The effects of replacement of fishmeal by Motiv on growth, hepatopancreatic histology and nutrient digestibility of Pacific white shrimp, *Litopenaeus vannamei*

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ABSTRACT

A 12-week feeding trial was conducted to evaluate the effects of replacing fishmeal with Motiv, a fermented corn protein concentrate, on growth performance, hepatopancreatic health, nutrient digestibility, and pigmentation in *Litopenaeus vannamei*. Diets containing 0%, 6%, 9%, and 12% Motiv were tested in a recirculating aquaculture system. Growth metrics (WG, ADG, FCR, survival) showed no significant differences among treatments, indicating fishmeal could be replaced up to 12% without compromising performance. Shrimp fed the 6% Motiv diet exhibited superior antioxidant status (higher SOD activity, lower TBARS values) and nutrient digestibility. Carotenoid analysis revealed increased astaxanthin and xanthophyll concentrations in Motiv-fed groups, correlating with enhanced shell coloration. Histological examination showed improved hepatopancreatic morphology in Motiv-fed shrimp. These findings suggest Motiv is a functional feed ingredient that supports shrimp health, pigmentation, and partial fishmeal replacement.

1. PURPOSE

The study evaluated the effects of replacement of fishmeal by Motiv on growth, hepatopancreatic histology and nutrient digestibility of Pacific white shrimp, *Litopenaeus vannamei*.

2.1 EXPERIMENTAL DIET

Experimental feeds were provided by Motiv Products, Cargill Inc. Motiv was used to replace fishmeal protein at 0, 6, 9 and 12%. Feed formulation and proximate composition were presented in Table 1.

2.2 ANIMAL TREATMENT

The SPF shrimp was purchased from Dr. Chu-Fang Lo's lab in National Cheng-Kung University, Taiwan (broodstock from Sheng-Long Biotech Co., Viet Nam). Upon arrival, the shrimp was acclimated in a 3,500 L FRP tank for 2 weeks.

At the beginning of the experiment, 25 shrimp (mean initial weight 1.6 g) were stocked in each of 15 FRP tanks (300 L). The diets were randomly assigned to groups of shrimp. The control group was conducted as three replicates, whereas Motiv6%, Motiv9% and Motiv12% groups were conducted as four replicates. Each aquarium was part of a closed recirculating system with a common reservoir of blackish water at 20±1 ‰ salinity. The system consisted of a common filter, biofilter, protein skimmer and UV light to maintain the water quality. The water temperature of the rearing system was controlled at 28±1ºC. One third of the water in the system was exchanged every two week. Two air stone were put in each tank to make sure water DO≥6 mg/L.

The shrimp were fed diets for apparent satiation 4 times per day at 0800, 1200, 1600 and 2000 h. The uneaten feed was immediately collected by siphon, then dried and weighed to determine the feed intake during the feeding period. The shrimp were counted for survival once every two weeks, then 6-8 shrimp were randomly sampled and weighed. The shrimp were fed the experimental diets for 12 weeks.

At the end of the feeding trial, the shrimp were bulk weighed and the growth performance including the weight gain (WG), average daily growth (ADG), feed conversion rate (FCR) and the survival rate were calculated. The equations used are shown below:

WG = (final body weight – initial body weight)/initial body weight × 100
ADG = (final body weight – initial body weight)/days × 100
FCR = feed intake/(final body weight – initial body weight)/
Survival = 100 × (final shrimp number/initial shrimp number)

After weighing, hepatopancreas from three shrimp were removed and pooled for morphology changes, thiobarbituric acid reactive substance (TBARS) values, superoxide dismutase (SOD) activity and carotenoids concentration determination. Another three shrimp were sampled for whole body proximate composition and carotenoids concentration. Hepatopancreas was fixed in 10% formalin and send to Veterinary Hospital of NPUST for the histological analysis.

After weighing and sampling, the remaining shrimp in each tank were fed diets with 0.5% chromic oxide (Cr_2O_3, Sigma Chemical) as an inert indicator. The digestibility trial was conducted for 3 week. The feeding regime was similar to the growth trial. The uneaten feed and fecal residue were removed at 10:00 h and the feces were collected at 11:00, 12:00, 13:00, 14:00 and 15:00 h using a fecal collection column. The proximate composition of feed and feces were determined by the AOAC (1995) method, chromium (Cr) concentration with an atomic absorption spectrophotometer (ZA-3000, Hitachi Co., Tokyo, Japan).

The calculation of the apparent digestibility coefficient (ADC) is shown below:

ADC-DM (%)=100×(%Cr in feed/%Cr in feces)
ADC-N (%)=100×[1-(%Cr in feed/%Cr in feces)×(%N in feces/%N in feed)]
DM: dry matter; N: nutrients, including protein and lipid.

After digestibility trial, the remaining shrimp were cooked in boiling water for three minutes, and then took photo to compare the body color.

2.3 STATISTICAL ANALYSIS

The results were analyzed by a one-way analysis of variance (ANOVA) by using the SAS/PC statistical software (SAS Inst. Inc., Cary, NC, USA). When the ANOVA identified differences among groups, multiple comparisons were made among the means using the Duncan's multiple range test. Statistical significance was determined by setting the aggregate type I error at $P<0.05$.

3. RESULTS AND DISCUSSION

After 12-wks feeding trial, final weight, weight gain, average daily growth and feed conversion rate showed no significant ($P>0.05$) differences among all dietary treatments (Table 2). Shrimp fed diets with Motiv at 6-12% (fishmeal from 260 to 100 g/kg) showed similar growth performances of the control group, suggesting that Motiv can be used to partially replace fishmeal up to 12% inclusion level without any negative effects on growth performance of the species.

Hepatosomatic index (HSI) was similar among all dietary treatments (Table 3). Hepatopancreatic superoxide dismutase (SOD) activity was higher in shrimp fed the control and Motiv6% diets than that in shrimp fed the Motiv12% diets. Shrimp fed the Motiv12% diet had the highest thiobarbituric acid reactive substance (TBARS) value, followed by shrimp fed the control diet, and lowest in shrimp fed the Motiv6% and Motiv9% diets. Shrimp fed the Motiv12% diet had higher ether extract (lipid) than shrimp fed the Motiv9% diet (Table 4). Moisture, ash and crude protein content were not different among all dietary treatments.

Shrimp fed the Motiv6% diet showed high antioxidative enzyme (SOD activity) and low oxidative status (TBARS value). Similar result was found in our previous work that white shrimp fed diets with Lactobacillus spp. fermented soybean meal. It might be caused by the functional peptides or organic acids derived from the fermented products. However, the shrimp fed Motiv12% presented low SOD activity but high TBARS value. The reason is unclear. More study regarding of this phenomenon is needed.

Apparent dry matter digestibility was higher in shrimp fed the control Motiv6% diets than that in shrimp fed the Motiv9% and Motiv12% diets (Table 5). Shrimp fed the Motiv6% diet had the higher protein digestibility than other dietary treatments. Lipid digestibility was higher in shrimp fed the control and Motiv6% diets than that in shrimp fed the Motiv9% and Motiv12% diets. Overall, it seems to find the best nutrient digestibility in shrimp fed the Motiv6% diet. The Motiv is a kind of fermented product. The fermentation processing will degrade protein and some antinutritional factors such as phytic acid, glycinin and conglycinin. In addition, the metabolite produced from fermentation processing, i.e. lactic acid, also can improve nutrient digestibility. So digestibility enhancement effect is expectable. However, high levels of Motiv (9 and 12%) in diet also depressed nutrient digestibility.

As the shrimp color after cooking, it clearly indicated that shrimp fed diets with Motiv (Motiv6%, Motiv9% and Motiv12%) showed redder color compared with shrimp fed the control diet (Figure 1). When measuring the carotenoids concentration in whole body and hepatopancreas, whole body astaxanthin concentration was the higher in shrimp fed the Motiv6%, Motiv9% and Motiv12% diets than that in shrimp fed the control diet (Table 6). Whole body xanthophyll concentration and hepatopancreatic astaxanthin concentrations were the highest in shrimp fed the Motiv9% and Motiv12% diets, followed by shrimp fed the Motiv6% diet, and the lowest in shrimp fed the control diet. Shrimp fed the Motiv12% diet had the highest hepatopancreatic xanthophyll concentration, followed by shrimp fed the Motiv9% diet, and shrimp fed the control and Motiv6% diets had the lowest xanthophyll concentration.

The carotenoids metabolism in crustacean is still unclear. In the past reports, β-carotene, lutein and zeaxanthin have demonstrated to convert astaxanthin in crustacean. But xanthophyll, major pigment in corn product, is still unknown. The present study clearly demonstrated white shrimp is able to utilize xanthophyll as astaxanthin source to meet their requirement. Thus, when the shrimp fed diets with Motiv, shrimp showed high astaxanthin concentration and also showed redder color in shell. It suggests that the use of Motiv product can spare the cost of astaxanthin supplementation for white shrimp.
Morphology changes of hepatopancreas in shrimp fed different diets for 12 weeks were showed in Figure 2. The shrimp fed the control diet showed unhealthy hepatopancreas compared with shrimp fed the Motiv6-12% diets. For example, most hepatocytes showed transformed and expanding lumen. Adding Motiv into the control diet can improve this phenomenon. Soybean meal is considered to induce some injury in animal intestine due to its antinutritional factors. The diets containing high levels of soybean meal (348 g/kg for control group) may cause dysfunction of hepatopancreas of the shrimp. The Motiv, a fermented corn protein, contains lactic acid. In land animal or fish study, organic acid showed benefit effects on intestinal health of the animals. This may explain the healthy hepatopancreas found in shrimp fed diets containing Motiv.

4. CONCLUSION

The study indicated that fishmeal can be partially replaced by Motiv up to 12% without any negative effects on growth performance for white shrimp. Shrimp fed the Motiv6% diet showed better SOD activity, TBARS values and nutrient digestibility compared with the control group. Moreover, shrimp fed diets containing Motiv showed redder shell color and healthier hepatopancreas histology. It suggests that the Motiv is a functional feed ingredient for white shrimp.

Formulation and proximate composition of the experimental diets.				
	Control	Motiv 6%	Motiv 9%	Motiv 12%
Ingredients (g/kg)				
Anchovy fishmeal	260.00	180.00	140.00	100.00
High quality SBM (46-48% protein)	348.00	348.00	348.00	348.00
Motiv	0.00	60.00	90.00	120.00
Fish oil	16.50	18.70	19.80	20.90
Wheat Flour	302.00	292.00	289.60	286.60
Krill meal ArkerBio	0.00	20.00	30.00	40.00
Wheat gluten	20.00	20.00	20.00	20.00
Choline chloride	10.00	10.00	10.00	10.00
Lecithin-Soy	20.00	20.00	20.00	20.00
Cholesterol	0.50	0.62	0.65	0.70
MCP	7.55	12.18	12.48	13.35
Sodium Chloride (NaCl)	7.00	7.00	7.00	7.00
Stay-C 35%	1.25	1.25	1.25	1.25
Shrimp Premix (Mix of Vitamin + Minerals)	3.75	3.75	3.75	3.75
Methionine	2.30	2.75	2.92	3.20
Threonine	0.00	1.40	1.50	1.60
Lysine	0.00	1.20	1.90	2.50
Powder mold inhibitor	0.75	0.75	0.75	0.75
Anti-oxidant	0.40	0.40	0.40	0.40
Proximate composition (%)				
Moisture	8.39	8.14	6.03	6.19
Ash	6.83	6.68	6.74	7.24
Crude protein	40.62	40.01	40.83	40.61
Ether extract	7.11	7.24	7.18	7.30

Table 1.

Growth performance of shrimp fed different diets for 12 weeks						
	Initial weight (g)	Final weight (g)	Weight gain (%)	Average daily growth (%)	Feed conversion rate	Survival (%)
Control	1.60±0.00	11.24±0.39	601.52±24.23	11.47±0.46	2.88±0.13	86.67±2.31
Motiv6%	1.62±0.02	12.36±1.17	662.49±80.73	12.79±1.41	2.62±0.28	88.00±5.66
Motiv9%	1.61±0.02	11.28±0.59	599.87±34.34	11.51±0.69	11.51±0.69	86.00±5.16
Motiv12%	1.60±0.01	11.83±0.55	637.82±29.89	12.17±0.64	2.70±0.13	88.00±3.27
Values are mean ± SD of three to four groups of shrimp (n=3-4).						

Table 2.

Hepatosomatic index (HSI), hepatopancreatic superoxide dismutase (SOD) and thiobarbituric acid reactive substance (TBARS) values of shrimp fed different diets for 12 weeks			
	HSI (%)	SOD (U/mg protein)	TBARS (nmol malonaldehyde/g tissue)
Control	3.28±0.33	1.79±0.05^{b}	145.85±17.19^{b}
Motiv6%	3.18±0.49	2.08±0.16^{b}	106.56±8.9^{a}
Motiv9%	3.06±0.79	1.50±0.51^{ab}	76.59±29.18^{a}
Motiv12%	3.36±0.67	1.40±0.32^{a}	188.10±19.29^{c}
Different superscripts a,b,c in the same column indicate significant (P<0.05) difference between different dietary treatments. Values are mean ± SD of three to four groups of shrimp (n=3-4).			

Table 3.

Whole body proximate composition (%) of shrimp fed different diets for 12 weeks				
	Moisture	Ash	Crude protein	Ether extract
Control	75.25±3.23	2.90±0.52	19.93±1.33	5.76±0.36^{ab}
Motiv6%	76.49±1.70	2.94±0.45	18.88±0.89	5.68±0.49^{ab}
Motiv9%	76.46±2.10	2.91±0.61	18.64±0.43	4.99±0.55^{a}
Motiv12%	75.71±1.50	3.04±0.52	19.76±0.90	6.44±0.49^{b}
Different superscripts a,b in the same column indicate significant (P<0.05) difference between different dietary treatments. Values are mean ± SD of three to four groups of shrimp (n=3-4).				

Table 4.

Apparent digestibility (%) of dry matter, crude protein and crude lipid of shrimp fed different diets for 12 weeks			
	Dry matter	Crude protein	Crude lipid
Control	78.06±1.35^{ab}	80.28±1.67^{a}	88.34±1.94^{b}
Motiv6%	79.48±0.77^{b}	85.64±0.80^{b}	87.68±0.75^{b}
Motiv9%	75.10±1.05^{a}	81.28±2.84^{a}	82.67±2.22^{a}
Motiv12%	76.02±2.07^{a}	81.82±1.62^{a}	82.84±0.97^{a}
Different superscripts a,b in the same column indicate significant (P<0.05) difference between different dietary treatments. Values are mean ± SD of three to four groups of shrimp (n=3-4).			

Table 5.

Whole body and hepatopancreas astaxanthin and xanthophyll concentration of shrimp fed different diets for 12 weeks				
	Whole body (mg/g tissue)		Hepatopancreas (mg/g tissue)	
	Astaxanthin	Xanthophyll	Astaxanthin	Xanthophyll
Control	0.12±0.02^{a}	0.008±0.005^{a}	0.70±0.55^{a}	0.19±0.03^{a}
Motiv6%	0.54±0.10^{b}	0.027±0.003^{b}	3.69±1.00^{b}	0.33±0.04^{a}
Motiv9%	0.66±0.13^{b}	0.059±0.009^{c}	6.34±1.11^{c}	0.53±0.07^{b}
Motiv12%	0.61±0.10^{b}	0.047±0.009^{c}	8.90±1.70^{c}	0.77±0.17^{c}
Different superscripts a,b,c in the same column indicate significant ($P<0.05$) difference between different dietary treatments. Values are mean ± SD of three to four groups of shrimp (n=3-4).				

Table 6.

Figure 1. Body color of shrimp after cooking in boiling water for 3 minutes.

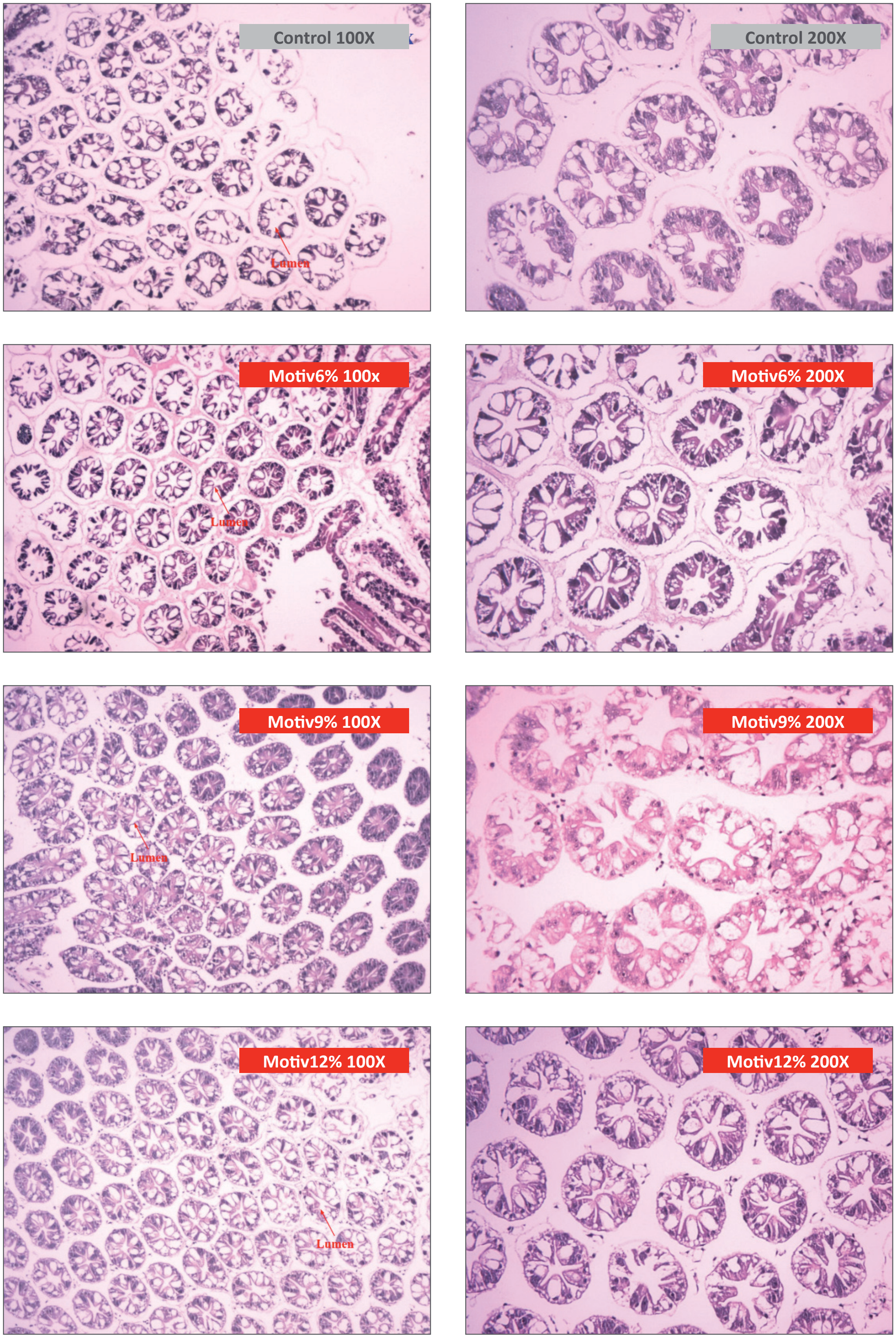

Figure 2. Histology of hepatopancreas of white shrimp fed different diets for 12 weeks.