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EDITED BY
Helena Peres,
University of Porto, Portugal

REVIEWED BY
Hongliang Zuo,
Sun Yat-sen University, China
Hongwei Shan,
Ocean University of Chinainstitution
Qingdao, China
Chengbo Sun,
Guangdong Ocean University, China

*CORRESPONDENCE
Wen Huang
huangwen549@126.com

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Effect of dietary soybean meal on growth performance, apparent digestibility, intestinal digestive enzyme activity and muscle growth-related gene expression of *Litopenaeus vannamei*

Kai Peng[1], Xiaoying Chen[1], Huijie Lu[1], Jichen Zhao[1], Yihong Chen[2], Chaozheng Li[3,4], Huo Li[5] and Wen Huang[1,4]*

[1]Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture and Rural Affairs, Guangdong Provincial Key Laboratory of Animal Breeding and Nutrition, Guangzhou, China, [2]School of Life Science, South China Normal University, Guangzhou, China, [3]State Key Laboratory of Biocontrol, Institute of Aquatic Economic Animals and the Guangdong Province Key Laboratory for Aquatic Economic Animals, Sun Yat-Sen University, Guangzhou, China, [4]Maoming Branch Center of Guangdong Provincial Laboratory of Lingnan Modern Agricultural Science and Technology, Maoming, China, [5]Guangdong Provincial Engineering and Technology Research Center, Guangdong Jinyang Biotechnology Co. Ltd., Maoming, China

Soybean meal is one of the major components of aquatic animal diets, whereas little information is available about the evaluation of soybean meal growth suppression mode of action. A 42-day feeding trial was performed to assess the effects of dietary soybean meal on growth performance, apparent digestibility, intestinal digestive enzyme activity, and muscle growth–related gene expression of *Litopenaeus vannamei*. A total of 600 shrimp were randomly distributed into 20 tanks with 30 shrimp per tank and four tanks per group. The soybean meal was added to the diets at the rate of 20% (T20), 28% (T28), 35% (T35), 42% (T42), and 50% (T50), respectively. Shrimp were fed with apparent satiation three times daily. Results indicated that the final body weight, weight gain rate, specific growth rate, feed intake, intestinesomatic index, dressed weight percentage, and the apparent digestibility coefficients of dry matter, crude protein, crude lipid, and ash were linearly decreased ($p < 0.05$), but feed coefficient was linearly increased ($p < 0.05$) as dietary soybean meal increased from 20% to 50%. The intestinal trypsin and amylase activities were decreased ($p < 0.05$) as dietary soybean meal increased from 20% to 50%, and reached significance at the level of 35%, 42%, and 50%. Shrimp fed with T20 had higher ($p < 0.05$) intestinal lipase activity than those fed with other diets. The mRNA relative expression of growth hormone, myogenic regulatory factor 5, and target of rapamycin was downregulated ($p < 0.05$) as dietary soybean meal

increased from 20% to 50%. To conclude, dietary soybean meal exceeded 28% significantly inhibited growth performance of *L. vannamei*, mainly due to the negative impact of soybean meal on digestion and feed utilization and also the inhibition on the muscle growth and related gene expressions.

KEYWORDS

fishmeal, soybean meal, growth, digestion, muscle growth, *Litopenaeus vannamei*

Introduction

Fishmeal is one of the most important feed protein sources in aquatic feed. It has been reported that more than two-thirds of fishmeal is used as aquatic feed in the world and about 60% in China (Mai et al., 2021). As the global population increase, the demand of people for aquatic products consumption is unabated. However, the production of fishmeal is maintained at around 5 million tons in recent years, which could no longer continue to increase in the further. Combined with the increasing need of fishmeal in the development of aquaculture, professionals have spared no effort to find alternatives to fishmeal and solutions to improve the utilization of non-fishmeal protein. Over the last decade, the plant protein such as soybean meal has been widely used to replace fishmeal in aquafeed and exhibits as a promising alternative in various species (Jiang et al., 2015a; Chakraborty et al., 2019; Shukla et al., 2019; Bae et al., 2020; Bruce et al., 2022; Wu et al., 2021; Zhao et al., 2021; Ding et al., 2022). Even so, soybean meal is considered to have deficiencies including the presence of anti-nutritional factors (e.g., soybean agglutinin, glycinin, and β-conglycinin), imbalance of essential amino acids, and absence of certain fishmeal components (e.g., taurine, hydroxyproline, and vitamin D_3) (Mai et al., 2021), which may result in poor palatability and digestibility of diets and lead to the reduced growth performance of animals.

Litopenaeus vannamei belongs to crustacean species that is being widely cultured and welcomed in China. In 2020, China contributed 1.19 million tons of the farmed *L. vannamei*, which account for 70% of shrimp production (Fishery Administration of Ministry of Agriculture and Rural Affairs, 2021). Although replacing fishmeal with soybean meal in *L. vannamei* diet has been well reported, the suitable substitution ratio is inconsistent among studies. For comprehensive assessment from growth performance and nutrient utilization of *L. vannamei*, Amaya et al. (2007) indicated that fishmeal could be totally replaced by soybean meal (dietary concentration at 39.5%); Lim and Dominy (1990) suggested that the suitable substitution ratio of fishmeal (dietary concentration at 11.3%) by soybean meal is 60%; and the suitable substitution ratio were proposed to be 28% (dietary fishmeal at 38.2%) (Xu et al., 2021) and 20% (dietary fishmeal at 30.0%) (Yang et al., 2015). On the other hand, high dose of dietary soybean meal caused inhibition of growth and digestion of *L. vannamei*, whereas the reason and related mechanism are still not fully understood. Previous studies documented that the inhibition effect of dietary soybean meal on growth of aquatic animals is mainly attributed to endogenous anti-nutritional factors (Elumalai et al., 2019; Zhao, 2021; Zhou, 2021). A recent study screened the growth and development related functional genes (e.g., myogenic regulatory factors *Myf5* and *MyoG*) in the muscle of *L. vannamei* by transcriptome sequencing, but the information regarding the effects of dietary soybean meal on the muscle growth–related gene expressions is rare. Therefore, this study is conducted to evaluate the effects of dietary soybean meal on growth performance, apparent digestibility, intestinal digestive enzyme activity, and muscle growth–related gene expression of *L. vannamei*.

Materials and methods

Diet preparation

The ingredients and proximate compositions of experimental diets were shown in Table 1. The soybean meal (crude protein 46%) was added to the diets at the rate of 20% (T20), 28% (T28), 35% (T35), 42% (T42), and 50% (T50), to replace fishmeal (crude protein 66%) at the rate of 0%, 20%, 40%, 60%, and 80%, respectively. All ingredients were ground (AHZC1265 Hammer Mill, Buhler Machinery Co., Ltd., Guanghzou, China) to pass through a 320-μm sieve, mixed (AHML2000 Mixer, Buhler Machinery Co., Ltd., Changzhou, China) thoroughly and then extruded (SLX-80 Twin-screw Extruder, South China University of Technology Machinery Factory, Guangzhou, China) into 2-mm pellets, dried (HMO-205 Oven Dryer, Haiming Electronic Technology Co., Ltd., Dongguan, China) at 55°C for 12 h, and stored at −20°C until use.

TABLE 1 Ingredients and proximate compositions (g/kg DM) of experimental diets.

Items	Diets				
	T20	T28	T35	T42	T50
Ingredients					
Fishmeal (Peru, crude protein, 68%)	250	200	150	100	50
Soybean meal (crude protein, 46%)	200	280	350	420	500
Peanut bran	120	120	120	120	120
Chicken meal	100	100	100	100	100
Wheat flour	220	200	180	160	130
Fish oil	20	20	20	20	20
Soy lecithin	20	24	27	30	34
Monocalcium phosphate	15	15	15	15	15
Vitamin premix	2	2	2	2	2
Mineral premix	5	5	5	5	5
Lysine	0	0	0.5	1	1.5
Methionine	2	3	3.5	4.5	5.5
Choline chloride	2	2	2	2	2
Salt	3	3	3	3	3
Sodium alginate	12	12	12	12	12
Cellulose	29	14	10	5.5	0
Total	1,000	1,000	1,000	1,000	1,000
Proximate composition					
Moisture	72	71	72	74	73
Crude protein	414	420	418	420	416
Crude lipid	84	84	83	82	82
Ash	75	77	74	75	77
Lysine	23	23	23	23	23
Methionine	8.8	9.0	8.7	8.8	9.0

T20–T50, diets containing 20%–50% of soybean meal.
One kilogram of diet provided: VA, 3,230 IU; VD, 1,600 IU; VE, 160 mg; VK_3, 4 mg; VB_1, 4 mg; VB_2, 8 mg; VB_6, 4.8 mg; VB_{12}, 0.016 mg; nicotinic acid, 28 mg; pantothenic acid calcium, 16 mg; biotin, 0.064 mg; folic acid, 1.285 mg; inositol, 40 mg; Ca, 1,150 mg; K, 180 mg; Mg, 45 mg; Fe, 50 mg; Zn, 40 mg; Mn, 9.5 mg; Cu, 7.5 mg; Co, 1.25 mg; I, 0.16 mg; Se, 0.25 mg.

Experimental design and feeding management

The protocol (No. GAAS20210501) and all procedures performed in this study were approved by the Institutional Animal Care and Use Committee of Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Guangdong, China). A 42-day feeding trial was conducted by randomly distributing 600 *L. vannamei* (initial body weight, 5.8 ± 0.15 g) into 20 tanks (30 shrimp per tank) with four tanks being assigned to each diet. Shrimp were hand-fed with apparent satiation (approximately 4% of body weight per day) three times daily (08:00, 14:00, and 20:00). Uneaten feed was collected in 1 h after each meal, analyzed for dry matter, and subtracted from feed offered (dry matter basis) to calculate feed intake (FI) as described by Wang et al. (2019). During the feeding trial, water was continuously aerated and filtered through sand filter system at a rate of 1.6 L/min. Water temperature ranged from 25°C to 27°C, dissolved oxygen was above 5.0 mg/L, pH ranged from 7.6 to 8.0, salinity was 5‰–6‰, ammonia nitrogen and nitrite were below 0.01 mg/L, and the photoperiod regime was 12-h light and 12-h dark.

Sampling

Feces were continuously collected from each tank daily at the last 14 days of the feeding trial according to the siphon method described by Gumus (2011). The intact feces were picked out with a tweezers and stored at −20°C. Fecal samples collected from each tank were pooled and dried at 105°C to a constant weight to determine the apparent digestibility coefficients (ADCs) of dry matter, crude protein, crude lipid, and ash. Indigestible acid insoluble ash (AIA) in diet ingested and in feces samples was used as marker to estimate nutrient digestibility according to Peng et al. (2021).

At the end of the feeding trial, all shrimp were fasted and anesthetized with MS-222 (40 mg/L; Sigma, USA) prior to sampling. Shrimp per tank were counted and weighted to analyze for survival rate (SR), final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR), and feed coefficient (FC). Six shrimp per tank were randomly selected, determined individual body and muscle weight, and slaughtered to analyze the hepatosomatic index (HSI), intestinesomatic index (ISI), and dressed weight percentage (DWP).

Intestines of three shrimp in each tank were sampled to determine trypsin (Ultraviolet colorimetry), lipase (colorimetry), and amylase (colorimetry) activities using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the corresponding instructions of the manufacturer.

The dorsal muscle of three shrimp in each tank was collected and immediately stored at −80°C for subsequent RNA extraction and real-time qPCR analysis of muscle growth–related gene expression.

Laboratory analyses

The nutrient compositions of experimental diets including moisture, crude protein, crude lipid, and ash were measured following the AOAC methods (AOAC, 1999). The contents of lysine and methionine were analyzed by chromatography using an automated amino acid analyzer (L8900, Hitachi, Japan) with a lithium high-performance column.

Total RNA of muscle samples was isolated using the RNAiso Plus kit (TaKaRa Biotechnology Co., Ltd.). The real-time qPCR analysis method was the same as described by Xin (2016). The primer sequences of the growth hormone (*GH*), myogenic regulatory factors (*Myf5* and *MyoG*), and target of rapamycin

(*TOR*) were listed in Table 2. The $2^{-\Delta\Delta CT}$ method was used to analyze the gene expression levels (Livak and Schmittgen, 2001).

Data calculations

Data were summarized and averaged for each tank. Growth performance parameters were calculated as follows:

SR (%) = 100 × (final number of shrimp/initial number of shrimp)

WGR (%) = 100 × [final body weight (g) − initial body weight (g)]/initial body weight (g)

SGR (%/d) = 100 × [ln final body weight (g) − ln initial body weight (g)]/days

FI (g/shrimp) = feed intake (g)/[(final number of shrimp + initial number of shrimp)/2]

FC = total feed intake (g)/[final body weight (g) − initial body weight (g)]

HSI (%) = 100 × hepatopancreas weight (g)/body weight (g)

ISI (%) = 100 × intestinal weight (g)/body weight (g)

DWP (%) = 100 × muscle weight (g)/body weight (g)

ADCs (%) = 100 × [1 − (a × b)/(c × d)]

where a and c are the AIA concentration in diet ingested and feces, respectively; b and d are the nutrient concentration in feces and diets ingested, respectively. a and d were calculated as difference between the diet offered and orts.

TABLE 2 Primers used for real-time qPCR.

Target Gene	Primer Sequence	Genbank Accession No.
GH	F: 5′-GATGGTTTGGGATCTGAGG AACA-3′ R: 5′-GGAAACTTATGGCATTAACAGGGA-3′	EU492542
Myf5	F: 5′-GGAACAACTACAACTTTGAAG CACA-3′ R: 5′-TCCCATCGCAACTCCTGTATCT-3′	KP715152
MyoG	F: 5′-AACCACCAACGCTGACCG-3′ R: 5′-CTGGTTGGGGTTGTTGGAAG-3′	KP715154
TOR	F: 5′-GACGGCAGTGCTCTATGA-3′ R: 5′-TGTTTGTGAGGCTTGGTG-3′	MK116884
β-Actin	F: 5′-TTGTACGAGGATCGAGTGGA-3′ R: 5′-ATGCTTTCGCAGTAGGTCGT-3′	GK26736

GH, growth hormone; Myf5 and MyoG, myogenic regulatory factors; TOR, target of rapamycin.

Statistical analysis

All data were subjected to normality test and homogeneity of variance by using Shapiro–Wilk and Levene's test for equal variance, respectively. If the data conform to the normal distribution, one-way ANOVA by using SPSS 17.0 analysis software (SPSS(Statistical Packages for Social Sciences, 2007) for Windows followed by the Tukey test with tank as statistical unit and treatment as fixed effect. Polynomial contrasts were used to determine linear and/or quadratic responses to the dietary soybean meal levels. Differences were regarded as significance when $p < 0.05$.

Results

Growth performance

All shrimp had similar ($p > 0.05$) SR and HSI regardless of the treatments (Table 3). The FBW, WGR, SGR, FI, ISI, and

TABLE 3 Growth performance of *L. vannamei* fed with experimental diets.

Items	Diets					SEM	*p*-value		
	T20	T28	T35	T42	T50		*P*	*L*	*Q*
SR, %	86.67	88.33	86.67	85.83	85.83	4.91	0.996	0.786	0.890
FBW, g	15.89^{a}	14.89^{b}	14.62^{b}	14.29^{b}	13.62^{c}	0.44	0.010	0.007	0.286
WGR, %	174.02^{a}	156.81^{b}	152.04^{b}	146.38^{b}	134.40^{c}	7.65	0.010	0.007	0.286
SGR, %/day	2.52^{a}	2.36^{b}	2.31^{b}	2.25^{b}	2.13^{c}	0.08	0.013	0.008	0.314
FI, g/shrimp	19.93^{a}	19.44^{ab}	19.07^{ab}	18.77^{ab}	18.30^{b}	0.53	0.183	0.018	0.953
FC	1.93^{c}	2.19^{b}	2.17^{b}	2.22^{b}	2.28^{a}	0.12	0.049	0.026	0.376
HSI, %	5.52	5.67	5.59	5.50	5.47	0.18	0.258	0.949	0.079
ISI, %	0.50^{a}	0.45^{b}	0.42^{b}	0.43^{b}	0.31^{c}	0.03	0.017	0.032	0.146
DWP, %	51.02^{a}	50.72^{a}	50.53^{a}	50.02^{ab}	49.23^{b}	0.57	0.164	0.048	0.831

T20–T50, diets containing 20%–50% of soybean meal.
SR, survival rate; FBW, final body weight; WGR, weight gain rate; SGR, specific growth rate; FI, feed intake; FC, feed coefficient; HSI, hepatosomatic index; ISI, intestinesomatic index; DWP, dressed weight percentage; SEM, mean standard error; P, overall effect; L, linear effect; Q, quadratic effect.
Different letters within a row indicate significant differences ($p < 0.05$).

DWP were linearly decreased ($p < 0.05$), but FC was linearly increased ($p < 0.05$) as dietary soybean meal increased from 20% to 50%.

ADCs of nutrients

The ADC of dry matter, crude protein, crude lipid, and ash was linearly decreased ($p < 0.001$) as dietary soybean meal increased from 20% to 50% (Table 4).

Intestinal digestive enzyme activities

The intestinal trypsin and amylase activities were decreased ($p < 0.05$) as dietary soybean meal increased from 20% to 50% and reached significance at the level of 35%, 42%, and 50% (Figure 1). Shrimp fed with T20 had higher ($p < 0.05$) intestinal lipase activity than those fed with other diets.

Muscle growth–related gene expression

All shrimp has similar ($p > 0.05$) mRNA relative expression of *MyoG* among diets (Figure 2). The mRNA relative expression of *GH*, *Myf5*, and *TOR* was downregulated ($p < 0.05$) as dietary soybean meal increased from 20% to 50%. Compared with T20, T28, T35, T42, and T50 had lower ($p < 0.05$) mRNA relative expression of GH and TOR, and T35, T42, and T50 had lower ($p < 0.05$) mRNA relative expression of *Myf5*.

Discussion

Growth performance

The similar SR among diets in this study indicated that dietary soybean meal at the rate of 20% to 50% did not affect survival of *L. vannamei*. However, the decreased SGR and FI suggested that the increased dietary soybean meal depressed feed palatability and growth performance of shrimp. This is consistent with the report by Yun et al. (2017) that the replacement ratio of dietary fishmeal with soybean meal at 33% reduced growth of *L. vannamei*. Xu et al. (2021) also reported that dietary fishmeal replaced by soybean meal at 56% reduced FI of *L. vannamei*. Similar results were also observed in various species, such as European catfish (*Silurus glanis*) (Kumar et al., 2017), Tiger puffer (*Takifugu rubripes*) (Lim et al., 2011), sharpsnout seabream (*Diplodus puntazzo*) (Hernandez et al., 2007), red snapper (*Lutjanus campechanus*) (Davis et al., 2005), *Pseudobagrus ussuriensis* (Wang et al., 2016), stellate sturgeon (*Acipenser stellatus*) (Emdadi et al., 2013), Jian carp (*Cyprinus carpio* var. Jian) (Jiang et al., 2015b), rainbow trout (*Oncorhynchus mykiss*) (Harlioglu, 2011), *Channa argus* (Zhang et al., 2020), *Epinephelus Fuscoguttatus* (Liu et al., 2018), bullfrog (*Rana catesbeiana*) (Ding et al., 2019; Wang et al., 2020), and Chinese mitten carb (*Eriocheir sinensis*) (Liu et al., 2021). In this study, the increased FC with increasing dietary soybean meal indicated that supplementation of soybean meal decreased feed utilization; this may account for the depressed growth performance of shrimp. It has been reported that FI is generally regulated by both digestibility and palatability of diet (Peng et al., 2016). The decreased FI as dietary soybean meal increased may partly attribute to decreased apparent digestibility of nutrients as observed in this study. In addition, soybean meal contains several anti-nutritional factors, e.g., soybean agglutinin, glycinin, and β-conglycinin, which have been well reported to reduce the palatability of diet and thereby decrease FI and growth performance of aquatic animals (Feng, 2006; Elumalai et al., 2019; Duan, 2019; Zhao, 2021; Zhou, 2021). Moreover, the decreased ISI and DWP as dietary soybean meal increased suggested that the inclusion of soybean meal in diet inhibited growth and development of the intestine and muscle of *L. vannamei*. This inhibition effect on the intestine may be due to the injury directly caused by soybean meal or indirectly induced by anti-nutritional factors. Zhang et al. (2020) documented that replacing 30% to 60% fishmeal with soybean meal induced intestinal injury of *Channa argus*. Ding et al. (2019) indicated that replacing 50% to 100% fishmeal with soybean meal damaged intestinal villus structure and caused enteritis of bullfrog (*Rana catesbeiana*). Zhou (2021) reported that dietary glycinin and β-conglycinin induced intestinal

TABLE 4 Apparent digestibility coefficients (ADCs) of nutrients by *L. vannamei* fed with experimental diets.

Items	Diets					SEM	*p*-value		
	T20	T28	T35	T42	T50		*P*	*L*	*Q*
ADC of dry matter, %	73.15^{a}	69.98^{b}	68.95^{b}	65.83^{c}	64.25^{c}	0.80	<0.001	<0.001	0.667
ADC of crude protein, %	92.28^{a}	88.73^{b}	86.48^{b}	85.50^{b}	81.50^{c}	0.82	<0.001	<0.001	0.306
ADC of crude lipid, %	87.68^{a}	84.68^{ab}	83.38^{ab}	81.25^{b}	77.28^{c}	2.46	<0.001	<0.001	0.490
ADC of ash, %	79.07^{a}	75.25^{b}	75.15^{b}	73.25^{b}	70.75^{c}	1.11	<0.001	<0.001	0.781

T20–T50, diets containing 20%–50% of soybean meal.
SEM, mean standard error; P, overall effect; L, linear effect; Q, quadratic effect.
Different letters within a row indicate significant differences ($p < 0.05$).

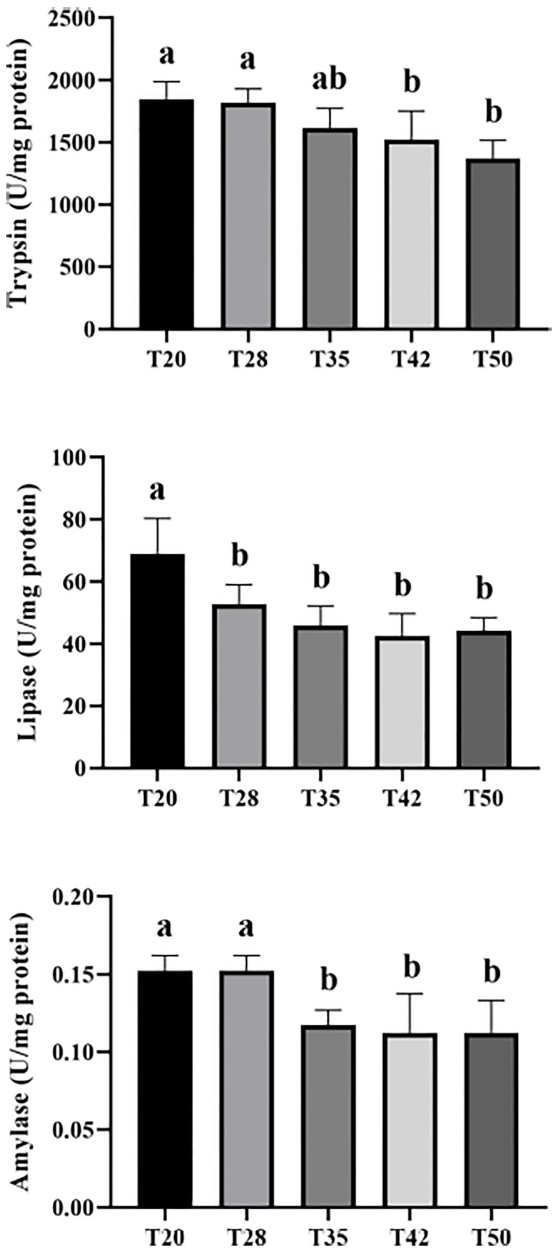

FIGURE 1
Intestinal digestive enzyme activities of L. vannamei fed with experimental diets. Different letters above the bars denote significant differences among diets ($p < 0.05$). T20–T50, diets containing 20%–50% of soybean meal..

damage and inflammation and thus inhibited growth and development of intestine in Songpu mirror carp by upregulating mRNA levels of apoptosis-related genes. Similar result was also reported by Duan (2019), in which the inclusion of β-conglycinin in grass carp diet caused enterocytes apoptosis and significantly reduced the ISI of fish. Similarly, Lu et al. (2018) also indicated that totally replacement of fishmeal by soybean meal in crayfish (*Cherax quadricarinatus*) diet significantly decreased crude protein content and depressed muscle growth. Xu et al. (2021) reported that replacing 28% to 70% fishmeal with soybean meal reduced the muscle nutrient compositions of *L. vannamei*.

Apparent digestibility and digestive enzyme activity

Digestibility of nutrients as reflected by the ADC values of crude protein, crude lipid, and ash usually indicates digestion and utilization of diets. These are closely related to the corresponding digestive enzyme activities in the intestine, because intestinal digestive enzymes, e.g., trypsin, lipase, and amylase, are commonly used as critical indicators to evaluate the changes in diets (Santigosa et al., 2008). In this study, the decreased ADCs of crude protein, crude lipid, and ash as dietary soybean meal increased suggest that the supplementation of soybean meal in shrimp diets decreased the digestion and utilization of diets. This is most likely due to the decreased intestinal digestive enzyme activities of trypsin, lipase, and amylase as observed in this study. The inhibition effect of intestinal digestive enzyme activities by replacing fishmeal with soybean meal in aquatic feed is a common phenomenon that has been well reported in previous studies. For instance, replacing 57% and 100% of fishmeal by soybean meal in Chinese mitten crab (*Eriocheir sinensis*) diets significantly reduced the intestinal digestive enzyme activities of trypsin, lipase, and amylase (Liu et al., 2021). Similar result was also reported by Zhang et al. (2015) that replacing 12% to 48% fishmeal with soybean meal significantly decreased the intestinal lipase and amylase activities of rice filed eel (*Monopterus albus*). Furthermore, Hernandez et al. (2007) documented that the inclusion of 60% soybean meal (approximately replacing 68% fishmeal) in sharpsnout seabream (*Diplodus puntazzo*) diet significantly reduced the ADCs of dry matter and crude protein. Yang et al. (2015) indicated that replacing 40% and 60% of fishmeal by soybean meal significantly decreased ADCs of dry matter and crude protein of *L. vannamei*. Tibaldi et al. (2006) also documented that substitution of 50% fishmeal by soybean meal significantly decreased ADCs of dry matter, crude protein, crude lipid, and ash. Although the reason why supplementation of soybean meal inhibited the intestinal digestive enzyme activities is not fully understood, this may be attributed to the increasing levels of the anti-nutritional factors (such as soybean agglutinin, glycinin, and β-conglycinin) with the increase of soybean meal in diets (Ding et al., 2019; Liu et al., 2021). Yue et al. (2012) also reported that anti-nutritional factors in soybean meal reduced the digestive enzyme activities in the gut of *L. vannamei*.

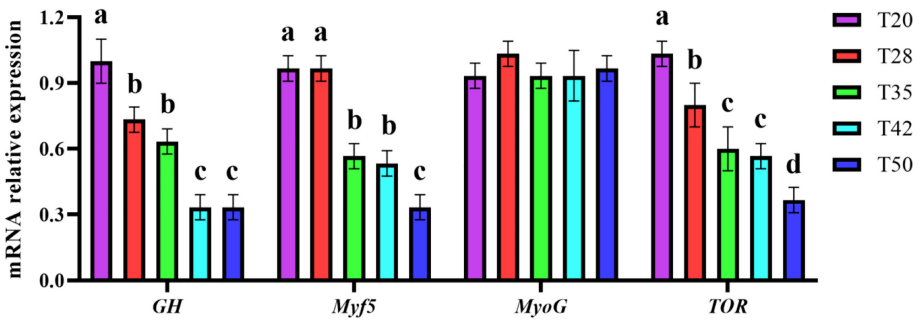

FIGURE 2
Muscle growth–related gene expression of *L. vannamei* fed with experimental diets. *GH*, growth hormone; *Myf5* and *MyoG*, myogenic regulatory factors; *TOR*, target of rapamycin. Different letters above the bars denote significant differences among diets ($p < 0.05$). T20–T50, diets containing 20%–50% of soybean meal.

Muscle growth–related gene expression

The growth and development of muscle that involves hyperplasia and hypertrophy is generally controlled by diverse genetic factors, such as growth hormone (*GH*), myogenic regulatory factors (*Myf5* and *MyoG*), and target of rapamycin (*TOR*) (Xin, 2016; Asaduzzaman et al., 2017). Therefore, the effects of formulated diets on muscle growth of aquatic animals were mainly investigated from the aspects of these muscle growth–related genes (Alami-Durante et al., 2018; Wei et al., 2020a; Wei et al., 2020b; Yang et al., 2021). *GH* stimulates the growth of muscle through inducing proliferation of myogenic cells and muscle hyperplasia and hypertrophy (Asaduzzaman et al., 2017). Myogenic regulatory factors, e.g., *Myf5* and *MyoG*, are the critical muscle development– and growth–related transcription factors (Funkenstein et al., 2007). *TOR*, an important regulatory factor in cellular central control system, plays a key role in cell growth and proliferation (Liang et al., 2020). Therefore, all of these genes act as a positive regulator of muscle growth that promote hyperplastic and hypertrophic muscular growth. In this study, the downregulated mRNA levels of *GH*, *Myf5*, and *TOR* as dietary soybean meal increased suggested that supplementation of soybean meal in shrimp diets decreased the growth and development of muscle. This may also account for the concomitantly decreased DWP as observed in this study. To our best knowledge, this study is the first to assess the effects of dietary soybean meal on these muscle-related gene expressions of *L. vannamei*. This is similar with the observation by Hu et al. (2018) that replacing 40% and 50% of fishmeal by fermented soybean meal in *Nibea albiflora* diets significantly decreased mRNA levels of muscle growth–related gene (insulin-like growth factor I). Ulloa et al. (2013) also reported that the dietary inclusion of plant proteins reduced the muscle growth–related gene expressions in the male zebrafish (*Danio rerio*). Despite the reason why the dietary inclusion of soybean meal downregulated the gene expression of muscle growth–related genes is not clear, this is most likely ascribed to the adverse effects of anti-nutritional factors exist in soybean meal on the proliferation of intestinal epithelial cells and thereby decreased digestion and absorption of nutrients in shrimp diet to provide necessary energy serving as basis contents for muscle growth. Numbers of studies documented that anti-nutritional factors extracted from soybean meal injured intestinal epithelial cells of animals (Feng, 2006; Guo, 2006; Xu, 2009; Duan, 2019; Peng, 2020; Zhou, 2021). These studies combined with the observation that supplementation of soybean meal inhibited nutrient digestibility of shrimp in this study may be the potential mechanisms to support this hypothesis. Further study is still needed to confirm this.

Conclusion

Dietary soybean meal that exceeded 28% significantly inhibited growth performance of *L. vannamei*. This growth inhibition effect may partly attribute to the negative impact of anti-nutritional factors that exist in soybean meal on the digestion and utilization of nutrients in shrimp diets and also the inhibition on the muscle growth and development as reflected by the decreased DWP and muscle growth–related gene expressions. This study provides a caution for the application of soybean meal in aquaculture, and further study is needed to clarify mechanisms regarding the inhibition effect of soybean meal on muscle growth of shrimp.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences, Guangzhou, China.

Author contributions

KP and WH conceived and designed the experiments. KP, XC, HJL, JZ, YC, CL, and HL performed the experiments. KP analyzed the data and wrote the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author HL is employed by Guangdong Jinyang Biotechnology Co. Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer (HZ) declared a shared affiliation with the author (CL) to the handling editor at the time of review.

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