Bacterial Protein Meal Digestibility, Digestive Enzyme Activity And Kinetics In Barramundi *Lates calcarifer* Juveniles

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Background

The rapid growth of aquaculture industries in recent decades has led to the scarcity and increasing cost of fish meal, which has traditionally been used as primary protein source in aquafeeds. Consequently, alterative protein sources are being explored to partially or fully replace fish meals. Bacterial protein meals (BPMs) have emerged as one of sustainable alternative protein sources for aquafeeds, however their ingredient apparent digestibility coefficients (IADCs) remain insufficiently characterized. This objective of this study was to evaluate the digestibility and digestive enzymatic responses to a BPM derived from beverage by-products in Asian seabass (*Lates calcarifer*).

Methodology

Feed Formulation

BPM was produced from aerobic heterotrophic bacteria grown in the wastewater of beverage processing factory. Experimental diets were formulated and mixed with a 60L mixer (Hobort) before being dried at 65°C.

Table 1: Formulation of experimental diets.

	Control	BPM
Fish meal	259.5	181.6
Bacteria protein meal	-	300.0
Soybean meal	381.3	266.9
Soy protein concentrate	187.2	131.0
Soy lecithin	30.0	21.0
Rice flour	89.8	62.9
Sardine oil	43.8	30.7
Vitamin premix	5.0	3.5
Mineral premix	2.5	1.7
Yttrium oxide	1.0	0.7

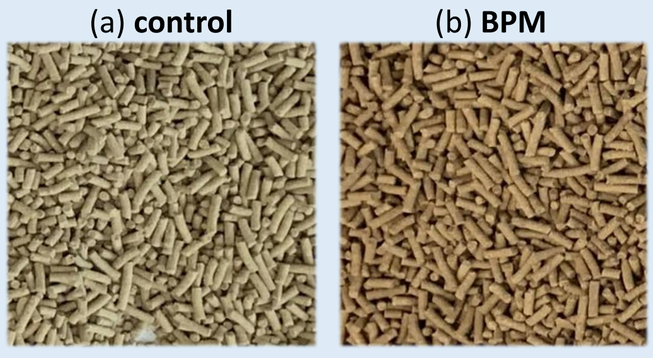
(a) (b) (c)



Fig. 1: Feed preparation steps (a) mixing, (b) pelletizing, (c) drying.

Feed Composition

(a) **control** (b) **BPM**



(c)

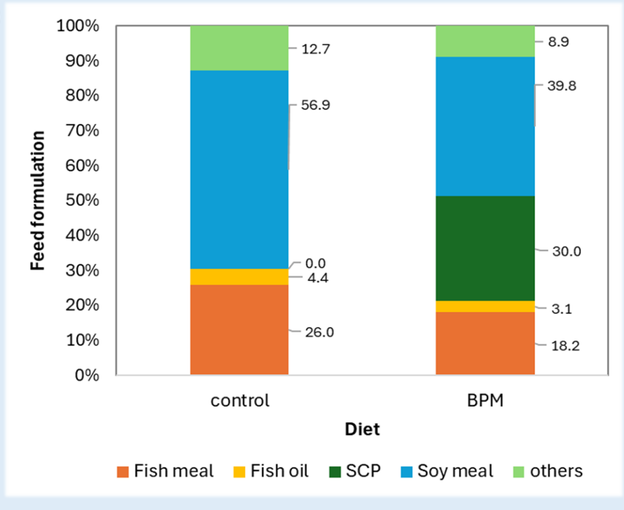


Fig. 2: (a) control diet (b) BPM diet, (c) Compositions of control and BPM diets.

Feeding Trial

A total of 225 fish (48.9 ± 4.2 g at 96 dph) were reared in a recirculating aquaculture system at 28°C and 5 ppt salinity at TLL, Singapore. Water quality parameters were monitored daily, such as temperature, salinity, pH, dissolved oxygen, nitrate, nitrite and total ammonia. The fish were individually weighed at the beginning and end of the feeding trial.

Feces were collected in a custom-designed trap for the determination apparent digestibility coefficient (ADC).

Tissue samples were collected 1 hour post-feeding for the analysis of digestive enzyme assays.

(a) (b)



Fig. 3: (a) Recirculating brackish-water system, (b) fecal samples were collected in a custom-designed trap at the base of each tank.

Results and Discussion

Apparent digestibility coefficients (ADC)

The apparent digestibility coefficient (ADC) is commonly used to estimate the digestibility of nutrients and energy in diets or test ingredients. Yttrium oxide (Y_2O_3) was used as a marker to determine the ADCs of the diet and ingredient.

How digestible is the test ingredient for fish?

ADC of nutrient/ energy in diet

$$\textbf{Apparent digestibility coefficients (ADC) of nutrient in the diet } (\%) = \left[1 - \left(\frac{\text{Concentration of marker in feed} \times \text{Nutrient concentration in feces}}{\text{Concentration of marker in feces} \times \text{Nutrient concentration in feed}}\right)\right] \times 100$$

Cho et al (1982)[1]

ADC of the test ingredient

$$\textbf{ADC of ingredients } (\%) = \text{ADC of test diet} + \left(\frac{(1-s) \times \text{Nutrient in reference diet}}{(s) \times \text{Nutrient in ingredient}}\right) \times (\text{ADC of test diet} - \text{ADC of reference diet})$$

Where,
s = nutrient contribution of test ingredient to nutrient content of test diet

Bureau et al (1999)[2]

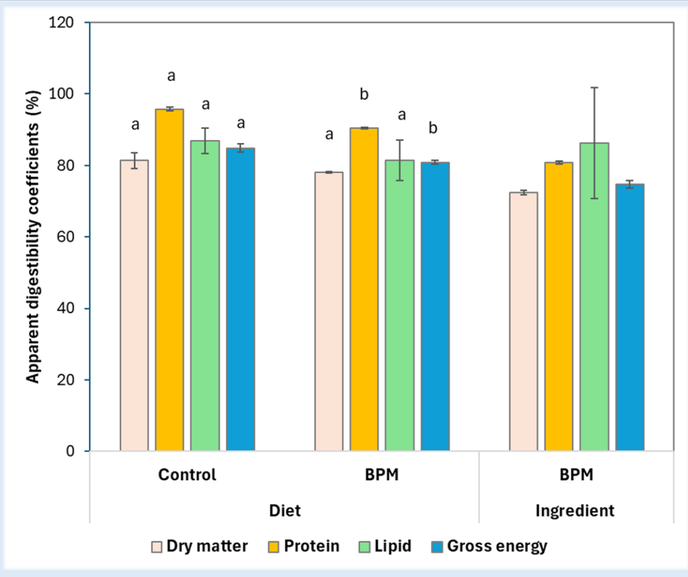


Fig. 4 Apparent digestibility coefficients of experimental diets and bacterial protein meal (BPM) ingredient in seabass.

Growth performance

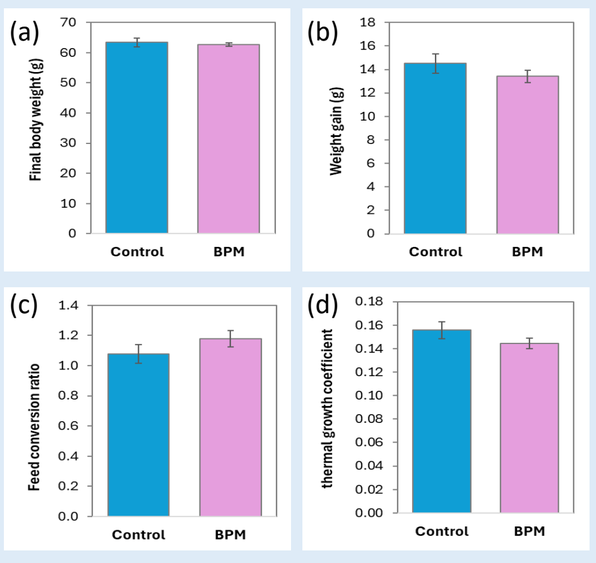


Fig. 5: Growth performance on (a) final body weight, (b) weight gain, (c) feed conversion ratio, and (d) thermal growth coefficient.

Table 2: Compositions (g kg-1 DM) of BPM and control diets.

	BPM	CV (%)
Crude lipid	111.4 ± 35.2	31.6
Crude protein	552.5 ± 37.2	6.7
Ash	100.8 ± 30.5	30.2
Nitrogen-free extracts	222.8 ± 33.6	15.1
Gross energy	21.5 ± 1.0	4.8

Effect of BPM on digestive enzymes

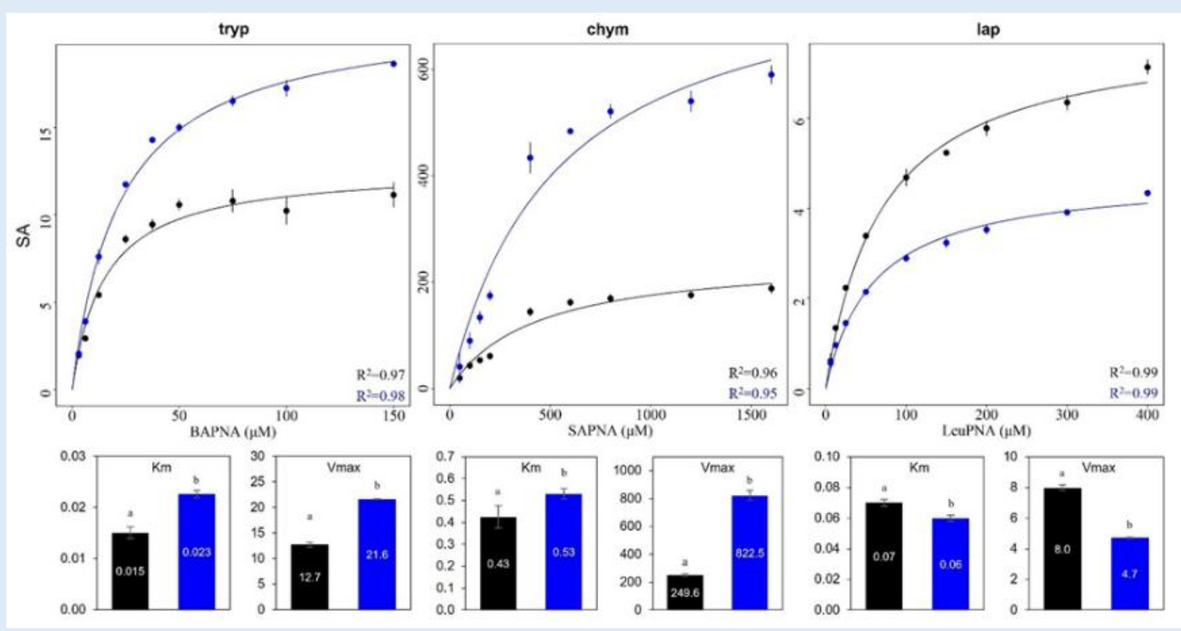


Fig. 6: Kinetic parameters of trypsin (tryp), chymotrypsin (chym), and leucine aminopeptidase (lap) extracted from the gut of seabass fed experimental diets.

Three fish from each tank were sampled, and the whole guts were homogenized for digestive enzyme activity analysis. The substrates used for trypsin, chymotrypsin, and leucine aminopeptidase-like enzymes were BAPNA, SAPNA, and LeuPNA, respectively. Proteolytic activities were recorded at 410 nm using a spectrophotometer.

Calculation of Kinetic parameters

Velocity of the reaction = Maximum velocity([substrate concentration]/ (Km + [substrate concentration]))

Km = Michaelis–Menten constant[3]

Conclusion

In this study, the tested BPM exhibited high digestibility and exerted minimal effects on gut enzyme functionality. The integration of waste-derived microbial protein production with validated animal performance as a promising and sustainable approach for future aquafeed development. Overall, BPMs could potentially become an alternative protein sources for seabass feed formulation but require careful choice of the species.

References

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2. Bureau, D. P., Harris, A. M., Cho, C. Y. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (Oncorhynchus mykiss). Aquaculture, 180(3-4), 345-358
3. Atkins, G.L., Nimmo, I.A. 1980. Current trends in the estimation of Michaelis-Menten parameters. Analytical Biochemistry 104, 1–9

Acknowledgements

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