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Study of Enzymolysis Kinetics with Four Kinds of Protein Feeds in *Megalobrama amblycephala in vitro*

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Abstract: This research provides a comparative study on the ability of protein digestion and enzymolysis with 4 kinds of common feed ingredients in *Megalobrama amblycephala in vitro*. About 12 *Megalobrama amblycephala* with were temporarily fed a day for eliminating the contents of digestive canal. The intestine and hepatopancreas were took out for the preparation of the digestive (I've juice sample) for digesting four kinds of common feed ingredients (fish meal, soybean meal, rapeseed meal and cottonseed meal) by enzymatic hydrolysis *in vitro*. The results showed that compared with the hepatopancreas, the intestine of *Megalobrama amblycephala* possessed better enzymolysis ability for protein in feed ingredients. Moreover, the enzymolysis ability of post intestine was the highest and the lowest in former intestine. The enzymolysis rate of fish meal (6.543 mg h^{-1}) in intestine within 0-4 h was higher than that of other feeds also the enzymolysis rate of fish meal (1.781 mg h^{-1}) was the highest in hepatopancreas than others ($p<0.05$).

Key words: Digestive *in vitro*, feed ingredients, enzymolysis kinetics, *Megalobrama amblycephala*, hepatopancreas, China

INTRODUCTION

Blunt snout bream (*Megalobrama amblycephala*) a herbivorous freshwater fish native to China has been widely favored as a delicacy and become currently the most widely cultivated fish in China. This species was originated in Lake Liangzi in Ezhou (Hubei, China) and its main distribution is in the middle reaches of the Yangtze river (Tsao, 1960).

Aquaculture of this fish in China has expanded rapidly during the last decade because of its fast growth, use of natural foods, tender flesh and high diseases resistance (Ke, 1986; Zhou *et al*., 2008). In 2005, the total production of bream reached 552,990 tons and is about 7% higher than in 2004 (MAPRC, 2005). Because this species can adapt well to the local environment, it is also distributed in North America (Northern Canada to Southern Mexico), Africa, Europe and other Asian countries. It thus has a bright future for culture worldwide (Ke, 1986).

Blunt snout bream feed mainly on *Vallisneria natans*, *Hydrilla verticillata* and zooplankton in their natural environment (Hong-Wen, 1975). However, it can accept formulated feeds. Traditional production of this fish relies on diets formulated for grass carp (*Ctenopharyngodon idella*) supplemented with some aquatic weeds. Little information on the nutritional requirements of this species has been reported.

Blunt snout bream requires 27-30% protein for optimal growth when water temperature is about 20°C and the optimum protein requirement ranges from 25.6-41.4% when water temperature varies from 25-30°C (Shi *et al*., 1988). In addition, blunt snout bream required a diet containing 2-5% lipids and the best growth performance was obtained when the dietary lipid level was about 3.6% (Liu *et al*., 1992). This is in agreement with who stated that optimal growth of blunt snout bream occurred with diets containing 4-6% lipid (Zhou *et al*., 1997). The traditional method is to conduct long-term growth experiments for assessing the performance of ingredients and feeds. This is likely to be the most dependable method but feeding trials with blunt snout bream are difficult, time consuming and expensive so that only a limited number of variables can be tested during each growing season. The need to

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rapidly screen large numbers of feed ingredients and to test a variety of feeds is especially important for the rapid development of viable feeds for blunt snout bream and other species new to aquaculture. As a result complementary research methods are being assessed in order to more rapidly achieve a viable manufactured blunt snout bream feed (Van Barneveld *et al*., 1997; Carter *et al*., 1998).

The difficulties in working with blunt snout bream exclude the routine use of *in vivo* digestibility procedures. Since, ingredient digestibility has a major effect on nutrient utilisation and growth the use of *in vitro* digestibility methods has potential for use in the development of blunt snout bream feeds.

A considerable amount of research has been conducted on the use of *in vitro* systems for predicting the digestibility of feeds and feed ingredients. Research has focused on farm animals (Boisen and Eggum, 1991; Fuller, 1991) particularly ruminants and it is clear that the development of systems for studies on fish requires modifications although, the general principles are the same. The major issues relate to the sensitivity of the assays whether they have the potential to separate different feed ingredients and how accurately they predict *in vivo* digestibility values. A number of studies have demonstrated significant correlations between *in vitro* and *in vivo* digestibility (Eid and Matty, 1989; Dimes and Haard, 1994; Dimes *et al*., 1994a, b).

The aims of this research were to screen feeds and ingredients with potential for inclusion in feeds for blunt snout bream and to provide ingredient digestibility data to be used in formulating new fish feeds. This involved the measurement of *in vitro* digestibility of a range of ingredients and feeds using different enzyme systems based on intestine and hepatopancreas digestive enzymes in blunt snout bream. Comparisons between these *in vitro* digestibility values and between *in vivo* digestibility measurements from bream were made to assess their performance.

MATERIALS AND METHODS

Experimental materials: About 12 of the blunt snout bream (average weight, 712±38 g) were caught at XinFa aquaculture base in Xinxiang (Henan, China). Prior to the experiment, fish were kept fasting in room aquarium for 24 h to clean their digestive tract contents.

Feed ingredients: Four feed ingredients, fish meal, soybean meal, rapeseed meal and cottonseed meal were used as protein sources for digesting experiment *in vitro*, respectively. Proximate composition of the feed ingredients is measured using conventional methods after grinding through 80 mesh sieve (Table 1). All ingredients were stored at 42°C in plastic-lined bags until used.

Table 1: Content of nutrient composition of trail feed ingredients (air-dry ,%)

Feed ingredients	Fish meal	Soybean meal	Rapeseed meal	Cottonseed meal
Crude protein	56.12±0.21	44.56±0.12	38.25±0.10	36.45±0.18
Crude lipid	13.54±0.13	18.01±0.14	15.33±0.09	14.89±0.13
Crude ash	10.82±0.06	6.04±0.09	6.24±0.14	6.15±0.09
Moisture	11.15±0.10	12.32±0.11	13.05±0.15	13.29±0.17

Digestive enzyme extraction: Digestive enzyme is extracted according to the method reported by Yuantu *et al*. (2003). The intestine (former bowel, midgut and posterior intestine) after removing fat and mesentery and hepatopancreas of bream were selected with conventional anatomy, then they were weighed after drying them with filter paper and homogenized quickly using the glass frozen homogenizer and ten times of the volume, 0.2 M phosphate buffer (pH 7.4) was added. Homogenate was centrifuged at 10,000 g for 20 min at 4°C and the supernatant was removed for further analysis.

Enzymolysis assays: The enzymolysis assay was conducted described by Yuantu *et al*. (2003), four feed ingredients was weight. The enzyme reaction mixture consisted of 30 mL, 0.2 M phosphate buffer (pH 7.4) and 10 mL digestive enzyme from various parts of the digestive tract as previously described which mixed the four feed ingredients (weight, 0.3000±0.0002 g) in the 100 mL flask with the plug, respectively. Furthermore, 150 IU mL^{-1} penicillin and streptomycin sulfate were added to each group of the enzyme reaction mixture, respectively for preventing microbial contamination and add one every 4 h. The reaction mixture were incubated and enzymed in the shaker for 12 h at 28±1°C, 50 times min^{-1}.

The recation was terminated after 12 h and the crude extract was filtered and washed 3-4 times with 30°C hot water. The residues (including filter) were baked at 105°C until constant weight in the oven, the crude protein content of residues was measured according to the national standards in China (GB/T 6432-1994, method for the determination of crude protein in feedstuffs). The crude protein digestibility from four protein feeds was calculated as follows:

$$\text{Digestibility}(\%) = \frac{\text{Feed sample weight} - \text{Residues weight}}{\text{Feed sample weight}}$$

$$\text{Crude protein digestibility}(\%) = \frac{\text{Feed sample weight} \times \text{Feed crude protein} - \text{Residues weight} \times \text{Residues crude protein}}{\text{Feed sample weight}} \times 100$$

Measuring and calculating of digestibility of the blunt snout bream *in vitro*: In the enzymatic digestion of the process *in vitro* within the first 4 h as 2.4, 0.2 mL supernatant of each test group was taken every 30 min then 10% trichloroacetic acid was immediately added to fix proteins, homogenate was centrifuged at 6000 r min^{-1} for 25 min, the 0.2 mL supernatant was removed and mixed with 3.0 mL distilled water, 2.0 mL ninhydrin reagent. The mixture was placed in a boiling water bath for10 min then cooled to room temperature. The leucine-A standard curve was made with without the sample group as the blank control and Leucine (Leu) as the standard solution. Each sample was made seven parallel tests and each treatment group was repeated five times, the absorbance (A value) was measured with a 723 spectrophotometer at 570 nm for quantitative analysis. Amino acid generating rate by dietary protein digestion is calculated as the method by Chong *et al.* (2002).

The regression line was made with the amount of amino acid hydrolysis which change along with variation of time therefore, the slope of the curve was the amino acid production rate.

Statistical analysis: Data were analyzed using the SPSS General Linear Models (GLM) procedure (SPSS 7.5, Michigan Avenue, Chicago, IL, USA) for significant differences among groups. If significant ($p<0.05$) differences were found in factors, Duncan's Multiple Range Test (MRT) was used to rank the means. All data were presented as means±SEM (Standard Error of the Mean) of three replications.

RESULTS AND DISCUSSION

The affect on digestibility of four high-protein feed ingredients in the blunt snout bream *in vitro*: The *in vitro* digestibility of crude protein in different organizations of bream: compared with the hepatopancreas, the intestine of *Megalobrama amblycephala* possessed better enzymolysis ability for protein in feed ingredients. Moreover, the enzymolysis ability of posterior intestine was the highest followed by the midgut, former bowel was minimum (Table 2). Furthermore, there are also differeces in the digestibility of dry matter among the feeds and feedstuffs: in terms of hepatopancreas digestibility, fish meal was maximum followed by the rapeseed meal and the third was soybean meal, the difference was not significant between soybean meal and cottonseed meal ($p>0.05$) however, the digestibility of the intestinal to the various parts of protein feeds, the fish meal was the highest followed by soybean meal, cottonseed meal was the minimum, the difference was not significant between soybean meal and cottonseed meal in the midgut and posterior intestine ($p>0.05$) but the difference was significant in the midgut and former bowel ($p<0.05$) taking for the digestibility of pancreatic and intestinal, fish meal was maximum followed by the soybean meal and the third was rapeseed meal, the difference was not significant ($p>0.05$) (Table 3).

The comparison of enzymolysis rate with four kinds of protein feeds in intestine and hepatopanceas in *Megalobrama amblycephala*: The total amount of amino acids are fish meal>soybean meal>rapeseed meal> cottonseed meal which are generated with the protein feed ingredients enzymolysis by whether intestinal or hepatopanceas digestive enzyme fluids in 0-4 h however, the total amount of amino acids are hepatopanceas> intestinal whatever feed ingredients are (Table 4).

The mean velocity of generating amino acids is: fish meal: 5.352 mg h^{-1}; soybean meal: 4.662 mg h^{-1}; rapeseed meal: 4.662 mg h^{-1}; cottonseed meal: 3.92 mg h^{-1}. Therefore, the mean velocity of generating amino acids is fish meal>soybean meal>rapeseed meal>cottonseed meal enzymolied by digestive enzyme fluids of digestive tract in *Megalobrama amblycephala*; moreover, the speed of generating amino acids is maximum (6.543 mg h^{-1}) which digested fish meal by intestinal digestive enzyme but the speed of generating amino acids is not very different in the hepatopanceas, the fish meal is 1.781 mg h^{-1}; cottonsed meal is 1.522 mg h^{-1} ($p>0.05$). There is a certain linear relationship with changes in the 0~4 h with formation of amino acids produced by hydrolysis and the time.

The comparison of enzymolysis rate with four kinds of protein feeds in different parts of the intestin in *Megalobrama amblycephala*: The total amount of amino acids are posterior intestine>midgut>former bowel

Table 2: The digestibility *in vitro* for *Megalobrama amblycephala* to the crude protein of feed ingredients (Means±SE) (%) (air-dry, %)

	Digest enzyme sample				
Feed ingredients	Hepatopanceas	Intestine	Former bowel	Midgut	Posterior intestine
Fish meal	16.32±0.24^{a}	50.87±0.18^{e}	34.28±0.25^{b}	54.31±0.19^{e}	59.29±0.31^{d}
Soybean meal	13.39±0.72^{a}	48.26±0.24^{c}	30.12±0.41^{b}	51.42±0.26^{c}	57.35±0.27^{d}
Rapeseed meal	10.37±0.19^{a}	32.48±0.17^{c}	27.09±0.25^{b}	42.23±0.21^{c}	45.51±0.14^{d}
Cottonseed meal	9.98±0.23^{a}	30.12±0.31^{c}	24.06±0.23^{b}	41.15±0.52^{c}	44.17±0.32^{d}

Table 3: The digestibility *in vitro* for *Megalobrama amblycephala* to the dry matter of feed ingredients (Means±SE) (%) (Air-dry, %)

	Digest enzyme sample				
Feed ingredients	Hepatopanceas	Intestine	Former bowel	Midgut	Posterior intestine
Fish meal	20.32±0.24^{a}	33.54±0.14^{b}	29.47±0.25^{b}	41.56±0.19^{c}	45.98±0.14^{c}
Soybean meal	17.49±0.72^{a}	30.69±0.09^{b}	28.36±0.41^{b}	37.08±0.27^{c}	42.49±0.21^{c}
Rapeseed meal	19.37±0.19^{a}	30.12±0.43^{b}	26.98±0.21^{b}	34.85±0.35^{c}	40.56±0.09^{c}
Cottonseed meal	17.98±0.23^{a}	29.23±0.24^{b}	26.08±0.51^{b}	37.54±0.21	41.07±0.17^{c}

Table 4: The amount of amino acids produced from feed ingredients degraded by digestive enzyne in intestine and hepatopanceas of *Megalobrama amblycephala* (mg)

		Time (h)							
Digest enzyme sample	Feed ingredients	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Intestine	Fish meal	5.43±0.64	9.20±1.05	14.54±1.32	28.83±5.16^{a}	35.32±2.83^{a}	38.85±1.72	43.95±1.96	49.22±1.20
	Soybean meal	2.33±0.25	5.22±0.59	14.19±1.98	16.75±1.30	29.80±3.72	32.52±3.61	33.67±3.99	41.59±1.01
	Rapeseed meal	1.67±0.35	4.08±1.51	8.56±1.40	14.55±0.33	24.73±1.79	26.55±1.12	32.72±0.96	37.18±5.48
	Cottonseed meal	0.96±0.15	3.54±1.11	7.64±0.82	15.25±1.67	22.09±2.07	26.08±1.26	29.37±1.96	34.08±4.77
Hepatopanceas	Fish meal	1.58±0.17	2.48±0.29	4.63±0.46	8.98±1.48	10.57±0.95	10.63±0.73	10.96±0.48	14.12±0.78
	Soybean meal	0.33±0.04	2.51±0.28	3.04±0.17	6.19±0.48	6.22±0.31	8.76±0.39	11.94±0.36	13.24±0.45^{a}
	Rapeseed meal	0.78±0.49^{a}	1.66±0.32^{a}	4.07±0.26^{a}	5.06±0.08^{a}	5.68±0.11^{a}	7.15±0.25^{a}	10.08±0.35^{a}	12.55±0.29^{a}
	Cottonseed meal	0.75±0.35^{a}	1.61±0.21^{a}	2.26±0.26^{a}	5.48±0.19^{a}	6.04±0.11^{a}	7.40±0.32^{a}	9.78±0.32^{a}	12.64±0.17

Table 5: The quantity of amino acids produced in intestinal and liver liquid ezymolysing feed ingredients (mg)

		Time (h)							
Enzymes fluid samples	Feed ingredients	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Former bowel	Fish meal	3.292±0.46	6.50±0.56	8.68±0.17	27.70±3.390^{a}	30.466±1.49	30.52±2.390^{a}	37.52±0.670	39.370±3.67^{a}
	Soybean meal	0.780±0.13	1.31±0.37	3.52±0.53	7.45±0.500	20.560±0.61	23.52±0.810	25.22±1.260	26.030±1.19
	Rapeseed meal	0.970±0.07^{a}	2.94±0.41^{a}	7.81±0.53^{b}	13.34±0.480^{b}	20.770±0.34^{b}	25.39±0.270^{b}	28.09±0.810^{b}	29.700±0.73^{b}
	Cottonseed meal	1.360±0.16^{a}	4.41±0.32^{a}	12.69±0.74^{b}	15.85±0.450^{b}	26.970±0.40^{b}	29.41±0.320^{b}	29.92±0.780^{b}	32.480±0.78^{b}
Midgut	Fish meal	8.790±1.15	11.02±1.22	19.56±3.08	31.64±2.900	38.840±1.39	42.97±1.590	48.41±2.620^{a}	53.620±5.67^{a}
	Soybean meal	5.360±0.58	7.45±0.88	9.07±4.50	20.427±2.85^{b}	29.570±2.19	30.86±2.160	39.53±3.140^{b}	47.230±2.86^{b}
	Rapeseed meal	0.860±0.05^{a}	4.95±0.09^{b}	8.31±0.05^{b}	16.11±0.380^{b}	20.910±0.34^{b}	25.43±0.190^{b}	28.46±0.640^{b}	32.480±0.92^{c}
	Cottonseed meal	1.750±0.22^{a}	2.33±0.38^{a}	13.37±0.33^{b}	16.32±0.590^{b}	27.760±0.62^{b}	31.06±0.880^{b}	32.61±0.760^{b}	34.850±0.81^{c}
Posterior intestine	Fish meal	7.000±0.81	10.07±1.13	15.37±2.01	27.15±1.540	36.660±6.70^{a}	43.08±2.500	45.92±1.550	54.680±1.35
	Soybean meal	1.370±0.23	6.89±0.75	13.08±0.22	15.79±2.320	24.050±2.62	25.276±1.21	33.395±1.56	51.502±1.79
	Rapeseed meal	1.050±0.11^{a}	2.77±0.17^{a}	6.78±0.13^{b}	16.28±0.780^{b}	24.600±0.89^{c}	27.41±0.820^{c}	31.55±0.650^{b}	40.080±0.33^{d}
	Cottonseed meal	1.910±0.11^{a}	5.51±0.43^{a}	16.51±0.65^{c}	18.09±0.570^{b}	34.670±0.10^{c}	37.09±0.490^{c}	38.48±0.930^{c}	44.210±0.56^{d}

Values are means±SE of 7 replications. Means in the same row with different superscripts are significantly different ($p<0.05$)

which are generated with the protein feed ingredients enzymolysis in 0-4 h (Table 5). In the same kinds of feed ingredients, the enzymolysis rate of protein is maximum in the midgut (fish meal: 7.046 mg h^{-1}; soybean meal: 6.175 mg h^{-1}; rapeseed meal: 5.978 mg h^{-1}; cottonseed meal: 5.354 mg h^{-1}) followed by posterior intestine (fish meal: 6.941 mg h^{-1}; soybean meal: 5.850 mg h^{-1}; rapeseed meal: 5.10 mg h^{-1}; cottonseed meal: 4.700 mg h^{-1}) the minimum for foregut bowel (fish meal: 5.693 mg h^{-1}; soybean meal: 4.470 mg h^{-1}; rapeseed meal: 4.331 mg h^{-1}; cottonseed meal: 3.990 mg h^{-1}) (Table 6).

That's very important for the development of rapid and reliable methods for predicting the value of protein sources for immediate use in *M. amblycephala* feeds. It is the most useful to measure the digestibility and several methods *in vitro* have been used in the development of fish feeds (Eid and Matty, 1989; Dimes and Haard, 1994; Dimes *et al*., 1994a, b; Haard, 1995). The success of these methods can be judged by the use of *in vitro* digestibility to first, accurately rank ingredients according to their *in vivo* ingredient digestibility values; second, give an accurate prediction of *in vivo* ingredient digestibility; third, rank the relative growth performance growth rate or feed efficiency. Of the species under study and fourth, accurately predict the growth performance of the species fed feeds containing the ingredient. The principle aim of the current research was to develop a method for

Table 6: Amino acids production rate and the regression equation to generate the amount of amino acids

Enzymes fluid samples	Feed ingredients	Regression equation	R^2	Amino acid formation rate (mg h^{-1})
Former bowel	Fish meal	y = 5.6394x-8.0567	0.9298	5.6394
	Soybean meal	y = 4.4723x-6.9702	0.9834	4.4723
	Rapeseed meal	y = 4.3314x-7.2174	0.9678	4.3314
	Cottonseed meal	y = 3.9901x-7.9257	0.9027	3.9901
Midgut	Fish meal	y = 6.9418x6.3944	0.9837	6.9418
	Soybean meal	y = 5.85x-10.254	0.9307	5.8500
	Rapeseed meal	y = 5.0995x-7.5894	0.9493	5.0995
	Cottonseed meal	y = 4.6996x-6.3649	0.9515	4.6996
Posterior intestine	Fish meal	y = 7.0464x-8.5728	0.9846	7.0464
	Soybean meal	y = 6.175x-8.8875	0.9565	6.1750
	Rapeseed meal	y = 5.9783x-8.834	0.9688	5.9783
	Cottonseed meal	y = 5.3539x-9.9213	0.9628	5.3539
Liver pancreatic	Fish meal	y = 1.781x-1.7981	0.9490	1.7810
	Soybean meal	y = 1.6843x-2.684	0.9699	1.6843
	Rapeseed meal	y = 1.6014x-2.7961	0.9506	1.6014
	Cottonseed meal	y = 1.5224x-2.3482	0.9635	1.5224
Whole bowel	Fish meal	y = 6.5425x-7.6746	0.9780	6.5425
	Soybean meal	y = 5.5297-3.6505	0.9471	5.5297
	Rapeseed meal	y = 5.2728x-9.0045	0.9688	5.2728
	Cottonseed meal	y = 5.0376-5.2933	0.9859	5.0376

predicting the digestibility of ingredients to allow more accurate formulation of new tuna feeds. The results supported the use of *in vitro* digestibility methods for this task. The *in vitro* method produced data with a range of values that differentiated between ingredients that showed there were significant correlations between the different enzyme systems used and that showed there were significant correlations between *in vitro* and *in vivo* measures of digestibility although, there were differences due to the different enzyme systems and the calculation of apparent digestibility.

Proteins are the major source of energy for aquatic animals. As other animals, fish can uptake feed proteins from the environment and break down them into smaller peptides and amino in the digestive tract for further adsorption and utilization of the body. The digestion of feed proteins is mainly related to the activity of proteases in the fish's digestive tract. Moreover, the degradation of protein can be accomplished under the synergistic reaction of enzymes from the intestinal tract and hepatopancreas. As for stomachless fish, the anterior intestine is slightly intumescent and considered as the stomach of these fish but this intumescent structure can only function as the food depot and can not act as the pepsin secernent.

Consequently, the preliminary digestive way of feed proteins for stomachless fish species is different from that of non-ruminants or fish species with stomach. Grass carp fish is an herbivorous and stomachless fish and has a longer intestinal tract than sarcophagous and omnivcrous fish species. Due to the reabsorption function, the protease activity of the end-gut for grass carp fish is higher than that of the foregut and midgut. *Megalobrama amblycephala* also belongs to an herbivorous fish and the zymolytic activity of their intestinal tract on protein feedstuff is in the order of end-gut>midgut>foregut>hepatopancreas which is in agreement with the Huang's report about the preliminary study on protease activity of the intestinal tract and hepatopancreas (Yaojian and Yongjian, 1988).

In this study, *M. amblycephala* had well digestive and zymolytic abilities on fishmeals and could quickly produce amino acids. Among four kinds of protein feedstuffs, the intestinal tract of *M. amblycephala* had well zymolytic abilities on soybean meal and rapeseed meal but it was worse as for hepatopancreas. The exsomatize digestion rate of crude proteins in four kinds of protein feedstuffs was in the order of fish meal>soybean meal>rapeseed meal>cotton seed meal, suggesting that the exsomatize eymolytic ability of *M. amblycephala* on four kinds of feed proteins was in agreement with their exsomatize digestion ability on four kinds of feeds. Moreover, it also kept the same trend with the formation rate of amino acids. The eymolytic rate and degree were related with the types and amino acids compositions of feedstuffs. Here, fish meal, soybean meal, rapeseed meal and cotton seed meal had different protein compositions. Subsequently, it would result in the difference at the digestion rate and generating rate of amino acids under the effect of the same protease due to the significant difference of zymolytic sites and number. Moyano compared the hydrolytic activities of animal purification enzymes and stomach digestive enzymes of red SeaBream on fish meal and soybean meal. The results indicated that both enzymes had better hydrolytic activity on fish meal than soybean meal and each enzyme had the same eymolytic activity on proteins of fish meal and soybean meal (Moyano and Savoie, 2001). Savoie *et al.* (1989) demonstrated that animal proteins could release much more free amino acids and peptides than

leguminous proteins. Additionally, the vegetal protein feeds such as soybean meal, rapeseed meal and cotton seed meal, contained different types and amounts of anti-nutritional factors, meanwhile the different amino acid compositions and content could cause different degrees of Maillard reaction with reducing sugars. It would further result in the difference at the release rate of amino acids under the effect of proteases. Parsons *et al.* (1992) considered that the utilization ratio of amino acids in soybean meal could be decreased after heat treatment for too long a time or at too high a temperature.

Shimei and Li (2008) found that Procypris rabaudi had better eymolytic activity on non-expanded soybean meal than expanded soybean meal but the activity was contrary to non-expanded and expanded rapeseed meal or cotton seed meal. Under the effect of pepsin and trypsin, animal protein could release much more free amino acids followed by leguminous proteins. Balance rations for dietary Essential Amino Acids (EAA) could enhance the growth of grass carp fish. Therefore, the better the balance rations of feed EEA was the easier the digestion of feed was. It suggested that the protein quality, amino acid compositions and processing of the feedstuff affected the protein digestion.

CONCLUSION

This study shows that *Megalobrama amblycephala* had a good ability to digest fish meal but the digestive enzymes to other feed ingredients are a bit poor.

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