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Effects of three different dietary plant protein sources as fishmeal replacers in juvenile whiteleg shrimp, *Litopenaeus vannamei*

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Abstract

Background: As the cost of fishmeal continues to rise, there will be a need to optimize the diet by minimizing dietary fishmeal inclusion in aquafeed. In this study, a 7-week experiment was conducted to evaluate soybean meal, fermented soybean meal (soytide), and sesame meal as fishmeal replacers in whiteleg shrimp, *Litopenaeus vannamei*.

Methods: A 30%-based fishmeal diet was considered as control (CON), six other diets were prepared by replacing 20% or 40% of fishmeal with soybean meal (SB_{20} and SB_{40}), fermented soybean meal (ST_{20} and ST_{40}), or sesame meal (SM_{20} and SM_{40}) from the CON diet. Twenty shrimp with average initial weight of 0.65 ± 0.05 g (mean ± SD) were randomly distributed into 21 tanks (45 L) and fed four times a day. Water temperature was controlled at 28 ± 1 °C and aeration was provided by air stones.

Results: Weight gain, specific growth rate, feed efficiency, and protein efficiency ratio of shrimp fed CON showed no significant differences compared to shrimp fed all the other diets. However, growth performance of shrimp fed ST_{20} diet was significantly higher than those of shrimp fed the SM_{20} and SM_{40} diets ($P < 0.05$). Superoxide dismutase activity (SOD) of shrimp fed CON, ST_{20}, and ST_{40} diets was significantly higher than those of shrimp fed the SB_{40} and SM_{40} diets. But there were no significant differences among shrimp fed CON, SB_{20}, ST_{20}, ST_{40}, and SM_{20} diets. Also, lysozyme activity of shrimp fed ST_{20} diet was significantly higher than those of shrimp fed the SB_{40} and SM_{40} diets. Although, lysozyme activity of shrimp fed the CON diet was not significantly different compared to shrimp fed all the other experimental diets.

Conclusions: Therefore, SB, ST, and SM could replace 40% of fishmeal based on growth performance and lysozyme. According to the SOD activity, SB and SM could replace 20% of fishmeal and ST could replace 40% of fishmeal in juvenile whiteleg shrimp *Litopenaeus vannamei*.

Keywords: Fishmeal, Soybean meal, Fermentation, Whiteleg shrimp, Sesame meal

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Background

Whiteleg shrimp (*Litopenaeus vannamei*) is an important species in aquaculture and is the most cultured prawn species in the world at 4,456,603 mt comprising over 84% of all cultured shrimp and prawn production at a value of over 26.7 billion USD (FAO 2017). This is in large part due to its rapid growth, disease tolerance, high stocking density tolerance, relatively low dietary protein requirement (30%), and broadness of supporting research (NRC 2011). However, as the body of knowledge about the dietary requirements of whiteleg shrimp grows and the cost of fishmeal continues to rise, there will be a need to optimize the diet by minimizing dietary fishmeal (FM) inclusion (Hamidoghli et al. 2018).

Over the last 10 years, FM production has declined as fisheries have become strained. In fact, from the year 2000 to 2018 the production of fishmeal fell from 7125 mt to 5130 mt, a 28% decrease. However, in the same period of time, FM price have continued to rise from 413 USD/mt in the year 2000, to 1546 USD/mt; a 73% increase (Kobayashi et al. 2015). This has led the feed industry to explore much less expensive alternatives to FM. Yet, when replacing FM with a more economic protein source, it is important to consider not just the price point, but to consider also other aspects such as nutritional value, digestibility, palatability, and the presence of anti-nutritional factors (Oliva-Teles et al. 2015).

One of the most successful plant-based protein crops in animal feed is soybean meal. This is due to its high content of protein (47%) and lipid (2.2%) along with its low price compared to FM ($400–500/ton). Likewise, sesame seed meal ($400/mt) also presents many of the same advantages found with soybean meal but with a much higher lipid content. Sesame seed meal typically contains around 42% protein making it comparable to soybean meal; however, it contains roughly five times the lipid content at 11.2% (NRC 2011). In recent years, there has been interest in the use of fermentation to increase the digestibility of plant-based proteins in many important aquaculture species. The fermentation process helps to break down more complex protein matter in the plant matter to make it more readily available for digestion. For example, fermented soybean meal has approximately 56% crude protein, whereas unfermented soybean meal has 47%. In a trial by Van Nguyen et al. (2018), over 25% of FM was successfully replaced in the diet of pacific whiteleg shrimp. Another important benefit in fermented products is the enrichment by the bacteria itself. A recent trial by Hamidoghli et al. (2019) showed that between 10 and 20% of FM could be substituted with single-cell protein obtained from *Corynebacterium ammoniagenes* in the diet of whiteleg shrimp. Many companies have been focusing on producing fermented plant protein products by a wide variety of microbes and processes such as batch, continuous, fed-batch, anaerobic, aerobic, surface, submerged, and solid-state fermentation. Therefore, it is important to investigate the effects of fermented plant-based protein sources as possible FM replacers in the diet of whiteleg shrimp.

Materials and methods

Experimental design and diets

The experimental feed formulation and proximate composition is shown in Table 1. Fishmeal (The feed Co., LTD, Seoul, Republic of Korea), soybean meal, poultry by-product, blood meal, meat and bone meal, squid liver powder, and wheat gluten meal were used as protein sources. Fish oil was used as the lipid source. Seven isonitrogenous diets were formulated to replace 20 and 40% of fishmeal by soybean meal (SB_{20} and SB_{40}), soytide (ST_{20} and ST_{40}), and sesame meal (SM_{20} and SM_{40}) included CON. Soytide is a commercial product (CJ Cheiljedang Bio, Seoul, Republic of Korea) of fermented soybean meal with *Bacillus subtilis* at 37 °C (Moniruzzaman et al. 2017). Every ingredient was well-powdered and mixed using electric mixer (Hanyoung Food Machinery, Gyeonggi-do,

Table 1 Formulation and proximate composition of 7 experimental diets (percent of dry matter basis)

Ingredients (% in diet)	Diets[1]						
	CON	SB_{20}	SB_{40}	ST_{20}	ST_{40}	SM_{20}	SM_{40}
Fishmeal (Chille)	30.0	24.0	18.0	24.0	18.0	24.0	18.0
Soybean meal	15.0	23.8	32.5	15.0	15.0	15.0	15.0
Soytide	0.00	0.00	0.00	7.20	14.5	0.00	0.00
Sesame meal	0.00	0.00	0.00	0.00	0.00	9.70	19.6
Poultry by-product	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Blood meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Meat and bone meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Squid liver powder	3.30	3.30	3.30	3.30	3.30	3.30	3.30
Wheat gluten meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Wheat flour	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Corn starch	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Fish oil	3.60	3.90	4.20	4.00	4.40	3.00	2.40
Others	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Total	100	100	100	100	100	100	100
Proximate analysis (% of DM)							
Moisture	9.10	9.10	8.80	8.10	8.80	9.30	8.50
Protein	41.8	42.0	41.9	41.8	41.9	41.0	41.9
Lipid	9.80	9.80	9.80	9.70	9.90	9.70	9.90
Ash	10.4	9.70	9.70	9.10	9.50	9.80	9.60

CON control diet (the basal diet), SB_{20} soybean meal at 20% FM replacement, SB_{40} soybean meal at 40% FM replacement, ST_{20} soytide at 20% FM replacement, ST_{40} soytide at 40% FM replacement, SM_{20} sesame meal at 20% FM replacement, SM_{40} sesame meal at 20% FM replacement

Republic of Korea). Fish oil was added according to feed formulation. The mixture was passed through a pellet machine (Shinsung, Seoul, Republic of Korea) with a 0.2 cm die. The prepared experimental diets were dried in the drying room for 48 h then stored at − 20 °C.

Experimental fish and feeding condition

Juvenile whiteleg shrimp *Litopenaeus vannamei* were carried from Pal-ttak shrimp farm (Go-seong, Rep. Korea) and stocked at 250 L rectangular tanks at Feed & Foods Nutrition Research Center (FFNRC, Pukyong National University, Busan, Rep. Korea). Twenty juvenile whiteleg shrimp (0.7 ± 0.03 g mean ± SD) were randomly distributed in each 21 tanks (45 L) as a triplicate groups. Filtered sea water with 1.3 L/min was supplied to each tank in a semi-recirculating system and water temperature was maintained at 28 ± 1.0 °C for the whole experimental period. Whiteleg shrimp were fed four times a day with 7% of wet body weight (Xie et al. 2017) during the 7 weeks of experiment. The amount of total feed was changed according to mortalities. The feces were removed by siphoning every day.

Sample collection and analysis

After the 7 weeks of feeding trial, every shrimp was counted and weighted in each aquarium and taken and measured to calculate growth performance including weight gain, specific growth rate, feed efficiency, protein efficiency ratio, and survival percent according to Mohanty (1999):

$$\text{Weight gain, WG}\ (\%) = [\text{final weight}\ (\text{g}) - \text{initial weight}\ (\text{g})] \times 100/\text{initial weight}\ (\text{g})$$

$$\text{Specific growth rate, SGR}\ (\%/\text{day}) = 100 \times [\ \ln \text{final weight}\ (\text{g}) - \ln \text{initial weight}\ (\text{g})]/\text{days}$$

$$\text{Feed efficiency, FE}\ (\%) = (\text{final weight}\ (\text{g}) - \text{initial weight}\ (\text{g})) \times 100/\text{feed ration}\ (\text{g})$$

$$\text{Protein efficiency ratio, PER} = \text{wet weight gain}\ (\text{g})/\text{protein intake}\ (\text{g})$$

$$\text{Survival}\ (\%) = 100 \times (\text{final number of fish}/\text{initial number of fish})$$

Proximate composition analysis of every experimental diet and whole body of shrimp were conducted following the Association of Official Analytical Chemists (AOAC 2005). Every sample was grounded after freeze-drying (Advantage 2.0, VirTis, New York, USA) for 48 h. Moisture and crude ash content were determined by drying to constant weight at 105 °C for 24 h and combustion at 550 °C in a muffle furnace for 3 h, respectively. The Kjeldahl method (2300 Autoanalyzer, Foss Tecator. AB, Hoganas, Sweden) was used after acid digestion to measure the nitrogen (N × 6.25) content. Soxtec system 1046 (Tecator AB, Hoganas, Sweden) was used with ether extraction to measure crude fat content.

Four shrimp were randomly selected from each aquarium for the hemolymph biochemical analysis. 0.3 ml of hemolymph was taken from the ventral sinus in the first pleomere using a 1-ml syringe that had a hypodermic needle with 2 mm of thickness. Hemolymph samples were centrifuged at 5000×*g* for 10 min and the serum was separated and stored at − 70 °C for determination of hemolymph biochemical parameters. These parameters were glutamic oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), total protein (TP), glucose (GL), superoxide dismutase (SOD), and lysozyme (Lys) activities. SOD was determined using an assay kit (Enzo ADI-900-157, Enzo Life Sciences, Inc.) and following the manufacturer's instructions. This method is based on inhibition against Water Soluble Tetrazolium dye and determination of SOD enzyme activity. The absorbance was monitored at 450 nm after incubating samples for 20 min at 37 °C using a multi-well spectrophotometer. Lysozyme activity was determined by reaction against *Micrococcus lysodeikticus* and spectrophotometric (Sunrise TECAN, Männedorf, Switzerland) analysis with 530 nm absorbance. Serum (20 μL), Hanks Balanced Salt Solution (HBSS), 3,3′,5,5′ tetramethylbenzidinedihydrochloride (TMB, 20 mM; Sigma-Aldrich), H_2O_2 (5 mM), and 4 M sulphuric acid were diluted in a 96-well plate. The color changes were measured at 450 nm in a microplate reader (Sunrise TECAN, Männedorf, Switzerland). Also, serum was used for the biochemical parameters including glutamic oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), total protein (TP), and glucose (GLU). These biochemical parameters were measured by a chemical analyzer Fuji DRICHEM 3500i (Fuji Photo Film Ltd., Tokyo, Japan).

Statistical analysis

After collection of every data, it was analyzed by one-way ANOVA test using SAS Program Version 9.4 (SAS Institute, Cary, NC, USA) to analyze the differences of each treatment group. When a significant difference was observed, a least significant difference (LSD) test was used to compare means. Treatment effects were considered significant at confidence level of $P < 0.05$.

Results

Growth performance

At the end of feeding trial, result of growth performance is shown in Table 2. Weight gain (WG) and specific growth rate (SGR) of shrimp fed CON diet showed no significant differences among shrimp fed the other experimental diets ($P > 0.05$). However, WG and SGR of shrimp fed the ST_{20} diet were significantly higher than

Table 2 Growth performances of juvenile whiteleg shrimp fed with 7 experimental diets for 7 weeks

Diets								Pooled SEM[6]
	CON	SB_{20}	SB_{40}	ST_{20}	ST_{40}	SM_{20}	SM_{40}	
WG[1]	391^{ab}	379^{ab}	370^{ab}	426^{a}	380^{ab}	358^{b}	334^{b}	10.8
SGR[2]	3.54^{ab}	3.48^{ab}	3.44^{ab}	3.69^{a}	3.47^{ab}	3.38^{b}	3.25^{b}	0.05
FE[3]	124^{ab}	135^{ab}	126^{ab}	115^{a}	130^{ab}	137^{b}	106^{b}	3.43
PER[4]	2.97^{ab}	2.93^{ab}	2.80^{ab}	3.23^{a}	2.88^{ab}	2.70^{b}	2.53^{b}	0.08
SR[5]	70.0^{ns}	68.3	68.3	68.3	70.0	66.7	68.3	0.43

Values are means from triplicate groups of fish (n = 3) where values in each row with different superscripts are significantly different (P < 0.05)
[1]Weight gain (WG) = (final weight − initial weight) × 100/initial weight
[2]Specific growth rate (SGR; %/day) = (in final weight − ln initial weight) × 100/d
[3]Feed efficiency (FE; %) = wet WG (g) × 100/dry feed intake (g)
[4]Protein efficiency ratio (PER) = wet weight gain/protein intake
[5]Survival (SR; %) = (total fish − dead fish) × 100/total fish
[6]Pooled standard error of mean = SD/√n

those of shrimp fed the SM_{20} and SM_{40} diets (P < 0.05). Feed efficiency (FE) and protein efficiency ratio (PER) of shrimp fed CON diet showed no significant differences among those fed the other experimental diets (P > 0.05). However, FE and PER of shrimp fed the ST_{20} diet were significantly higher than those of shrimp fed the SM_{20} and SM_{40} diets (P < 0.05).

Non-specific immune responses

The results of non-specific immune responses fed seven experimental diets were shown in Table 3. SOD activity of shrimp fed CON, ST_{20}, and ST_{40} were significantly higher than those of shrimp fed SB_{40} and SM_{40} diets (P < 0.05). However, there were no significant differences among the shrimp fed CON, SB_{20}, ST_{20}, ST_{40}, and SM_{20} diet (P > 0.05). Lysozyme activity of shrimp fed CON diet showed no significant differences among shrimp fed all experimental diets (P > 0.05). Although, ST_{20} diet was significantly higher than those of shrimp fed the SB_{40} and SM_{40} diets (P < 0.05).

Whole body proximate composition

Whole body composition of shrimp fed seven experimental diets were showed in Table 4. There were no significant differences in whole body crude protein, lipid, ash and moisture of shrimp fed every experimental diets (P > 0.05).

Table 3 Non-specific immune responses of juvenile whiteleg shrimp fed with 7 experimental diets for 7 weeks

	Diets							Pooled SEM[3]
	CON	SB_{20}	SB_{40}	ST_{20}	ST_{40}	SM_{20}	SM_{40}	
Lys[1]	1.62^{ab}	1.62^{ab}	1.54^{b}	1.70^{a}	1.63^{ab}	1.61^{ab}	1.55^{b}	0.02
SOD[2]	87.1^{a}	84.4^{ab}	81.8^{b}	87.7^{a}	87.2^{a}	84.9^{ab}	81.7^{b}	0.95

Values are means from triplicate groups of fish (n = 3) where in each row with different superscripts values are significantly different (P < 0.05)
[1]Lysozyme (U/l)
[2]Superoxide dismutase (% inhibition)
[3]Pooled standard error of mean = SD/√n

Table 4 Whole body proximate composition of juvenile whiteleg shrimp fed with 7 experimental diets for 7 weeks (% dry matter basis)

	Diets							Pooled SEM[5]
	CON	SB_{20}	SB_{40}	ST_{20}	ST_{40}	SM_{20}	SM_{40}	
Mo[1]	6.08^{ns}	6.43	6.12	6.48	6.42	6.19	6.02	0.07
CP[2]	76.3^{ns}	76.0	77.1	76.2	75.2	75.7	76.2	0.22
CL[3]	1.84^{ns}	1.97	1.68	1.67	1.77	1.97	1.98	0.05
As[4]	14.2^{ns}	13.7	13.7	13.2	14.0	14.1	16.6	0.16

Values are means from triplicate groups of fish (n = 3) where in each row with different superscripts values are significantly different (P < 0.05)
[1]Mo, moisture content of whole body composition of juvenile rainbow trout in different diets
[2]CP, crude protein present under different dietary treatments
[3]CL, crude lipid, present in whole body proximate composition of juvenile rainbow trout after feeding trial
[4]As, ash content of whole body proximate composition of fish in different diets
[5]Pooled SEM, pooled standard error of mean = SD/√n

Hemolymph parameters

Hemolymph parameters of shrimp fed seven experimental diets are presented in Table 5. There were no significant differences in serum glutamic oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), total protein (TP), and glucose of shrimp fed every experimental diets (P > 0.05).

Discussion

The diets prepared for this experiment were readily accepted by the shrimp and almost no uneaten feed was observed in the tanks 1 h post feeding. Also, the survival percentage of each tank at the end of experiment was normal considering the initial weight of shrimp and the experimental period. Based on weight gain, specific growth rate, feed efficiency and protein efficiency ratio, 20% and 40% replacement of fishmeal with soybean meal, sesame meal, or fermented soybean meal did not

Table 5 Hematological parameters of juvenile whiteleg shrimp fed with 7 experimental diets for 7 weeks

	Diets							Pooled SEM[5]
	CON	SB_{20}	SB_{40}	ST_{20}	ST_{40}	SM_{20}	SM_{40}	
GL[1]	51.7^{ns}	48.2	50.0	52.5	45.5	49.0	46.5	4.54
TP[2]	10.6^{ns}	10.1	10.2	10.3	10.2	9.57	9.60	0.58
GOT[3]	63.3^{ns}	64.8	67.2	65.2	64.7	69.8	65.3	4.51
GPT[4]	15.0^{ns}	13.3	15.0	15.3	14.5	15.4	14.3	1.15

Values are means from triplicate groups of fish (n = 3) where in each row with different superscripts values are significantly different (P < 0.05)
[1]GL, glucose (mg/dl)
[2]TP, total Protein (g/dl)
[3]GOT, glutamic oxaloacetic transaminase (U/l)
[4]GPT, glutamic-pyruvic transaminase (U/l)
[5]Pooled SEM, pooled standard error of mean = SD/√n

show significant differences compared to the control group. Although, growth performance of shrimp fed the 20% fishmeal replacement (ST_{20}) with fermented soybean meal was significantly higher than the sesame meal fed group (SM_{20} and SM_{40}). According to previous studies, soybean meal is an appropriate candidate to replace fishmeal in aquafeed due to high protein level, balanced amino acid content, and acceptable price (Francis et al. 2001; Azarm and Lee 2014). But the anti-nutritional factors present in soybean meal, such as protease inhibitors, lectin, and anti-vitamin components, decrease the nutritional value of this ingredient and reduce the digestibility (Francis et al. 2001; Shiu et al. 2015). Fermentation of plant ingredients can reduce the anti-nutritional factors and enhance the digestibility (Jannathulla et al. 2017; Moniruzzaman et al. 2017). The significant differences observed in the present study with growth performance of shrimp fed the fermented soybean meal is probably due to the partial elimination of anti-nutritional factors and increased digestibility of ingredients. Similarly, Jannathulla et al. (2017) compared fermented and non-fermented plant proteins such as soybean meal, groundnut oil cake, rapeseed meal, and sunflower oil cake in the diet of whiteleg shrimp. Results indicated higher apparent digestibility coefficient of fermented soybean meal along with higher amino acid digestibility. These results are in agreement with the findings of our study where fermented soybean meal increased the growth performance of whiteleg shrimp compared to sesame meal, although we did not observe major differences between fermented and non-fermented soybean meal groups. Another explanation for the higher growth performance of fermented soybean meal could be attributed to the probiotic (*Bacillus subtilis*) compounds available in this ingredient. In a similar study, fermented soybean meal with *B. subtilis* increased the apparent digestibility coefficient of crude protein in whiteleg shrimp (Van Nguyen et al. 2018). Also, Seong et al. (2018) replaced 30% of fishmeal with *B. subtilis*-fermented soybean meal in olive flounder (*Paralichthysolivaceus*) without adverse effects on growth performance. Probiotics can benefit shrimp by exclusion of pathogenic bacteria and production of some digestive enzyme that can facilitate the digestion process (Liu et al. 2009; Zokaeifar et al. 2012). Although further studies regarding the effects of probiotic-fermented plant proteins on growth performance of shrimp are required.

Shrimp are among the species that lack adaptive immunity and therefore their health condition depends largely on non-specific immune responses (Sakai 1999; Farzanfar 2006). Superoxidase dismutase (SOD) and lysozyme are enzymes that neutralize radical oxygen species and breakdown cell walls of pathogenic organisms, respectively (McCord and Fridovich 1969; Samarakoon et al. 2013). Our results for SOD activity showed higher values for shrimp fed the fermented soybean meal (ST_{20} and ST_{40}) compared to 40% replacement by both soybean meal (SB_{40}) and sesame meal (SM_{40}). These results corresponded with serum lysozyme activity where 20% of fermented soybean meal (ST_{20}) was significantly higher than 40% of soybean meal (SB_{40}) and sesame meal (SM_{40}). These results are in agreement with previous findings that reported enhanced non-specific immune responses of fish fed fermented plant protein sources (Azarm and Lee 2014). Also, Kim et al. (2010) and Kader et al. (2012) demonstrated that fermented plant ingredients increased the antioxidant activity in olive flounder. These authors explained the mechanism of actions by higher bioavailability and access to soy isoflavones through the fermentation process.

Conclusions

The results of present study demonstrate that soybean meal, fermented soybean meal and sesame meal could replace 40% of fishmeal based on growth performance and lysozyme activity. But based on superoxide dismutase activity, soybean meal and sesame meal could replace up to 20% of fishmeal and fermented soybean meal could replace up to 40% of fishmeal in juvenile whiteleg shrimp *Litopenaeus vannamei.*

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Authors' contributions

JB designed and conducted the experiment and wrote the paper. AH prepared the manuscript. MSD designed and conducted some parts of the analysis and experiment. SM conducted some parts of the analysis and experiment. AH conducted some parts of the analysis and experiment. IS prepared the ingredients and feeding trial. NWF conducted analysis of sample. SCB supervised the study. All authors read and approved the final manuscript.

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Availability of data and materials

Please contact author for data requests

Ethics approval and consent to participate

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Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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