Challenges associated with carrying out a meta-analysis of essential amino acid requirements of fish

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High cost of fishmeal, the volatility in the price of agricultural commodities and the stagnant price of aquaculture products are constraining aquafeed manufacturers to pay very close attention to the cost-effectiveness of their feeds.

Nutritionists are required to formulate to lower or narrower essential nutrient specifications in order to minimise feed cost. At the same time, these feeds must sustain high growth, feed efficiency, health and product quality of the animals at the farm.

Nutritionists also are relying on an increasingly diverse portfolio of 'economical' protein sources, with different amino acid profiles.

Thus, formulating cost-effective aquafeeds requires increasingly precise information on essential amino acid (EAA) requirements of aquaculture species.

Dynamic field of research

Aquaculture nutrition is a very dynamic field of research. A very large number of studies have been conducted on EAA nutrition of teleost fish and penaeid shrimp over the past 50 years and the body of knowledge on EAA requirements of aquaculture species is continuously expanding.

One of the problems resides in keeping up with progress and developing a wholesome understanding of the 'state-of-the-art'.

The great diversity of methodological approaches used and animal species and ingredients studied as well as the multitude of opinions with regards to optimal levels and modes of expression of EAA requirements limits the ability of manufacturers to meaningfully improve the cost-effectiveness of feeds and/or adapt formulations to an ever changing commodities market.

A number of scientific reviews and publications have attempted to summarise the body of knowledge on EAA nutrition and requirements of aquaculture species (Wilson, 1989; NRC, 1993; Cowey, 1994; Lall and Anderson, 2005; Bureau and Encarnação, 2006; Hernandez-Llamas, 2009; NRC, 2011), and some concluded that the state-of-the-art on EAA nutrition of aquaculture species is still quite shallow.

The choice of the mode of expression (percent dry diet, percent crude protein, g/kJ digestible energy, ideal protein, etc.) of EAA requirement is a matter of much debate, and reflects the conflicting assumptions authors make when considering what affects the requirements or not (Bureau and Encarnação, 2006; Bureau, 2008).

Together with methodological issues (notably limitations of the experimental design used), the variability in achieved growth and feed efficiency, as well as differences in the mathematical and statistical approaches used to analyse data, these result in high variability in estimates of EAA requirements.

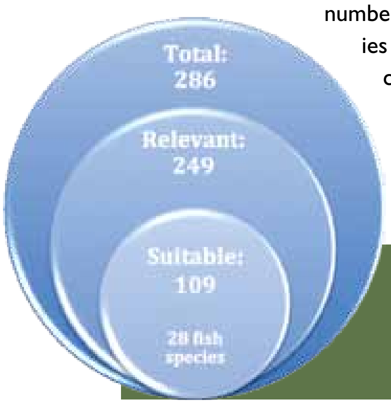


Figure 1: Diagram illustrating the screening of studies, from the total number found to the number of suitable studies that constituted our working data set

Understanding the reasons underpinning this great inconsistency is important for developing more reliable and practical estimates of EAA requirement of aquaculture species.

All these issues point toward a need for the systematic integration and analysis of information from the large number of studies that have been published so far on EAA requirements of aquaculture species. Statistical meta-analysis offers a mean to realize by integrating and standardizing information and allowing meaningful comparisons.

The goal of this project was to carry out a meta-analysis of EAA requirement of fish through the construction of a dataset gathering all available data on EAA requirements of teleost fish.

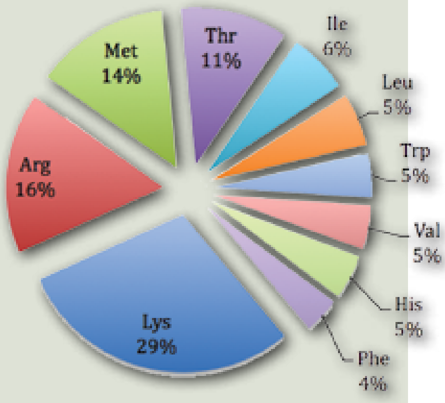
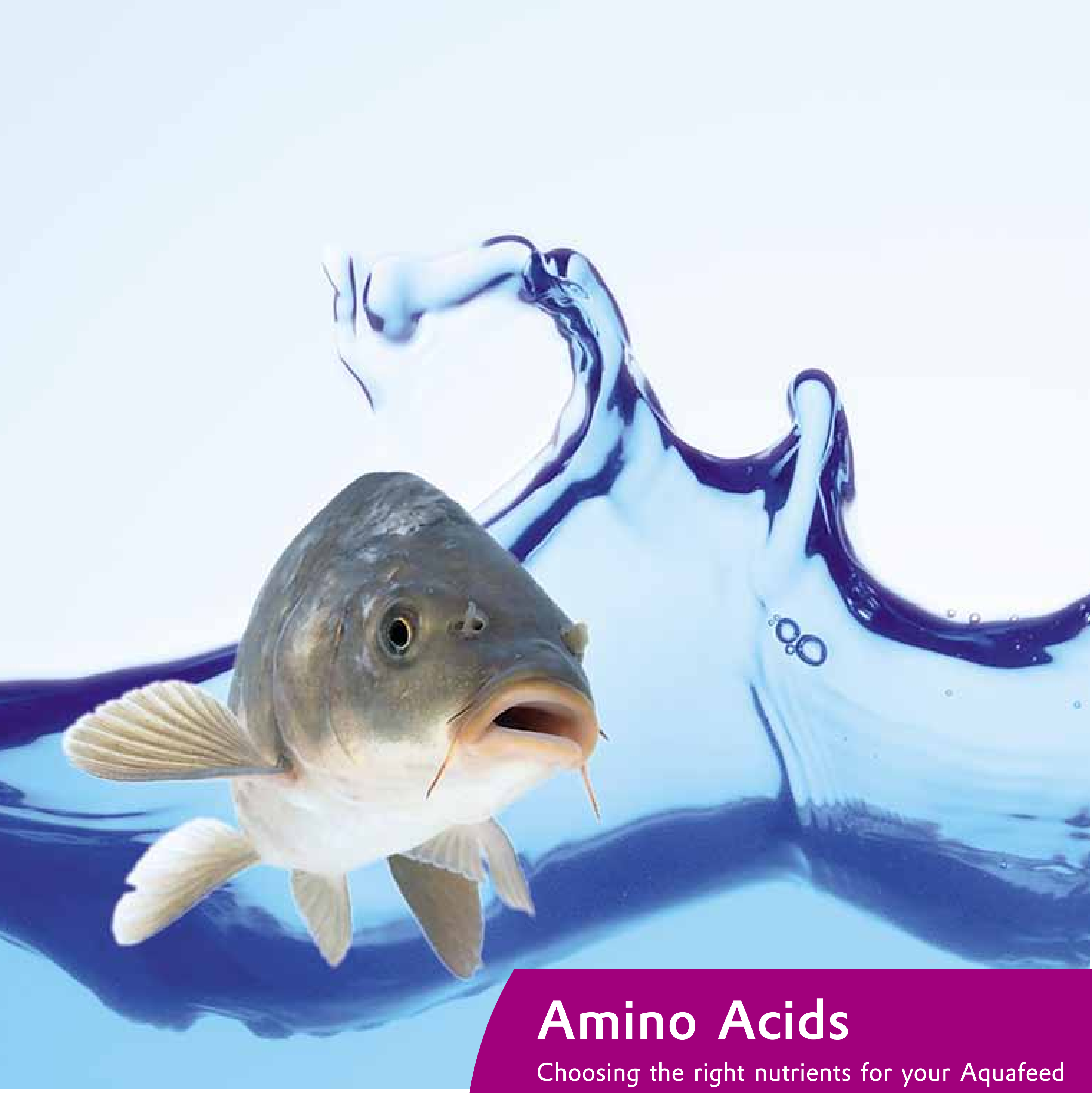


Figure 2: representation of EAA across the dataset



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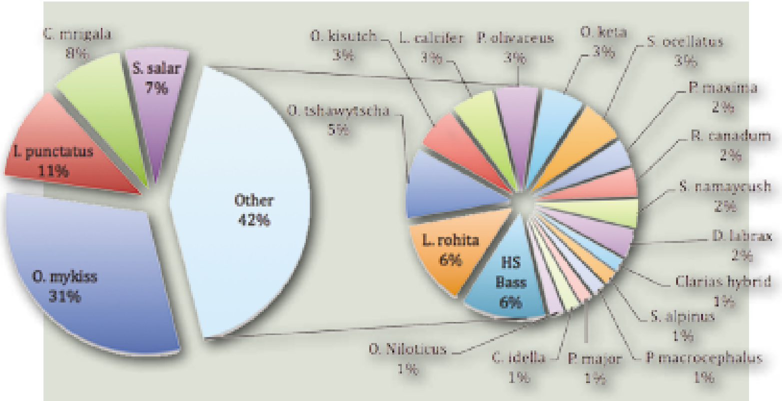

Figure 3: representation of species across the datase

The main objectives of this effort were identifying factors that may affect estimates of requirement, highlighting the shortcomings in the existing body of knowledge, and providing guidelines for future research.

Building a working dataset

A comprehensive search of papers on EAA nutrition of commercially relevant teleost species (for example, salmonidae, cychlidae, cyprinidae) published in peer-reviewed journals and other technical publications was carried out.

This search yielded 286 papers of which 249 were original research studies focusing on EAA requirement of teleosts.

As expected, a great variety of objectives, experimental designs, and analytical methodologies were employed in these studies. Selection criteria were therefore applied to the original dataset to identify studies suitable for a meta-analysis.

Amongst selection criteria, studies had to have at least five or more experimental diets with graded levels of an EAA. This criterion was established in order to ensure the accuracy of the non-linear regressions analyses.

Studies had to report information on water temperature, experiment duration, diet composition (dry-matter basis), initial and final individual body weight, and feed intake. Screening of the studies with these selection criteria yielded a final dataset comprised 109 studies, which covered all 10 EAA in 28 teleost species (see Figure 1).

A fragmented & diluted body of knowledge

It is striking that less than half of the 249 original studies could be considered for the working dataset.

This highlights the limited scope of many studies and/or scientific manuscripts and the uneven quality of the research effort. An important cause of rejection of studies was simply a lack of reported information, which precluded us from calculating the various variables.

Simple parameters, such as feed intake (feed served) and the dry matter content of the diets, were frequently not reported by authors. Other major motives for rejecting studies included the use of too few graded levels of nutrient studied or poor growth performance achieved during the trial.

The large number of species studied and the large number of EAA resulted in a very fragmented dataset (see Figures 2 and 3).

For example, the dataset only includes four studies on phenylalanine requirements, which were all conducted on different species. Great differences in the body weight of fish used (<1g to more than 600g) introduce some challenges for standardisation of data.

Almost half of the studies did not report information on carcass composition.

Protein gain (retention), an important response variable for EAA requirements, could only be computed for a limited number of studies.

Finally, only 16 percent of the studies in the working data set included some evaluation of protein digestibility.

Consideration of digestibility would greatly strengthen and refine our understanding of EAA requirements in fish by taking account of some of the variability due to bioavailability of EAA in different ingredients.

In order to standardise the data onto a common ground, two different modes of expression (percentage of EAA of interest in the dry diet, amount of EAA per MJ of digestible energy) were computed and two different growth response variables (weight gain per kg of metabolic body weight and thermal-unit growth coefficient).

Therefore, for each study we obtained four pairs of variables, each of which was analysed using four mathematical models: the broken-line model (BLM), the quadratic model (QM), the broken-quadratic model (BQM) and the saturation kinetic model (SKM) (see Figure 4) in each study to allow estimation of EAA requirements.

Preliminary results and perspective for future studies

Figure 5 presents the computed arginine requirement for rainbow trout using six studies for which models fitted correctly, depending on the three modes of expression.

It illustrates that even after selecting suitable studies and standardising data, very large discrepancies in estimates between the studies remain. Variations around estimates based on ingested EAA remain high (coefficient of variation between 20 and 35 percent overall,

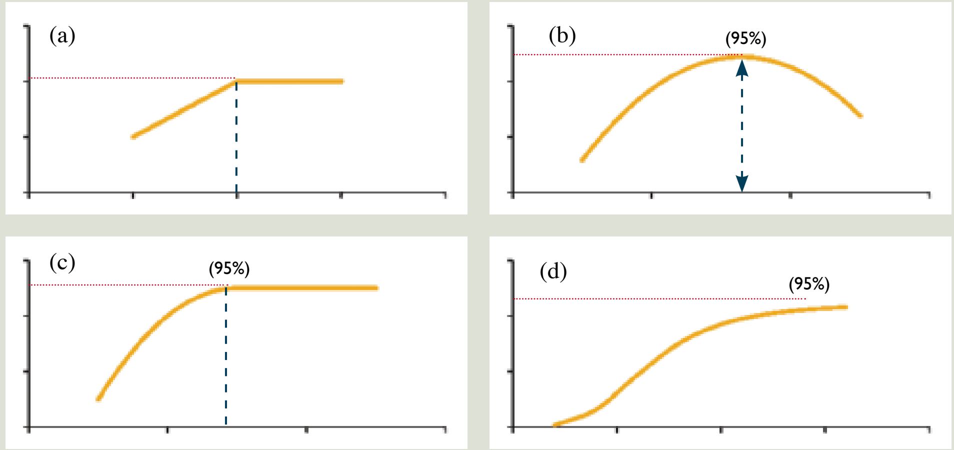

Figure 4: Diagrams illustrating the typical shape of the four models: (a) broken-line model (BLM), (b) quadratic model (QM), (c) broken-quadratic model (BQM), and (d) saturation kinetic model (SKM). Dashed line indicates the determination of the requirement for each model

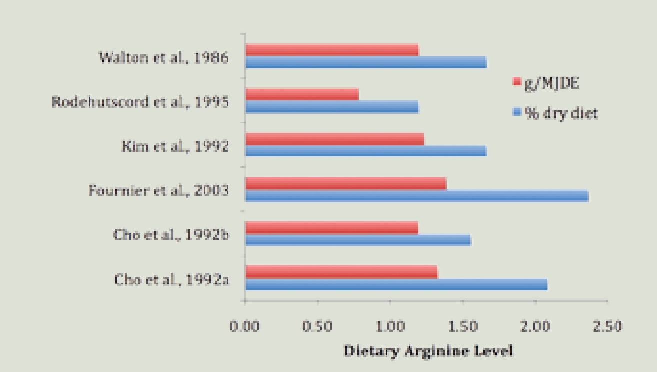

Figure 5: Estimated arginine requirements for rainbow trout according to the mode of expression

14 and 23 percent for arginine requirement of rainbow trout).

This shows that much remains to be done to reconcile the existing data and determine factors underpinning variability.

Nevertheless, several valuable points arose from the data standardisation.

The accuracy of the models was impacted by the shape of the curve they are meant to fit. Since all of these models are based on the law of diminishing returns, they attempt to fit curves shaped as those illustrated in Figure 4.

Therefore experiments should be designed to produce such result curves.

For example, data points of a given study will approach the horizontal line if there is little difference between treatments, which will cause the model to fail.

Our meta-analysis shows that if the final body weight of the slowest-growing group is higher than 70 percent of the fastest growing group, then the probability of obtaining a good fit decreases significantly. When using the SKM, this number is closer to 50 percent.

Additionally, if the curve only increases but does not plateau, then models can only guess where the curves plateaus or peaks. Since the requirement is estimated by the EAA level where the curve plateaus or peaks, such an estimation should not be considered reliable.

Studies which simultaneously incorporated very deficient and obviously adequate levels of EAA produced a clear 'diminishing return' pattern and consequently had vastly improved chances of estimating the requirement accurately.

Conclusions and recommendations

In this study we highlighted important variations in requirement estimates despite an attempt to standardise our working dataset.

Simple steps can be taken to improve the quality and relevance of future studies and allow us to develop more precise estimate of EAA requirements. Experiments should not ideally use fewer than six experimental diets, and report sufficient information on diet composition, growth performances and husbandry information.

The BLM has now been clearly demonstrated to underestimates requirements, and thus should be avoided. Instead, the QM or BQM offer a better balance between accuracy and practicality (that is, parsimony and ease of fit).

Most importantly, studies should be designed so results fit a clear 'diminishing return' pattern. The graded dietary levels of the test EAA should include clearly adequate levels so an obvious plateau can be observed.

Concomitantly, equally clearly deficient levels should also be included to ensure sufficient differences between treatments receiving suboptimal levels.

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