CONSIDERATIONS OF EXPERIMENT DESIGN FOR SOY COMPARISON IN AQUACULTURE APPLICATION

by Min Xue, PhD

Feed Research Institute
Chinese Academy of Agricultural Sciences (CAAS)

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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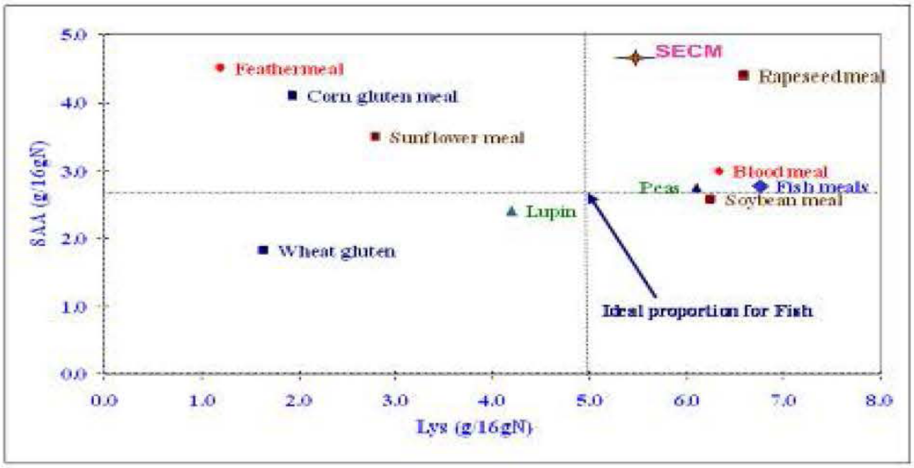
Introduction

Compared to human nutrition the research history on fish and shellfish is fairly recent. It generally is seen to have started 70 years ago with the first article published by Prof. John Halver in 1950. In most intensive aquaculture systems feed accounts for about 60% of total production costs, and therefore plays an essential role in the success of aquaculture businesses. Fish meal (FM) is the most important and one of the most expensive protein sources in aquaculture diet formulations. Due to a generally fixed supply volume, industry expects that FM will continue to command high prices into the future. Finding alternative protein sources has become an inevitable requirement for eco-friendly feed and to further sustainable aquaculture (FAO, 2020). Plant proteins from cereals and legumes represent an economical and nutritious resource for fishmeal replacement in aquafeed, which have high protein content (Liang et al., 2017; 2019), but may have poor palatability and heat-stable anti-nutritional factors (ANFs). Before optimally utilizing a plant protein, including SBM, in commercial fish feed formulas, we at least need to consider five aspects; digestibility, ideal amino acid profiles, feed intake, pre-processing and nutrient functions on animal health as discussed in the following sections.

Lys and SAA contents of selected protein sources compared with the requirements by fish.



Kaushik & Seiliez, 2010

Methodologies Consideration for Soy Protein Evaluation

Digestibility

Digestible and metabolizable nutrients of ingredients are the basis for precise formulation of animal feed. However, compared to terrestrial livestock this information for aquatic animals is still absent or incomplete in many cases. Even for the same aquatic species, we can expect a 15-20% error rate (NRC, 2011) in nutrient digestibility values for a certain feedstuff from different literatures. Concerning digestibility experiments, the related methodological considerations that should be included, but not limited to:

1) A clear ingredient source background, with defined processing methods and

storage conditions, origin information, and definitely NO ADULTERATION.

2) Adapted feces collection systems with specific feces collector or siphoning system, with only feces samples collected at peak times being acceptable to avoid over-estimating of digestibility (Dong et al., 2012). Some species, like Asian seabass (*Lates calcarifer*), must use fecal stripping to get valid samples due to the dispersed nature of the feces, although this still might underestimate the results.

3) Well-accepted inner markers and determination methods. Chromic oxide (Cr_2O_3) is a commonly used inner marker for digestibility determination. Additionally, several rare earth oxides: Y_2O_3, Yb_2O_3, La_2O_3, Sm_2O_3, Nd_2O_3, Gd_2O_3, and Ti_2O_3 could be good markers that do not cause a physiological and color disturbance to animals (Xue and Cui, 2001). Our lab has compared the stability and reliability using three methods to determine that two inert markers (Cr_2O_3 and Y_2O_3) worked well when included in diets and in recovery from fish feces. The results indicated that these markers are much more stable with lower variability with ICP-OES than Acid-digestion Colorimetry (AC) analysis methods in these samples.

4) A mathematically correct approach. The earliest mathematical equation for digestibility was developed by Professor Young Cho (1973). A simplified equation (detailed in the Nutritional Requirements of Fish and Shrimp - NRC 2011) is now generally used.

$$ADC_{test\ ingredient} = ADC_{test\ diet} + \left[\left(ADC_{test\ diet} - ADC_{ref.diet}\right) \times \left(\frac{0.7 \times D_{ref}}{0.3 \times D_{ingredient}}\right)\right]$$

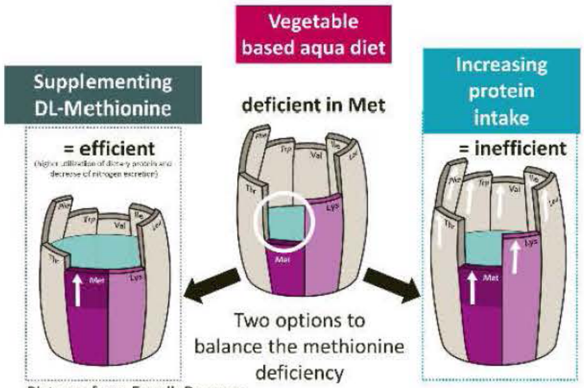
Where D_{ref} is the percentage of nutrient or J/g gross energy of the reference diet, and $D_{ingredient}$ is the percentage of nutrient or J/g gross energy of the ingredient. The values of 0.7 (70%) and 0.3 (30%) are used for levels of the reference diet and ingredient in the combined test diet.

Ideal Amino Acid (AA) Profile

Fish generally require 5% of lysine (Lys) and 2-3% sulfur-containing AA of crude protein. FM is abundant in Lys and sulfur amino acid (SAA) but most plant proteins are essential amino acid (EAA) deficient. Particularly when only considering the methionine (Met), but not SAA based on the digestible level, almost all plant proteins are Met deficient (Kaushik & Seiliez, 2010). A famous bucket effect diagram on AA balance theory (Figure 1) helps to illustrate this. Plant protein source ingredients, such as soybeans, have been successfully demonstrated to a certain level in substituting fishmeal in various aquaculture feed, such as trout, seabass, and shrimp with balanced essential AAs. When FM was replaced by a plant protein, Met is deficient. There are two options to balance the Met deficiency. One is supplementing dietary Met, and the other one is increasing protein intake. Obviously, the first approach could be more efficient. There are several methionine sources in the market with various relative bio-availability values (RBV). Two models are widely used for RBV studies. The first one is linear model. Take for example the comparison of DL-Met and Met-Met. The di-peptide Met-Met has a much higher RBV than DL-Met, about 1.5 times higher (Yin et al., 2017). The non-linear model, like exponential model is commonly used as well. Met hydroxy analog [MHA] (2-hydroxy-4-methylthiobutanamide salt) could be 75-80% of RBV of DL-Met on an equimolar basis for animals (NRC, 2011). In the CAAS lab, we also found that FM can be completely replaced by soy protein concentrate (SPC) by increasing feeding frequency in Nile tilapia (GIFT) under an ideal AA profile (Zhao et al., 2010), but only 50% of FM could be replaced in the diets of spotted seabass and Amur sturgeon (Liang et al., 2017; Wei et al., 2019, 2020) due to the different EAA requirements by different species.

Feed Intake Regulation

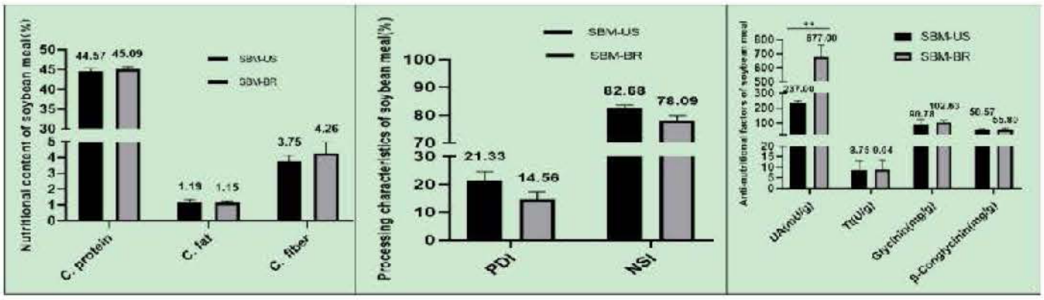
Feed intake should first consider feed design. FM is generally considered to be highly palatable for fish, but lower cost alternative proteins from plant or territorial animals with less palatability may impact the feed intake behavior of fish. We conducted several studies in the last decade on feed intake regulation with a carnivorous fish, Spotted Seabass (*Lateolabrax maculatus*) by various body sizes. In general, the specific growth rate (SGR) of spotted seabass showed the same trend as the feed intake, higher performance could be observed with higher feed intake (Hu et al., 2013; Liang et al., 2017, 2019). In an earlier study, a 16-week growth trail was conducted. The results showed that when the plant protein (SPC) levels increased, the SGR decreased during the first eight weeks. However, in the next eight-week period obvious adaptation and higher intake was shown in the plant protein group. Thereafter, with the decreased use of fishmeal in commercial feeds, the ability of adaptation to plant protein feeds for spotted sea bass is growing, which could be related to the gustatory system, like the umami taste receptor T1Rs (G protein–coupled taste receptors) degeneration (unpublished data). The fish gustatory system showed remarkable adaptive change to the plant protein, revealing that carnivorous fish T1Rs participate in nutrient sensing, digestion and absorption, which is of great significance for the efficient utilization of plant proteins, and could be a biomarker gene for plant-protein tolerance strain selection.



Sources and Processing

The genetic varieties and processing conditions of heat-labile anti-nutritional components of soybeans are a key concern when evaluating the quality of soybean meal in animal feed. There are large variations in SBM quality between different batches. We can visually see the different colors of these samples, and different responses from fish farmers with regards to the feed quality. There are some articles reporting very different feeding results when using these SBMs made from soybeans from these two origins. However, these reports did not compare results of fish growth and health when these different origin soybeans were processed into SBM using beans processed on the same equipment under controlled conditions. Our lab conducted a trial on the effects of using US and Brazilian SBM (processed under the same conditions using the same processing line in Qingdao, China) in feeds with Nile tilapia (GIFT strain). Much lower protein dispensability index (PDI) and nitrogen solubility index (NSI) in Brazilian soybeans could be related to the pre-heating damage by drying before oil pressing (Batal et al., 2000). US beans are largely air dried, while Brazilian bean are heat dried by natural gas. We also found the different physio-chemical properties between the FM and soy proteins. Some plant ingredients, such as SBM and/or SPC have higher expansion potential than fishmeal in extrusion process for floating pellet manufacture. The expansion potential from those plant ingredients could improve feed pellet hardness, oil absorption and reducing oil leakage. Higher pellet hardness might benefit for pellet durability index, however, it could prolong the digestion period and postpone the time for emptying intestine content, which could reduce the appetite of fish (Kraugerud et al., 2011; Zhang et al., 2019).

SBM from same processing line with US and Brazil beans



Nutrient composition Physio-chemical properties Anti-nutrition factors

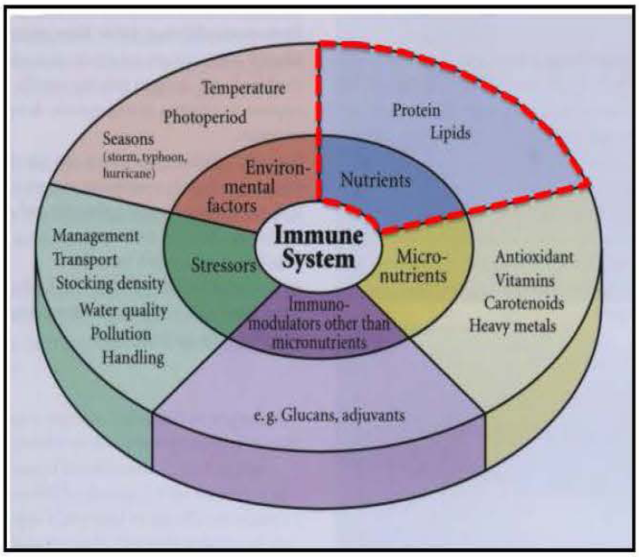
Nutrition and Relationships to Liver and Intestinal System Health

The liver and intestinal system physical conditions are one of the key concerns for aquatic animal health. When fishmeal is replaced by soymeal in a significant amount, some reported symptoms, such as enteritis and/or liver damage are commonly seen problems.

An Amur sturgeon nutrition study indicated distinct damage of intestine valves, poor energy metabolism, and low proliferation ability in intestinal cells causing high mortality when diets completely replaced fishmeal by plant protein (Wei et al., 2020).

In recent studies published in the Journal of Nutrition reported that *Cetobacterium* could be the most important bacteria supporting the balance of the inner-gut environment. In our recent studies in last year, we found that Brazilian SBM increased the frequency of serious enteritis, and in these fish, the prebiotic bacterial *Cetobacterium* decreased 62.55%, and the potential pathogenic bacterium, *Aurantimicrobium* increased 35.83%. Identifying regulatory mechanisms of nutritional metabolic disorders and conducting reasonable nutritional interventions are the inevitable ways to ultimately resolve the efficient utilization of plant proteins.

Nutrition is one of key factors on health



Summary

Considerations of experiment design for soy comparison in fish:

1) Improved digestibility: A correct protocol for digestibility determination is important, in which, the facilities, inner markers, determination methods and the formulations are key factors affecting the results.

2) Balance EAA: The EAA, like Lys, Met and Thr should be balanced when using SBM replace FM in fish diet. The RBV should be considered when select various sources of commercial amino acids.

3) Feeding stimulant / physio-properties by extrusion: we need to consider the palatability of feed with various ingredients. When FM is replaced by SBM, the feeding stimulant and proper pellet physio-properties, like hardness and hydration time should be considered for the acceptance of feeding behavior.

4) Better processing: A proper pre-treating of soy, and the SBM processing is important. The soybeans should be treated the same in processing to SBM to determine their relative value as nutrient providers.

5) Pre and Pro-biotics: When FM is replaced by soybean meal in significant proportions, the commonly reported symptom is enteritis, which may sometimes be accompanied by liver damage. Pre- or pro-biotics that could work as immunity enhancers could be considered for use in high SBM diets for fish.

About the Author

Dr. Min Xue
Prof. at Feed Research Institute, CAAS
Haidian District, Beijing, China

Title, Position, Works: Director of the Lab of National Feed Safety Evaluation Center, Feed Research Institute, Chinese Academy of Agriculture Sciences. She mainly focuses on the study of efficacy and tolerance test of feed and feed additives in aquatic target animals.

Alternative protein and lipids; Feed processing and Feeding technology; Start feeding and larval fish nutrition.

The group had published more than 80 publications in international and local language journals and she is working as the reviewer of more than ten International Journals.

Experience

Feed Research Institute, CAAS, Beijing, China
Feed Research Institute, CAAS
Lab of National Aquafeed Safety Assessment Center
Jul 2004 – Present, 16 years

Education

Ph.D Hydrobiology Institute, the Chinese Academy of China
Degree: Philosophy (PhD) in Hydrobiology
1998 – 2001

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