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Project Title: Redefining Essential Fatty Acid Requirements of Fishes in the Context of Rendered Fat-based Aquafeeds

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INDUSTRY SUMMARY

1. Introduction

Aquaculture's demand for marine-derived feedstuffs continues to increase despite record-high pricing for fish meals and oils. The Food and Agriculture Organization has indicated that for many aquaculture species, replacing or sparing fish oil may prove a more significant challenge than finding alternatives to fish meal, noting that while alternative sources of essential amino acids abound, there are few alternative sources of the essential fatty acids found in marine-derived ingredients. Furthermore, fish meal sparing typically exacerbates the fish oil 'bottleneck' by increasing the need for fish oil as a dietary source of long-chain polyunsaturated fatty acids (LC-PUFA) required by many fishes. The issues of least-cost feed formulation and providing of adequate levels of LC-PUFA are further complicated by incomplete knowledge of the LC-PUFA requirements of many fishes. Quantitative essential fatty acid requirements are lacking for many commonly cultured fishes, and none of the studies conducted to-date have taken the interactive effects of overall dietary profile into account. Data generated by the Principal Investigator's laboratory suggests that saturated fatty acid (SFA) and monounsaturated (MUFA)-rich alternative lipids, including rendered animal fats, can make utilization of available LC-PUFA more efficient. We hypothesized that using SFA- and MUFA-rich beef tallow as the primary alternative to fish oil may effectively reduce minimum LC-PUFA requirements and allow for greater fish oil sparing in aquafeeds.

2. Objective

Determine the relative requirements of long-chain polyunsaturated fatty acids EPA and DHA in beef tallow-based feeds for Atlantic salmon *Salmo salar*, hybrid striped bass *Morone chrysops* x *M. saxatilis*, and pompano *Trachinotus carolinus*.

3. Industry Summary

We evaluated growth performance and tissue fatty acid profiles of juvenile hybrid Striped Bass *Morone chrysops* × *M. saxatilis*, Pompano *Trachinotus carolinus*, and Atlantic Salmon *Salmo salar* fed diets containing menhaden fish oil (FISH ONLY), beef tallow (BEEF ONLY), or beef tallow amended with purified sources of eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) to achieve levels corresponding to 50% or 100% of those observed in the FISH ONLY feed (BEEF + 50% EPA, BEEF + 100% EPA, BEEF + 50% DHA, BEEF + 100% DHA, BEEF + 50% BOTH, BEEF + 100% BOTH). Diets were randomly assigned to replicate tanks of fish (*N* = 3-4, 10 fish/tank), and fish were fed assigned diets for a period of 8-10 weeks. Results suggest that beef tallow may be used as a direct replacement for menhaden fish oil in practical diets for a number of fish species, but performance may be improved by supplementation with LC-PUFA, particularly DHA. The three trials described herein have expanded our knowledge and understanding of fatty acid requirements in fish and have demonstrated that beef tallow has considerable value—perhaps even strategic value compared to traditional, plant-derived oils—as an ingredient in aquafeeds. Presentations of these results at national and international meetings have generated great interest in the aquaculture community; manuscripts are in various stages of completion (several in preparation, one in review) and, once published, will increase the awareness of and interest in beef tallow as an ingredient in aquafeeds.

Deliverables To-date

Professional Presentations

G.M. Turchini, J.A. Emery, J. Trushenski, F. Norambuena. 2014. DISTINGUISHING THE NUTRITIONAL REQUIREMENTS AND PHYSIOLOGICAL FATE OF DIETARY EPA AND DHA IN ATLANTIC SALMON. Journal of Nutrition & Intermediary Metabolism. 1, pg 9 (Conference abstract) http://dx.doi.org/10.1016/j.jnim.2014.10.023

G.M. Turchini, J.A. Emery, J. Trushenski, F. Norambuena. 2014. DISTINGUISHING THE NUTRITIONAL REQUIREMENTS AND PHYSIOLOGICAL FATE OF DIETARY EPA AND DHA IN ATLANTIC SALMON. 38th National Conference of the Nutrition Society of Australia. Nutrition: Challenges & Opportunities 26-28 November, 2014, Hobart, Tasmania, Australia

J.A. Emery, F. Norambuena, C. Trullas, J. Trushenski, G.M. Turchini. 2014 EPA AND DHA SUPPLEMENTATION IN ATLANTIC SALMON: REDIFINING ESSENTIAL FATTY ACID REQUIREMENTS. 16th International Symposium on Fish Nutrition and Feeding, ISFNF2014, 25-30 May 2014, Cairns, Queensland, Australia

Rombenso, A.N., and J.T. Trushenski. 2014. MARINE FINFISH LC-PUFA REQUIREMENTS: INSIGHTS GAINED FROM YELLOWTAIL, WHITE SEABASS, AND FLORIDA POMPANO. World Aquaculture 2014, Adelaide, Australia.

Trushenski, J.T., J. Bowzer, and C. Jackson. 2015. Beef tallow as an alternative to fish oil in hybrid striped bass feeds. Aquaculture America, New Orleans, LA.

Trushenski, J.T. 2015. Update on nutrition research at Southern Illinois University Carbondale, Invited presentation to Striped Bass Growers' Association. Aquaculture America, New Orleans, LA.

Trushenski, J.T. 2015. OPPORTUNITIES FOR RENDERED PRODUCTS IN AQUACULTURE: ADVANCING SCIENCE AND INDUSTRY THROUGH PARTNERSHIP. International Production and Processing Expo, Atlanta, GA.

Trushenski, J.T. 2014. THE ROLE OF RENDERED PRODUCTS IN AQUACULTURE FEEDS. International Production and Processing Expo, Atlanta, GA.

Trushenski, J.T. 2014. Opportunities for rendered products in aquaculture: advancing science and industry through partnership, Invited. National Renderers Association Annual Convention, Palm Springs, CA.

Manuscripts

Bowzer, J., C. Jackson, and J. Trushenski. HYBRID STRIPED BASS FEEDS BASED ON FISH OIL, BEEF TALLOW, AND EPA/DHA SUPPLEMENTS: INSIGHT REGARDING FISH OIL SPARING AND N-3 LONG-CHAIN POLYUNSATURATED FATTY ACID REQUIREMENTS. North American Journal of Aquaculture, *in review.*

MANUSCRIPT SECTIONS

Scientific Abstract

Previous research suggests that saturated fatty acid (SFA)-rich lipids, including beef tallow, can make utilization of available long-chain polyunsaturated fatty acids (LC-PUFAs) more efficient in aquatic livestock. We hypothesized using beef tallow as an alternative to fish oil may effectively reduce LC-PUFA requirements of hybrid Striped Bass *Morone chrysops* × *M. saxatilis*, Pompano *Trachinotus carolinus*, and Atlantic Salmon *Salmo salar* and allow for greater fish oil sparing. Accordingly, we evaluated growth performance and tissue fatty acid profiles of juvenile fish fed diets containing menhaden fish oil (FISH ONLY), beef tallow (BEEF ONLY), or beef tallow amended with purified sources of eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) to achieve levels corresponding to 50% or 100% of those observed in the FISH ONLY feed (BEEF + 50% EPA, BEEF + 100% EPA, BEEF + 50% DHA, BEEF + 100% DHA, BEEF + 50% BOTH, BEEF + 100% BOTH). Diets were randomly assigned to replicate tanks of fish (*N* = 3-4, 10 fish/tank), and fish were fed assigned diets for a period of 8-10 weeks. With respect to Hybrid Striped bass, survival (98—100%) was equivalent among treatments, but weight gain (117—180%), specific growth rate (1.1—1.5% body weight/d), feed intake (1.4—1.8% body weight/d), and feed conversion ratio (FCR; 1.1—1.4, dry matter basis) varied. Except for FCR, no differences were observed between the FISH and BEEF treatments, but performance was generally superior among fish fed the BEEF + 100% DHA, BEEF + 50% BOTH, and BEEF + 100% BOTH diets. Regarding Pompano, production performance was largely unaffected by dietary lipid source and fatty acid composition. Weight gain (183-223%), specific growth rate (1.85-2.09% body weight/d), feed intake (3.37-3.71% body weight/d), and FCR (1.60-1.85, dry matter basis) varied slightly among dietary treatments, but not significantly. Fish fed the negative control feed BEEF ONLY exhibited equivalent performance as those fed the positive control feed FISH ONLY. Supplementing beef tallow-based diets with DHA alone had a greater effect on growth than supplementing in combination with EPA or with EPA alone. With respect to Atlantic salmon, (*N* = 3, 30 fish/tank, 14-week feeding period) survival (99—100%) was also equivalent among treatments. Weight gain was consistent, apart from BEEF ONLY out-performing BEEF + 50% EPA 50% (~250%), though no difference in specific growth rate (~1.3% body weight/d) was observed. Feed intake was highest in BEEF ONLY and lowest in BEEF + 100% BOTH (3.0—2.2% body weight/d). Feed conversion ratio was lowest among fish fed the FISH ONLY feed, but equivalent in all other treatments (0.9—1.0) varied. Organosomatic indices did not vary. Tissue fatty acid composition was significantly distorted in favor of SFAs and monounsaturated fatty acids among fish fed the beef tallow-based feeds; however, changes in fatty acid profiles were generally less overt than those typically induced by C_{18} polyunsaturated fatty acid-rich plant oils. Results suggest that beef tallow may be used as a direct replacement for menhaden fish oil in practical diets for a number of fish species, but performance may be improved by supplementation with LC-PUFA, particularly DHA.

Key Words: Hybrid Striped Bass, Pompano, Atlantic Salmon, long-chain polyunsaturated fatty acid, beef tallow

Introduction

State of World Aquaculture

Traditional fisheries are increasingly unable to meet seafood demand. To balance the deficit between fisheries landings and demand, aquaculture has emerged as a source of quality seafood and is consequently the fastest growing sector of livestock production. According to the Food and Agriculture Organization (United Nations), global aquaculture production has increased at an average annual rate of 8.9% since 1970, and currently, 45% of the fish consumed worldwide is farm-raised. Assuming per capita seafood consumption is maintained, aquaculture production must double to keep pace with human population growth over the next 20 years (FAO 2010).

Further expansion of aquaculture will require expansion of the aquafeed industry. Traditionally, aquafeeds contained large quantities of fish meal and fish oil because of their nutritional value as feedstuffs, palatability to aquatic livestock, and historically competitive pricing and availability. Fish meal and fish oil production reached its peak in the late 1980s at roughly 6 million metric tons (MT) and 1 million MT, respectively, yet demand continues to increase. Increased demand and fluctuating productivity led to a volatile fish meal/oil market. Throughout the early 2000s, prices were ~$650/MT, but in 2006, prices spiked to nearly $1400/MT; current prices fluctuate between $1600 and $1800/MT. Aquaculture is the single largest consumer of fish meal and fish oil, and the industry must reduce its reliance on these feedstuffs to maintain economic viability.

Challenges of Sparing Marine Feedstuffs in Aquafeeds

Given the high protein content of most aquafeeds (30-50%) and the high cost associated with the protein fraction of any livestock feed, the search for fish meal alternatives has been a higher priority than the search for alternatives to fish oil. The prioritization of fish meal sparing research was also supported by the fact that moderate levels of fish oil sparing are unlikely to result in reductions in aquafeed acceptance or growth performance. However, as fish nutrition has progressed, the importance of fish oil as a source of long-chain polyunsaturated fatty acids has become increasingly apparent, along with the need to perhaps reprioritize research endeavors to include greater emphasis on lipid nutrition and fish oil sparing. Although alternative proteins may not contain the same levels or optimal balance of essential amino acids found in FISH MEAL, the essential amino acids themselves are abundant in a range of potential feed ingredients. Conversely, there are few sources of long-chain polyunsaturated fatty acids not sourced from marine fisheries; those fungal, algal, and other non-fish meal/fish oil sources of LC-PUFA that do exist are quite costly and are not economically viable ingredients for aquafeeds at this time. Based on this information and aquaculture's increasing monopoly of global fish oil supplies, the FAO has suggested that fish oil will be a more serious constraint than fish meal for some aquaculture species (FAO 2010).

Species whose natural diet includes nutrient-dense food items like marine fishes and invertebrates tend to have greater demands for protein and lipid, and often exhibit requirements for nutrients, including fatty acids, which are considered only conditionally essential or expendable for other taxa. The species for which fish oil sparing is especially problematic are those species with requirements for LC-PUFA ARA (arachidonic acid, 20:4n-6), EPA (eicosapentaenoic acid, 20:5n-3), and DHA (docosahexaenoic acid, 22:6n-3) in addition to the essential C_{18} PUFA required by all vertebrates, linoleic acid (18:2n-6) and alpha-linolenic acid (18:3n-3). Whereas some species are capable of producing the bioactive LC-PUFA from their physiologically inert precursors, others cannot complete the necessary biosynthetic transformations at rates sufficient to meet demand for LC-PUFA. These species, including most marine fishes and some carnivorous freshwater taxa, must be provided with intact LC-PUFA in the diet to ensure normal growth and survival. Although it is likely that most if not all species would benefit from the direct provision of LC-PUFA, most carnivorous taxa simply cannot do without these essential nutrients. As the pressures of least-cost formulation encourage greater and greater levels of fish oil sparing, it will become increasingly important to accurately define the minimum LC-PUFA requirements for these species.

Previous Research on Subject by Investigators:
It is difficult to meet the nutritional needs of demanding fishes while minimizing feed cost and reliance on limited marine resources. This challenge is further complicated by the unknown quantitative nutrient requirements for many species and the scarcity of alternative sources of nutrients currently provided by marine ingredients. This problem is particularly well-illustrated by the complications which arise when sparing or replacing fish oil in feeds for carnivores, such as cobia *Rachycentron canadum*. We observed cobia growth performance to be reduced when fish oil is replaced with soybean oil (Trushenski et al. 2011a). Based on this study and the work of others, it has been suggested that cobia require both EPA and DHA, but only combined 'requirement' estimates have been reported (Chou et al. 2004; Trushenski et al. 2011a). Our knowledge of LC-PUFA requirements is similarly vague for freshwater carnivores, including rainbow trout *Oncorhynchus mykiss* and hybrid striped bass *Morone chrysops* x *M. saxatilis*: although complete fish oil replacement typically impairs growth and survival (Lewis and Kohler 2008), only approximate 'total n-3' fatty acid requirements have been reported (NRC 2011) However, in subsequent research with cobia (Trushenski et al. 2012), we demonstrated that soybean oil can effectively replace fish oil in cobia feeds if partially augmented with DHA (Figure 1). This study also revealed that cobia require little-to-no EPA in the diet—a major finding which suggests that fish oil sparing may be more feasible than originally thought given that nutritionists need only concern themselves with maintaining adequate dietary levels of DHA, not EPA and DHA or total n-3 LC-PUFA. Given the number of purified and semi-purified sources of LC-PUFA that are now available, it is possible to reevaluate the fatty acid requirements of fishes with greater precision. This, in turn, will allow for greater precision in feed formulation and fish oil sparing.

Going beyond the idea of absolute fatty acid requirements, it is possible that the amount of LC-PUFA needed in the diet to meet the fish's requirements may vary based on the alternative lipid used to spare fish oil. We have observed the extent of tissue fatty acid profile modification to differ among hybrid striped bass (Trushenski et al. 2008), Nile tilapia (Trushenski et al. 2009), and rainbow trout (Trushenski et al. 2011b, 2011c) fed different alternative lipids. We have observed the reduction in fillet LC-PUFA content typically associated with fish oil sparing to be attenuated when saturated fatty acid (SFA) –rich alternative lipids are used. More specifically, we have observed improved retention of LC-PUFA within the tissues when feeding reduced fish oil diets containing coconut oil, palm oil, SFA-enriched soy oil, as well as various SFA-rich rendered fats (Trushenski et al. 2011c). We have suggested that this is the result of optimized selective fatty acid metabolism: providing SFA instead of the monounsaturated (MUFA) and C_{18} polyunsaturated fatty acids (C_{18} PUFA) associated with most plant oils prevents MUFA and C_{18} PUFA from outcompeting LC-PUFA for tissue deposition and other uses. Most recently, we have observed this phenomenon in white seabass *Atractoscion nobilis*, wherein the LC-PUFA conservation associated with dietary provision of SFA has translated into differences in production performance (Figure 2; Trushenski et al., *accepted*), adding further credibility to our hypothesis that SFA-rich oils effectively reduce the LC-PUFA requirements of fishes. If undesirable competition between the less desirable MUFA/ C_{18} PUFA and the beneficial LC-PUFA can be avoided, it may be possible to meet the essential fatty acid requirements of fish with fewer of these precious nutrients. In short, if the appropriate alternative lipids can be identified, it may be possible to meet LC-PUFA requirements and maintain survival and growth performance of cultured fish using less fish oil.

Various rendered lipid sources contain substantial amounts of SFA and MUFA, and these differences may give rise to differences in apparent LC-PUFA requirements. These products, including pork lard, yellow grease, beef tallow, and others, are readily available and may represent more economical and strategic choices for aquafeed formulation. Although these products have been dismissed by some aquaculture nutritionists because of their relatively low PUFA content, more recent data suggests this characteristic may be what sets rendered fats apart as ideal alternative lipids for aquafeeds. Although beef tallow, pork lard, and poultry fat have all yielded excellent results in our work with rendered fats to-date, recent results we have generated using different rendered fats in feeds for cobia and rainbow trout suggest that beef tallow is the most appropriate choice in terms of maintaining growth performance and limiting the loss of LC-PUFA from the tissues. Accordingly, our objective was to determine the relative requirements of EPA and DHA in rendered fat-based feeds for Hybrid striped bass *Morone chrysops* x *M. saxatilis*, Pompano *Trachinotus carolinus*, Atlantic Salmon *Salmo salar*.

Experimental Procedures (Materials and Methods)

Previously validated practical feed formulations were used as the control formulations for the feeding trials (Table 1). These feeds contained a moderate level of menhaden fish meal, and menhaden fish oil (Virginia Gold™, Omega Protein, Inc., Houston, Texas) served as the primary lipid source in the positive control feeds ("FISH ONLY"). Similar feeds containing beef tallow as the primary lipid source were used as negative control feeds ("BEEF ONLY"). Experimental feeds were developed from the negative control formulations by supplementing the rendered fat base with LC-PUFA concentrates (Incromega DHA 500TG and Incromega EPA 500TG; Croda, Inc., East Yorkshire, United Kingdom) to achieve dietary concentrations of EPA and/or DHA equivalent to 50% or 100% of the levels found in the fish oil based positive control feed as follows: "BEEF + 50% EPA", "BEEF + 100% EPA", "BEEF + 50% DHA", "BEEF + 100% DHA", "BEEF + 50% EPA + DHA", and "BEEF + 100% EPA + DHA". All feeds were analyzed in triplicate to confirm moisture/dry matter, protein, lipid, and ash content according to standard methods. Reserved crude lipid samples were transmethylated and analyzed to determine fatty acid composition via standard GC methodology.

All culture and husbandry methods, as well as euthanasia and sample collection procedures described, were conducted under the direction and approval of the Southern Illinois University Institutional

Animal Care and Use Committee (IACUC), Deakin University IACUC, and/or Virginia Tech IACUC as appropriate.

The Hybrid Striped Bass trial was conducted at the Center for Fisheries, Aquaculture, and Aquatic Sciences (CFAAS; Carbondale, IL) in recirculating aquaculture system consisting of fiberglass tanks equipped with bead filters, temperature control, and a diffusion aeration system. The Pompano trial was conducted at the Virginia Seafood Agricultural Research and Extension Center (VSAREC; Hampton, VA) in a recirculating aquaculture system consisting of fiberglass tanks, equipped with fluidized-bed biofilters, bubble bead filters, protein skimmers, UV sterilization, immersion titanium heaters and a diffusion aeration system. The Atlantic Salmon trial was conducted at the Deakin University Aquaculture Research Facility (DUARF; Warrnambool, Australia) in a recirculating aquaculture system consisting of replicated culture tanks, and a physical and biological filtration plant. Water temperature and dissolved oxygen (DO), and pH were monitored daily using standard water quality monitoring meters. Total ammonia-, nitrite- and nitrate-nitrogen were quantified weekly by spectrophotometric analysis. Throughout each trial, water quality was maintained within optimal ranges for the relevant taxon.

Experimental feeds were randomly assigned to replicate tanks (*N* = 3-4, depending on the trial) and fish were fed assigned diets daily to apparent satiation for a period of 8-10 weeks. At harvest, all fish were counted and group weighed by tank, and standard metrics of growth performance were calculated as follows:

$$\text{Weight Gain (\%)}=100\times\frac{\text{(average final weight-average initial weight)}}{\text{average initial weight}}$$

$$\text{Feed Conversion Ratio (FCR)}=\frac{\text{average individual dry matter feed intake}}{\text{average individual weight gain}}$$

$$\text{Specific Growth Rate (SGR, \% body weight/day)}=100\times\frac{\ln\text{average final weight}-\ln\text{average initial weight}}{\text{days of feeding}}$$

$$\text{Feed Intake (\% body weight/day)}=100\times\frac{\text{average individual dry matter feed intake}}{\text{(initial individual weight}\times\text{final individual weight)}^{0.5}\Big/\text{days of feeding}}$$

Fish were subsampled from each tank and euthanized by tricaine methanesulfonate (MS-222) overdose (exposure to sedation solution until cessation of opercular movement, ~ 5 minutes), and then dissected to remove liver, white muscle, eye, and brain tissue samples. Liver samples will be weighed in order to calculate Hepatosomatic Index, and then all tissue samples were frozen and stored at -80°C prior to analysis. Fatty acid profile of the harvested tissues was analyzed in a manner similar to that described for feeds.

Although multiple fish will were sampled from each tank, replicate tanks served as the experimental units for all statistical analyses (*N* = 3-4). Data were analyzed by one-way analysis of variance (ANOVA) to determine significance of treatment effects. For parameters exhibiting significant treatment effects, Tukey's HSD tests were used for pairwise comparison of means to determine significance of differences among dietary treatment groups. For all statistical procedures, differences will be considered significant at $p < 0.05$.

Results

<u>Hybrid Striped Bass</u>

Survival (range = 98—100%) was equivalent among treatments, but weight gain (117—180%), specific growth rate (1.1—1.5% body weight/d), feed intake (1.4—1.8% body weight/d), and feed conversion ratio (1.1—1.4, dry matter basis) of juvenile hybrid Striped Bass varied with dietary lipid source and fatty acid composition (Table 2). No differences were observed between the FISH ONLY and BEEF ONLY treatments with respect to these parameters, but performance was generally superior among fish fed the BEEF + 100% DHA, BEEF + 50% BOTH, and BEEF + 100% BOTH diets. Supplementation with DHA alone or in combination with EPA had a greater effect on growth than with supplementation with EPA alone.

Tissue fatty acid profiles largely reflected the fatty acid composition of the feeds. Tissues of fish fed beef tallow generally contained higher levels of MUFA (32.1-40.3 vs. 30.4 g MUFA/100 g FAME; particularly 18:1n-9), and lower levels of LC-PUFA (15.4-25.9 vs. 27.2 g LC-PUFA/100 g FAME; particularly EPA and DHA), except when the diet had been supplemented with these fatty acids. In general, fillet levels of 18:1n-9 decreased with increasing supplementation of EPA and/or DHA in the diet, though the levels remained significantly elevated compared to those observed in the FISH ONLY treatment (22.8-29.4 vs. 15.7 g 18:1n-9/100 g FAME). Fillet levels of n-6 fatty acids varied somewhat among the treatments (11.0-13.8 g n-6/100 g FAME) as did the composition of the n-6 fraction, with tissues of fish fed the beef tallow feeds typically containing more 18:2n-6 and less 20:4n-6 unless supplemented with LC-PUFA concentrates. Fillet levels of n-3 fatty acids followed the pattern observed for LC-PUFA, with increasing EPA and/or DHA supplementation reversing the reduction in n-3 fatty acid content associated with feeding beef tallow (18.7-25.3 vs. 15.1 g n-3/100 g FAME) in lieu of fish oil (27.0 g n-3/100 g FAME). Overall, fillet PUFA levels were lower among fish fed the beef tallow-based feeds compared to those fed the FISH ONLY feed (30.2-38.6 vs. 40.2 g PUFA/100 g FAME), though C_{18} PUFA levels were significantly elevated in the BEEF ONLY treatment (13.8 vs. 10.9-12.0 g C_{18} PUFA/ 100 g FAME). These trends were observed in all tissues, but peripheral tissues (i.e., fillet, liver, eye, and intraperitoneal fat) were more strongly influenced by dietary fatty acid composition than the central tissue (i.e., brain) as evidenced by the higher ranges of Coefficient of Distance (D_{jh}) values associated with the former.

<u>Pompano</u>

Production performance of Pompano was largely unaffected by dietary lipid source and fatty acid composition (Table 3). Weight gain (183-223%), specific growth rate (1.85-2.09% body weight/d), feed intake (3.37-3.71% body weight/d), and feed conversion ratio (1.60-1.85, dry matter basis) varied slightly among treatments, but not significantly. Juvenile Pompano fed the negative control feed BEEF ONLY exhibited equivalent performance as those fed the positive control feed FISH ONLY, however, fish fed BEEF + 50% DHA, and BEEF + 100% DHA feeds exhibited the highest growth, numerically. Supplementing beef tallow-based diets with DHA alone had a greater effect on growth than supplementing in combination with EPA or with EPA alone.

Tissue fatty acid composition was affected by dietary treatments mirroring dietary fatty acid profiles. In general, fish fed beef tallow-based diets exhibited greater content of MUFA (33.1-39.6 g MUFA/100 g FAME, particularly 18:1n-9) and lower levels of LC-PUFA (15.1-21.7 g LC-PUFA/100 g FAME) in their tissues compared to those fed the positive control FISH ONLY diet (29.3 g MUFA/100 g FAME; 23.1 g LC-PUFA/100 g FAME). However, this effect was less overt with increasing supplementation of EPA and/or DHA. Although 18:1n-9 was somewhat reduced with supplementation of EPA and/or DHA in fillet tissue, it was still elevated compared to FISH ONLY group (24.7-30.7 vs. 15.4 g 18:1n-9/100 g FAME). With regard to fillet LC-PUFA deposition, EPA and/or DHA supplementation was able to restore and also surpass LC-PUFA levels observed among fish fed the FISH ONLY feed. Fillet levels of n-3 fatty acids and PUFA exhibited similar trend with BEEF + 100% EPA feed restoring these fatty acids levels, and BEEF + 100% BOTH feed surpassing those levels. Generally, beef tallow-based diets were associated with higher fillet levels of n-6 fatty acids, particularly 18:2n-6, compared to the positive control FISH ONLY diet. The addition of EPA and/or DHA reduced this effect with BEEF + 100% BOTH diets, resulting in equivalent fillet levels of these fatty acids as observed among fish fed the FISH ONLY feed. No difference was observed regarding fillet C_{18} PUFA content. A greater degree of tissue profile distortion was observed in peripheral tissues such as fillet, liver, and eye tissue than was noted for the centralized tissues of the brain.

<u>Atlantic Salmon</u>

All experimental diets were readily accepted by fish, mortality was low (< 1%) and equivalent between diets. Fish gained on average ~250% of their initial body weight. Significant differences in growth between treatments were recorded for final average weight and weight gain (Table 4). Final average weight and weight gain of fish fed the BEEF ONLY feed was the highest recorded (203g), and significantly greater than the final weight of fish fed the BEEF + 50% EPA and BEEF + BOTH 100%; no differences were present between other treatments. Fish fed the FISH ONLY feed displayed a significantly lower FCR (0.9) than those fed the BEEF + 50% EPA feed; no difference was observed between BEEF ONLY and FISH ONLY nor were any other differences recorded for FCR. Organosomatic indices did not vary among treatments.

Fatty acid composition of fillet tissue mirrored that of the feeds. Fillet levels of SFA were low in the BEEF + 100% DHA and BEEF + 100% BOTH (201 and 211 mg/g lipid, respectively) compared with all other treatments (~237 mg/g lipid). Fillets of fish fed the BEEF ONLY feed contained the highest levels of MUFA (430 mg/g lipid), whereas fish fed the FISH ONLY and BEEF + 100% BOTH contained the lowest levels (258 and 274 mg/g lipid, respectively). This primarily corresponded to differences in dietary levels of 18:1n-9 (340, 156 and 211, mg/g lipid, respectively). Fillet levels of 18:2n-6 were lowest in the BEEF ONLY treatment (33 mg/g lipid), with significantly higher levels recorded in BEEF + 100% DHA, BEEF + 50% BOTH, and BEEF + 100% BOTH treatments (37, 38 and 37 mg/g lipid, respectively). The BEEF + 100% BOTH yielded significantly higher fillet levels of 20:4n-6 compared to all other treatments (9.1 mg/g lipid), whereas the FISH ONLY, BEEF + 100% EPA, and BEEF + 50% BOTH feeds yielded significantly higher levels compared to the BEEF + 50% DHA, which yielded the lowest content (7.7, 7.2, 7.0 and 5.7 mg/g lipid, respectively). There was no significant difference in fillet EPA content between the BEEF ONLY, BEEF + 50% DHA and BEEF + 100% DHA treatments, these treatments exhibited the lowest content with 8.6, 9.4 and 12.0 mg/g lipid respectively. Fillets of fish fed BEEF + 100% BOTH contained significantly higher levels of EPA than BEEF + 100% EPA, though this feed yielded higher levels than the FISH ONLY feed (65, 59 and 55 mg/g lipid, respectively); no difference was recorded between BEEF + 50% EPA and BEEF + 50% BOTH (33.3 and 33.3 mg/g lipid, respectively). Fillets of fish fed the BEEF + 100% BOTH feed contained a significantly higher level of DHA than all other treatments including FISH ONLY, FISH ONLY was also higher than DHA 100% (109, 99 and 88 mg/g lipid respectively). BEEF+BOTH 50% contained higher levels of DHA than BEEF+DHA 50% (77 and 65 mg/g lipid respectively), no difference was present between BEEF+EPA 50% and BEEF+DHA 100% (67 and 65 mg/g lipid, respectively). BEEF ONLY contained the lowest level of DHA (34 mg/g lipid). Fillet levels of total n-3 LC PUFA followed similar dietary trends, though the BEEF + 100% BOTH feed yielded significantly higher levels of these fatty acids in comparison with the FISH ONLY feed (215 and 194 mg/g lipid, respectively). The BEEF + 50% EPA feed yielded higher fillet levels of n-3 LC-PUFA than the BEEF + 50% DHA and BEEF + 100% DHA feeds (129, 94 and 110 mg/g lipid, respectively). Fillet n-3:n-6 ratios essentially followed the same trends as were present in the respective diets: the FISH ONLY and BEEF + 100% BOTH treatments yielded the two highest n-3:n-6 ratios (4.1 and 4.0, respectively) followed by BEEF + 100% EPA (3.3). No differences were present between BEEF+EPA 50 % and BEEF+BOTH 50% (2.7 and 2.6, respectively), nor BEEF+DHA 50% and BEEF+DHA 100% (1.8 and 2.0 respectively); the BEEF ONLY treatment yielded the lowest n-3:n-6 (1.2).

Discussion

Although our results are somewhat surprising, it is encouraging that the virtually the same response has been observed in each trial. The lack of difference between our positive and negative controls is somewhat unusual, but consistent with our previously observed results with a SFA-rich, hydrogenated soybean oil fed to White Seabass and beef tallow fed to Rainbow Trout. We anticipated that using SFA-rich beef tallow would effectively 'reduce' the essential fatty acid requirements of Pompano, Atlantic Salmon, and Hybrid Striped Bass; what we didn't anticipate is that the requirements were reduced to the point of being adequately met by residual lipid from fish meal. Although we caution readers to take the short-term nature of these feeding trials and the number of taxa we have tested into consideration when attempting to extrapolate the results we have generated, we believe our results indicate beef tallow is suitable for use in aquaculture feeds as a primary lipid source and may be a strategic choice among alternatives to fish oil.

Conclusions

The three trials described herein have expanded our knowledge and understanding of fatty acid requirements in fish and have demonstrated that beef tallow has considerable value—perhaps even strategic value compared to traditional, plant-derived oils—as an ingredient in aquafeeds.

Presentations of these results at national and international meetings have generated great interest in the aquaculture community; manuscripts are in various stages of completion (several in preparation, one in review) and, once published, will increase the awareness of and interest in beef tallow as an ingredient in aquafeeds.

Figures/tables

Table 1. Positive (FISH ONLY) and negative (BEEF ONLY) control feed formulations. Formulations are based on previously validated feeds for each taxon (Trushenski et al. 2008, 2011a-d; Riche et al. 2009; G. Turchini, personal communication).

Ingredient	FISH ONLY (g/kg)	BEEF ONLY (g/kg)
Pompano Formulations		
Fish meal	350	350
Soy bean meal	235	235
Fish oil	115	0
Blood meal	100	100
Wheat bran	95	95
Corn gluten meal	75	75
Carboxymethyl cellulose	20	20
Choline chloride	6	6
Micronutrient premixes	4	4
Beef tallow	0	115
Atlantic Salmon Formulations		
Fish meal	394	394
Fish oil	173	0
Beef tallow	0	173
Wheat flour	115	115
Soy protein concentrate	100	100
Blood meal	60	60
Poultry byproduct meal	51	51
Wheat gluten	50	50
Pregel maize starch	40	40
Micronutrient premixes	7	7
Monosodium phosphate	5	5
Methionine chloride	1	1
Hybrid Striped Bass Formulations		
Wheat bran	202	202
Fish meal	200	200
Corn gluten meal	140	140
Fish oil	98	0
Beef tallow	0	98
Soybean meal	300	300
Carboxymethyl cellulose	20	20
Sodium phosphate	15	15
Dicalcium phosphate	15	15
Choline chloride	6	6
Micronutrient premixes	4	4

Table 2. Production performance of Hybrid Striped Bass by dietary treatment. Values represent means ± pooled SE. Means with common letter labels are not significantly different (*P* > 0.05); absence of letter labels indicates the omnibus ANOVA test failed to reveal a significant treatment effect.

Performance parameter	FISH ONLY	BEEF ONLY	BEEF + 50% EPA	BEEF + 100% EPA	BEEF + 50% DHA	BEEF + 100% DHA	BEEF + 50% BOTH	BEEF + 100% BOTH
Initial weight (g)	23.9 ± 0.3	24.1 ± 0.3	23.6 ± 0.3	23.7 ± 0.3	23.9 ± 0.3	23.7 ± 0.3	23.7 ± 0.3	23.5 ± 0.3
Final weight (g)	58.0 ± 3.3 abc	53.9 ± 3.3 bc	59.4 ± 3.3 abc	51.6 ± 3.3 c	59.4 ± 3.3 abc	66.2 ± 3.3 a	64.1 ± 3.3 ab	65.2 ± 3.3 a
Weight gain (%)	142 ± 14 ab	124 ± 14 b	152 ± 14 ab	117 ± 14 b	148 ± 14 ab	180 ± 14 a	170 ± 14 a	177 ± 14 a
Feed Intake (% bw/d)	1.56 ± 0.11 ab	1.72 ± 0.11 ab	1.57 ± 0.11 ab	1.41 ± 0.11 b	1.74 ± 0.11 ab	1.73 ± 0.11 ab	1.77 ± 0.11 a	1.71 ± 0.11 ab
FCR	1.17 ± 0.05 bc	1.44 ± 0.05 a	1.14 ± 0.05 bc	1.23 ± 0.05 bc	1.29 ± 0.05 ab	1.11 ± 0.05 c	1.18 ± 0.05 bc	1.11 ± 0.05 c
SGR	1.28 ± 0.08 ab	1.17 ± 0.08 b	1.33 ± 0.08 ab	1.12 ± 0.08 b	1.31 ± 0.08 ab	1.49 ± 0.08 a	1.44 ± 0.08 a	1.47 ± 0.08 a
Survival	97 ± 2	100 ± 2	97 ± 2	100 ± 2	97 ± 2	100 ± 2	100 ± 2	100 ± 2

Table 3. Production performance of Pompano by dietary treatment. Values represent means ± pooled SE. Means with common letter labels are not significantly different (*P* > 0.05); absence of letter labels indicates the omnibus ANOVA test failed to reveal a significant treatment effect.

Performance parameter	FISH ONLY	BEEF ONLY	BEEF + 50% EPA	BEEF + 100% EPA	BEEF + 50% DHA	BEEF + 100% DHA	BEEF + 50% BOTH	BEEF + 100% BOTH
Initial weight (g)	41.3 ± 0.3	41.3 ± 0.3	41.2 ± 0.3	40.7 ± 0.3	41.1 ± 0.3	40.6 ± 0.3	41.1 ± 0.3	40.7 ± 0.3
Final weight (g)	126.3 ± 7.4	120.8 ± 7.4	122.2 ± 7.4	115.3 ± 7.4	133.8 ± 7.4	130.7 ± 7.4	124.8 ± 7.4	125.0 ± 7.4
Weight gain (%)	206 ± 18	192 ± 18	197 ± 18	183 ± 18	223 ± 18	222 ± 18	203 ± 18	207 ± 18
Feed Intake (% bw/d)	3.42 ± 0.14	3.71 ± 0.14	3.47 ± 0.14	3.37 ± 0.14	3.61 ± 0.14	3.49 ± 0.14	3.40 ± 0.14	3.45 ± 0.14
FCR	1.64 ± 0.12	1.85 ± 0.12	1.71 ± 0.12	1.75 ± 0.12	1.63 ± 0.12	1.60 ± 0.12	1.63 ± 0.12	1.64 ± 0.12
SGR	1.99 ± 0.11	1.91 ± 0.11	1.94 ± 0.11	1.85 ± 0.11	2.09 ± 0.114	2.08 ± 0.11	1.98 ± 0.11	2.00 ± 0.11
Survival	100	100	100	100	100	100	100	100

Table 4. Production performance of Atlantic Salmon by dietary treatment. Values represent means ± SD. Means with common letter labels are not significantly different (*P* > 0.05); absence of letter labels indicates the omnibus ANOVA test failed to reveal a significant treatment effect.

Performance parameter	FISH ONLY	BEEF ONLY	BEEF + 50% EPA	BEEF + 100% EPA	BEEF + 50% DHA	BEEF + 100% DHA	BEEF + 50% BOTH	BEEF + 100% BOTH
Initial weight (g)	53.3±1.8	53.2±0.9	53.6±2.9	52.9±0.9	53.1±2.4	53.1±1.1	53.3±1.4	53.1±0.5
Final weight (g)	187.2±3.3 ab	203.3±6.3 b	171.1±10.6 a	191.5±3.6 ab	199.2±3.3 ab	191.6±7.6 ab	177.1±8.0 ab	167.3±7.4 a
Weight gain (%)	251.8±8.9	282.4±8.2	220.1±16.9	261.9±7.5	276.5±17.6	260.8±7.9	233.0±22.0	215.1±15.7
Feed Intake (% bw/d)	1.06±0.03 ab	1.21±0.05 b	1.11±0.05 ab	1.14±0.02 ab	1.19±0.03 ab	1.12±0.02 ab	1.06±0.04 ab	1.03±0.03 a
FCR	0.93±0.01 a	1.01±0.04 ab	1.04±0.02 b	0.99±0.00 ab	1.01±0.02 ab	0.97±0.00 ab	0.97±0.01 ab	0.98±0.01 ab
SGR	1.28±0.03	1.37±0.02	1.18±0.05	1.31±0.02	1.35±0.05	1.31±0.02	1.22±0.07	1.17±0.05
Survival	98.9±1.1	100.0±0.0	97.8±1.1	98.9±1.1	97.8±1.1	98.9±1.1	100.0±0.0	100.0±0.0

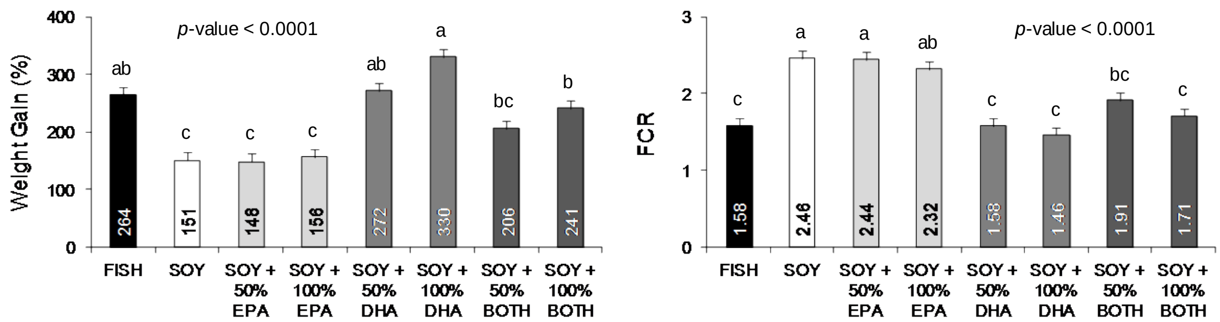

Figure 1. Weight gain and feed conversion ratio (FCR) of juvenile cobia fed diets containing fish oil (FISH), soy oil (SOY ONLY), or soy oil supplemented with purified sources of eicosapentaenoic acid (SOY + 50% EPA, SOY + 100% EPA), docosahexaenoic acid (SOY + 50% DHA, SOY + 100% DHA), or both (SOY + 50% BOTH, SOY + 100% BOTH) to achieve 50% or 100% of the levels of these fatty acids typically found in fish oil. Columns represent least square-means (specified as numerals at the base of each column) and error bars represent pooled standard error throughout. *P*-values generated by one-way ANOVA tests are provided; means with common letters are not significantly different ($P > 0.05$) . Data illustrate the critical need for DHA supplementation to maintain production performance in juvenile cobia, and the apparent expendability of EPA supplementation in this context.

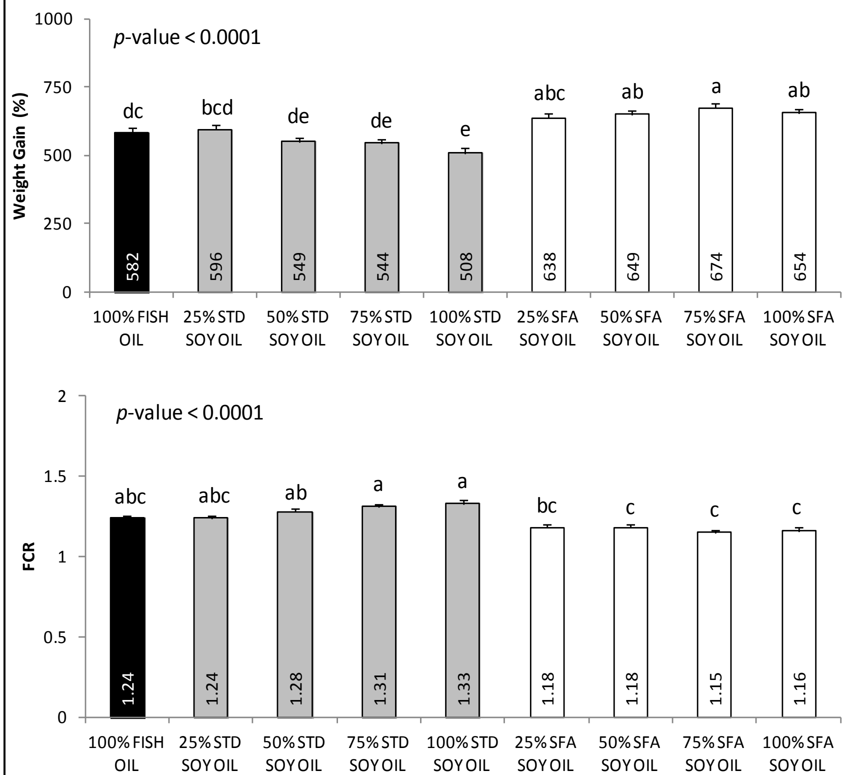

Figure 2. Weight gain and feed conversion ratio (FCR) of juvenile white seabass fed diets containing fish oil or increasing amounts (25, 50, 75, or 100% fish oil replacement) of standard soybean oil (grey columns) or saturated fatty acid-enriched soybean oil (white columns). Columns represent least square-means (specified as numerals at the base of each column) and error bars represent pooled standard error throughout. *P*-values generated by one-way ANOVA tests are provided; means with common letters are not significantly different ($P > 0.05$) . Data illustrate the negative effects of replacing fish oil with standard soybean oil, but not with saturated fatty acid-enriched soybean oil. These data underscore the likelihood that requirements for long-chain polyunsaturated fatty acids found in fish oil may be effectively reduced by provision of saturated fatty acid-rich alternative lipids.

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