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Effects of crystalline amino acids, phytase and fish soluble supplements in improving nutritive values of high plant protein based diets for kuruma shrimp, *Marsupenaeus japonicus*

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ABSTRACT

Plant proteins are the most important alternatives to fishmeal in shrimp diets. However, there is a general trend of reducing feed intake and shrimp performance with dietary inclusion of high levels of plant proteins. Supplementation of feed additives could overcome the negative effects of high plant protein based diet. Therefore, an experiment was conducted to assess the effectiveness of different feed additives such as crystalline amino acids (CAA), phytase (PT) and fish soluble (FS) in enhancing the utilization of high levels of plant proteins in the diets of kuruma shrimp, *Marsupenaeus japonicus*. Six isocaloric diets (19 kJ g^{-1}) were formulated where diet 1 was 40% fishmeal based control diet (FM). Diets 2 to 6 were prepared as follows, by replacing 60% fishmeal protein with a plant protein blend (soybean meal and canola meal; 6:4) alone (PP); protein blend and 1.5% CAA (PAA); protein blend and 0.04% phytase (2000 FTU kg^{-1}) (PPT); protein blend and 10% FS (PFS); and protein blend and a mixture of CAA, phytase and FS (PMX) respectively. Triplicate groups of shrimp (1.75 ± 0.40 g; mean initial body weight ± SD) were stocked in 54-l rectangular tanks at a rate of 15 shrimp per tank. The tanks were maintained under natural light/dark regime in a flow-through sea water system. Shrimp were fed the respective test diets at a rate of 8–10% of the body weight daily for 56 days. At the end of the feeding trial, final body weight (g), weight gain (%) and specific growth rate (% day^{-1}) were significantly ($P < 0.05$) lower in shrimp fed PP diet. However, these growth parameters recovered when fed diets supplemented with CAA and FS. Although, growth parameters were slightly improved by the supplementation of PT, the differences in growth parameters between FM and PPT were still significant. The fastest growth was found in shrimp fed PMX among the dietary treatments. The growth results were mostly reflected by feed intake (FI). Significantly lowest FI was found in PP group, while similar values were found among the rest. Similarly, protein gain (g kg weight $gain^{-1}$) and protein retention (%) were significantly decreased in PP and PPT groups. Whole body crude protein was significantly lowest in PP group and highest in PMX group. Protease activity (unit mg^{-1} protein) in the digestive tract of shrimp was lowest in PP and PAA groups; and the values were comparable among the rest. Overall, the best results for most of the parameters were found in shrimp fed PMX diet.
Based on the overall performance of shrimp, it can be concluded that CAA, PT and FS are effective supplements in high plant protein diets for juvenile kuruma shrimp. A mixture of these supplements in appropriate ratio could further reduce the fishmeal levels in shrimp diets.

1. Introduction

Commercial shrimp feeds are often rich in fishmeal (25–50% of the diet), the preferred protein source due to its unique nutritional specifications including essential amino acids (EAA), essential fatty acids, cholesterol, vitamins, minerals, attractants and many other finite micronutrients (Bulbul et al., 2015; Hardy, 2010; Suarez et al., 2009; Tacon and Metian, 2008). However, the continuous increasing demand in contrast to limited supply have soared the fishmeal price almost double during the last few years (http://www.globefish.org). Furthermore, it has been predicted that future demand for fishmeal is expected to increase as aquaculture production expanded. Therefore, replacement of fishmeal with cost-effective alternative protein sources is the prerequisite for profitable aquaculture venture.

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The use of plant proteins as alternative protein sources to replace fishmeal has become the focus of studies in shrimp feeds around the world (Gatlin et al., 2007). However, the higher dietary inclusion of plant protein has negatively affected the dietary amino acid composition, palatability and antinutritional components of diets, thus decreased feed intake (FI) and performance of shrimp (Bulbul et al., 2013, 2015; Lim and Dominy, 1990). Therefore, one of the prerequisites for effective utilization of higher levels of plant proteins in shrimp feed is to recover depleted FI. Addition of feeding stimulants/feed additives could play a positive role in inducing adequate feed consumption through maintaining feed attractiveness in high plant protein based diet. Commonly recognized feeding stimulants are relatively small soluble molecules, such as certain amino acids (taurine, glycine, arginine, glutamic acid and alanine), betaine, nucleotides and organic acids which are rich in marine organisms (Grey et al., 2009; Kader et al., 2010; Smith et al., 2007). In comparison, plant proteins contain less of these substances. Nevertheless, plant proteins also contain some unpalatable substances such as antinutritional components, toxins etc. These lead to decreased palatability of plant protein rich diets for fish and shrimp. Supplementations of feeding stimulants and palatability enhancers in shrimp feeds are important when developing diets containing large amounts of plant proteins in order to maintain feed attractiveness and induce adequate feed consumption rate by shrimp (Mendoza et al., 2001; Nunes et al., 2006). Crystalline amino acid (CAA), phytase (PT) and fish soluble (FS) are effective supplements in high plant protein based diets for fish and shrimp (Biswas et al., 2007; Kader et al., 2010, 2012; Mendoza et al., 2001). Methionine and lysine are the limiting amino acids in most of the plant proteins which need to be supplemented during the inclusion levels of plant proteins exceeding certain levels. Phosphorus is stored as phytic acid in plant proteins which is not available to monograstric animals and therefore accumulated to the aquatic environment and caused water pollution (Hardy, 2010). PT is an enzyme chemically known as myo-inositol-hexaphosphate phosphohydrolase. Supplementation of PT in diets containing high levels of plant protein substantially increases phosphorus digestibility, reduces phosphorus leaching from faces and improves growth performance as observed in rainbow trout (Cain and Garling, 1995), *Pangasius* catfish (Debnath et al., 2005) and tiger shrimp (Biswas et al., 2007). FS is a by-product of bonito flake processing industry which is proven as a natural feeding stimulant in fish (Kader and Koshio, 2012; Kader et al., 2010, 2012; Kousoulaki et al., 2009). Kader et al. (2012) reported that supplementation of FS could significantly improve FI of red sea bream fed high SBM based diet which was even better than fishmeal based control diet.

In the previous study, it was found that up to 50% of fishmeal could be replaced (fishmeal from 40% down to 20%) with a blend of soybean meal (SBM) and canola meal (CM) (6:4) without affecting growth performance of kuruma shrimp (Bulbul et al., 2013), while these two plant proteins could individually replace only 45% (Bulbul et al., 2015) and 20% (Bulbul et al., 2014) of dietary fishmeal respectively. In our previous studies (Bulbul et al., 2013, 2015), it was also found that FI and growth performance were significantly decreased by replacing 60% fishmeal which might be related to imbalanced amino acids, decreased palatability or presence of antinutritional factors in plant proteins. Supplementation of CAA, PT or FS might improve the dietary deficiency and support higher fishmeal replacement levels. Although CAA was supplemented in kuruma shrimp diets by some investigators (Alam et al., 2002), no study is available to utilize PT and FS in this shrimp. Comparative evaluation or combined effects of these supplements were also not made yet. In addition, research on alternative protein sources for kuruma shrimp is scarce. The present study was conducted to investigate the effects of supplementation of CAA, PT and FS in high plant protein and low fishmeal based diets on FI, growth performance, body composition and enzymatic activity of juvenile kuruma shrimp.

2. Materials and methods

2.1. Preparation of test diets

The ingredient composition and chemical analysis of the test diets were shown in Table 1. All the dietary components were obtained commercially, except for FS, which was provided by "Makurazaki Fish Processors Cooperatives, Kagoshima, Japan". It has been using as a potent feeding stimulant in fish diets (Kader et al., 2010, 2012). The dietary components and the basal diet were the same as used previously (Bulbul et al., 2013, 2014, 2015). High quality brown fishmeal was used as the major protein source in the basal diet and a blend of SBM and CM at 6:4 ratio was used to replace fishmeal in other test diets. Six isonitrogenous (44% crude protein), isolipidic (15% total lipid) and isocaloric (19 kJ g^{-1} gross energy) diets were formulated; where diet 1 was a 40% fishmeal based control diet (FM). Diets 2 to 6 were prepared as follows, by replacing 60% fishmeal protein with the plant protein blend alone (PP); plant protein blend and 1.5% CAA (PAA); plant protein blend and 0.04% PT (2000 FTU kg^{-1}) (PPT); plant protein blend and 10% FS (PFS); and plant protein blend and a mixture of CAA, phytase and FS (PMX), respectively. Squid liver oil, soybean lecithin, cholesterol and n-3 highly unsaturated fatty acids (HUFA) were supplied as lipid sources.

Table 1
Ingredient composition and proximate analysis of the experimental diet (% dry matter basis).

Ingredient	Diet groups					
	FM	PP	PAA	PPT	PFS	PMX
Fish meal[a]	40.00	16.00	16.00	16.00	16.00	16.00
Dehulled soybean meal[b]	–	20.00	18.30	20.00	11.80	10.00
Canola meal[b]	–	18.30	16.70	18.30	10.80	9.20
Krill meal[a]	5.00	5.00	5.00	5.00	5.00	5.00
Squid meal[a]	10.00	10.00	10.00	10.00	10.00	10.00
Squid liver oil[c]	2.00	4.00	4.00	4.00	4.00	4.00
Soybean lecithin[c]	3.00	3.00	3.00	3.00	3.00	3.00
n-3 HUFA[d]	0.50	0.50	0.50	0.50	0.50	0.50
Cholesterol[e]	1.00	1.00	1.00	1.00	1.00	1.00
Starch	7.00	2.00	2.00	2.00	2.00	2.00
Dextrin	3.00	2.00	2.00	2.00	2.00	2.00
Vitamin mixture[f]	1.70	1.70	1.70	1.70	1.70	1.70
Mineral mixture[g]	8.60	8.60	8.60	8.60	8.60	8.60
Activated gluten	5.00	5.00	5.00	5.00	5.00	5.00
Amino acid[h]	–	–	1.50	–	–	1.50
Phytase[i]	–	–	–	0.04	–	0.04
Fish soluble[j]	–	–	–	–	10.00	10.00
CMC	1.00	1.00	1.00	1.00	1.00	1.00
Attractants[k]	1.40	1.40	1.40	1.40	1.40	1.40
α-cellulose	10.80	0.48	2.35	0.44	6.22	8.05
Proximate composition (% dry matter basis)						
Crude protein	44.28	44.87	44.60	44.80	44.95	45.03
Total lipid	15.28	15.16	15.20	15.36	15.96	15.10
Ash	14.74	13.13	13.05	13.20	14.67	14.60
Gross energy (kJ g^{-1})[l]	19.37	19.44	19.59	19.64	19.55	19.36

[a] Nippon Suisan Co. Ltd., Tokyo, Japan.
[b] J. Oil Mills, Japan.
[c] Riken Vitamin, Tokyo, Japan.
[d] Poweash A, Oriental Yeast Co, Ltd., Tokyo, Japan.
[e] Takeda Kagaku Siriyo, Japan.
[f] Vitamin mixture (g 100 g^{-1}): ρ-Amino benzoic acid, 9.48; D-Biotin, 0.38; Inositol, 379.20; Niacin, 37.92; Ca-pantothenate, 56.88; Pyridoxine-HCl, 11.38; Riboflavin, 7.58; Thiamin-HCl, 3.79; L-ascorbyl-2-phosphate-Mg, 132.00; Folic acid, 0.76; Cyanocobalamine, 0.08; Menadione, 3.80; Vitamin A-palmitate, 17.85; α-Tochopherol, 18.96; Calciferol, 1.14.
[g] Mineral mixture (g 100 g^{-1} diet): K_2PO_4, 2.011; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 2.736; $MgSO_4 \cdot 7H_2O$, 3.05; $NaH_2PO_4 \cdot 2H_2O$, 0.795.
[h] Amino acid (g 100 g^{-1} diet): Lysine, 1.00; Methionine, 0.05.
[i] Ronozyme P 5000, DSM Nutrition Japan K.K., Shizuoka, Japan.
[j] Makurazaki Fish Processor Cooperatives, Kagoshima, Japan
[k] Attractants (g 100 g^{-1} diet): Glucosamine HCl, 0.80; Sodium succinate, 0.30; Sodium citrate, 0.30.
[l] Calculated using combustion values for protein, lipid and carbohydrate of 236, 395 and 172 kJ kg^{-1}, respectively.