

EVALUATION OF SOY OPTIMIZED FEEDS FOR WARM WATER FISH AND SHRIMP

by **D. Allen Davis**

School of Fisheries, Aquaculture and Aquatic Sciences
203 Swingle Hall, Auburn AI 36849-5419



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U.S. Soybean Export Council (Southeast Asia) Ltd

541 Orchard Road, #11-03 Liat Towers, Singapore 238881

Tel: +65 6737 6233, Fax: +65 6737 5849

Email: Singapore@ussec.org, Website: www.ussec.org

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Introduction

We are all familiar with the world population expanding and the rise of the middle class all leading to an increased demand for food. The world's population needs a food supply that is safe, sustainable, and healthy for human consumption. This means that all sectors of food production must work together to meet the increased need. This also means expansion of both animal and plant based agricultural production systems. Irrespective of the system we need to consider sustainability as a critical component. When considering sustainability of aquaculture my view is that we are equal or better than other animal production systems when it comes to the conversion of protein to protein. However, we are considerably better in producing protein per unit of energy intake which is one of aquaculture's primary advantages. Other advantages include a relatively low use of water, and the water that is used can be used for other purposes such as irrigation. Multi-trophic production systems can also be implemented to further conserve or recycle nutrients. Finally, marine production systems often use land that is not suitable for row crops and are using the marine environment to expand our food production. Like all industries aquaculture has both strengths and weaknesses. Our strength is that we are able to convert a range of protein sources in combination with cereal grains into a high-quality food product that is desired by the world's population.

Presently, aquaculture is producing over half of the world's seafood. We are clearly meeting the needs to expand seafood production, but we need to make sure it is in a sustainable manner. One of our biggest weaknesses is our use of marine ingredients which are limited in supply. Aquaculture is using around 60% of the fish meal and 90% of the fish oil. What is interesting is that some of the biggest consumers for fishmeal, for example shrimp, really do not need the fishmeal in the feed formulations. Similarly, fish oil is often used as the default oil despite that

it may not be needed nutritionally in the feed formulations. In reality, the use of marine ingredients in aquaculture feed formulations is often, what I would call, a perceived need. In that there is no nutritional requirement for the nutrients contained within the protein or oil source it is simply a perceived need requested by the farmer or nutritionist.

Marine protein and lipid sources are exceptional sources of required nutrient and should be used when appropriate. However, due to their limited supply we need to use them more prudently. Of course, there is a range of different species whose requirements also vary. But if aquaculture is to continue to expand, we need to prudently use ingredients that are in limited supply or find alternatives to them. Hence, the reduction and/or removal of marine ingredients has been a priority for the continued expansion of aquaculture.

As fisheries harvests plateaued and aquaculture expanded, there has been a slow and steady increase on the reliance of plant-based proteins, and alternative animal-based proteins (e.g., poultry by product meal) for aquaculture diets. Indeed, many diets formulated for aquaculture use about half of the amount of fishmeal that was once utilized (Boyd and McNevin, 2014). If we think about fish meal use, worldwide, aquaculture had expanded to the point that is now used more as an alternative protein than a primary protein. Current estimates of aquatic feed mill production are around 50 to 60 million metric tons of feed in the world. There is probably around 15 million metric tons of soybean use that means average inclusion is around 25% of the diet. If you look at the current use of fishmeal in aquaculture it is about 4.5 million metric tons or 7.5% of the diet. Consequently, the perception that fishmeal is the primary protein source in aquaculture is no longer valid. Clearly both marine and terrestrial animal-based proteins are an excellent ingredient and as agriculture grows the

production of these by products will also increase. However, we need to minimize the use of all animal proteins as their rate of growth is not likely to be as fast as that of row crops which are slowly replacing animal proteins as the primary protein source in agriculture feeds. If you look at protein sources worldwide, such as oil seed you find that there are around 330 million tons of soy products whereas there is only around 7 million tons of fishmeal so we are looking at orders of magnitude more plant based proteins available to agriculture. Because of the nutritional quality and availability of soy-based products, they are the most utilized plant protein source in agriculture.

Despite high levels of incorporation, there are challenges to using soy-based products in fish and shrimp feeds. Solvent extracted soybean meal (referred to here on as soybean meal) which is by far the most utilized meal, has a moderate level of protein and there are potential anti-nutritional factors. In fact, most ingredients have some form or level of anti-nutrient. Some of the push back for use of soy or plant-based ingredients are perceived issue (i.e. issues that may be real or not) towards anti-nutrients and potential palatability issues. Quite often, poor performance of an animal is blamed on an ingredient when the real issue is the feed formulation is improperly restricted or nutritional deficiencies were not accounted for.

Nutritional Perspective

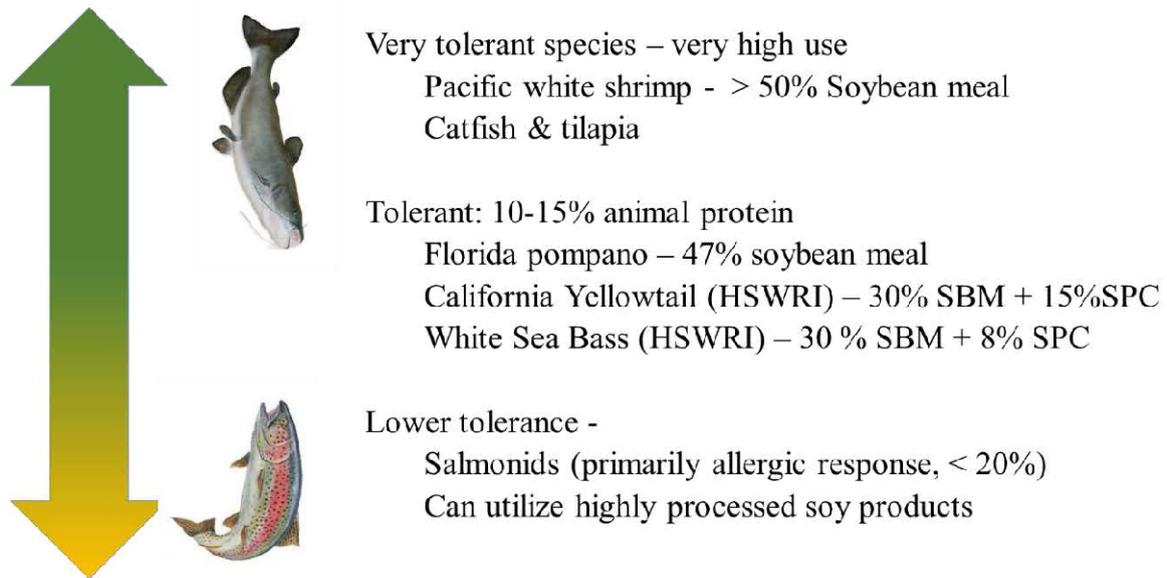
If your training is from the perspective of the catfish or tilapia industry, you are used to dealing with diets containing moderate levels of protein, high levels of carbohydrates and are using plant-based ingredients as the primary protein source. Whereas if you are trained in the salmonid industry, your perspective is one that feed formulations are high in protein, lipid and animal protein and low in carbohydrates with minimal levels of plant-based ingredients. As anti-nutrients in soybean meal can cause enteritis, salmonid nutritionists are trained to avoid soybean meal. Similarly, feeds in Asia and the Americas tend to have different characteristics for the same species and life stage.

Neither of these perspectives are correct nor are they wrong. I would suggest that people's views on soy or plant-based feeds are also influence by their perspective. Based on my experience, the tolerance to soybean meal is not a yes no answer it is a continuum which is represented in Figure 1. If we take, catfish, tilapia and the Pacific white shrimp as examples the limit for inclusion are more dictated by the ability to process the diet than nutritional restrictions due to anti-nutrients. Hence, such diets can contain as much as or more than 50% soybean meal in combination with other protein sources to balance the nutrient profile. Other species are tolerant of soybean meal but require a quality animal protein source at some minimal level (e.g. 10-15% of the diet) to meet nutritional requirements. Then at the other end of the spectrum are species less tolerant to plant-based ingredients or the use of soybean meal in their diets quite often due to allergic responses. In these species soybean meal use is restricted (e.g. <20% of the diets). However, other soy-based products such as enzyme treated, fermented or soy protein concentrates which have reduced levels of anti-nutrient can be utilized. Hence, when one has a good understanding of the nutritional requirements and the tolerance to various ingredients one can formulate a feed using a range of ingredients to work within the tolerance and needs of the animal.

Developing Feed Formulation

If you consider the species that utilize low fishmeal feed formulations, these have been developed because we have systematically defined the nutritional requirements and restrictions for the species. With this information we can balance the nutrition in particular levels of protein or essential amino acids. However, we must also consider essential fatty acids, trace minerals and vitamins as these are all found in fishmeal. When replacing fishmeal, we cannot simply replace it with another ingredient and expect it to work. We must use multiple sources of nutrients to balance the diet and meet the nutritional requirements of the species.

Figure 1. Continuum of tolerance to soy-based feeds



There are many freshwater and marine species that are tolerant of soy-based feeds. These include our carp, catfish, and tilapia from the freshwater side as well as barramundi, cobia, pompano, red drum, and yellowtail on the marine side to name a few. If we take shrimp as an example, you find that there is a huge amount of research that has been developed over the years. This starts in the laboratory where conditions are controlled, and then as nutrient requirements are defined and feed ingredient preference identified, the results are transferred to the industry. Nutrient requirement trials are conducted in clear water systems as we want to control all food sources, water quality, and have good replication. We then move to outdoor green water tanks where some natural productivity is available and environmental conditions are more in line with production conditions but is more controlled and easier to replicate than ponds. Then we can move to demonstration in research ponds under more commercial type conditions. Of course, the final goal is then adaptation by the industry.

Throughout my career I have worked towards the improvement of commercial feed formulations which often means optimizing soy-based feeds. Such lines of research are based on numerous people work but for easy of discussion, I often point to a series of studies

from my laboratory. In work conducted by Davis and Arnold (2000) a number of protein source were evaluated as a replacement for fish meal. This was one of our first experiences using poultry by product meal as a replacement for fish meal, which seems to work across multiple species (see review by (Galkanda-Arachchige *et al.*, 2020)). In this series we also looked at a co-extruded soybean poultry by product meal which worked well yet it was primarily soy with only a small amount of by product. Hence, this was to me our first indication that we could do a low animal protein diet or possibly completely plant-based proteins. This was followed up later with outdoor tank trials (Samocha *et al.*, 2004) which again demonstrated the efficacy of high soybean meal based feed formulations with around 50% soybean meal. This then lead to a series of tank and pond production trials where we demonstrated that fish meal could be replaced with poultry by product meal (Amaya *et al.* 2007b, Amaya *et al.* 2007a) with the idea that farmer are more likely to accept a substitution of fishmeal with poultry meal. We then took this to the next step and replaced the poultry by product meal to produce an all plant based diet (Markey *et al.*, 2010). This was then followed up by a series of studies demonstrating the efficacy of high soy feed formulations using various alternative protein sources (Roy *et al.*,

2009, Sookying, 2010, Sookying and Davis, 2011, Sookying and Davis, 2012) and demonstration of soy-based feed formulations at various stocking densities (Sookying *et al.*, 2011). This line of work and that of others was then summarized in a review paper (Sookying *et al.*, 2013).

In more recent work (Galkanda Arachchige *et al.*, 2019, Galkanda-Arachchige *et al.*, 2019, Galkanda-Arachchige and Davis, 2020). We used the Pacific white shrimp as the model species to evaluate a range of genetic lines,

processes, and country of origin for various soybean meals. This work demonstrates that numerous factors can influence soybean quality and that improvements to genetic lines as well as processing have the potential to further improve soy products by increasing the level of protein, improving the amino acid balance and reducing the levels of anti-nutrients. In general, quality soybean meal is a highly digested protein source. Average amino acid digestibility of Pacific white shrimp for a range of soybean meals obtained from the US are presented in Table 1.

Table 1: Average apparent amino acids (AA) digestibility (n=5) for solvent extracted soybean meal from the U.S. in Pacific white shrimp using 70:30 replacement technique. Apparent digestibility coefficients (%) of dry matter, protein and energy were 59.7±10.3, 68.7±8.6, 90.5±2.2, respectively.

Alanine	85.1 ± 4.3	Methionine	85.2 ± 3.4
Arginine	92.4 ± 1.6	Phenylalanine	89.7 ± 2.3
Aspartic Acid	90.3 ± 2.0	Proline	88.7 ± 2.4
Cysteine	79.4 ± 2.8	Serine	88.4 ± 2.9
Glutamic Acid	91.7 ± 1.6	Threonine	84.0 ± 3.4
Glycine	82.1 ± 6.7	Tryptophan	93.6 ± 1.2
Histidine	89.9 ± 2.2	Tyrosine	92.2 ± 1.8
Isoleucine	90.0 ± 2.0	Valine	86.5 ± 3.2
Leucine	88.3 ± 2.3	Total AA	89.2 ± 2.4
Lysine	91.9 ± 1.9		

In current lines of research, we have been looking at high density mixotrophic or bio-floc type systems stocking at 120 shrimp per meter. In this work all diets contained 42% soybean meal with the basal diet containing 20% fishmeal which was incrementally reduced to 0% by replacing the fishmeal on an isonitrogenous basis with a combination (1:1 ratio) of advanced soy protein (Nutrivance, Midwest Ag Enterprises) and corn protein concentrate (Empyreal75, Cargil Inc) which allowed us to balance the amino acid profile as fishmeal was replaced. Results at the end of the 8-week trial were excellent with weight gain ranging from 10.23 to 10.59g or 3905 to 4120% with no significant differences in weight gain. In this example, survival was slightly lower in the 0% fishmeal feed (93.7 vs 88.0%) which also caused the FCR to be slightly lower (1.04 vs 1.15) as feed inputs are standardized. Quite

often we have no difference in performance but on occasion we will get slight reductions in some parameter. The interpretation that this is just variation across studies, or indication that a low level of animal protein is helpful, it is up to you to interpret. In the case of most commercial feed formulations, it does not make economic sense to remove all animal protein from a feed formulation. Hence, maintaining a low level of animal-based protein in a feed is reasonable.

No matter what species you are working with as you move from a fishmeal-based feed formulation you will run into nutritional roadblocks. Quite often when nutritionists are using plant based ingredient's they simply blame the poor response to anti-nutrient as opposed to defining what the core issue is, which is often times a nutritional deficiency. When shifting protein sources, it is very important to

understand the amino acid requirements as an imbalance in amino acids is often one of the first issues. When dealing with marine fish, it has become clear that a major limitation in the use of many protein sources is a deficiency of taurine. There are numerous fish species which cannot or have limited ability to synthesize taurine (Salze and Davis, 2015). This has been identified as one of the first limiting amino acid when shifting protein sources from animal to plant based ingredients which typically do not contain taurine. Of course, once we solved this limitation, we simply run into another which must be worked out.

Using the Florida pompano as another example, once we identified taurine as a limiting nutrient (Salze *et al.*, 2014), we then went back to re-evaluate the use of soy based ingredients in their diet. This led to work using various forms of soy-based proteins to see if performance in high soybean meal based diets could be improved by using more advanced soy-based products produced using as enzyme treatment, fermentation and/or fractionation technologies (Novriadi *et al.*, 2018, Novriadi *et al.*, 2019a, Novriadi *et al.*, 2019b, Quintero *et al.*, 2012). These studies also included work that evaluated histological shifts in the hind gut. Albeit shifts in histology were quantifiable as diets were shifted there were no clear indications of adverse effects on the health of the fish. Many of these animal meal free diets performed statistically equal to those containing poultry by product meal or fishmeal albeit these diets were consistently lower in performance. This line of work also looked at the effects of various attractants, hydrolysates and acidification of the feed on performance of the fish on low animal meal diets (Novriadi *et al.*, 2017, Novriadi *et al.*, 2020). Overall, my conclusion is that soybean meal in combination with other plant based proteins such as corn protein concentrate work fine in this species but if you need room in the formulation or you desire to bring in biologically active compounds, such as those produced by fermentation, that advanced soy proteins can be used. This work as a whole confirms that we are very close to developing a practical diet using primarily plant-based protein sources for this species.

Enzyme Supplements

Another consideration as we move to higher levels of plant-based ingredients, we increase the level of low digestible or undigestible compounds in the feed. Hence, we may be able to increase digestibility and improve performance of the animal through the use of select enzyme supplements.

One of the most widely, studied enzymes is phytase for which there is an excellent review by Lemos and Tacon (2017). In general, I think phytase should be used in all feed formulations as it enhances phosphorus bioavailability but it also improves the availability of trace minerals (e.g. Cu, Fe, Zn) and you get an added benefit of improved protein digestions. In previous work with shrimp, we evaluated diets with moderate levels of fishmeal (12% diet) and in a soybean meal based diet and found that in both cases phytase improved digestibility coefficients (Qiu and Davis 2017). These results demonstrate that even in fishmeal-based feeds that the addition of phytase is appropriate and that in addition to improved mineral availability there is also about 5% improvement in protein digestibility. Do not forget, that when using phytase in feed formulations that we need to adjust P levels of the diet down as it now has a higher availability, we are also able to reduce protein levels of the diet as there is also an increase in protein digestibility.

There are also a number of proteases and carbohydrase's that have been evaluated with varying degrees of success and reviewed by Castillo and Gatlin (2015). My take on this is that the response to these supplements is very much dependent on the substrate or ingredient matrix of the diets. As an example Amoroch (2018) conducted a series of studies looking at both proteases and carbohydrase's in tilapia. In this work one of the studies evaluated the supplementation of beta-xylanase and beta-glucanase in practical diets for tilapia. Two series of diets (low and high fiber) were formulated, both with 2% fishmeal and 6% poultry by-product meal. For the low fiber diet, the remaining protein was supplied by soybean

meal (49%) and the high fiber diet contained 30% distillers dried grains with solubles and 30% soybean meal. Significant improvement in weight gain were observed in the high fiber diets with the addition of carbohydrase to the diets. Thus, confirming that these are viable supplement but that the most benefits will be seen when there is substrate in the diet.

My view on the use of the various carbohydrase's that are available, is that they often improve protein digestibility so from a digestible protein point of view they improve the diet. We have not necessarily seen improvement in fish performance. Hence, there is clear advantage in the theoretical improvement in protein digestibility and cost effectiveness of the feed formulations but not always a clear improvement in performance in terms of fish growth.

There are many ways to improve a feed formulation to make them more cost effective and sustainable. This is a continual cycle of improvement and refinement which occurs in all animal species as we learn more about their requirements, as ingredient cost and quality shifts so must our feed formulations. One also must remember that developing a high-quality feed is only part of the equation. If feed management is not improved, we are not getting the most out of the diets.

Feed Management

In terms of fish, feed management is generally pretty good as most production systems rely on floating feeds which is a good tool to help with feed management. Clearly improvements can be made in fish system's but given the time constraints we will concentrate on shrimp as feed management is often more problematic. In shrimp there is considerable variability between farms and there are numerous management limitations. Consequently, considerable effort has been spent on feed management and its effects on production. Shrimp are grazers with limited capacity to store feed inside their digestive tract, which results in slower continued ingestion of small quantities of feed. Hence, multiple studies have shown enhanced growth

performance of Pacific white shrimp with multiple feedings through the day (Carvalho and Nunes, 2006, Ullman *et al.*, 2018, Ullman *et al.*, 2019). However, multiple daily feeding applications if done by hand can be very labour intensive and economically impracticable in many regions. Consequently, there is a move to automated feeding systems. In general, the more feedings one can give the better the performance as more feed can be consumed and the feed has not been in the water very long minimizing nutrient loss.

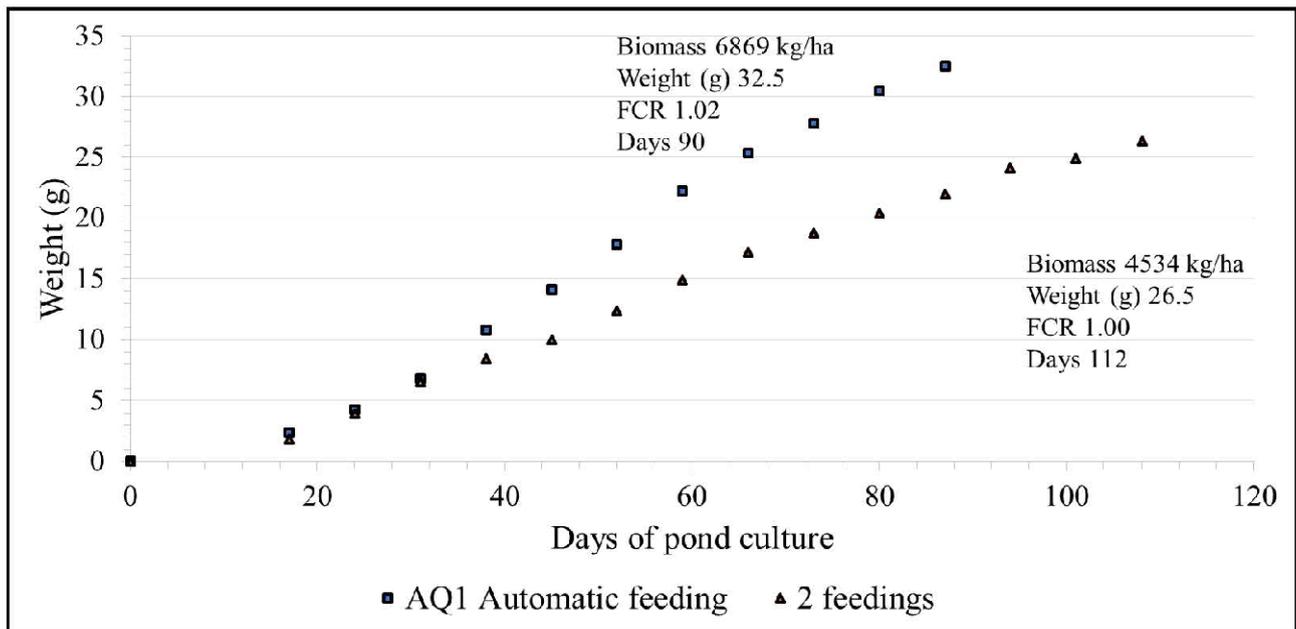
A unique feature of shrimp is that they externally masticate their feed. This particular feeding activity is acoustically active producing a distinctive clicking noise as food is shredded. This has led to the development of an acoustic demand-feeding system which has been proven to be a reliable tool in shrimp farming. Previous works by Jescovitch *et al.* (2018), Ullman *et al.* (2018), and Ullman *et al.* (2019) have shown improvements in growth performance by application of acoustic demand-feeding system in comparison to two hand-feeding per day or timer based feeders which deliver feed multiple times per day. Over the last two years at our research production facility we have transitioned from two feedings per day running a 120-day production cycle to the use of automated feeding systems running a 90-day production cycle (Figure 2). Despite reducing the production cycle by 30 days we are producing a larger biomass and shrimp of larger size. This is accomplished as the automated feeding systems allow for more feeding and thus higher consumption of feed which in turn promotes faster growth. Such systems have been used across pond sizes and production densities across the globe.

In our most recent pond production trials we have tested four practical diets containing 50-57.5% soybean meal in combination with various proteins including fishmeal, poultry meal and corn gluten meal (60% protein). Four feeds were designed to be nutritionally equivalent and designated as plant (no animal protein); 8% poultry meal; 8% fishmeal and 12% fishmeal. The feeds were offered to shrimp in outdoor ponds over a 90 day production cycle

using AQ1 passive acoustic monitoring system to allow the shrimp to have free choice in food consumption. At the conclusion of the trial, there were no statistical differences in production parameters including weekly growth rate (1.64 to 1.72 g/week), final mean weight

(21.0-22.59g), survival (80.9-93.0%), yield (5355-6276 kg/ha) or FCR (1.21-1.54). These results confirm that a range of feed formulations either all plant or with animal protein can be used to produce Pacific white shrimp under pond production conditions.

Figure 2. Historical perspective – changing feed management from 2 feedings per day to self-regulating feed intake using a passive acoustic feeding system with shrimp stocked at 35/m²



The use of passive acoustic feedback is not only an exceptional tool for managing the feed under production conditions, but it is also an excellent research tool that can help us understand the feeding habits of shrimp and their response to various feed ingredients and potential attractants. We are presently using this tool to look at the characteristics of the feed which may influence noise production such as the type of processing (pelleted vs extruded) as well as length and diameter of the pellet. Numerous studies have validated the correlation of acoustic energy to feed intake so we can also use this as a tool to study the response to various attractants. As this is a quantitative measure that is non-invasive we feel this will be an excellent tool for evaluating possible attractants and feeding stimulators.

Summary

As we continue to expand and advance aquaculture, we must continually work to improve our feed formulations and feed management strategies to enhance economic returns while also protecting the natural resources we rely on. The advancement of our industry is based on a huge number of researchers, producers and consumer working together to improve our production and the quality of our products. Hopefully, working together our industry will continue to grow and prosper providing safe and sustainable food for the world.

About the Author



D. Allen Davis is an Aquatic Animal Nutritionist holding the position of Professor with the School of Fisheries, Aquaculture and Aquatic Sciences. Auburn University.

He is a warm water nutritionist specializing in applied nutrition and feed management. He has authored numerous publications and also serves as associate editor for The Journal of the World Aquaculture Society and is on the Editorial Board for Aquaculture Nutrition.

Throughout his career he has dedicated his research and teaching efforts to improve technologies for the culture of marine (Florida pompano, red drum, red snapper, white seabass, California yellowtail and Pacific white shrimp) and freshwater (catfish and tilapia) species for stock enhancement and aquaculture. His primary activities have emphasized, graduate student education, the development and improvement of commercial feeds and feed management strategies, as well as providing continuing education opportunities to the industry. Over his career, he has co-authored over 192 journal articles, 11 book chapters, and has contributed to over 74 articles and symposium proceedings.

Soy In Aquaculture Program

This technical paper was created through the USSEC Soy In Aquaculture (SIA) program and the USSEC Southeast Asian Regional Program. USSEC works with target audiences in Southeast Asia and globally to show the utility and benefits of using United States soybean products in aquaculture diets.

The SIA program replaces the Managed Aquaculture Marketing and Research Program (the AquaSoy Initiative, funded and supported by the United Soybean Board and American Soybean Association) which was designed to remove the barrier to soybean meal use in diets fed to aquaculture species.

The objective of the SIA is to optimize soy product use in aquaculture diets and to create a preference for U.S. soy products in particular, including but not limited to U.S. soybean meal, soybean oil, soybean lecithin, and “advanced soy proteins” such as fermented soy and soybean protein concentrate.

This paper follows the tradition of USSEC to provide useful technical materials to target audiences in the aquaculture industry.

For more information on soybean use in aquaculture and to view additional technical papers, please visit the Soy-In-Aquaculture website at www.soyaqua.org.

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U.S. Soybean Export Council Headquarters

16305 Swingley Ridge Road, Suite 200

Chesterfield, MO 63017, USA

TEL: +1 636 449 6400

FAX: +1 636 449 1292

www.ussec.org



USSEC INTERNATIONAL OFFICES

USSEC AMERICAS

Carlos Salinas
REGIONAL DIRECTOR –
AMERICAS (AM)
U.S. Soybean Export Council
16305 Swingley Ridge Road,
Suite 200
Chesterfield, MO 63017-USA
CSalinas@ussec.org
TEL: +52 331 057 9900

USSEC GREATER CHINA

Xiaoping Zhang
REGIONAL DIRECTOR -
GREATER CHINA
U.S. Soybean Export Council
Suite 1016
China World Office #1
China World Trade Center
No. 1 Jianguomenwai Avenue
Beijing 100004
People's Republic of China
XPZhang@ussec.org
TEL: +86 106 505 1830
FAX: +86 106 505 2201

USSEC NORTH ASIA

Rosalind Leeck
SENIOR DIRECTOR -
MARKET ACCESS AND
REGIONAL DIRECTOR -
NORTH ASIA
16305 Swingley Ridge Road,
Suite 200
Chesterfield, MO 63017
RLeeck@ussec.org
TEL: +1 314 304 7014
FAX: +1 636 449 1292

USSEC SOUTH ASIA

Kevin Roepke
REGIONAL DIRECTOR -
SOUTH ASIA
16305 Swingley Ridge Road,
Suite 200
Chesterfield, MO 63017-USA
KRoepke@ussec.org
TEL: +1 314 703 1805

USSEC GREATER EUROPE, MIDDLE EAST/NORTH AFRICA

Brent Babb
REGIONAL DIRECTOR -
GREATER EUROPE AND
MIDDLE EAST/NORTH
AFRICA (MENA)
16305 Swingley Ridge Road,
Suite 200
Chesterfield, MO 63017
BBabb@ussec.org
TEL: +1 636 449 6020
FAX: +1 636 449 1292

USSEC SOUTHEAST ASIA AND OCEANIA

Timothy Loh
REGIONAL DIRECTOR -
SOUTHEAST ASIA
U.S. Soybean Export Council
541 Orchard Road
#11-03 Liat Towers
Republic of Singapore 238881
TLoh@ussec.org
TEL: +65 6737 6233
FAX: +65 737 5849