Availability and effectiveness of free amino acids in aquaculture

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Abstract

While free amino acids are routinely used in feeds for terrestrial farm animals, it is – except for few aqua species – not commonly practiced in finfish and shrimp nutrition. Reasons for not using free amino acids are often related to doubts on the efficiency and availability of these ingredients. This paper discusses aspects on the availability and efficiency of supplemental amino acids in fish and shrimp nutrition and compiles recent studies but also references from literature providing evidence that free amino acid in aqua feed are available to the animals and thus effective. Moreover, question of the availability of supplemental amino acids compared to protein bound amino acids is addressed. Research presented provides evidence that supplemental amino acids are effective in aqua species including finfish and shrimp. This provides that these amino acids were available to the animals. Although research indicates a high efficiency of supplemental amino acids more research is needed in order to quantify the availability compared to amino acids from protein ingredients.

Keywords: *amino acids, efficiency, availability*

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Amino acid balance – key for optimising fish nutrition

Like terrestrial animals also fish and shrimp require dietary amino acids for metabolic purposes and growth. One of the major purposes of amino acids is as building blocks for body protein synthesis (e.g. building muscle, organs and functional proteins such as enzymes, hormones, or immunoglobulins). Some of the amino acids such as lysine, methionine, threonine, tryptophan, arginine, valine, isoleucine, leucine, histidine and phenylalanine are considered essential because they cannot be synthesised by the animal and need therefore to be provided with feed. In addition, some amino acids are required not only as building block but have other metabolic functions in addition to building protein. For example, methionine has a central role as methyl-group (CH_3) donator.

In order to meet but not exceed the requirement for all these above mentioned purposes, the animals need particularly the essential amino acids in a specific quantity and ratio to each other. The body lacks the ability to store amino acids per se, so any absolute but also relative surplus of individual amino acids will limit the body's ability to build protein and will thus reduce their efficiency. As a consequence amino acids not used for protein syntheses will utilised as energy source which should be avoided from the economical point of view. The quantitative and qualitative requirement of animals thus defines an ideal dietary amino acid composition known as "Ideal Protein". Knowledge about the Ideal Protein needs of aquaculture species is crucial as it provides a means to minimize potential deficiencies or excesses of the essential amino acids. This will play an increasingly important role in aqua-nutrition in the light of current strategies to replace fishmeal replacement and to reduce dietary protein reduction. Both of these strategies are of environmental and economic importance as they can reduce nitrogen pollution and minimise use of expensive raw materials (fishmeal) and nutrients (protein) while maintaining performance.

Provided the requirements of the target species and the nutritional value of ingredients are known, optimisation of the dietary amino acid level and composition can be done by an intelligent combination of different ingredients. Least cost formulation will help to find the best

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solution at lowest feed price. However, often not enough different protein sources are available and in this situation use of free amino acid sources such as DL-Methionine, Biolys® (Lysine sulphate), L-Threonine, L-Tryptophan and others can be helpful. While in nutrition of terrestrial farm animals the use of free amino acids is common practice, this is established only in a few aqua-species including salmonids. Major reasons for not using free amino acids are concerns whether they would be available to fish and shrimp and whether they thus would be effective. The objective of this paper is to discuss if supplemental amino acids are available and effective in aqua-species in order to provide a basis for decisions for least cost feed formulation – which finally will reveal if the use of free amino acids is cost-effective.

Effectiveness of supplemental amino acids

To avoid any misunderstandings at the beginning: Commercially available supplemental amino acids are effective and are consequently somehow available to fish. Many examples can be found in the literature and a few will be given in the following. However, remaining question might be how their effectiveness compares to that of protein bound amino acids or so called protected amino acid sources.

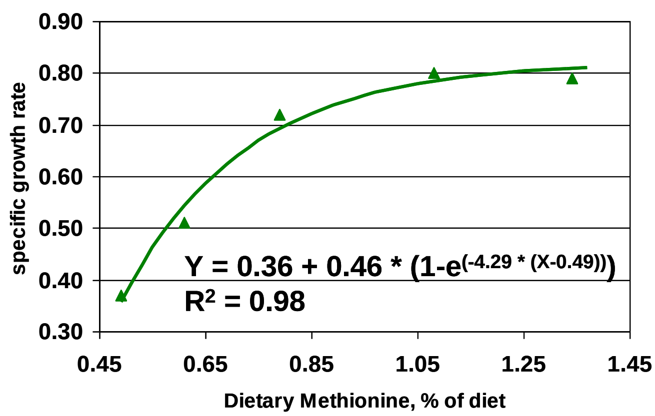

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Figure 1: Response of common carp with initial body weights of 552 g/fish to graded levels of dietary methionine achieved by DL-Methionine supplementation on specific growth rate after an experimental period of 85 days (adopted from Schwarz *et al*., 1998).

Many examples can be found in the scientific literature where free amino acids were used. In a trial by Schwarz *et al*. (1998) common carp of about 550 g initial body weight were fed experimental diets composed mainly of peas, isolated soybean protein, wheat starch and gelatine and supplemented with no or five graded levels of DL-Methionine. Responses to dietary treatments on growth expressed as specific growth rate provide clear evidence that supplemental methionine had an effect on growth (Figure 1). These data also provide evidence that supplemental amino acids are available to fish. This experiment is a nice example that amino acid responses in fish are basically identical to what has been described for terrestrial species. The non-supplemented diet resulted in the lowest growth and increasing dietary methionine resulted in a substantial improvement until achieving a maximum performance. The nonlinear response followed the law of diminishing returns. Re-evaluation of the original data by exponential regression suggested an optimal dietary methionine level of 1.19% (Figure 1). Schwarz *et al*. (1998) using the broken-line analysis in their original paper came up with an optimum at 0.86% methionine in dry matter opening a discussion on how to describe dose-response data best. This has been addressed in detail by Rodehutscord and Pack (1999) and will thus not be discussed in this article. Anyway, these data support that under conditions of deficiency supplementation of free amino acids allow for maximising performance.

More recently, in a trial conducted at Yangze River Fisheries Research Institute, China, juvenile tilapia with initial weights of 5.7 g were fed experimental diets with graded levels of DL-Methionine three times a day (Zhu *et al*., 2010). Dietary protein level was reduced (27.5% i. DM) compared to a positive control with 32.4% i. DM. After the experimental period of 56 days, specific growth rate (and also feed conversion ratio, not shown) improved significantly with increasing methionine supplementation, even above the performance achieved by the positive control (Figure 2, left).

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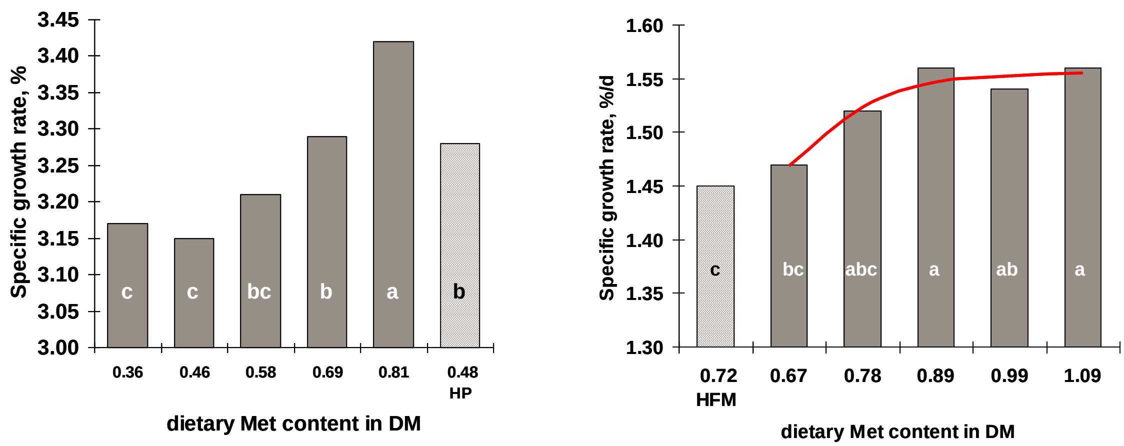

Figure 2**:** Specific growth rate of juvenile tilapia fed a positive control and low protein diets supplemented with graded DL-Methionine levels (left, Zhu *et al*., 2010) and specific growth rates of gibel carp fed a diet with standard fishmeal inclusion and fishmeal reduced diets with increasing levels of methionine achieved by DL-Methionine supplementation (right).

In another trial with gibel carp done in facilities of a Chinese aqua feed producer, fishmeal was reduced from 15% to 10% and the low fishmeal diet was supplemented with graded levels of DL-Methionine (Figure 2, right). Fish with an initial weight of 18.6g responded up to a dietary methionine level of 0.79% i. DM corresponding to a DL-Methionine inclusion of 0.19% again indicating that supplemental methionine was effective. A number of further studies with shrimp, rainbow trout, tilapia, grass carp, and catfish which were recently presented confirm such findings (Fox *et al*., 2009, Ho *et al*, 2010; Jintasataporn *et al*., 2010; Liebert *et al*., 2010; Liu *et al*., 2010, Sangsue *et al*., 2010, Wang *et al*., 2010).

Factors affecting effectiveness of supplemental amino acids

From all this it can be concluded that supplemental amino acids are effective in fish. However, questions remain to the effectiveness compared to protein-bound amino acids. In this context the most prominent concerns include losses of supplemental amino acids due to leaching and reduced availability of supplemental amino acids due to fast absorption of free amino acids.

The impact of leaching is of minor importance for most of the aqua-species. Under normal circumstances feed is consumed immediately after supply leaving no time for leaching. In shrimp the situation is different as they eat rather slowly. Cruz-Suarez *et al*. (2005, 2009) investigated the amino acid leaching of commercial shrimp feeds which were not supplemented with free amino acids. In both trials, these authors reported substantial losses of dietary amino acids with dry matter losses ranging from 5% to 18%. On average, methionine and lysine disappeared at higher rates than for other essential amino acids. These data show that leaching is not necessarily restricted to supplemental amino acids. However, supplemental amino acids might be especially endangered because of their comparably high solubility. This of course per se might influence availability of dietary amino acids for shrimp. Cruz-Suarez *et al*., (2009) also reported that when part of a reference diet was replaced by one of five soybean products leaching losses were reduced suggesting that leaching depends on diet composition. It should be noted that fishmeal often contains relatively high amounts of free amino acids.

If not taken into account, the impact of leaching may also lead to an overestimation of digestibility. Cruz-Suarez *et al*. (2009) reported an apparent methionine digestibility of 84%, but after correction for leaching losses this number was reduced to 78%. This demonstrates that leaching may lead to an overestimation of digestibility if not considered. To minimise this error, difference methodology should be applied which was done for determination of amino acid digestibilities for various soybean products.

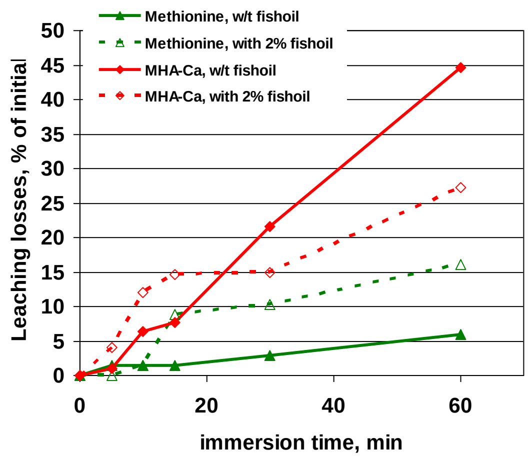

Figure 3**:** Leaching losses of a pelleted fish diet (2.5mm) supplemented with either DL-Methionine (triangles) or methionine hydroxy analogue calcium salt (rectangles) and added with no (solid lines) or 2% fishoil (dotted lines)

The extent of nutrient leaching is very much dependent on pellet quality and components used for the feed. Today, expectation for water stability of shrimp pellets is higher than reported in the above mentioned studies. Accordingly, dry matter loss shall be below 10% after two hours immersion (personal communication). Fogle *et al*. (1999) reported that leaching of supplemental amino acids interacted with pellet size, feed processing technology, and fat content of the diet. More recent investigations confirm those interactions. Either 0% or 2% fishoil were added to pelleted feed (2.5 mm; post-pelleting application) consisting mainly of soybean meal, wheat, corn, rapeseed meal and fishmeal. Leaching behaviour of 0.15% supplemental DL-Met or of 0.17% of methionine hydroxy analogue-calcium salt (MHA-Ca) was investigated using freshwater with 25°C (Figure 3). Results suggest that there are interactions between methionine sources and fat coating. The addition of fishoil increased leaching of both methionine sources during the first 15 minutes. Over time, however, the MHA-Ca generally showed a higher leaching, which might be explained by higher solubility (85 g/L vs 33 g/L for MHA-Ca vs DL-Methionine at 20°C). After 60 minutes 6% to 16% of supplemental DL-Methionine were leached whereas 27% to 44% of MHA-Ca were lost. Apart from diet composition, pellet quality is the key factor for minimising those losses. Fox *et al*. (2010) reported that in their study leaching losses of supplemental L-Methionine were minimal which probably can be attributed to pellet quality.

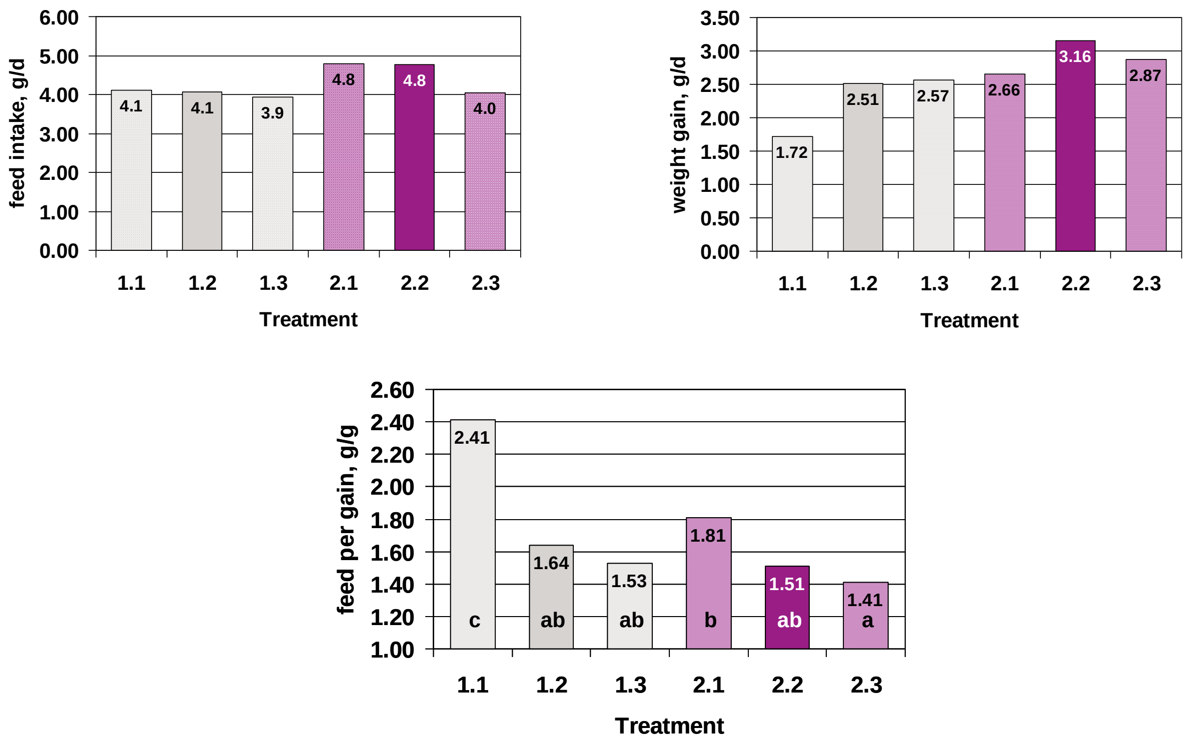

Figure 4: Feed consumption (top left), daily weight gain (top right), and feed conversion ratio (bottom) of common carp after an 81-day feeding trial. Treatments: 1.x: 0.45% i. DM dietary methionine; 2.x: 0.86% i. DM by supplemental DL-Methionine; x.1: twice daily feeding until satiation; x.2: continuous feeding of same amount as x.1 (pair feeding); x.3: continuous feeding of fixed feeding rate (1.4% of body weight)

The other major concern of aqua nutritionists is that supplemental amino acids are absorbed faster than protein-bound amino acids limiting the efficiency of utilisation because of a considerable catabolism of the free amino acids. Indeed, free amino acids are absorbed faster than protein-bound amino acids. This has been demonstrated by several researchers examining blood plasma concentrations after feeding diets containing free amino acids compared to non-

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supplemented amino acids (e.g. Ambardekar *et al*., 2009; Schuhmacher *et al*., 1997). Does this finding impact the utilisation of the supplemented amino acids? Earlier research indicated that in growing swine the utilisation of supplemental amino acids might be reduced only at once-a-day feeding while with more than one meal daily this phenomenon disappeared (Batterham and Bayley, 1989; Batterham and Murison, 1981). In this context a 81-day feeding trial with common carp was conducted by Schwarz *et al*. (2010) in order to investigate whether there is an interaction between the dietary methionine supply (low or enhanced with supplemental DL-Methionine) and feeding strategy (twice daily feeding until satiation, feeding the same amount of feed continuously using band-feeders (pair feeding), feeding 1.4% of body weight (adjusted fortnightly)). The results confirmed that feed intake between twice daily feeding and continuous feeding of the same amount of feed was similar in the corresponding treatments, however, enhanced dietary methionine significantly increased feed intake (Figure 4). Interestingly, weight gain and consequently also feed conversion ratio improved significantly ($p<0.05$) with increased dietary methionine as well as with changing from twice-daily feeding to continuous feeding independent of dietary methionine supply. This finding suggests that increasing feeding frequency while maintaining the feed volume clearly improves feed and thus nutrient utilisation – which by the way was also confirmed by Lemme and Elwert (2010). Moreover, this effect occurred at low (unsupplemented) dietary methionine as well as at 0.4% supplemental DL-Methionine suggesting that a lower performance at twice daily feeding is not due to reduced utilisation of the supplemental amino acid but due to a generally reduced utilisation of dietary nutrients. So, if fish are fed at least twice a day, supplemental amino acid will be utilised very well.

As pointed out above availability and effectiveness of supplemental amino acids might be influenced by certain factors. However, if good pellet quality is ensured and animals are fed at least twice a day, high effectiveness can be assumed.

Outlook

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Still, despite those results, further research on the availability of free amino acids particularly compared to protein bound amino acids but also to other amino acid sources is required.

The slope ratio approach descirbed by Littell *et al*. (1997) is one of the promising methods regarding determination of the relative bioavailability of nutrient sources. This relative bioavailability allows to quantify the effectiveness of any amino acid source (free amino acids, ingredients) and thus to rank them. Comparisons of different sources of free amino acids where free amino acids were supplemented in graded levels to a deficient basal diet have been reported. For example, Rodehutscord *et al*. (2000) compared two lysine sources in trout by means of the slope ratio technique. Lemme (2010) compiled data from literature on the comparison of methionine sources.

Not much slope ratio research on supplemental free amino acids versus amino acids from intact proteins in aqua species is available. This might be attributed to some methodological hurdles as e.g. increasing levels of an amino acid bound in an intact protein is accompanied by an increased dietary protein level which in turn may influence the response. On the other hand, increased inclusion levels of a protein source at the expense of another protein source and maintaining dietary protein level might be an alternative strategy. However, in this case effectiveness of the free amino acid can only be related to the whole diet but not to one particular ingredient as differences of amino acid availabilities between ingredients should be assumed. Once these issues have been solved effectiveness of supplemental amino acids can be quantified which is difficult to achieve in other experimental setups.

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