)	14.6 ab	16.2 a	16.1 a	13.8 ab	11.7 bc	13.0 abc	12.8 abc	15.2 ab	11.8 bc	9.1 c	0.8	0.0002
n-3	25.6 ab	27.0 a	25.2 ab	21.7 abc	19.6 c	20.8 bc	19.9 c	24.1 abc	18.9 c	13.5 d	1.0	<0.0001
Total LC-PUFAs	24.7 ab	26.2 a	24.9 ab	21.6 abc	19.2 cd	20.5 bc	19.8 bc	24.1 abc	19.0 cd	14.1 d	1.1	<0.0001
Total MC-PUFAs	10.9	11.0	10.4	10.0	10.6	10.2	10.9	9.9	10.5	10.0	0.3	0.0929

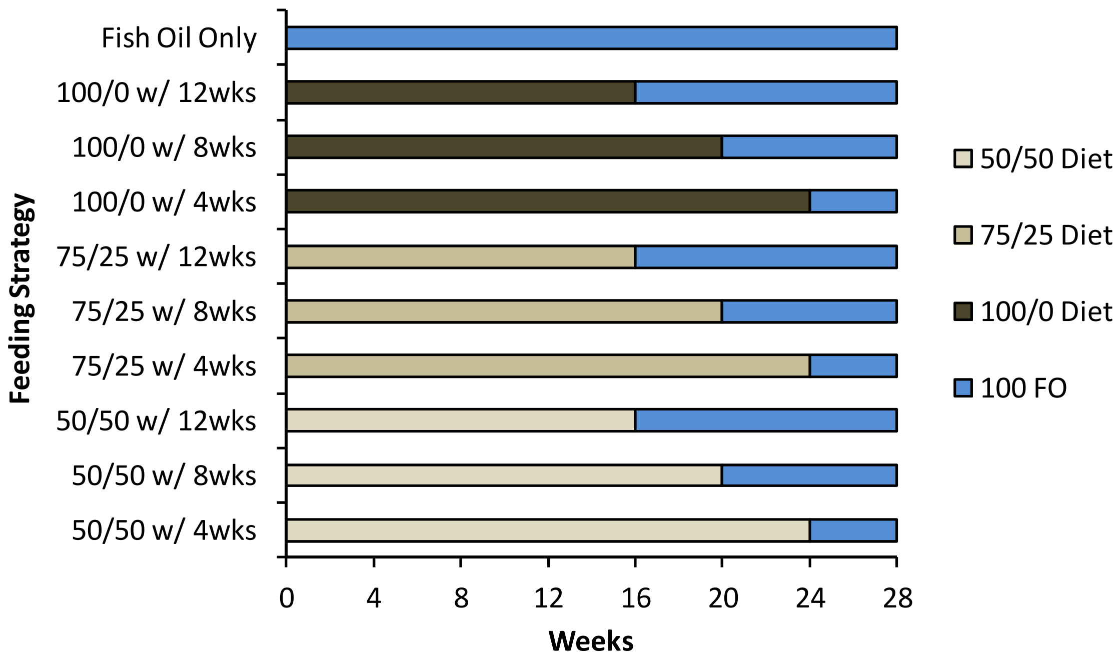

Figure 1. Schematic illustrating experimental feeding regimens. Experimental regimens represented feeding the grow-out feeds based on fish oil (100 FO), beef tallow (100/0 Diet) or blends of beef tallow and fish oil (50/50 Diet and 75/25 Diet) throughout the feeding trial or in combination with 4, 8, or 12 weeks of finishing period with the control 100 FO feed. The control regimen represented feeding the 100 FO feed throughout the entire feeding trial.