



## Review

## Practical supplementation of shrimp and fish feeds with crystalline amino acids



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## ABSTRACT

In 2011, aquaculture accounted for almost 64 million MT of fish, crustaceans and mollusks (FAO, 2012). Fish meal has historically been the protein source of choice in aquatic feeds, but global supplies have reached a plateau making it less available and more expensive. As a consequence, the use of cheaper proteins made from processed plant proteins, byproducts from agriculture, fisheries or the slaughtering of terrestrial production animals was popularized within the aquaculture feed industry. Although these alternative ingredients may contain a crude protein (CP) content comparable to fish meal, they may be less digestible and deficient in one or more of the ten essential amino acids (EAAs). This has required the adoption of more modern formulation approaches which take into account nutrient availability, especially in regard to EAAs. EAA requirements have been established for a number of species of fish and shrimp. Methionine (Met) followed by lysine (Lys) are the first limiting amino acids in plant and rendered animal byproducts. Formulating for EAAs by simply increasing the dietary inclusion levels of the feedstuffs that contain intact sources of the targeted EAAs can lead to overformulated feeds with excessive levels of CP and other nutrients. A more rational approach is to supplement the diet with crystalline amino acids (CAAs). A first step to the application of a nutrient-based formulation approach is knowledge of the digestible EAA content of the ingredients available for use in the formula. It is highly desirable to formulate on a CP, EAA and energy digestibility basis in line with current formulation practices of most modern feed companies. Further expansion of current EAA digestibility values across ingredients and farmed aquatic species will further enable nutrient based formulation. By formulating on an EAA basis across farmed aquatic species, nutritionists have realized least cost formulation opportunities with the use of CAAs which are now available throughout the world at accessible prices. The CAAs mostly used by the animal feed industry, in order of usage, are the following: DL-methionine or Met analogs, L-lysine, L-threonine, L-tryptophan, L-isoleucine and L-valine. Met and Lys are the most currently used due to their wide availability and importance as most limiting EAAs in plant protein ingredients, such as soy and corn based meals. There is a relatively wide variation in the dietary Met and Lys requirement values for farmed fish and shrimp. This is due to differences in species requirements, culture systems, developmental stage and composition of experimental diets. The dietary Met requirements for fish and shrimp range from 0.5 to 1.5% and from 0.7 to 0.9% of the diet, respectively (NRC, 2011). The dietary Lys requirements range from 1.2% to 3.3% of the diet for cultured fish and from 1.6 to 2.1% of the diet for cultured shrimp. Appropriate dietary Met and Lys levels improve the use of other EAAs because they have the ability to reduce the oxidation rate of other amino acids.

The present review discusses bioavailability, requirements and sources of EAAs and practical considerations for their application in aquaculture feeds. The appropriate supplementation of crystalline amino acids in feeds for fish and shrimp represents an opportunity to reduce formulation costs in the face of the volatile commodity market of protein ingredients and the short supply of fish meal. Competitiveness in the aquatic animal feed industry today depends upon application of modern and environmentally-sound formulation techniques based on nutrient value and supplementation with crystalline EAA to meet animal nutrient requirements.

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## 1. Introduction

In 2011, almost 64 million MT of fish, crustaceans and mollusks were harvested from aquatic farms worldwide. More than 75% of this production was achieved through the use of industrially-manufactured feeds. This contrasts markedly with aquaculture in the 1980s when production was dominated by extensive practices, low-trophic level fish and filter-feeding species (FAO, 2012). Today, due to an increasing global consumer demand, fish and shrimp are farm-raised to reach the market up to twice as fast while allowing yields as much as 10 times higher than in the early days of aquaculture.

Much of the development of modern aquaculture was achieved through improvements in farm management, genetics, disease control and a better understanding of nutrient requirements of farmed animals, ingredient processing and feed manufacturing. In the recent past, it was not uncommon to find under- or overformulated feeds. Today, commercial diets need to be fine-tuned to meet species' nutrient requirements targeting specific stages of development, culture system and farm production levels.

Aquatic feeds for farmed fish and shrimp are made from raw materials similar to those used in the feeding of livestock animals. The major difference is the need for protein-rich ingredients since the dietary concentration of crude protein (CP) in aquatic feeds is generally higher compared to feeds for terrestrial animals. A large number of protein ingredients can be used to manufacture aquatic feeds, but historically fish meal has been the protein source of choice. The reason is due to its nutrient value, especially in regard to the high levels of digestible CP and the balanced essential amino acid (EAA) profile that approximate fish and crustacean requirements. However, with the rapid growth of aquaculture, global fish meal supplies have reached a plateau. As a consequence, it has become less available and more expensive to use as a major protein component in aquatic feeds.

Hence, there has been a compelling need to increase the dietary use of alternative protein sources. Logical choices for fish meal replacement have included byproducts from agriculture, fisheries or the slaughtering of terrestrial production animals (for example: soybean meal, canola meal, corn gluten meal, meat and bone meal, soybean or pea protein concentrates, poultry by-product meal, feather meal, blood meal, and fisheries by-catch and processing waste meals). Some of these ingredients can contain CP levels comparable to fish meal, but values usually range from 40 to 75% (as is basis).

Prior to arrival at feed mills, these raw materials need to be processed by drying and grinding, by the application of chemicals to extract part of the nutritional components, or by cooking or fermentation. The way ingredients are processed, their origin and nature determine the amount of protein available and their resulting amino acid composition. Not all the protein in an ingredient is available for a certain species of fish and shrimp. The digestibility and amino acid profile are what ultimately determine the nutritional and economic value of a protein.

Therefore, selection of a protein ingredient also involves knowing its amino acid profile and bioavailability. Having all essential amino acids (EAAs) present at balanced and biologically available levels that meet

the targeted species nutrient requirements is the most desirable condition. However, this is often not the case since most alternative ingredients commonly used in fish and shrimp diets are deficient in one or more of the ten EAAs. Methionine (Met) followed by lysine (Lys) are the first limiting amino acids in plant and rendered animal byproducts. Traditionally, fish meal and other marine proteins have been used to assure adequate Lys, Met and other EAA levels in feeds. However, attempting to drive up Met and Lys levels through the use of fish meal or other marine protein sources is usually too costly today due to price and market constraints. A common approach is to supplement the deficient amino acids with synthetic sources.

Commercial feeds for aquatic animals are typically high in protein with many formulations containing excessive amounts of dietary crude protein (CP). The use of the ideal protein concept in formulating EAA requirements is gaining popularity especially in reducing the dietary CP content and supplying EAA requirements through synthetic amino acids. The present article discusses different formulation approaches to meet EAA requirements of fish and shrimp through supplementation of crystalline amino acids.

## 2. Formulating for essential amino acids (EAAs)

Hundreds of studies have been carried out on protein and amino acid nutrition involving a very large number of fish and the most commercially-relevant shrimp species. A review chapter on protein and amino acid nutrition in fish and shrimp was compiled as part of the recently published National Research Council review entitled Nutrient Requirements of Fish and Shrimp (NRC, 2011). The chapter provides a review of the nutritional biochemistry of proteins and amino acids. Essential amino acids are discussed in the framework of factors which can affect utilization efficiencies. Literature on quantitative amino acid requirements of many commercially important fish and shrimp species is briefly summarized in Table 1. The chapter discusses methodological approaches and challenges associated with defining essential amino acid requirements and applying them to practical diet formulation in fish and shrimp.

EAA requirements established for fish and shrimp are derived from studies carried out with purified or semi-purified diets made from high-quality ingredients. These diets contain low levels of anti-nutritional factors (ANFs), avoiding negative effects on the digestibility and absorption of the tested EAAs, and the proteins used have high levels of digestibility. These experimental feeds are often designed to meet the exact animal nutrient requirements.

In a real scenario, commercially available ingredients have significant amounts of ANFs and digestibility of the proteins is highly variable. In feed mills, nutrient losses can also occur during feed manufacturing and further losses can occur due to improper storage of feed at the farm level. For this reason, a safety margin of 5% or more above the animal EAA requirements should be set to accommodate these conditions. Yet no universal safety margin can be proposed for all situations due to the wide variation in ingredient composition and culture conditions (e.g., water quality, feed management, stocking density). Any excess

**Table 1**  
Quantitative estimates for key limiting essential amino acids in some representative species. Data are modified from those given in NRC (2011).

Species	Initial body weight (g)	Dietary CP (% as is)	Requirement (% of diet)	Reference
<i>Lysine</i>				
Atlantic salmon	4.7	50	2.0%	Anderson et al. (1993)
	642	44	2.2%	Espe et al. (2007)
Channel catfish	200	24–30	1.2–1.5%	Wilson et al. (1977) and Robinson et al. (1980)
Common carp	1.5	48	2.2%	Nose (1979)
European sea bass	0.9	50	2.2%	Tibaldi and Lanari (1991)
Nile tilapia	0.04	28	1.4%	Santiago and Lovell (1988)
	118	25	1.3–1.4%	Furuya et al. (2004)
	5.7	30	1.44%	Furuya et al. (2006)
Black tiger shrimp	0.2	40	2.1%	Millamena et al. (1998)
	2.4	34	2.0%	Richard et al. (2010)
Pacific white shrimp	0.10	35–45	1.6–2.1%	Fox et al. (1995)
<i>Methionine</i>				
Atlantic salmon	493	43	0.7% (with 0.6% Cys)	Espe et al. (2008)
Channel catfish	200	24	0.6%	Harding et al. (1977)
Common carp	24	48	0.8% (with 2% Cys)	Nose (1979)
European sea bass	134	44	0.8–0.9% (with 0.4% Cys)	Tulli et al. (2010)
Nile tilapia	0.06	28	0.8% (with 0.2% Cys)	Santiago and Lovell (1988)
	1.3	30.6	1.1% (Met + Cys)	Furuya et al. (2001)
	1.28	28	0.85% (Met + Cys)	Nguyen and Davis (2009)
Black tiger shrimp	0.02	37	0.9% (with 0.4% Cys)	Millamena et al. (1996)
	2.4	34	0.9% (0.1–0.3% Cys)	Richard et al. (2010)
<i>Threonine</i>				
Atlantic salmon	1.8	40	1.1%	Bodin et al. (2008)
Channel catfish	195–205	24	0.5%	Wilson et al. (1978)
Common carp	15	40	1.5%	Nose (1979)
European sea bass	7.5	49	1.1–1.3%	Tibaldi and Tulli (1999)
Nile tilapia	0.05	28	1.1%	Santiago and Lovell (1988)
Black tiger shrimp	0.05	40	1.4%	Millamena et al. (1997)
<i>Arginine</i>				
Atlantic salmon	383	42	2–2.2%	Berge et al. (1997)
	110	40	1.6%	Lall et al. (1994)
Channel catfish	200	24	1.01%	Robinson et al. (1981)
Common carp	0.6	48	1.7%	Nose (1979)
European sea bass	21	46	1.8%	Tibaldi et al. (1994)
Nile tilapia	0.02	28	1.2%	Santiago and Lovell (1988)
Black tiger shrimp	0.02	40	1.9%	Millamena et al. (1998)
	0.32	45	2.5%	Chen et al. (1992)
<i>Tryptophan</i>				
Channel catfish	195–205	24	0.1%	Wilson et al. (1978)
Common carp	1.8	48	0.3%	Nose (1979)
Nile tilapia	0.06	28	0.28%	Santiago and Lovell (1988)
Black tiger shrimp	0.02	35–40	0.2%	Millamena et al. (1999)

of amino acids ingested by the animal will be catabolized as energy. Conversely, having a diet deficient in one or more EAAs will restrain protein synthesis, compromise the retention of other EAAs and suppress animal growth.

In general, there are two ways to improve the levels of EAAs in the diet: (1) to increase the dietary inclusion levels of the feedstuffs that contain intact sources of the targeted EAAs, or (2) to supplement the diet with crystalline amino acids (CAAs). The first approach can be accomplished by either raising the dietary inclusion of the main protein source in the formula or by adding a new protein concentrate to the formula in order to obtain a high protein mix (NRC, 2011).

The first mode of action is so-called the “ingredient-based formulation” and the last one as the “nutrient-based formulation”. The two approaches are not equally efficient, the former having some undesirable side-effects. Firstly, when the targeted levels of the dietary EAAs are reached through the increase of protein ingredients, which are a complex mixture of many substances, several other nutrients are raised simultaneously in the formula. The ingredient-based formulation approach may also increase the dietary CP level to excessive levels. This may increase feed cost and also raise the risk of water pollution in farming systems. Also, as the targeted EAA is met, other nutrients

(EAAs and non-EAAs, essential fatty acids, minerals and digestible energy) may become unbalanced. These conditions often require broad changes in the inclusion of other ingredients in the formula.

For these reasons, formulators often adopt the nutrient-based approach. The method uses low supplementation levels of CAAs to cost effectively reach targeted dietary EAA concentrations through a least costing exercise. Presently, feed mills regularly supplement aquatic feeds with CAAs, mainly Met and Lys, because those are the two main deficient EAAs in low-fish meal diets. Using the nutrient-based formulation approach, rapid dietary adjustments can be performed through CAA supplementations with minimum changes in the dietary levels of other macro and micro ingredients while achieving the desired levels of dietary EAAs.

If for example, one wishes to design a grower diet for the tiger shrimp, *Penaeus monodon*, containing at least 38.0% CP and 0.89% Met, the application of the two formulation approaches will provide different outputs. Assuming that only three protein sources are available, fish meal, soybean meal and poultry byproduct meal, by locking the latter two at 35.0 and 20.0% dietary inclusion in the formula, a total of 25.0% fish meal will be required in the diet applying the ingredient-based approach (Table 2). Although this will meet the targeted Met level of 0.89%, it will provide an excess of 10% CP and fill up 80% of the formula

**Table 2**Example of the ingredient-based versus nutrient-based approaches in a grower diet for *P. monodon* by locking dietary levels of Met in the formula.

	Nutrient content (% as is)		Dietary inclusion (% of diet, as is)	
	Crude protein	Methionine	Ingredient basis	Nutrient basis
Target in diet	38.0 (min.)	0.89 (min.)	–	–
Fish meal	60.0	1.75	25.0	19.0
Soybean meal	45.0	0.65	35.0	35.0
Poultry byproduct meal	55.0	1.12	20.0	20.0
Synthetic methionine	–	88.00	–	0.12
Achieved in total	–	–	80.0	74.1
Ingredient basis	41.8	0.89	–	–
Nutrient basis	38.2	0.89	–	–

space. By adopting a nutrient-based approach, the 38.0% CP content can be met by reducing fish meal inclusion to 19.0%, while meeting the missing Met levels with 0.11% synthetic methionine. This formulation approach will reduce fish meal usage by 24% while providing more formula space.

A similar solution will be reached in plant-based diets for omnivorous species, such as the Nile tilapia (Table 3). In this example, a finisher diet is formulated for this species containing a minimum of 24.0% CP and 1.4% Lys, using a nutrient based approach. This method will spare 5.8% of the dietary CP and 12.3% of formula space by requiring only 25.0% instead of 35.0% of corn meal in the formula, in combination with 0.1% of a synthetic lysine source.

Today, one of the major challenges of a feed mill nutritionist is to keep feed costs acceptable in the face of raw material price fluctuations. Formulating on a nutrient-basis provides room to reduce or eliminate the dependence/reliance on high-priced and non-renewable ingredients, such as fish meal, while making diets performance-equivalent and more cost-competitive.

### 3. Applying the nutrient-based formulation approach with crystalline EAAs

The increasing use of plant and rendered animal byproducts in complete fish and shrimp feeds has required feed mill formulators to pay more attention to the total and digestible levels of EAAs. The dietary deficiencies in EAAs, mainly of the total sulfur amino acids (TSAAs, Met plus cysteine), increase proportionally when the levels of plant ingredients, such as soybean meal, are raised in the formula (Goff and Gatlin, 2004). If corn-based proteins are used, for example, Lys could be limiting. Therefore, unlike the early days of aquaculture, today's least cost formulations using low- or no-fish meal diets pose an enormous challenge to aqua feed nutritionists, especially in regard to the digestible EAA composition.

A first step to apply the nutrient-based formulation approach is to know the EAA content of the ingredients available for use in the formula. This can be predicted by applying regression equations based on the

CP value of the feedstuff, estimated with feed composition tables, such as the ones provided by the new NRC (2011, see Chapter 19, Feed Composition Tables), or determined through chemical methods or NIR (near-infrared spectroscopy). It is also highly desirable to formulate on a CP, EAA and energy digestibility basis, in line with how most modern feed companies are formulating aquatic feeds nowadays. In order to do that, it is necessary to know the digestibility coefficients (DCs) of the available feedstuffs for the targeted species.

A direct transformation of the DC value of the CP of a feedstuff into the DC of the EAA is not appropriate as there can be significant differences between the two. For example, in a recent study, Wang et al. (2012) working with juveniles of the Japanese sea bass determined that fish meal had a crude protein digestibility coefficient (CPDC) of 91.2%. However, these authors reported that the essential amino acid digestibility coefficient (EAADC) ranged from 86.3% (leucine) to 96.0% (arginine). Kitagima and Fracalossi (2011) have observed that a poultry by-product meal (PBPM) had a CPDC of 90.6% for juveniles of the channel catfish. The EAADC for the same ingredient ranged from 80.8% (isoleucine) to 98.2% (methionine + cysteine). Therefore, if the CPDC is indiscriminately used to calculate the digestible EAAs, there could be under- or overestimation.

The in vivo methodology employed under controlled culture conditions for determining the EAADC for a fish or crustacean species is the same as the one used for protein and energy. Briefly, a reference diet (RD) containing chromium, yttrium or another inert marker formulated with purified or high-quality practical ingredients is made. Test diets are formulated by mixing known proportions of the RD and the protein ingredients under evaluation (70:30, for example). Feces collections are then carried out over the course of the study and their EAA concentrations determined by an appropriate method. The EAADC of the tested ingredient is then calculated by the use of established equations and their further modifications. For example, Sugiura et al. (1998) and Foster (1999) have demonstrated the need to take into account the relative contribution of the tested ingredient and the reference diet when measuring the nutrient or energy digestibility of a single ingredient. In order to achieve greater accuracy in calculating ADC values, Bureau et al. (1999) and Bureau and Hua (2006) have proposed a simplified equation to correct for differences in the dry matter content of the test ingredient and the reference diet. In the case of CAAs, no experimentation would be needed because they are considered 100% digestible (Wang et al., 2012).

Therefore, determining the EAADC of all protein ingredients that will be used in the formula is desirable to correctly supplement diets with CAAs. However, this is nearly impossible to carry out in a commercial feed mill setting. A small-size feed mill will manufacture not less than 500 MT of aquatic feeds monthly, having more than a dozen feed types made from a mix of protein ingredients coming from several different suppliers. While the quality and nature of the feedstuffs used in experimental versus commercial feeds may be different, published EAADC values is one of the only sources of information that a feed mill formulator can depend on to formulate on a digestible basis.

**Table 3**

Example of the ingredient-based versus nutrient-based formulation approaches in a low-protein finisher diet for Nile tilapia, containing a minimum of 1.4% Lys.

	Nutrient content (% as is)		Dietary inclusion (% of diet, as is)	
	Crude protein	Lysine	Ingredient basis	Nutrient basis
Target in diet	24.0 (min.)	1.40 (min.)	–	–
Soybean meal	45.0	2.78	42.0	42.0
Corn meal	8.5	0.25	35.0	25.0
Cottonseed meal	32.0	1.24	12.0	10.0
Synthetic lysine	–	49.0	–	0.1
Achieved in total	–	–	89.0	77.1
Ingredient basis	25.7	1.40	–	–
Nutrient basis	24.2	1.40	–	–

As opposed to the previous NRC report (NRC, 1993) which provided EAADC values of only 11 protein ingredients for two species (Atlantic salmon and channel catfish), the new NRC (2011) report has listed EAADC values of 31 protein ingredients for more than 10 species, including commercially significant and emerging species (salmon, rainbow trout, Nile tilapia, cobia, Pacific white shrimp, striped bass, yellow-tail, etc.). With this information, a database can be created to develop coefficients or mathematical equations able to predict the biological value of a feedstuff for selected EAAs. This data can assist formulators to discriminate between what is digestible from what is available in EAA content of their formulation matrix. The sum of the digestible EAA content of all protein ingredients used in the diet is the starting point to perform cost-effective supplementation of CAAs. Unfortunately, the EAADCs for some farmed aquatic species are still scarce, so in this case, the industry often relies on EAADCs derived from species with correlated feeding habits (i.e., omnivorous, carnivorous, herbivorous).

#### 4. Major issues with nutrient based formulation

Currently, the nutrient-based formulation approach can be applied by the aqua feed industry because CAAs are now available throughout the world at accessible prices. The CAAs mostly used by the animal feed industry, in order of usage, are the following: DL-methionine or Met analogs, L-lysine, L-threonine, L-tryptophan, L-isoleucine and L-valine. However, a debate still remains among the aqua nutritionists on CAA bioefficacy (biological efficiency). While some studies have demonstrated that the bioefficacy of the CAAs is similar to that found in protein-bound AAs (Hu et al., 2008; Williams et al., 2001), other researchers have found lower bioefficacy values for the former (Dabrowski et al., 2010; Zarate et al., 1999). The lowest CAA bioefficacies were found in studies with shrimp due to water leaching problems. This can be explained by the slow-feeding habit of shrimp when compared to fish.

The CAAs appear to have faster gastrointestinal absorption rates than the protein-bound AAs. As such, the plasma concentrations of CAAs are higher than those observed for the protein-bound AAs. Some of the plasma CAAs may not be rapidly taken up by the tissues when the cell capacity to suitably metabolize absorbed AAs for protein synthesis is exceeded. Consequently, it is possible that some of these CAAs will be used for other catabolic purposes such as donating its carbon chain during the oxidation process as an energy source.

The following techniques are used to overcome the faster CAA absorption and leaching problems: encapsulation, pre-coating and polymerization. In addition, CAA can also be chemically bound to a protein, such as soy protein. The pH adjustment of the diet, which typically goes down after CAA supplementation, is another way to maximize the CAA bioefficacy. It is advisable to use efficient feed binders and attractants, and increase the daily feeding frequency at the farm level in order to improve assimilation of CAAs by fish and especially by shrimp due to their slow feeding habit.

#### 5. Practical supplementation with crystalline EAAs

Today there is a widespread view that replacement of fish meal with cheaper protein sources is needed to drive down the price of commercial aquatic feeds. In this case, one approach to meet the EAA requirements of farmed fish and shrimp while reducing feed costs is achieved through dietary supplementation of CAAs (Table 4).

There is a relatively wide variation in the dietary Met and Lys requirement values for farmed fish and shrimp. This is due to differences in species requirements, culture systems, developmental stage and composition of experimental diets. The dietary Met requirements for fish and shrimp range from 1.3 to 3.3% (minimum–maximum) and 1.4 to 2.9% of the dietary CP (dry matter basis), respectively. In terms of the percentage of the total diet, values range from 0.5 to 1.5% for cultured fish and from 0.7 to 0.9% for cultured shrimp (NRC, 2011). The dietary Lys requirements range from 3.0 to 8.6% of the dietary CP (1.2%–3.3% of the diet) for cultured fish; and from 3.8 to 5.8% of the dietary CP (1.6–2.1% of the diet) for cultured shrimp (NRC, 2011). Appropriate dietary Met and Lys levels improve the use of other EAAs because they have the ability to reduce the oxidation rate of other amino acids (Kerr and Easter, 1995).

Lys is limiting in many plant feedstuffs commonly used in fish and shrimp artificial diets (Gatlin et al., 2007). Ingredients of animal origin produced under harsh conditions may also be Lys deficient (NRC, 2011). This is critical because Lys is the EAA with the highest concentration in the carcasses of many fish species (Ahmed and Khan, 2004). Among one of the few metabolic uses of Lys is carnitine biosynthesis which is required for normal lipid oxidation in mitochondria (Harpaz, 2005). Therefore, crystalline Lys supplementation may be required in plant-based diets for normal growth of fish and shrimp. The L-lysine HCl is the most popular form of synthetic Lys used by the animal feed industry.

Met can be partially spared by cysteine (Cys) since Cys is synthesized from Met (NRC, 2011). Cys can represent 40% to 60% of the total sulfur amino acid requirement (Met + Cys). Met and Cys are considered to be the principal sulfur-containing amino acids. In order to spare Met, formulators should seek the maximum sparing rate of Cys possible in the formula which is species-specific. On average, Cys in excess of 3 g/kg of the diet has no Met sparing effect. However, most practical feedstuffs available for aquatic feeds can supply more than this limit.

The overlapping function of Met and Cys and the fact that cysteine can spare methionine confound the determination of the Met requirement. As a result, many studies determine the single TSAA (total sulfur amino acid) requirement (i.e., Met + Cys) and not the Met requirement alone. The TSAA dietary requirement for marine shrimp (*Marsupenaeus japonicus*, *P. monodon* and *Litopenaeus vannamei*) ranges between 2.6 and 4.0% of the dietary CP. For fish, such as salmonids, channel catfish, hybrid striped bass, Indian major carps and Nile tilapia, the quantitative dietary requirement of TSAA varies between 0.6 and 1.4% of the diet (see Table 1).

**Table 4**  
Example of the cost reduction in a marine shrimp diet with 35% CP driven by progressive decreases in fish meal levels. As the dietary fish meal inclusion reduced, there was a need to raise the amount of soybean meal and poultry byproduct meal to meet dietary CP. Dietary Met levels were kept consistent in all diets at 1.00% through CAA supplementation.

Ingredients	% Met	USD/kg	Dietary inclusion (% of diet, as is)					
Fish meal	1.75	1.3	0.0	5.0	10.0	15.0	20.0	25.0
Soybean meal	0.65	0.4	45.0	40.0	35.0	30.0	25.0	20.0
Soy protein concentrate	0.80	1.0	12.0	12.0	12.0	12.0	12.0	12.0
Poultry byproduct meal	1.12	0.7	14.0	12.6	11.2	9.9	8.5	7.1
Synthetic methionine	88.00	5.0	0.52	0.47	0.43	0.38	0.34	0.29
Formula cost (USD/MT)	–	–	424	457	490	523	556	589
Difference in cost (USD/MT)	–	–	166	133	99	66	33	0
Cost reduction (%)	–	–	39.1	29.1	20.3	12.7	5.9	0

## 6. Sources of crystalline Met

Traditionally, the crystalline Met used to supplement complete animal diets is DL-methionine (DL-Met), a racemic mixture of D- and L-isomers of Met (Goff and Gatlin, 2004). More recently, a functional and economical source of crystalline Met has been gaining the attention of the aquaculture industry: DL-2-hydroxy-4-methylthiobutanoic acid (HMTBa) or methionine-hydroxyl analog. Both HMTBa and DL-Met are sources of methionine activity with the D- and L-isomers of HMTBa and D-isomer of DL-Met being converted to L-methionine, the form that is used in protein synthesis. The recent National Research Council (NRC, 2011) review of protein and amino acid requirements of fish and shrimp suggested that DL-HMTBa is not as available or efficacious as other methionine sources such as DL-Met or L-Met. The NRC review summarized the papers published in the literature in aquaculture and poultry that address DL-HMTBa's relative bioavailability to DL-Met and concluded a range of 75% to 80%. When generating an unbiased range of bioavailability estimates that represents the information published in the literature, it is critical that the range published encompasses all available literature. The NRC (2011) report omitted several publications where the relative bioavailability of DL-HMTBa to DL-Met in poultry and aquaculture species were higher than the range reported. The literature includes several meta-analysis publications from large numbers of poultry trials that report predicted DL-HMTBa bioavailability estimates of 81%–86% (Vedenov and Pesti, 2010) and 100% (Vázquez-Añón et al., 2006a). These were not included in the report nor were they reflected in the reported range of relative bioefficacy.

The NRC (2011) review cites differences in absorption dynamics between HMTBa and DL-methionine as a cause for differences in biological efficiency and uses the poultry literature as an example (Drew et al., 2003). However, contrary to this view, there are several peer-reviewed publications that demonstrate the efficient absorption, availability, metabolism and biological efficiency of HMTBa in numerous livestock species (Dibner, 2003; Dibner and Knight, 1984; Dibner et al., 1998; Elkin and Hester, 1983; Garlich, 1985; Harms and Buresh, 1987; Knight and Dibner, 1984; Knight et al., 1998; Lapierre et al., 2007; Lobley et al., 2006a, 2006b; Martín-Venegas et al., 2008; Richards et al., 2005). A recent study with the turbot, *Psetta maxima* (Ma et al., 2013a) indicates that HMTBa appears in the circulation faster than L-methionine reaching similar maximum serum concentrations, thus indicating that HMTBa is absorbed as well as L-methionine in fish.

For aquatic species, the body of literature comparing the percent relative bioavailability of DL-HMTBa to DL-Met is not as extensive as in terrestrial livestock species. The NRC report identified six publications where comparisons of DL-HMTBa to DL-Met were conducted. Two of the six publications reported bioavailability values for DL-HMTBa higher than 80%. Goff and Gatlin (2004) reported equal bioavailability for DL-HMTBa in two experimental trials which directly compared L-Met, DL-Met, and DL-HMTBa. Other trials available in the literature but not cited report equal or better relative bioavailability for juveniles of the whiteleg shrimp *L. vannamei*, for the Jian carp *Cyprinus carpio* and for turbot *P. maxima* (Forster and Dominy, 2006; Ma et al., 2013b; Xiao et al., 2010). In addition, a series of papers demonstrate the efficacy of DL-HMTBa for supplementation of reduced fishmeal aquafeeds for shrimp (Browdy et al., 2012; Huai et al., 2010) and fish (Boonyoung et al., 2013; Hu et al., 2008; Kuang et al., 2012; Savolainen and Gatlin, 2010; Shen et al., 2007; Xiao et al., 2012; Yang et al., 2010; Zhao et al., 2010). It is therefore reasonable to believe the upper value of the range of relative bioavailability should be higher than 80%, and include 100%.

The wide range of bioavailability estimates reported in the literature for DL-HMTBa has been a source of debate in the scientific community for the last five decades. Although DL-Met and DL-HMTBa are sources of methionine activity, their chemical structure, manner and site of absorption (Knight and Dibner, 1984), transport in the body and conversion to L-methionine by the tissues (Dibner, 2003; Lobley

et al., 2006a, 2006b) are quite different. Because of these differences, the two compounds do not follow the same form of dose response (González-Esquerria et al., 2007; Kratzer and Littell, 2006; Vázquez-Añón et al., 2006b), due primarily to differences in feed intake at the extremes of the dose response curves (Knight et al., 2006a). There have been multiple individual studies that have demonstrated performance differences under specific conditions that have favored each compound, which has contributed to the controversy. The wide range of bioavailability estimates reported by the different meta-analyses (Jansman et al., 2003; Sauer et al., 2008; Vázquez-Añón et al., 2006a; Vedenov and Pesti, 2010) of large numbers of poultry studies is partly driven by the different statistical dose response models used to determine the relative bioavailability. Since DL-Met and HMTBa are different compounds (one an amino acid, the other an organic acid) and are metabolized very differently in the body once absorbed, the assumption that they should have identical dose response curves cannot be made without testing. Furthermore, it has been statistically demonstrated that HMTBa and DL-Met do not always follow the same form of dose response and therefore, one would obtain differing bioavailability estimates depending on where in the dose response curve the estimates are made (González-Esquerria et al., 2007; Kratzer and Littell, 2006; Vázquez-Añón et al., 2006b). It is because of this fact that there is disagreement in the literature with respect to the relative bioavailability of DL-HMTBa and DL-Met and such a wide range of published estimates. It becomes critical therefore, when reviewing the HMTBa literature, that all published statistical approaches are at least considered when reporting an unbiased range of relative bioavailability, and in so doing, a value of 100% relative bioavailability for DL-HMTBa would certainly be included in that range.

## 7. Conclusion

The correct supplementation of crystalline amino acids in feeds for fish and shrimp represents an opportunity to reduce formulation costs in the face of the volatile commodity market of protein ingredients and the limited supply of fish meal. While fish meal use may still remain economically competitive at strategic inclusion levels, for specialty diets (starters, anti-stress/transition, and premium) and certain markets, there has been a permanent movement in grower feeds towards very low inclusion of marine proteins. The market perception that all feeds with high levels of fish meal are high performers and more profitable is quickly fading away as confidence in formulating on an ingredient basis evolves. Aqua feed mill nutritionists are abandoning old-fashioned formulation approaches that rely solely on ingredient EAAs. In order to improve competitiveness and effectively apply advancements in aquaculture nutrition, formulators are now adopting modern and environmentally-sound formulation techniques based on nutrient value, on supplementation with crystalline EAAs and on animal nutrient requirements.

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