Project Title: RFP 1463 - A Study on the Potential Economic Benefits of Manufacturing and Use of Taurine for Inclusion in Aquafeeds in the U.S.
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Objective 1: Document details about current Taurine production, supply, and use in aquaculture.

Taurine background and current uses
Taurine (2-aminoethanesulfonic acid) is an organic acid (CAS #107-35-7) that has a variety of functions including constituent of bile, osmoregulation, cell membrane stabilization, antioxidation, and early development of visual, muscular and neural systems (Huxtable, 1992). Taurine is not an amino acid, since it lacks a carboxyl group and has not been found within any known protein structure but is often classified as an amino acid. Taurine is the most abundant free amino acid in animal tissues, accounting for the following percentages of the free amino acid pool in plasma, 25% in liver, 50% in kidney, 53% in muscle and 19% in brain (Brosnan and Brosnan, 2006).

Taurine is typically synthesized from methionine and cysteine. Taurine exists naturally in animals including oysters, mussels and fish but is not found in terrestrial plants. People consuming low amounts of animal products may have a tendency to become taurine deficient than people on omnivore diets (Laidlaw et al., 1998). Supplementation of taurine in diets for select species of fish containing high levels of plant proteins may be necessary as de novo synthesis may be limiting. There are many known uses for taurine, as chemical reagents for food additives; it is added to cat food, chicken feed, energy drinks, infant formula, dietary supplements, cosmetics, inert ingredients in pesticides and pharmaceuticals.

Approximately 5,000-6,000 tons of taurine (synthetic and natural) were produced in 1993; 50% for pet food manufacture, 50% in pharmaceutical applications (Tully, 2000). In 2008, China's total taurine output reached 33,800 tons, exporting 90% of production (China Chemical Reporter, 2010). In China alone, there are more than 40 manufacturers licensed to produce taurine but the industry is dominated by four major producers. Natural taurine can be extracted from ox bile, large muscle of abalone, oysters and octopus. Synthetic taurine is obtained from isethionic acid, ethyl chain sulfonic acid containing hydroxyl group. It has also been synthesized via reaction of 2-aminoethyldulfuric acid (prepared from monoethanolamine and sulfuric acid) with sodium sulfite (Bondareva et al., 2008).

According to manufactures, taurine products are crystalline powders and are more than 98.5% pure, to conform to standards of the United States, Japanese and European Pharmacopoeia. Heavy metal contents are less than 10ppm and arsenic less than 2 ppm. Eleven commercially available products were analyzed for the concentrations of taurine, mercury, arsenic and selenium. The results were compared with label claims for compliance with US Food and Drug Administration (FDA) guidelines, and no substantial amount of contamination was found in any products (Bragg et al. 2009). It seems that specifications for pharmaceutical grade, food grade, and feed grade are all based on the 98.5% purity. There are some products that are claiming a higher standard of 99% or higher purity and even less contaminants.

There is little regulation of the substance, if any by various governmental agencies. Taurine compounds are used as inert ingredients in pesticides but are not specifically regulated by the US Environmental Protection Agency (EPA). They are not included in the EPA list of inert pesticide ingredients. Taurine is sold as a nutritional supplement and included in a number of food or drink products. It is not included in several FDA, Code of Federal Regulations; Food for human consumption as supplemental vitamins and minerals (21 CFR 104.2), direct (21 CFR 184) or indirect food substances (21 CFR 186) affirmed as generally recognized as safe. It is however used as food additives permitted in feed and drinking water of animals (21 CFR 573.980) in accordance with the following conditions, it is used as a nutritional supplement in the feed of growing chickens so the total taurine content does not exceed 0.054 %. Taurine has been shown to be an essential dietary requirement for cats. It is now a requirement of the Association of American Feed Control Officials (AAFCO) for cats and any dry or wet food product labeled approved by the AAFCO should have a minimum of 0.1% taurine in dry food and 0.2% in wet food. In 2009, the US cat food tonnage produced was 1,399,174.1 metric tons dry food and 660,275.8 metric tons wet food. According to the Pet Food Institute, in 2008 domestic retail sales of pet food totaled over $17 billion and U.S. pet food companies exported an additional $1.3 billion in products.

Another large use of taurine is the addition in infant formulas. In 2005, taurine was added to the List of CODEX specifications for food additives. CODEX defined taurine as optional ingredients and no low limit was set. It is also included in the CODEX Advisory list of amino acids and other nutrients for use in foods for special dietary uses intended for infants and young children and standard for infant formula and formulas for special medical purposes intended for infants. The maximum taurine in formula however was set to be 12 mg/100 kcal. Infant formula shall contain (per 100 ml) not less than 60 kcal and not more than 70 kcal. The EU Opinion 1999, estimated the mean daily intake of taurine from omnivore diets was about 58 mg (range from 9 to 372 mg) and to be low or negligible from a strict vegan diet. The European Food Safety Authority (EFSA) 2009, stated the No Observed Adverse Effect Level (NOAEL) was observed at 1,000 mg/kg bodyweight per day. This is equivalent to 60,000 mg/day or taurine to an adult at 60 kg of bodyweight. A large amount of taurine is used as a supplement in energy drinks. The popularity of energy drinks continues to grow, in 2005 there were more than $3.5 billion in energy drink sales. An energy drink contains an average of 1,000 mg per serving.

Taurine in aquaculture

In addition to the human and animal nutrition uses already mentioned, there is a growing interest for use in aquaculture diets. In the EU and China it is an authorized ingredient for fish feeds, the authorization allows it to be used for all species. However, there is no current regulation in the U.S. that states it is acceptable or unacceptable for aquaculture.

There is increasing interest of the replacement of fish meal in aquaculture diets with alternative proteins. Plant proteins, specifically soybean meal has received considerable consideration. Soybean meal has a good amino acid profile with the exception of methionine which may be limiting and taurine is absent. Hence, supplementation of taurine in diets containing high levels of plant proteins may be necessary in some fish species.

There is increasing evidence that indicates some marine fish have a conditional requirement for taurine. Many of the protein sources and attractants used in marine fish diets are high in Taurine; hence, the taurine content may be a primary contributor to the positive response of these ingredients. In fish, the ability to synthesize taurine varies among species due to differences in cysteinesulphinate decarboxylase activity and during ontogenesis (Yokoyama et al., 2001, Goto et al., 2001, Kim et al., 2005a). Research has been conducted on several species of larval and juvenile fish including Japanese flounder (Park et al. 2002, Kim et al 2003, Kim et al., 2005a,b), European sea bass (Martinez et al., 2004), red sea bream (Goto et al. 2001, Takagi et al 2006a, 2010, Matsunari et al., 2008), yellowtail (Matsunari et al., 2006, Takagi et al., 2005, 2006b, 2008), cobia (Lunger et al., 2007), Florida pompano (Rossi Jr. 2011) and sole (Pinto et al., 2010) with results suggesting that taurine may be essential. Freshwater species such as rainbow trout (Gaylord et al. 2006, 2007), and tilapia (Goncalves et al. 2011) have positively responded to taurine whereas other such as Common carp and Atlantic salmon (Espe et al. 2008) did not show a response. The supplementation may not only improve growth and performance but also to reduce nutritional diseases such as green liver disease and low hematocrit levels which seem to be characteristic of a deficiency. A summary of species and critical response to taurine is reported in Table 1.

Marine protein sources are rich sources of taurine. As these meals are removed from marine fish feeds, we are finding an increasing number of species which appear to have a conditional requirement for taurine. Deficiencies are often characterized to include green liver syndrome, reduced hematocrit readings and of course poor growth and reduced survival. The response to taurine supplements seems to be species specific with some only requiring a small supplement whereas others continue to respond positively to relatively high supplement. Consequently, as marine ingredients are removed from practical diets the supplementation of low levels of taurine may be required.

Objective 2: Perform a cost/benefit analysis using Florida pompano (*Trachinotus carolinus*) as an initial model species to evaluate the response of Taurine supplemented high soy diets as compared to more traditional fish meal based diets.

In 2010, US annual landings of pompano were valued over 1 million dollars, averaging $8.55/kg (National Marine Fisheries Service, 2011). Fillets of pompano are reported to cost between $35 to 45/kg (Weirich, 2011), and thus the considerable interest in commercial culture of the Florida pompano. A part of this is due to the successful culture of a similar species, the golden pompano, in Asia. If the production of marine species is to continue to expand fish meal must be replaced and feed prices reduced. Previous studies indicate that when fish meal is removed from Florida pompano feed formulations a taurine deficiency is exhibited. However, supplementing taurine back to the diets has resulted in good growth under short term laboratory studies. Pompano was used as a model marine species to evaluate the cost/benefit analysis of two diets. The goal was to compare two commercially produced feed formulations one with fishmeal and the other without fishmeal, a soy based diet with Taurine supplementation.

To evaluate these feeds, two growth trials were conducted in recirculating systems at the Claude Peteet Mariculture Center, Gulf Shores, AL, USA. The large outdoor system contained six 30 m^3 tanks (Trial 1). The second indoor greenhouse system contained twelve 1 m^3 tanks (Trial 2). Juvenile pompano (11 g) were stocked into both systems, trial 1 at 170 fish/25 m^3 and trial 2 at 20 fish/1m^3 tank. Natural light was provided for both trials. Dissolved oxygen, temperature, salinity and pH were measured twice daily and total ammonia nitrogen twice a week. Two diets were formulated to compare the growth response of pompano, one utilized maximum levels of soybean meal supplemented with taurine (diet 1) and the other moderate levels of fish meal (diet 2, Table 2). The diets were manufactured using extrusion conditions at Kansas State University Extrusion Lab. Trial 2 also used a commercial diet, with a similar protein and lipid profile as a reference diet (diet 3) to the two experimental diets (Table 1). Fish in both trials were fed four times daily. Fish were sampled every two weeks to collect growth and survival data. At termination, blood samples of four fish from each tank were used to determine hematocrit (%) levels.

The parameters used to evaluate the growth performance of the fish were: weight gain (%) = [(final body weight – initial body weight)/ (initial body weight × 100)]; feed conversion ratio FCR = (dry feed intake/wet weight gain); and the economic component was measured through feed cost and production, as: feed cost/fish gain ($feed/kg fish) = ((cost of each diet x total feed)/final biomass). As the feed cost is really the only variable cost it was focused on to show the relative difference in costs for the 2 (or 3 in Trial 2) treatments; other production costs including electrical, chemical and labor were the same for each treatment and becomes a constant. Since we are using experimental tank and pond scale it is difficult to extrapolate results from this research up to a commercial scale, thus our focus on the feed cost per quantity of pompano produced as a relative cost efficiency measure.

The fish in trial 1 (outdoor) fed aggressively and had good growth performance through week 6 (Table 3). The fish were 96.6 g and 100.5 g fed diets 1 and 2, respectively with FCR of 1.62 and 1.45. The survival at this sampling was 92.6 and 95.7%. The fish is the 3 tanks were infected with *Amyloodinium* and the percent survival decreased to 30-35%. After 10 weeks in Trial 1, the fish were 131.4 g and 144.4 g fed diets 1 and 2, respectively. The FCR remained good 2.15 and 2.04 for diets 1 and 2 in the 3 tanks not infected with *Amyloodinium*. There were very few fish alive at the 14 week sampling. The final weights averaged 191.7 and 169.6 g for diets 1 and 2, respectively. The mean treatment hematocrit percentages were similar, 59.4 and 54.9%. Since the survival was poor in both systems, the final FCRs were also poor. After 16 weeks in Trial 2, the fish were 140.3 g, 139.5 g and 118.3 g fed diets 1, 2 and reference, respectively (Table 4). The overall FCR for trial 2 was 2.01, 2.14 and 2.14 for treatments 1, 2, and 3, respectively. Survival for trial 2 was 93, 89 and 96% for treatments 1, 2 and 3, respectively. This study indicates that Florida pompano perform well when fed soy based diet that is supplemented with Taurine.

Several studies have been conducted to evaluate the partial replacement of fishmeal for pompano diets (Lazo *et al.*1998; Williams *et al.* 1985; Riche 2009; Riche & Williams 2011), for which some of these studies have indicated a taurine supplements may be needed. The requirement has been confirmed and is required for proper growth in pompano when fishmeal is completely removed from the diet (Rossi, 2011). However, this study did not compare the soy based diet to a diet containing fishmeal. In the current study, we compared a fishmeal free diet supplemented with taurine (diet 1) with a fishmeal based diet (diet 2). Florida pompano performed equally well when fed the fishmeal free, soy based diet that is supplemented with taurine. The cost/benefit analysis of the two feeds was conducted. The feed cost is really the only cost that is variable assuming the other production cost including electrical, chemical and labor would be the same for the treatments.

Not only did we make a fishmeal free diet, but the cost of the soy based diet supplemented with taurine was less expensive than the diet containing fishmeal (Table 2). Based on industry discussions, there appears to be unutilized capacity for the synthesis of taurine. Hence, one would expect that increased demand could easily be met and prices are not likely to increase due to new uses. Under the current price structure, the addition of taurine to fishmeal free diets did not substantially increase the cost of producing diets. The feed cost for diet 1 and diet 2 was $0.908 per kg ($0.4122/lb) and $ 0.967/kg ($0.4388/lb) (the cost for a traditional reference feed with fish meal, diet 3, was 1.265/kg ($0.5741/lb).

While the cost to manufacture the diets was not significantly different, the feed cost per kg of fish produced was significantly less when fed the soy based diet. There were no significant differences in the growth performance of pompano when fed either diet 1 or diet 2 for both trials. As expected the growth rate slowed down as fish grew, in trial 1 the cost of feed for the quantity of fish produced for the first 6 week trial for diet 1 averaged $1.13/kg fish (includes feed costs only) and for diet 2 $1.15/kg with fish reaching 97g and 101g respectively. For trial 2, fish that were grown for 16 weeks, the average feed cost per quantity of fish produced was $1.73/kg, $2.10/kg and $2.47/kg for diets 1, 2 and 3 respectively with fish mean weights of 140g, 140g and 118g respectively. The market size of pompano is 450g, and fish were fed for an additional 8 weeks with only a few fish reaching this desired size.

Fish were reared in two different systems. The indoor clear water system had the most control but also relatively small tanks which may not support maximum growth rates. The second trial was conducted in the outdoor system which is a much larger system but we also have far less control over water quality and environmental conditions. While the measured water quality parameters were suitable for fish production, they were probably suboptimal and contributed to the difficulty in managing disease problems which occurred late in the production cycle. The susceptibility of this species to various diseases may be an issue for commercial production.

In conclusion, Florida pompano growth performance was similar for both the fishmeal and soy based diets. The soy based diet supplemented with taurine was a less expensive feed formulation and the overall feed cost per unit of fish produced was significantly less than the traditional diet containing fishmeal. It is expected that the price of fishmeal will continue to increase and complete substitution with alternative proteins is viable for pompano as long as amino acids and taurine are supplemented. More research with fish larger than 150g needs to be conducted to determine production cost to market size.

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Table 1. Summary of species and critical response to taurine.

Species	Size range (g)	Dietary Taurine g/100g	Response Criteria	Reference
Cobia *Rachycentron canadum*	9.8 - 150	0.4 – 0.6	Growth, survival	Lunger *et al.* (2007)
Common dentex *Dentex dentex*	40 - 100	0.3 – 0.4	Growth	Chatzifotis *et al.* (2008)
Florida pompano *Trachinotus carolinus*	5 - 80	0.5 – 0.75	Growth, survival	Rossi Jr *et al.* (2011)
Japanese flounder *Paralichthys olivaceous*	0.9 - 15	1.4	Growth	Park *et al.* (2002)
	0.2 - 15	1.6	Growth, feeding behavior	Kim *et al* (2005a, 2005b)
Rainbow trout *Oncorhynchus mykiss*	18.4 - 220	0.85 – 1.45	Growth	Gaylord *et al.* (2007)
Red Drum *Sciaenops ocellatus*	2.5-200	1.5	Growth	McGoogan and Gatlin (1997)
Red Sea bream *Pagrus major*	153 - 560	0.26 – 0.5	Growth, green liver	Takagi *et al.*(2006a)
	2.5 - 20	0.42 – 1.6	Growth	Matsunari *et al.* (2008)
	580 - 1049	0.6 – 2.82	Growth, green liver	Takagi *et al.* (2010)
Sea bass *Dicentrarchus labrax*	0.8 – 3.0	0.45 – 5.35	Growth, diet selectivity	Martinez *et al.* (2004)
Sole *Senegalese sole*	Larvae	0.9	Growth, metamorphosis success	Pinto *et al.* (2010)
Tilapia *Oreochromis niloticus*	0.3-17.0	0.6-0.8	Growth	Goncalves *et al.* (2011)
Yellowtail *Seriola quinqueradiata*	0.5 - 11	1.3 – 2.3	Growth	Matsunari *et al.* (2006)
	~ 6100	1.2	Reproductive performance	Matsunari *et al.* (2006)
	250 – 1000	3.4 – 7.2	Growth, survival, green liver, hematocrit	Takagi *et al.* (2006b)

Table 2. Composition of two diets manufactured at Kansas State University Feed Extrusion Lab.

	Diet 1 (SoyTau)	Diet 2 (FM)	Diet 3* (Reference)
Menhaden Fishmeal [1]	0.00	**20.00**	
Poultry by product meal [2]	**15.00**	0.00	
Meat and Bone Meal [3]	0.00	**10.00**	
Soybean meal solvent extracted [4]	**47.50**	**35.00**	
Menhaden Fish Oil [1]	4.80	3.90	
Whole Wheat/Corn/Sorghum [5]	24.44	29.24	
Corn Gluten meal [6]	4.00	0.00	
UFWS Trace Mineral premix [7]	0.10	0.01	
UFWS Vitamin premix w/o choline[8]	0.40	0.40	
Choline chloride [5]	0.20	0.20	
Stay C 250 mg/kg using 25% [9]	0.10	0.10	
CaP-diebasic [5]	1.50	0.00	
Lecithin [5]	1.00	1.00	
Methionine[5]	0.06	0.00	
Taurine [5]	**0.75**	0.00	
Mold inhibitor	0.15	0.15	
Crude Protein (%)	41.6	43.9	40.0
Acid Hydolysis Fat (%)	10.6	10.7	10.0
Cost ($/kg)	0.908	0.967	1.265

*Proprietary information

[1] Omega Protein Inc., Reedville, Virginia, USA.

[2] Griffin Industries, Inc., Mobile, Alabama, USA.

[3] 65% MBM, Midsouth Milling, Memphis, Tennessee, USA.

[4] De-hulled solvent extracted soybean meal, Faithway Feed Co. Inc., Guntersville, Alabama, USA.

[5] MP Biochemicals Inc., Solon, Ohio, USA.

[6] Grain Processing Corporation, Muscatine, IA, USA.

[7] US fish and wildlife formulation, Rangen Inc, Buhl, Idaho, USA.

[8] US fish and wildlife formulation, Rangen Inc, Buhl, Idaho, USA.

[9] Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

Table 3. Response of juvenile Florida pompano in Trial 1 (outdoor) after 6 weeks being fed experimental diets.

Diet Number	Initial weight (g)	Final weight (g)	Weight gain (%)	Survival (%)	FCR	Hematocrit* (%)	Cost/kg fish ($/kg)
1.SoyTau	21.8	96.6	361.4	92.6	1.62	59.4	1.13
2. FM	21.8	100.5	343.5	95.7	1.45	54.9	1.15
PSE[1]	0.013	3.92	17.898	3.147	0.1733		0.176
P value	0.9106	0.6315	0.6471	0.3310	0.1441		0.8477

*hematocrit were measured at termination (14 weeks); [1]Pooled Standard Error

Table 4. Response of juvenile Florida pompano in Trial 2 (indoor) after 16 weeks being fed experimental diets.

Diet Number	Initial weight (g)	Final weight (g)	Weight gain (%)	Survival (%)	FCR	Hematocrit (%)	Cost/kg fish ($/kg)
1.SoyTau	11.0	140.3	1177.0	92.5	2.01	56.5	1.73[a]
2.FM	11.1	139.5	1158.2	88.8	2.14	50.3	2.10[b]
3.Reference	11.1	118.3	964.9	96.3	2.14	50.0	2.47[c]
PSE[1]	0.038	14.407	135.561	4.330	0.0883	4.279	0.426
P value	0.9754	0.1821	0.1990	0.6482	0.2320	0.1288	0.0036

[1]Pooled standard error