

## Potential, Implications and Solutions Regarding the Use of Rendered Animal Fats in Aquafeeds

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**Abstract: Problem statement:** In the past, aquafeeds were comprised largely of fish meal and fish oil derived from marine reduction fisheries. In addition to being highly palatable and readily digested by cultured fishes, these feedstuffs were historically inexpensive sources of protein, energy and essential nutrients. However, increasing cost and concerns over safety and sustainability have greatly incentivized the transition from fish meal and oil to alternative sources of protein and lipid for aquafeed formulation. Fish oil replacement is proving more difficult than originally anticipated, particularly for marine carnivorous species. **Approach:** If complete fish oil replacement is not a viable goal for fish nutritionists and aquafeed manufacturers, at a minimum, we must strive for judicious use of limited marine-derived resources. In the present review, we explore the opportunities of using rendered fats as alternatives to marine-derived fish oils in aquaculture feeds, beginning with a discussion of the products themselves before reviewing the most recent literature and concluding with a discussion of the future of these products in aquafeed formulations. **Results:** Rendered fats have not been as intensively evaluated in aquaculture nutrition as grain and oilseed-derived lipids, although a number of recent publications on the subject suggest increasing interest in the use of rendered products in aquafeeds. **Conclusion:** Poultry fat, beef tallow, pork lard and to a lesser extent, yellow/restaurant grease and catfish oil, have been investigated individually or in combination with other lipids in feeds for a broad range of cultured taxa with generally acceptable results.

**Key words:** Aquaculture, fish oil, alternative lipid, rendering, fatty acid, fish nutrition

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### INTRODUCTION

In the past, aquafeeds were comprised largely of products derived from marine reduction fisheries, i.e., fish meal and fish oil. In addition to being highly palatable and readily digested by cultured fishes, these feedstuffs were historically inexpensive sources of protein, energy and essential nutrients. However, in the face of growing demand and relatively static landings, the price of fish meal and oil has grown steadily since the mid-1980s<sup>[1]</sup>. In addition to market volatility driven by seasonal and inter-annual variations in landings, the fish meal and oil market is also affected by fluctuations in the price of competing commodities such as soybean meal and oil. As global interest in biofuels began to drive the price of grains and oilseeds at the beginning of the 2000s, fish meal and fish oil prices skyrocketed. In

2000, fish meal and oil were both marketed at roughly \$400 USD/MT; today, the price of fish meal is \$1200 USD/MT and fish oil is priced in excess of \$1600 USD/MT<sup>[1]</sup>. Rising costs have been joined by the issues of sustainability and product safety in creating substantial incentives to reduce the use of marine-derived feedstuffs in aquafeeds: Environmental advocates have long-criticized aquaculture's reliance on capture fisheries and the practice of "feeding fish to fish"<sup>[2]</sup> and recently fish meal and fish oil have been linked with elevated levels of environmental contaminants in farm-raised seafood<sup>[3-5]</sup>.

The need to identify alternative raw materials for the burgeoning aquafeed industry is clear, but advances have been slowed by the difficulties in finding suitable alternatives to fish meal and particularly fish oil. Fish oil contains high levels of long-chain polyunsaturated

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fatty acids (LC-PUFA, No. of carbons  $\geq 20$ , No. of double bonds  $\geq 3$ ), such as 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA), which are known to have a positive impact on a variety of human health disorders and conditions and are required micronutrients for many finfishes. Excluding specialty oils derived from algal or fungal cultures, fish oil alternatives contain, at most, trace levels of these fatty acids. The Food and Agriculture Organization addressed this pitfall in the most recent State of World Fisheries and Aquaculture report, concluding that "...given the difficulty in replacing fish oils...it is clear that competition for fish oil is likely to be a more serious obstacle [than fish meal] for some sections of the aquaculture industry"<sup>[1]</sup>. It is likely that fish oil will always been used in aquafeeds to some extent-complete replacement may be simply unattainable for some taxa or life stages<sup>[6]</sup>. If complete fish oil replacement is not a viable goal for fish nutritionists and aquafeed manufacturers, at a minimum, we must strive for judicious use of limited marine-derived resources. In the present study, we explore the opportunities of using rendered fats as alternative to marine-derived fish oils in aquaculture feeds, beginning with a discussion of the products themselves before reviewing the most recent literature. We revisit issues raised in a previous review of rendered fats in finfish nutrition<sup>[7]</sup>, providing the most up-to-date information available on the subject and conclude with a discussion of the future of these products in aquafeed formulations.

#### **Production and characteristics of rendered animal fats:**

**Production of rendered animal fats:** Rendering is the process by which waste animal tissues-usually from the slaughtering industry-are converted into more stable products of higher quality. Rendering produces protein meals and fats and the fats are often refined further to produce products such as tallow and lard. Rendering processes are distinct for "edible" and "inedible" products, which refers to the suitability of the products for human consumption. Fats produced by either process are suitable for inclusion in animal feeds, but the cost of the inedible grades is usually lower and they are more commonly used in fish feeds. The vast majority of rendered fats are from mammalian or avian sources, but in the Southern US, enough catfish offal oil is produced as a byproduct of the channel catfish industry to include in commercial feeds for catfish<sup>[8]</sup>. Other sources of rendered animal fats include mortalities of farmed animals, pets and zoo animals, as well as butcher trimmings and restaurant grease<sup>[9]</sup>. On average, raw materials contain about 20% lipid before

the rendering process. In 2005, 10.9 billion pounds of fat was produced by the US rendering industry. Inedible tallow, primarily from beef, accounted for more than one third (3.9 billion pounds) of the total<sup>[9]</sup>.

The rendering process for edible products is often conducted in a continuous, "wet" system, whereby ground raw materials are heated at a low temperature (with or without steam). The water and fat are separated from the solids by centrifugation and then a second centrifugation separates the water from the fat. Rendering for inedible products is usually a "dry" method in which the raw material is ground and heated to release fats and remove water. The free fat is recovered, then additional fat is pressed from the remaining solids to increase yield.

**Lipid refinement:** Crude lipids contain impurities such as phospholipids, free fatty acids, aldehydes, ketones, water and pigments. Various degrees of purification are applied to the crude lipids to improve quality and stability. Common purification processes include degumming, neutralization, bleaching and deodorizing. Degumming removes phospholipids and water, neutralization removes water and bleaching removes pigments, minerals, free fatty acids, aldehydes and ketones. Deodorizing removes free fatty acids and other compounds that cause objectionable flavors<sup>[10]</sup>. Winterizing-a common form of dewaxing-improves transparency, brightness and palatability of lipids. Not all of these refining processes are applied to inedible oils, which are the primary type used in animal feeds. However, stability is an important issue for feed manufacturers and lipids of low palatability can reduce feed intake and fish performance irrespective of the lipid's nutritional value. Nearly all rendered fats used in animal feeds are refined so that they are predominantly triglycerides, but they often contain free fatty acids and specifications now allow the addition of free fatty acids to the products. Triglycerides consist of a glycerol backbone esterified to three fatty acids, which may be essential or non-essential. The energy they liberate depends both on the carbon chain length and the degree of unsaturation of the fatty acids. Longer chains yield more energy than shorter chains and saturated chains (with no double bonds) yield more energy than unsaturated chains.

#### **Description of animal fats that have been used in fish diets:**

**Poultry fat:** Obtained from the tissue of poultry in the commercial process of rendering or extracting, containing only the fatty matter natural to the product produced under good manufacturing practices.

**Lard:** Fat rendered from fresh, clean, healthy tissues of swine.

**Tallow (bovine or ovine):** Product obtained by rendering the clean, sound, fatty tissues (including trimming and cutting fats), attendant muscles and bones of bovine animals and/or sheep in good health at the time of slaughter

**Choice white grease:** A specific grade of mostly pork fat.

**Yellow grease:** Recovered restaurant greases from deep fryers; may include tallow.

**Catfish offal oil:** Produced from the clean, dried and ground tissue and bones of fresh catfish parts, consisting primarily of heads, fins, viscera, skins, fillet frames and trim parts obtained from freshwater farm-raised catfish in the US.

**Menhaden fish oil:** Oil extracted from fresh, whole menhaden in a wet reduction process.

**Chemical and physical properties of fats used in fish diets:** After rendered fats are produced and refined, their chemical and physical properties are characterized (Table 1). These features are used in manufacturing specifications, along with the fatty acid composition and can be used to determine the suitability of different lipids for different functions, including use as feedstuffs for animal diets. Lipid characteristics can also be used to determine whether a fat is pure, adulterated, or an intentional blend (such as an animal/vegetable blend).

Specific gravity is defined as the ratio of the density of a given solid or liquid substance to the density of water at a specific temperature and pressure. Different fats are very similar in specific gravity (Table 1). Viscosity is an internal property of a fluid that offers resistance to flow. Viscosity varies both among lipid types and with temperature. This property

can have implications for feed manufacturing, as more viscous fats might slow the extrusion process. Color is measured using a variety of methods (Table 1). The FAC color is based on standards of the Fat Analysis Committee of the American Oil Chemists' Society (AOCS). FAC colors of 1-9 indicate light-colored fats, FAC 11-series colors are considered very yellow, FAC 13-19 indicates dark, reddish fat, FAC 21-29 are greenish fats and FAC 31-45 are very dark fats. Color can be influenced by diet, age, condition, geographical location and breed of the animal from which the fat is rendered. Although additives can be used to alter the color of rendered fats, this is not usually done to fats destined for use in livestock feeds. The MIU stands for moisture, impurities and unsaponifiable matter. Moisture should be less than 1% because higher levels can reduce the energy of the fat and cause oxidation. Impurities are non-hazardous materials that impede the filtering process during refining. Unsaponifiable matter (including sterols and pigments) does not convert chemically to soap and is low in available energy. The iodine value is a measure of the unsaturation of a lipid. Lipids with fatty acids containing more double bonds such as menhaden fish oil have higher iodine values. Lipids with high iodine values typically have low melting points and titers (Table 1). The titer is the point at which a saponified fat resolidifies and is a measure of the "hardness" of a fat. Greases have titers below 40 and tallow has a titer above 40. The melting point is the temperature at which a solid transitions to a liquid state. Some fats such as fish oils are liquid at room temperature (25°C) and have melting points below that. Terrestrial animal fats are generally solid at room temperature and have higher melting points. Boiling point is defined as the temperature at which the vapor pressure of a liquid equals the environmental pressure surrounding the liquid. The boiling point of all fats is much higher than that of water (100°C) and in some cases the lipids do not boil but decompose instead. Temperatures used in extrusion production of floating fish feeds are well below the boiling point of lipids.

Table 1: Chemical and physical characteristics of rendered animal fats

Characteristic	Poultry fat	Lard	Tallow (beef or mutton)	Choice white grease	Yellow grease	Catfish offal oil	Menhaden fish oil
Specific gravity, 15-15.6°C	0.91	0.94	0.89-0.91	0.84	0.92	0.8	0.92
Viscosity, 90-100°C except where noted (cSt = cps = mPas)	8.36	62.1	8.97	25 (at 38°C)	8.45	60 (at 30°C)	10
Color (various methods)	Lt-brown liquid/pale brown solid	White	<3 red (AOCS Wesson)	Yellow liquid/pale brown solid; FAC color 13-11B	Med-brown liquid /lt. brown solid; FAC 39 (max)	Light-medium tan	7 (Gardner)
MIU (%)	1.3-2.8	0.25	0.44	0.78	1.17-1.35	1.10	≤0.5
Iodine value (Wijs)	83.9	57.6	3-50	64-66	104	89	172
Titer (°C)	34.98	38.0	42.40	36-37	30.25	NR <sup>1</sup>	32.0
Melting point (°C)	35.36	36-42	44.72	32.5	33.87	<20	13.0
Boiling point (°C)	Decomposes	<700	Decomposes	<700	Decomposes	>200	>250
Free fatty acids (%)	15 max	15 max	10 max	15 max	5-10	3-5	0.2
Gross energy (kcal kg <sup>-1</sup> )	9000	9020	9020	9350	9372	NR <sup>1</sup>	9020

<sup>1</sup>: Not reported

Table 2: Selected fatty acid composition (percent of total fatty acids by weight) of rendered animal fats<sup>1</sup>

Fatty acids	Poultry fat	Lard	Tallow (beef)	Tallow (mutton)	Choice white grease	Yellow grease	Catfish offal oil	Menhaden fish oil
10:0	ND	0.1	0.1	0.2	ND	ND	ND	ND
12:0	0.2	0.2	0.1	0.3	0.1	ND	ND	ND
14:0	0.9	1.3	3.7	4.5	1.5	2.43	1.4	8.48
16:0	21.6	23.8	24.9	21.5	30.3	23.24	17.4	17.52
18:0	6.0	13.5	18.9	19.5	7.5	12.96	6.1	3.10
SFA <sup>2</sup>	27.5	39.2	47.5	46.0	39.4	38.60	24.9	29.10
14:1	0.2	ND	0.2	0.3	ND	0.20	ND	ND
16:1	6.4	2.7	4.2	2.3	3.7	3.97	2.9	11.51
18:1 <sup>3</sup>	37.3	41.2	36.0	37.6	46.0	44.32	49.1	9.52
22:1	0.1	ND	ND	ND	ND	ND	ND	0.67
MUFA <sup>4</sup>	44.1	43.9	40.4	40.2	49.7	48.50	52.0	21.7
18:2n-6	19.5	10.2	3.1	5.5	9.7	6.97	11.9	1.96
18:3n-3	1.0	1.0	0.6	2.3	0.4	0.67	1.22	2.18
20:4n-6	0.1	ND	ND	ND	ND	ND	4.5	2.55
20:5n-3	ND	ND	ND	ND	ND	ND	0.99	12.73
22:5n-3	ND	ND	ND	ND	ND	ND	0.97	2.78
22:6n-3	ND	ND	ND	ND	ND	ND	1.09	12.31
n-6 <sup>5</sup>	19.6	10.2	3.1	5.5	9.7	6.97	16.41	4.95
n-3 <sup>6</sup>	1.0	1.0	0.6	2.3	0.4	0.67	4.27	33.3
n-3 LC-PUFA <sup>7</sup>	ND	ND	ND	ND	ND	ND	3.05	27.82
n-3/n-6 Ratio	0.05	0.10	0.20	0.40	0.04	0.10	0.26	6.73
Unsaturation Index <sup>8</sup>	86.5	67.3	48.4	58.1	70.3	64.4	113.8	208.2

<sup>1</sup>: Fatty acids present at  $\leq 0.1$  g/100 g are not included in the table, ND = not detected; <sup>2</sup>: Saturated fatty acids, total of all fatty acids without double bonds; <sup>3</sup>: Total of n-7 and n-9 isomers; <sup>4</sup>: Monounsaturated fatty acids, total of all fatty acids with a single double bond, includes 20:1 in addition to individually reported MUFA; <sup>5</sup>: Total n-6 fatty acids, includes 20:3n-6 in addition to individually reported n-6 fatty acids; <sup>6</sup>: Total n-3 fatty acids includes 18:4n-3 in addition to individually reported n-3 fatty acids; <sup>7</sup>: Long-chain polyunsaturated fatty acids in the n-3 series, total of all fatty acids with chain length  $\geq 20$  carbon atoms and double bonds  $\geq 3$ ; <sup>8</sup>: Unsaturation index =  $\sum$  [(weight percent of unsaturated fatty acid)  $\times$  (number of double bonds)]

Free fatty acids in fats result from partial hydrolysis of the triglycerides during processing and sometimes storage prior to use in diets. The FFA value is expressed as a percentage of a fatty acid common to the product being tested, such as oleic acid for tallows. Aside from indicating instability or spoilage, free fatty acids (along with aldehydes, ketones and other compounds) are primary contributors to “off-flavors” and reduced palatability. Therefore, specifications on feed lipids usually list maximum allowable inclusion rates for free fatty acids. Rates up to 15% may be allowed in lower quality fats, but most contain from 1-10% (Table 1). Gross energy is a measure of the energy liberated when a fat is burned in a bomb calorimeter. All fats are concentrated energy sources and most feed fats are highly digestible by fish (see digestibility and utilization below). The addition of fats can also reduce fines associated with pelleted feeds as well as enhance flotation of extruded pellets.

**Fatty acid composition:** Rendered animal fats from mammalian and avian sources differ in fatty acid composition from one another and from fish oils (Table 2). None of the mammalian or avian sources contain the high concentrations of n-3 LC-PUFA found in marine fish oils (around 30% 20:5n-3+22:6n-3).

Catfish offal oil contains more n-3 LC-PUFA than mammalian or avian fats, but less than marine fish oils. Marine fish oils also contain at least 3-fold more arachidonic acid (ARA, 20:4n-6) and approximately 5-fold more cholesterol than other animal fats. However, many animal fats do contain significant amounts of C<sub>18</sub> n-6 fatty acids (such as 18:2n-6) and low to moderate amounts of C<sub>18</sub> n-3 fatty acids (such as 18:3n-3). There are other minor differences among the terrestrial animal fats. For instance, pure beef tallow differs from pure ovine (goat and sheep) tallow in that ovine tallow contains decanoic (10:0) and lauric (12:0) acids while beef tallow does not. These differences have little bearing on the energy content of lipids, which are all similar. There are numerous combinations of lipids that could meet the essential fatty acid requirements of different fish<sup>[11]</sup> and provide energy. However, marine and carnivorous fishes generally require the n-3 LC-PUFA found in highest concentrations in marine fish oils and terrestrial animal fats do not provide these. Some carnivorous fish perform well on diets with very little fish oil, but the n-3 LC-PUFA content and health value of the product for human consumers decline as a result of feeding low LC-PUFA feeds. Rendered animal fats from terrestrial sources can potentially be used at higher concentrations in diets of omnivorous or herbivorous fishes.

**Lipid quality and stability-assessment and preservation:**

**Lipid decomposition:** As soon as a fat is produced, it begins to undergo a variety of chemical and physical changes. Lipid stability and quality can be compromised by factors such as moisture, heat, exposure to oxygen and exposure to metals or other damaging agents such as free radicals. The degree of unsaturation of a lipid is a major determinant of its tendency to oxidize or decompose. Unsaturated lipids contain double bonds that are targets for attack by free radicals, thus, highly unsaturated lipids such as marine fish oils are particularly vulnerable to attack. The byproducts of lipid oxidation, including peroxides and aldehydes, are associated with rancid flavors and aromas and can affect feed palatability and acceptance. Additionally, the oxidation process is progressive, with one free radical begetting another. Thus when added to a compound feed, rancid lipids can destroy vitamins and can affect the stability of proteins and carbohydrates.

There are a number of methods used to assess the integrity of lipids either before or after inclusion in feeds.

The AOM (Active Oxygen Method) predicts the stability of a fat by bubbling air through a solution of the fat using specific conditions of flow rate, temperature and concentration. At intervals, peroxides and hydroperoxides are measured by titration with iodine. The AOM value is defined as the number of hours required for the peroxide concentration to reach 100 meq kg<sup>-1</sup> of fat. The more stable the fat, the longer it will take to reach that level. This method is still used, but is very slow for stable fats (up to 48 h).

The Oxidative Stability Index (OSI) method is a more rapid, automated method to predict fat stability. Air is passed through a sample held at constant temperature, then bubbled through a reservoir of deionized water. Volatile acids produced by lipid oxidation are dissolved in the water, increasing its conductivity. Conductivity of the water is monitored continuously and the OSI value is defined as the hours required for the rate of conductivity change to reach a predetermined value.

In contrast to predictive methods, the Peroxide Value (PV), Anisidine Value (AnV) and the Thiobarbituric Acid Reactive Substances (TBARS) tests are used to assess the oxidation that has already occurred. Lipid decomposition is accompanied by characteristic compounds that appear at different stages of the process, or in different quantities depending on the severity of the decomposition<sup>[12]</sup>. Often, more than one test is used to determine the degree of lipid

decomposition, because each test is fairly specific for compounds that appear at different stages of decomposition. For instance, peroxides appear early in the oxidation process, but they degrade into secondary products that are not detectable with a peroxide test. Therefore, the PV of a lipid tested after several months of exposure to harsh conditions (such as high temperature) could be just as low as the PV done on a fresh, high-quality product, obscuring the greater degree of oxidation in the more degraded product. It is thus difficult to correlate a specific PV with rancidity, but fresh fats have very low PV's of 1-2 and fats with PV of 15-20 are usually considered rancid.

The TBARS test specifically measures Malondialdehyde (MDA), another secondary decomposition product derived from polyunsaturated fatty acids. The MDA combines with thiobarbituric acid, yielding the TBARS. This is a well-established method for screening and monitoring lipid peroxidation. A higher TBARS number corresponds to a greater degree of rancidity. In general, oils with TBARS above 1.0 ppm may be considered rancid.

The Anisidine Value (AnV) may be performed in conjunction with the PV test, because the AnV detects secondary products of lipid decomposition such as non-volatile aldehydes (e.g., 2-alkene). High anisidine values may indicate lipid oxidation even when TBARS and other aldehyde tests give low results, because volatile aldehydes may be removed during processing. Anisidine value is defined as 100 times the absorbance (at 350 nm) of a solution resulting from reaction of 1 g of fat in 100 mL of solvent. Choice white grease with an AnV of 10.6 was described as slightly rancid in one study<sup>[13]</sup>, but freshly produced peanut oil had an AnV of 20 in another study<sup>[14]</sup>. Therefore, it is difficult to compare the practical significance of AnVs among studies.

All of the methods of measuring lipid oxidation and rancidity have advantages and limitations due to the dynamic nature of the lipid decomposition process and the potential for interference by chemically similar products. Although more sophisticated tests are available to measure specific products of lipid peroxidation, they generally require expensive, sophisticated instrumentation (e.g., gas chromatograph/mass spectrophotometer) and may not be practical for routine quality control.

**Antioxidant content:** Both natural and synthetic compounds are used in feed lipids to prevent degradation due to chemical reactions with oxygen, or enzymatic reactions<sup>[15]</sup>. Although the FA composition of rendered fats make them more resistant to oxidation

than other, more unsaturated lipids, under unfavorable environmental conditions (i.e., high temperature, moisture, light, exposure to oxygen and metal salts) and in the absence of antioxidants, rancidity can develop. Rancid lipids have greatly reduced nutritional value and can compromise fish health and product quality<sup>[16-18]</sup>. Both fish tissues and most feed lipids contain vitamin E (tocopherols and tocotrienols) the primary fat-soluble antioxidant class in living cells. Vitamin E stored within the tissues will act to counteract free radicals ingested with rancid lipid until the vitamin E molecules themselves are depleted. If vitamin E stores are depleted and free radicals remain, the signs of vitamin E deficiency will manifest. These signs include fragile cell membranes, fluid accumulation in the abdomen (ascites), anemia, liver pathology, muscular dystrophy and reduced immune function<sup>[19,20]</sup>. Although animal tissues will contain vitamin E and other natural antioxidants (i.e., vitamin C and carotenoids), these compounds will be largely destroyed (by heat) or removed (i.e., by degumming or bleaching) during the rendering process. Accordingly, various antioxidants are added to rendered fats after processing to enhance stability and shelf-life. Although vitamin E is routinely added to fish feeds due to its essentiality as a vitamin, the form used most commonly is a synthetic racemic mixture of tocopherols in an esterified form (all-rac- $\alpha$ -tocopheryl acetate), although esterified natural source vitamin E (RRR- $\alpha$ -tocopheryl acetate) may also be used<sup>[21,22]</sup>. The esterified tocopherol molecules are more light- and heat stable and thus preferred for extrusion applications, but these compounds have no antioxidant activity until consumed and converted back to the bioactive alcohol form via gastric hydrolysis. Therefore, to protect feeds and feedstuffs from oxidation during storage, other antioxidants may be used in place of, or more commonly in conjunction with vitamin E. Common synthetic antioxidants approved by the US Food and Drug Administration (FDA) for use in animal feeds include ethoxyquin (dehydroacetic acid), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These antioxidants protect lipids from oxidation during and after manufacturing, thus extending the shelf life of the feed. Recently, numerous reports of antioxidant activity in a variety of natural products (i.e., extracts of fruits, seeds, spices and teas) have surfaced<sup>[23]</sup>. The 'green revolution' would favor more natural ingredients in human foods and in animal feeds, but synthetic antioxidants are usually less expensive and more consistent in terms of potency. Given the sharp increase in cost of diets with standard ingredients since 2008, higher-cost natural antioxidants may be limited to use in diets of high-

value species such as ornamentals and organically-produced fish and shellfish. Antioxidants are added to feed fats according to manufacturer or consumer specifications. The FAO Codex Standard for Named Animal Fats<sup>[24]</sup> shows typical inclusion rates for various antioxidants.

**Contaminant burden:** Animal fats do not exhibit hazardous characteristics (i.e., flammable, combustible, corrosive, explosive or radioactive) and they have been designated as Generally Recognized As Safe (GRAS) feedstuffs by the FDA. Normally, there are no hazardous components in animal fats that are irritating, toxic or carcinogenic, however, if the source animals are exposed to environmental contaminants, these compounds can accumulate within the tissues and contaminate terrestrial rendered products<sup>[25]</sup> in the same manner as rendered marine meal and oils. To assess the potential for contamination of livestock tissues with organic contaminants, the US Environmental Protection Agency and U.S. Department of Agriculture (USDA) conducted nationwide surveys of PCBs and dioxin-like compounds in pork, beef and poultry fats<sup>[26-28]</sup>. The results of these surveys indicated the range of contamination for the compounds assessed was less than 1  $\mu\text{g g}^{-1}$  lipid (ppb) toxic equivalence concentrations (TEQ). These concentrations are quite low considering that Hazard Analysis and Critical Control Point plans specify FDA certification of levels below maximum tolerances in the  $\mu\text{g g}^{-1}$  range (ppm). For example for DDT, DDD and DDE, 0.5 ppm; dieldrin, 0.3 ppm and PCBs, 2.0 ppm. In addition, the FDA sets limits on heavy metals such as lead (7 ppm). The FAO codex has more stringent standards, with limits of 0.1 ppm for lead or arsenic.

**Production rates and cost of animal fats:** Total production of selected animal fats in 2008, as well as current prices are shown in Table 3. Production of tallow exceeded that of all other rendered animal fats, followed closely by greases (Table 3). This pattern has been stable for decades. Poultry fats and lards are produced in smaller quantities, but their production is considered more sustainable than that of marine fish oils because the latter originate from wild fish. Catfish oil is not produced in large quantities compared to the other fats, but it could be a more sustainable source of essential fatty acids than menhaden oil, particularly for warm water fish raised in the southern US. The greater stability of catfish oil compared to menhaden oil is also attractive to feed producers, however, availability may become an issue as oil production has also decreased since 2008 due to significant declines in catfish production.

Table 3: Annual US production (2008) and current cost of rendered animal fats

Item	Poultry fat	Lard	Tallow (beef or mutton)	Choice white grease	Yellow grease	Catfish offal oil	Menhaden fish oil
Production (1000s of metric tons)	500-600	100-200	16,000-17,000	13,000 <sup>1</sup>	NR <sup>1</sup>	12.74	55.4
Cents/pound in 2009, to date <sup>2</sup>	25-30	31-33	23-28	26-28	23-27	26	31-69

<sup>1</sup>: Production of white and yellow greases is not reported separately (US Census Bureau); historically, yellow grease has comprised 55-60% of total grease production; <sup>2</sup>: Price ranges reflect differences in geographical origin and quality grades of fat

Although tallow supplies are strong, overall use in animal feeds has declined, possibly due to misguided concerns over Transmissible Spongiform Encephalopathies (TSEs) from animals fed ruminant products. There are no cases of humans or other animals contracting TSEs from fish and the infectious agents (prions) that cause TSEs are not present in rendered fats.

With the exception of fish oil, current prices of the other animal fats are similar. Prices for menhaden fish oil increased sharply in 2008 and early 2009, then declined. However, production is expected to decrease and the cost is likely to increase again in the near future. At the same time, there is a rapidly expanding market for fish oil for human nutritional supplements and functional foods that will increase competition for the limited supply of fish oil. As a result, there will be increasing pressure to reduce or eliminate marine fish oil from aquafeeds.

Increased use of poultry fat and lard in fish diets may be warranted if supplies are sufficient. However, there has been a global decrease in animal production since the onset of the economic crisis and supplies of all rendered animal fats may be restricted in the near future. Average prices for all terrestrial rendered fats have increased 20-30% since 2000 and prices typically increase as supplies dwindle. With increasing competition for many fats from the biofuels industry, the trends in supplies and costs are more difficult to predict. Nevertheless, rendered animal fats have considerable potential to replace most or all marine fish oil in fish diets due to their combination of nutritional qualities, reasonable costs and sustainable methods of production.

**Using rendered animal fats in aquafeeds:** Fish oil replacement is a broadly-researched theme in aquaculture nutrition and a wide variety of alternative lipids have been evaluated<sup>[29]</sup>. Rendered fats have not been as intensively evaluated as grain and oilseed-derived lipids, although a number of recent publications on the subject seems to suggest increasing interest in the use of rendered products in aquafeeds. Poultry fat, beef tallow, pork lard and to a lesser extent, yellow/restaurant grease and catfish oil, have been investigated individually or in combination with other lipids in feeds for a broad range of cultured taxa (Table 4) with generally acceptable results.

**Palatability and acceptance:** Palatability and acceptance are not commonly reported to vary with dietary lipid source. Fish oil replacement is occasionally associated with changes in feed intake, however, this is mostly likely a consequence of altered nutrient density or digestibility of the feeds rather than diminished olfactory or gustatory response to the feeds themselves. Preference for certain flavors or feedstuffs is difficult to quantify in fishes, but the reason for reduced consumption can be inferred if the feed intake is expressed as a percent of body weight. If percent body weight consumed is reduced, one can assume palatability is an issue; if total consumption is reduced but percent body weight intake is unaffected, one can assume the issue is one of reduced digestibility/utilization resulting lower weight gain and a concomitant reduction in nutrient demand. That said, feed intake does not appear to be substantially affected by incorporation of rendered fats in aquafeeds. A vast majority of published studies report no effect whatsoever on feed intake, expressed as either cumulative intake or as a percent of body weight. Although Du *et al.*<sup>[30]</sup> reported increased consumption of diets containing pork lard relative to fish oil-based feeds, when expressed as percent body weight, the differences in intake were minor (3.1 Vs 3.2% BW/day for feeds containing 2% supplemental lipid). Yilmaz and Genc<sup>[31]</sup> reported improved consumption of yellow grease-based diets by common carp, however, this was in comparison with diets containing soy acid oil, an apparently problematic dietary lipid source. Yildirim-Aksoy *et al.*<sup>[32]</sup> reported a reduction in cumulative consumption for Nile tilapia fed a beef tallow-based feed compared to a fish oil-based formulation. Although this was associated with a reduction in standardized feed intake as well, (~3.1 Vs ~2.4% BW/day), suggesting a greater preference for the fish oil feed, the authors attributed the reduction in intake to impaired digestibility and EFA deficiency. Regardless, as previously indicated, these effects are uncommonly reported and a wide variety of aquatic taxa accept rendered fat-based feeds readily.

Table 4: Summary of production performance effects of replacing dietary fish oil with rendered fats, expanded from Bureau *et al.*<sup>[7]</sup>

Species	Diet composition	Effect of replacement on production performance criteria				Reference
		Survival	Weight gain	Conversion efficiency	Consumption	
<b>Warm-water Taxa</b>						
Channel catfish <i>Ictalurus punctatus</i>	2.3% beef tallow+ 2.3% corn oil+2.3% Menhaden oil	=	↑ (at higher temp) = (at lower temp)			[33]
	7% beef tallow	=	↓ (at higher temp) = (at lower temp)			[33]
	2.3% beef tallow+ 2.3% corn oil+2.3% Menhaden oil		↓			[34]
	7% beef tallow		↓			[34]
	2% beef tallow	=	=	=		[97]
	2.3% beef tallow+ 2.3% corn oil+2.3% Menhaden oil		↑			[97]
	7% beef tallow		↓			[97]
	2% beef tallow (4.7%)	=	=			[98]
	4% beef tallow (6.7%)	=	=			[98]
	2% catfish oil (4.7%)	=	=			[98]
	4% catfish oil (6.7%)	=	=			[98]
	2% poultry fat+2% cod liver oil (4% lipid)	=	=	=		[99]
	4% poultry fat (4% lipid)	=	=	=		[99]
	2% beef tallow (~5% lipid)	=	=	=		[100]
	2% catfish oil (~5% lipid)	=	=	=		[100]
<b>Common carp</b>						
<i>Cyprinus carpio</i>	4.5% beef tallow+5% feed oil		↓	=	=	[101]
	9.5% beef tallow+5% feed oil		↓	=	↓	[101]
	12% beef tallow+1.4% linseed oil		=			[102]
	4, 8, or 13% yellow grease (4, 8, or 13% lipid)	=	↑ Compared to soy acid oil	↑	↑	[31]
<b>African sharptooth catfish</b>						
<i>Clarias gariepinus</i>	10% beef tallow		↓ Compared to sunflower oil			[103]
Blue tilapia <i>Tilapia aurea</i>	2-14% beef tallow (2-14% crude lipid)		=	=		[104]
	2.5, 5, 7.5, or 10% catfish oil (2.5-10% lipid)	=	=	=		[105]
Nile tilapia <i>Oreochromis niloticus</i>	2.3% beef tallow	=	=	=	=	[32]
	+2.3% linseed oil					
	+2.3% corn oil (7% lipid)					
	2.3% beef tallow+2.3% corn oil+2.3% fish oil (7% lipid)	=	=	=	=	[32]
	7% beef tallow (7% lipid)	=	↓	↓	↓	[32]
	4.9% poultry fat+4.9% fish oil (9.8% lipid)	=	=	=	=	[93]
Hybrid tilapia (blue × nile)	1.7% lard+1.7% corn oil	=	↑	↓		[106]
	+1.7% cod liver oil (5% lipid)					
	2.5% lard+2.5% cod liver oil (5% lipid)	=	↓	↓		[106]
	2.5% lard+2.5% corn oil (5% lipid)	=	↓	↓		[106]
	5% lard (5% lipid)	=	↓	↓		[106]
	8% lard (8% lipid)	=	↓			[107]
Red drum <i>Sciaenops ocellatus</i>	5.4% beef tallow+ 1% n-3 HUFA mix (6.4% lipid)	=	=	=		[79]
Grass carp <i>Ctenopharyngodon idella</i>	2, 6, or 10% pork lard (2, 6, or 10% lipid)		= (at 2% lipid) ↑ (at 6 and 10% lipid)	= (at 2% lipid) ↑ (at 6 and 10% lipid)	(at 2% lipid) ↑ (at 6 and 10% lipid)	[30]
Fathead minnow <i>Pimephales promelas</i>	10% poultry fat (10% lipid)	=	↑	↑		[108]
Golden shiner <i>Notemigonus crysoleucas</i>	2% poultry fat+2% cod liver oil (4% lipid)	=	=	=		[99]
	4% poultry fat (4% lipid)	=	=	=		[99]
Japanese sea bass <i>Lateolabrax japonicas</i>	5% beef tallow (10% lipid)	=	=	=	=	[109]



Table 4: Continued

	5% pork lard (10% lipid)	=	=	=	=	[109]
	5% poultry fat (10% lipid)	=	=	=	=	[109]
	3% beef tallow+1%	=	=	=	=	[109]
	Soy oil+1% fish oil (10% lipid)					
Largemouth bass	5 % chicken fat+5% fish oil (10% lipid)	=	=	=	=	[63]
<i>Micropterus salmoides</i>	10% chicken fat (10% lipid)	=	=	=	=	[63]
Surubim	4% pork lard	=	=	=	=	[77]
<i>Pseudoplatystoma coruscans</i>	+8% squid liver oil (~19% lipid)					
	6% pork lard+6% squid liver oil (~19% lipid)	=	=	=	=	[77]
	8% pork lard+4% squid liver oil (~19% LIPID)	=	=	=	=	[77]
	12% pork lard (~19% lipid)	=	=	=	=	[77]
<b>Cool-water Taxa</b>						
White sturgeon	5% pork lard+5% corn oil+	=	=	=	=	[73]
<i>Acipenser transmontanus</i>	5% cod liver oil (15% lipid)					
	15% pork lard (15% lipid)	=	=	=	=	[73]
Hybrid striped bass	4.9% poultry fat	=	=	=	=	[80]
<i>Morone chrysops</i> × <i>M. saxatilis</i>	+4.9% fish oil (9.8% lipid)					
	2.5 % catfish oil (2.5% lipid)	=	=	=	=	[110]
<b>Cold-water Taxa</b>						
Rainbow trout	4% hydrogenated beef	=	=	=	=	[111]
<i>Oncorhynchus mykiss</i>	tallow+6% cuttlefish liver oil (10% lipid)					
	6% hydrogenated beef tallow+					
	4% cuttlefish liver oil (10% lipid)	=	=	=	=	[111]
	10% hydrogenated beef tallow (10% lipid)	=	↓	↓		[111]
	4.8% lard+7.2% beef tallow (18% lipid)		↓			[7]
	6% chicken fat (13% lipid)		=	=		[112]
	6% pork lard (13% lipid)		=	=		[112]
	6% beef tallow (13% lipid)		=	=		[112]
	8% beef tallow+8% fish oil (21% lipid)	=	=	=		[7]
	8% beef tallow+8% herring oil (18% lipid)	=	=	=		[7]
	8% beef tallow+8% fish oil (16% lipid)		=	=	=	[36]
	8% beef tallow+8%					
	Alkitol omega-3 (16% lipid)		=	=	=	[36]
	10.2% poultry fat+5% fish oil (15.2% lipid)	=	=	=	=	[94]
Coho salmon	4% beef tallow+12% salmon oil		=			[113]
<i>Oncorhynchus kisutch</i>	8% beef tallow+8% salmon oil (16% lipid)		=			[113]
	12% beef tallow+4% salmon oil (16% lipid)		↓			[113]
	4.3% lard+4.3% canola oil (12% lipid)	=	=	=		[114]
	4.3% lard+4.3% herring oil (12% lipid)	=	=	=		[114]
	8.6% lard (12% lipid)	=	=	=		[114]
Chinook salmon	3% beef suet+1.5% linseed oil		=	=		[115]
<i>oncorhynchus tshawytscha</i>	+3.5% salmon oil (15% lipid)					
	5% beef suet+3% linseed oil (15% lipid)		=	=		[115]
	6.5 % beef suet+1.5% linseed oil (15% lipid)		=	=		[115]
	4.1% lard+4.1% canola oil (16% lipid)		=	=		[116]
	4.1% lard+4.1% herring oil (16% lipid)		=	=		[116]

Table 4: Continued

Atlantic salmon <i>Salmo salar</i>	8.2% lard (16% lipid)	=	=		[116]
	5% beef tallow+1.9% herring oil (20% lipid)	=	=		[117]
	4.5% lard+3.5% corn oil+1% canning oil (9% lipid)	=			[118]
	40-50% of total lipid as poultry fat (dietary lipid increased during the trial from ~30 to ~37% lipid)	=	=	=	[119]
	3.5% poultry fat+3.5% canola oil+16.5% anchovy oil (23.5% lipid)	=	=	=	[74]
Brown trout <i>Salmo trutta</i>	7% poultry fat+7% canola oil +9.5% anchovy oil (23.5% lipid)	=	=	=	[74]
	10.3% pork lard (20% lipid)	=	=	=	[70]
	10.3% poultry fat (20% lipid)	=	=	=	[70]

Note: ↓: Decrease relative to fish oil-based control feed, except where noted; ↑: Increase relative to fish oil-based control feed, except where noted

**Digestibility and utilization:** The digestive system of finishes is apparently robust to a wide range of dietary lipids, however, most taxa conform to broad conventions with respect to digestion and absorption of different lipid and FA classes. The ability of finfish to digest and utilize lipids is a function of the physical state of the lipid source (liquid or solid at environmental temperature), the structural form of the lipid (waxes, sterols, phospholipids and triglycerols) and its fatty acid composition, as well as the innate digestive capacity of the taxon considered (gut morphology and enzyme activities).

Although fishes undoubtedly regulate their body temperature (i.e., through behavior), as poikilotherms, their body temperatures will fluctuate more so than their homeothermic, terrestrial counterparts. Although adaptations to broader temperature ranges have developed (i.e., enzymes with broad thermal ranges of activity, isozymes with disparate thermal optima), this does not compensate for the effect of different temperatures on the physical state of digesta. In other words, a lipid which is in a liquid state within the gastrointestinal tract of a homeotherm may solidify in the gut of cool or cold-water species. Because of their high polyunsaturated FA (PUFA, No. of double bonds  $\geq 2$ ) content, most vegetable oils have freezing points below the ideal thermal range for most cultured fishes. Rendered fats, on the other hand, have much higher freezing points because of their predominantly saturated and monounsaturated fatty acid profile. These fats, particularly tallows and lards, may remain solid at normal culture temperatures, even for warm-water species (Table 1). As most chemical and enzymatic digestive processes are a function of surface area, solidification may dramatically impact digestibility as well as absorption of lipid. Although in one study, implementation of beef tallow in channel catfish feeds

reduced weight gain regardless of fish oil replacement level<sup>[33]</sup>, in a later study, reduced growth was noted at lower temperatures<sup>[34]</sup>. Conversely, channel catfish reared at cooler temperatures grew equally well on feeds containing menhaden or catfish oils, but at warmer temperatures, the menhaden fish oil-fed group outperformed the catfish oil-fed group<sup>[35]</sup>. It is somewhat surprising that these effects were observed in a warm-water species-one might anticipate that the influence of temperature on lipid digestibility would be more pronounced in cool-and coldwater species whose ideal water temperatures are nearer or below the melting points of many rendered fats. On the other hand, one could hypothesize that the digestive systems of these species compensate morphologically (greater surface area, particularly within the pyloric ceca) or enzymatically (lower thermal optima, greater enzyme activity or production) for the effects of temperature on lipid digestibility. The latter appears to be true: Although dry matter digestibility of a beef-tallow based feed was slightly improved among rainbow trout reared at 15°C compared to 7.5°C (apparent digestibility coefficient [ADC] = 82 Vs 79), lipid digestibility was unaffected by temperature (ADC for 7.5°C = 94, ADC for 15°C = 95)<sup>[36]</sup>. Although lipid solidification within the gut may impact digestibility and utilization, the available data suggest the potential influence of temperature on digestibility of rendered fats is not particularly problematic.

Lipid digestibility and utilization are also affected by fatty acid and lipid class composition. The apparent digestibility of phospholipids and wax esters is less than that of triglycerols<sup>[37,38]</sup> and in terms of digestion rates, triglycerols > cholesteryl esters > phospholipids<sup>[39]</sup>. Although perhaps less digestible than triglycerols, dietary phospholipid is nonetheless important for meeting dietary requirements for this nutrient class and

for enhancing lipid digestion and absorption via the emulsifying action of the byproducts of phospholipid hydrolysis<sup>[40]</sup>.

It has been suggested that polyunsaturated fatty acids are preferentially hydrolyzed within the gut<sup>[39]</sup> and fatty acid uptake by enterocytes increases with chain-length and degree of unsaturation<sup>[41,42]</sup>. Based on these data, it follows that the digestibility of PUFA would be greater than monounsaturated FA (MUFA, No. of double bonds = 1) and Saturated FA (SFA, No. of double bonds = 0)<sup>[38,43-45]</sup>, however, higher digestibilities of MUFA and SFA have also been observed<sup>[46,47]</sup>. Turchini *et al.*<sup>[48]</sup> and Francis *et al.*<sup>[49]</sup> also observed the digestibility and absorption of PUFA>MUFA>SFA, but included the negative effect of increasing chain length as a caveat. In these studies, apparent digestibility coefficients were typically in the ~85-95% range, though lower values (~65-75%) were noted for SFA, particularly for longer chain lengths and at lower temperatures. Although it would appear that the relative digestibility of fatty acids and fatty acid classes is controversial, given the variation in culture conditions, taxa and dietary formulations represented by these studies, it is perhaps not surprising that the digestibility values do not always agree. Furthermore, as discussed by Bureau *et al.*<sup>[7]</sup>, the digestibility of SFA may be enhanced by the presence of PUFA and/or MUFA. This appears to be the case: Digestibility of SFA was relatively high (~75-90%) when dietary PUFA levels were high<sup>[38, 47]</sup>, whereas the digestibility coefficients for SFA declined sharply when dietary PUFA content was reduced<sup>[45]</sup>. Utilization of FA for energy production appears to follow a more predictable path. Generally, fish utilize SFA>MUFA>PUFA for energy production via  $\beta$ -oxidation<sup>[50-53]</sup>, although surpluses of PUFA and may increase shunting of these FA into catabolic pathways<sup>[54,55]</sup>.

From these studies, we can conclude that lipid digestion, absorption and fulfillment of energy requirements would be maximized by provision of SFA and MUFA-rich triglycerols, with sufficient amounts of phospholipid and PUFA to meet requirements and facilitate digestion and utilization. From this perspective, rendered fats would seem to be relatively well-suited for use in aquafeeds. Rendered fats contain substantial amounts of SFA, MUFA and PUFA (Table 2), primarily in the form of triglycerols. The expectation that rendered fats would be well-utilized by finfish appears to be borne out in the literature evaluating the use of rendered fats in aquafeeds. In general, rendered lipid-based feeds yield equivalent growth performance to that of fish oil-based feeds (Table 4). Reduced growth performance has been reported, however, in a

majority of these cases, EFA deficiencies are likely to be contributing factors. Although PL may be a limiting factor for some rendered lipids, it is possible that supplementation with soy lecithin or other phospholipid-rich products may correct for reductions in overall lipid digestion and uptake.

**Survival and growth performance:** Survival does not appear to be impacted by incorporation of rendered fats in lieu of fish oil: of the studies reporting survival data, none demonstrated a significant effect of rendered fat source or dietary incorporation level. Though many of these studies were perhaps too short to allow nutritional deficiencies to develop and associated mortalities to occur, in most cases, the dietary formulations were not particularly aggressive with respect to known EFA requirements: i.e., most feeds still contained some basal amount of fish oil and intact LC-PUFA. Although survival encompasses many factors and is not necessarily responsive enough to characterize the nutritional sufficiency of a diet, particularly during the course of a short-term feeding trial, it is, of course, an important contributor to the “bottom line” of any aquaculture operation. The absence of differential mortality in these studies is encouraging and in keeping with complementary studies of plant-derived lipids.

As is the case with survival, growth performance is not typically affected by changes in dietary lipid source, so long as any basal energy and fatty acid requirements are met. Weight gain and conversion efficiency has been assessed for a wide variety of taxa fed diets containing various levels and blends of rendered fats. The results of these studies are summarized in Table 4 and overwhelmingly indicate partial, if not complete, replacement of marine-derived lipid with rendered fats is feasible for a wide range of finfishes.

**Comparative physiological effects:** A number of studies have been conducted to address the possible link between dietary lipid source and the immune system of fishes. Obviously, requirements for energy and essential fatty acids must be met to ensure normal immune function, however, dietary lipid content and composition may have immunological influence beyond basic nutritive value. Dietary lipid source may affect immunocompetence via two primary mechanisms: (1) by affecting membrane fatty acid composition and therefore fluidity, flexibility and function and/or (2) by affecting the inflammatory process and other cell-signaling pathways. Membrane strength and flexibility likely influence the ability of immunocompetent cells such as macrophages to phagocytose and destroy foreign cells and diet-related

changes in lymphocyte membrane composition has been linked to impaired phagocytic function<sup>[56]</sup>. As a biosynthetic precursor to the physiologically potent eicosanoids (2-series prostanoids and 4-series leukotrienes), ARA has emerged as being particularly relevant to immunonutrition of aquatic and other species<sup>[57]</sup>. In addition to absolute ARA levels, the balance between ARA and EPA (precursor to the less-potent eicosanoids, 3-series prostanoids and 5-series leukotrienes) in the tissues appears to influence physiological status in aquatic species. Given the relationship between dietary FA composition and tissue profile, it is not surprising that replacing fish oil with various plant-derived lipids influences eicosanoid production<sup>[58]</sup> and, in turn, processes regulated by eicosanoids such as immune function<sup>[56,59-61]</sup>. However, most of these studies, in spite of having compared feeds with radically different fatty acid profiles, have reported moderate or conflicting immunological effects. Although there would appear to be a relationship between dietary lipid composition and immune function and disease resistance in fishes, the relationship is apparently indirect and nuanced.

Studies of the immunological effects of replacing fish oil with rendered fats have been similarly inconclusive. Feeding pork lard or poultry fat to vundu *Heterobranchus longifilis* resulted in significant alterations of various hematological parameters compared to fish raised on cod liver oil-based feeds<sup>[62]</sup>. In the poultry fat group, circulating red and white blood cell densities were significantly elevated, as was hemoglobin content; the pork lard group was largely unaffected with respect to these response parameters. Numbers of circulating platelets and lymphocytes and mean corpuscular volume (larger red blood cells) were significantly elevated in both rendered fat groups, whereas neutrophils were reduced relative to control. The authors interpreted these results as indicative of an inability of vundu to effectively utilize n-3 LC-PUFA and an immunosuppressive effect of cod liver oil via attenuation of the inflammatory response in these fish (i.e., eicosanoid derivatives of n-3 LC-PUFA tend to be anti-inflammatory). Conversely, one could argue that the altered hematological profiles and serum enzyme activities suggest the rendered fat groups were undergoing some sort of physiological challenge. The authors also noted significant elevation in serum alanine aminotransferase (in both the poultry fat and pork lard-fed groups) and aspartate aminotransferase (in pork lard-fed group only), which may be indicative of hepatic cellular degradation related to LC-PUFA deficiency in these fish. Nonetheless, the authors concluded that all of the observed effects of FO

replacement were relatively mild and that all of the lipids evaluated were suitable for use in vundu feeds.

Lingenfelter *et al.*<sup>[35]</sup> assessed a suite of immunological parameters in channel catfish reared on menhaden fish oil- or catfish oil-based feeds at either 18 or 28°C, before and after vaccination for *Edwardsiella ictaluri*. Although diet did not influence antibody production, cell-mediated immune responses were influenced by diet and temperature. Whereas macrophage phagocytic activity was greater among the catfish oil-fed fish at 28°C, activity was greater in the menhaden oil-fed fish at 18°C; respiratory burst activity was consistently greater in the menhaden-oil fed group. The authors attributed these responses to differences in cell membrane fatty acid composition and fluidity. As did Babalola and colleagues<sup>[62]</sup>, Lingenfelter *et al.*<sup>[35]</sup> ultimately concluded that menhaden oil could be partially replaced by catfish oil in channel catfish feeds, at least at optimal culture temperatures, in spite of the immunological differences observed. Similarly, Subhadra *et al.*<sup>[63]</sup> observed a reduction in complement activity (alternative activation pathway) and some changes in thrombocyte and lymphocyte counts in largemouth bass fed poultry fat-based feeds, but concluded these effects did not necessarily preclude the use of this or other alternative lipids in largemouth bass feeds.

Although no dietary effects were noted for various hematological parameters and measures of nonspecific immunocompetence, Atlantic salmon fed diets containing a blend of poultry fat and canola oil as a partial substitute for anchovy oil demonstrated a marginally reduced response to vaccination with *Listonella anguillarum*<sup>[64]</sup>. The authors suggested reduced dietary availability of C<sub>20</sub> PUFA substrates may have limited eicosanoid production and thus affected the post-vaccination inflammatory response. Although a subsequent disease challenge was not conducted to determine whether these differences in clinical measures would give rise to differences in resistance to *L. anguillarum*, the authors concluded that the fish fed the alternative lipid-based feeds did not show any clear signs of impaired immunocompetence. Channel catfish fed beef tallow solely or blended with menhaden fish and soybean oils exhibited elevated hematocrit, but reduced numbers of circulating leucocytes (total) and thrombocytes<sup>[65]</sup>. The fish fed beef tallow also demonstrated reduced performance in an erythrocyte fragility test, likely resulting from altered red blood cell membrane composition. Although Klinger and colleagues<sup>[65]</sup> observed that dietary lipid source could influence immunological status of channel

catfish, they ultimately concluded that a 100% fish oil based formulation was not necessary.

Generally, the immunological effects of implementing rendered fats in aquafeeds, when observed, appear to be minor. Unfortunately, there is a dearth of disease challenge studies addressing the cumulative immunological effects of dietary lipid. In fact, we are aware of only a single recent study which employed a disease challenge to test the effects of rendered fat on practical disease resistance. In this study, Nile tilapia reared on feeds containing beef tallow as the sole dietary lipid source exhibited impaired production performance, reduced serum lysozyme and complement activities and significantly lower survival following experimental challenge with *Streptococcus iniae*<sup>[32]</sup>. However, these fish were exhibited moderate anorexia and significantly reduced survival prior to the disease challenge. These fish were likely immunocompromised as a result of generally poor nutrition, not as a specific consequence of feeding beef tallow. This conclusion is supported by the fact that equivalent immunocompetence was observed among normorexic tilapia fed beef tallow as an equal-parts blend with other oils. Although reduced feed intake is certainly problematic from a variety of perspectives, use of beef tallow in this study does not appear to have had a direct negative effect on immunocompetence and disease resistance.

Dietary lipid source may affect an assortment of other physiological processes and response criteria as well, though the effect of dietary supplementation with rendered fats is apparently minimal. For example, the reproductive performance of fishes is known to be influenced by maternal lipid nutrition, however, replacing fish oil with poultry fat in feeds for rosy red fathead minnows *Pimephales promelas* had no effect on egg size, percent hatch, or larval size<sup>[66]</sup>. Following a crowding stress challenge, golden shiners exhibit a classical cortisol response, but magnitude and time-course of this response was similar among fish raised on poultry fat or menhaden fish oil-based feeds<sup>[67]</sup>. Similarly, replacing dietary menhaden fish oil with poultry fat had no effect on reproductive performance of channel catfish<sup>[68]</sup>. Atlantic salmon fed a lard-based diet had a small but significant effect on circulating levels of astaxanthin, suggesting greater fillet pigmentation could be achieved by including lard in salmonid feeds<sup>[69]</sup>. Presumably astaxanthin levels increased in the bloodstream because the greater dietary levels of SFA and MUFA enhanced intestinal uptake of this fat-soluble pigment. Hepatic activity of carnitine palmitoyltransferase-I, an enzyme associated with shuttling of LC-PUFA into  $\beta$ -oxidation for energy

production, was significantly reduced among brown trout reared on pork lard-, but not poultry-fat-based feeds<sup>[70]</sup>. Though this may suggest reduced fatty acid catabolism and perhaps active sparing of LC-PUFA, these changes did not translate into altered growth performance or conservation of tissue levels of LC-PUFA. Atlantic salmon fed a predominantly poultry fat-based diet were significantly outperformed by those reared on an anchovy oil-based feed in an exhaustive swimming trial, but in a repeat trial with the same individuals, no difference in critical swimming speed was observed<sup>[71]</sup>. In a follow-up study, Wilson et al.<sup>[72]</sup> observed the swimming performance and cardiovascular capacity of Atlantic salmon fed anchovy or poultry-derived lipids and found no difference between the dietary treatments.

**Effects on tissue composition and quality:** The compositional plasticity of fish tissues with respect to fatty acid profile has been well-documented in a wide range of taxa<sup>[29]</sup>. Although differences in fatty acid uptake, trafficking and utilization give rise to minor differences between dietary and tissue profiles, composition is largely driven by fatty acid intake. Given that most rendered lipids are comprised predominantly of SFA and MUFA, one can expect tissue levels of these fatty acids to increase among fish raised on feeds containing tallows, lards and greases. These changes have been observed in a variety of species and studies.

Tissue proximate composition of grass carp<sup>[30]</sup> and white sturgeon<sup>[73]</sup> did not differ between fish fed fish oil and pork lard-or poultry fat-based feeds, but in both studies, fillet fatty acid profile changed to reflect increased intake of SFA and MUFA. Similar effects were observed in Atlantic salmon fed diets containing increasing amounts of a 1:1 blend of canola oil and poultry fat, though fillets did not become significantly enriched with SFA as the feeds were relatively consistent in terms of SFA content<sup>[74]</sup>. Monounsaturated fatty acids also became enriched within the fillet lipid of brown trout fed poultry fat or pork lard, but minor differences in dietary SFA were not reflected within the fillet profile<sup>[75]</sup>.

Carcass proximate composition of rainbow trout was unaffected by replacing 50% of dietary fish oil with beef tallow, however, the triglycerol profile of the tallow-fed fish reflected a substantial increase in 18:1n-9 intake<sup>[36]</sup>. Phospholipid profile was similarly affected, but in a less dramatic fashion, underscoring the strong preference of this lipid class for certain fatty acid chain lengths and levels of desaturation which typically results in a somewhat conserved fatty acid profile.

Tissue fatty acid composition was similarly affected in surubim<sup>[76,77]</sup> and white sturgeon<sup>[78]</sup> fed pork lard-based feeds: Substantial increases in SFA and MUFA were observed in neutral and/or total lipid, but these differences were somewhat attenuated in the polar lipid fraction. Conversely, feeding beef tallow to red drum had the opposite effect: Neutral and polar lipid became enriched with MUFA among tallow-fed fish, but the effect was most overt within the polar fraction<sup>[79]</sup>. It appears that if the fatty acids preferred for incorporation into phospholipid are available, composition can change rapidly within the lipid class, whereas if these fatty acids are not available, composition will be resistant to dietary modification<sup>[80]</sup>.

The concept of selective fatty acid metabolism and preferential inclusion of certain fatty acids within different lipid classes may hold special relevance for the use of rendered lipids in aquafeeds. As previously noted, dietary lipid composition will influence tissue composition and, in turn, the nutritional value of the resultant fillets to the human consumer. Although alternative lipids, including rendered fats, can be readily incorporated into aquafeeds, the resultant seafood products will contain less of the beneficial LC-PUFA. To balance the conflicting demands to reduce reliance on marine-derived lipids while maintaining product quality, aquaculture nutritionists have begun investigating the use of finishing feeds to compensate for the effects of alternative lipids on fillet fatty acid profile<sup>[81-92]</sup>. In these strategies, fish are raised on a low LC-PUFA content, alternative lipid based grow-out feed for a majority of the production cycle and then are switched to a high LC-PUFA content, fish oil-based finishing feed to modify fillet fatty acid profile immediately prior to harvest. These strategies have proven effective in significantly altering fillet composition in as little as 4 weeks<sup>[93]</sup>. However, the composition of the grow-out feed, i.e., the alternative lipid source used, can affect finishing success<sup>[80, 93]</sup>. In these studies, grow-out feeds rich in SFA and MUFA (provided by coconut oil or poultry fat) yielded the best results in terms of final fillet levels of EPA, DHA and total LC-PUFA. These results suggest rendered fats, as sources of SFA and MUFA, would be particularly appropriate for use during the pre-finishing grow-out period. Further research addressing the use of rendered lipids in context of finishing strategies is certainly warranted.

Manipulations of dietary lipid and fillet fatty acid profile may impact product organoleptic profile and sensory quality. Fillet texture, color, odor, flavor and shelf-life may be influenced by dietary lipid content, fatty acid composition and the presence (or absence) of

lipid soluble pigments. As was noted for immunological effects, though use of rendered fats in aquafeeds may influence production quality and consumer acceptance, in practice, the magnitude of these effects appears to be minimal. For example, feeding pork lard or poultry fat to brown trout affected the total amount and profile of volatile flavor compounds in the resultant fillets<sup>[70]</sup>, but these differences were not detected by consumers in a subsequent sensory analysis. Trained panel members noted no differences in the color or odor of fresh product and found the color, flavor and texture of cooked fillets to be equivalent<sup>[70]</sup>. Although consumer-based assessments of fillet color were slightly affected by feeding poultry fat-based diets to rainbow trout (consumers described the fillets as “darker” and “more brown” than fillets from fish oil-fed fish), analytical assessments of fillet color (tristimulus color test) did not reveal a significant effect of dietary lipid source<sup>[94]</sup>. Dietary lipid source did affect shelf-life in this study, but fillets from the poultry fat-fed group exhibited lower levels of TBARS (reduced rancidity) throughout the storage period. Ultimately, Liu et al.<sup>[94]</sup> concluded that the use of poultry fat had little meaningful influence on rainbow trout fillet quality or consumer acceptance.

**Future of rendered products in aquafeeds:** Based on the attributes of rendered animal fats, the acceptance and successful utilization of these products by fishes and the economics of aquaculture, the future for rendered animal fats in aquafeeds looks particularly bright. Rendered animal byproducts are the largest potential source of animal-derived feedstocks for the aquaculture industry and in 2000, Tacon described rendered products as “a necessity in aquafeeds for the new millennium”<sup>[95]</sup>. This statement appears to have been prescient: As of 2002, roughly 300,000 MT of non-marine animal protein meals were used annually in aquafeeds and this share is expected to grow rapidly<sup>[96]</sup>. Perhaps the biggest issue facing the use of rendered animal products in aquafeeds is public concern over the safety of feeding animal-derived feedstuffs to livestock intended for human consumption. But as we have described previously, these issues are not associated with rendered fats per se and extensive preventative measures have been implemented in the production, import/export and use of rendered animal products to avoid issues of microbiological or other contaminants entering the human food supply. Further, the risk, both real and perceived, of transmitting pathogenic material via feeding of animal processing byproducts is minimized when the phylogenetic distinction between the source animals and the target livestock is large.

Thus, the feeding of terrestrial rendered products to fish (and likewise, the feeding of rendered fishery products to terrestrial livestock) is considered quite safe. Although the use of fish meal and fish oil in aquafeeds has been considered benign in terms of disease transmission, a new type of rendered fishery product, i.e., derived from mortalities and processing wastes from the aquaculture industry and the potential safety issues they may pose have not been addressed. One can argue that the fish species being fed traditional marine reduction fishery products (salmonids and basses) are still quite distinct from the source animals (anchoveta and menhadens). If the fish meal and oil are being derived from, for example, catfish or salmon processing wastes, the issue becomes more complex. Although catfish oil is currently being used in feeds for catfish (US) and salmon oil is being fed to salmon (Chile) without biological consequences or public backlash, the possible risk for disease transmission and negative perception should not be dismissed out of hand. Disposal of aquaculture trimmings and offal is an increasingly important issue and certainly this growing source of feedstuffs should be used to maximal advantage. However, the risks of “intraspecies recycling” should be evaluated or, ideally, avoided altogether<sup>[6]</sup>.

## CONCLUSION

In closing, it is very likely that the current use of rendered animal fats in aquafeeds can be increased. However, further research will be needed to support expanded applications in aquafeeds and to avoid pitfalls that may arise when nutritionists begin to ‘push the envelope’ with rendered lipids in the feeding of fishes.

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