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Dietary protein requirements of kuruma shrimp *Marsupenaeus japonicas* in recirculating aquaculture system

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Abstract

The farming of *Marsupenaeus japonicas* in recirculating aquaculture system (RAS) is relatively recent, consequently, knowledge of their dietary protein requirements is still insufficient. Due to the specific ecological conditions of RAS, these systems may affect the nutritional requirements of shrimp. Therefore, this study aimed at assessing the optimal protein requirement of *M. japonicas* in RAS. Six experimental diets were devised to contain 35%, 40%, 45%, 50%, 55%, and 60% crude protein (P35, P40, P45, P50, P55 and P60, respectively), and were fed in triplicate groups of 20 shrimp (initial body weight 1.86±0.01g) at a feeding rate of 3.0% body weight per day for 56 days. At the end of the feeding period, the optimal protein requirement was estimated at 46.13% for weight gain rate, and specific growth rate with all groups exhibited high survival rates. The P45 diet increased the digestive enzymes activities, antioxidant indices and improved intestinal morphology, while the P50 diet showed the highest intestinal microbiome diversity. The experimental results showed that diets containing higher protein seemed to have compromised the antioxidant status of shrimp and did not necessarily result in better growth. Hence, results from this study suggested that the optimal protein requirement of *M. japonicas* fed in RAS was determined to be 46.13%, but protein levels up to 50% can be beneficial to the intestinal microbiome diversity.

Experimental diets

The test feed formulations are shown in Table 1. Six experimental diets were devised to contain 35%, 40%, 45%, 50%, 55% and 60% crude protein (P35, P40, P45, P50, P55, and P60, respectively) by increasing casein at the expense of starch. The energy values of experiment diets were about iso-energetic. These diets were subject to triplicate evaluations. All ingredients were thoroughly mixed, after which they were compressed into a singular pellet size (0.3 mm) using a press machine (JLA-125; Pinzheng Equipment Co. Ltd). Pellets were dehydrated in a ventilated oven to a moisture content of approximately 10%. All diets were packed and stored at -20 °C until utilization.

Results

Growth performances

Table 1 Growth performances of *M. japonicas* fed diets differing in protein levels

Parameters	Treatments					
	P35	P40	P45	P50	P55	P60
IBW(g)	1.86±0.01	1.86±0.01	1.87±0.01	1.87±0.01	1.86±0.01	1.86±0.01
FBW(g)	4.38±0.02^{d}	4.65±0.10^{bc}	5.28±0.08^{a}	4.79±0.13^{b}	4.52±0.02^{cd}	4.08±0.04^{e}
WGR(%)	135.43±1.07^{d}	152.38±3.09^{bc}	183.69±4.10^{a}	157.59±6.97^{b}	143.03±0.99^{cd}	119.64±1.96^{e}
SGR(%/d)	1.53±0.01^{d}	1.65±0.02^{bc}	1.86±0.03^{a}	1.69±0.05^{b}	1.59±0.01^{cd}	1.40±0.02^{e}
SR(%)	95.00±2.89	93.33±1.67	93.33±1.67	90.00±2.89	81.67±1.67	81.67±1.67

Values (Means ± SEM; n=3) in the same row with different superscripts show significant difference (P<0.05).

IBW: initial body weight, FBW: final body weight, WGR: weight gain rate, SGR: specific growth rate, SR: survival rate.

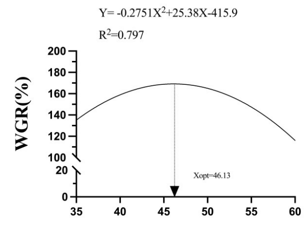

Fig. 1 Relationship between dietary protein levels (%) and weight gain rate (WGR) of *M. japonicas*. The optimal protein requirement, as estimated by the second-order polynomial regression analysis and represented by the abscissa value at the peak, is 46.13%.

Gut microbiota

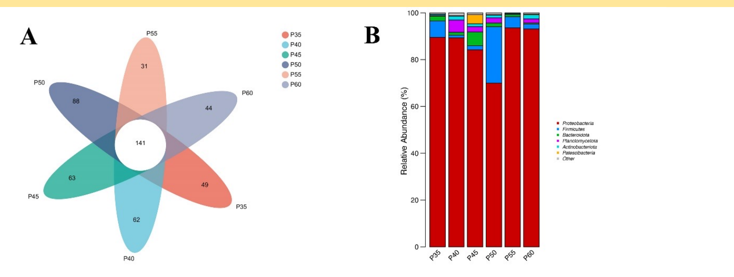

Fig. 2 **(A)** Venn diagram analysis for intestinal microbiota OTU distribution of *M. japonicas* fed diets differing in protein levels. **(B)** Composition and relative abundance of dominant microbes at the phylum level of *M. japonicas* fed diets differing in protein levels

Means with different superscripts are significantly different (P< 0.05). n = 3.

Intestinal morphology

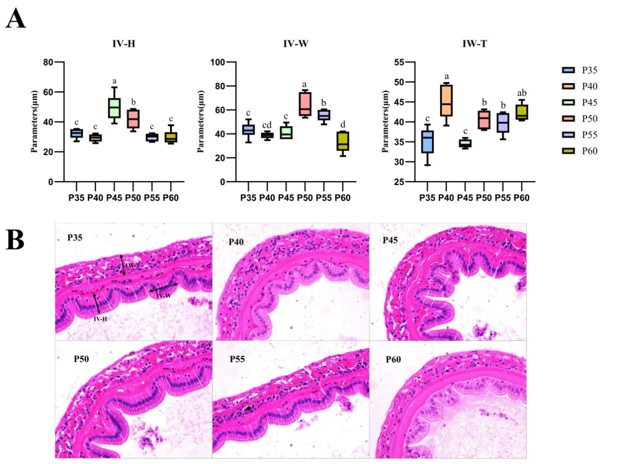

Fig. 3 **(A)** Intestinal (midgut) morphometric parameters of *M. japonicas* fed diets differing in protein levels for 8 weeks. **(B)** Midgut villus structure of *M. japonicas* fed diets differing in protein levels for 8 weeks.

IV-H: intestinal villus height, IV-W: intestinal villus width, IW-T: intestinal wall thickness.

The level of magnification in the images is 40×. Means with different superscripts are significantly different (P< 0.05). n = 3.

Antioxidant indices in the hepatopancreas of M. japonicas fed diets differing in protein levels

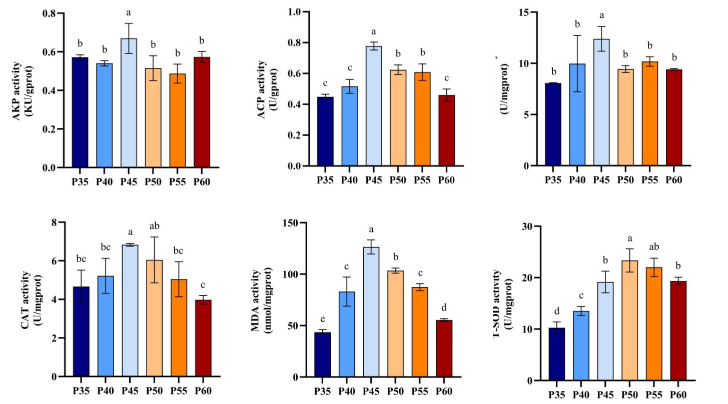

Fig. 4 Antioxidant indices in the hepatopancreas of *M. japonicas* fed diets differing in protein levels. Values (Means ± SEM; n=3) in the same row with different superscripts show significant difference (P < 0.05).

T-AOC: total antioxidant capacity, AKP: alkaline phosphatase, ACP: acid phosphatase, CAT: catalase, POD: peroxidase, T-SOD: total superoxide dismutase, MDA: malondialdehyde

Conclusion

The findings of this study validated that the optimal dietary protein requirement for *M. japonicas* was 46.13% when casein and fishmeal were utilized as the primary protein sources, which is recommended for *M. japonicas* fed in RAS. Furthermore, the results suggested that increasing protein levels up to 50% may enhance the diversity of the intestinal microbiome in kuruma shrimp. These findings will undoubtedly contribute to establishing a more precise understanding of the protein requirements for kuruma shrimp. On the other hand, further research on dietary amino acids and palatability factors should be conducted on sustainable shrimp feed development.