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Using a selectively bred nongenetically modified soybean meal to replace fishmeal in practical diets for the Pacific white shrimp *Litopenaeus vannamei*

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Abstract

Growth and digestibility trials were conducted to evaluate the use of nongenetically modified soybean meal (NMSBM) as a supplement in practical shrimp feeds. The apparent digestibility coefficient of dry matter, protein, and energy for the reference diet and NMSBM were 72.87% and 90.13%, 84.83% and 96.61%, and 85.26% and 96.10%, respectively. The 10-week growth trial was conducted to evaluate the replacement of fishmeal by NMSBM at the levels of 0%, 20%, 40%, 60%, 80% and 100%. The experimental diets were named as D0, D20, D40, D60, D80 and D100, respectively. Results showed that there were no significant differences in the specific growth rate among the D0-D80, and in the feed conversion ratio and body composition among all the treatments. However, the SGR in D100 was significantly lower than that in D0, D20 and D40. The feeding rate in D100 was significantly higher than that in D0, D20 and D60. The challenge test with *Vibrio parahaemolyticus* after the growth trial showed that no significant differences were found on the cumulative mortalities of shrimp. In conclusion, NMSBM can replace up to 80% fishmeal with no significant negative effects on the growth performance, body composition, immune response and disease resistance of shrimp *Litopenaeus vannamei*.

KEYWORDS
digestibility, feeds, fishmeal, growth, *Litopenaeus vannamei*, nongenetically modified soybean meal

1 | INTRODUCTION

Soybean meal (SBM) is often regarded as a cost-effective and nutritionally valuable protein source in shrimp and fish feeds. The popularity of SBM as a dietary protein source is the result of a relatively balanced nutrient profile, high digestibility, steady supply and availability of large quantities, expandable production and reasonable price (Amaya, Davis, & Rouse, 2007; Samocha, Davis, Saoud, & DeBault, 2004). However, using SBM as a sole protein source in shrimp feed is limited due to the low level of some essential amino acids (e.g., methionine, lysine and threonine) (Espe, Lemme, Petri, & El-Mowafi, 2006; Mai et al., 2006), available phosphorus (Dias et al., 2009; Yun et al., 2014) and the presence of many antinutritional factors (ANFs) that may cause an inhibition of digestive enzyme activity and its growth performance (Suárez et al., 2009). These disadvantages limited the utilization of SBM in aquafeed to

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a certain extent. The ANFs are compounds, which reduce the nutrient digestion and the utilization, such as saponins, tannins, trypsin inhibitors, oligosaccharides and flavonoids (Soetan & Oyewole, 2009). Some of the ANFs can be detoxified, reduced or eliminated by various manufacturing processes, such as fermentation and thermal treatment. However, some of them may still remain.

Continuing to improve the nutritive value of the SBM is a desirable progression. Genetically modified soybeans have reduced levels of ANFs and increased content of essential amino acids (Krishnan, 2005). However, genetically modified foods have resulted in heated scientific and public debate, and the issue with the safety of genetically modified foods remains controversial since the mid-1990s. New strains of selectively bred nongenetically modified soybean can be used to develop new soybean cultivars. A new strategy utilizes marker-assisted breeding programmes to improve nutritional characteristics of soybeans such as improving the levels of protein and amino acids and reducing of ANFs, which makes the SBM becomes the potential to attain a more complete fishmeal replacement (Watson, Buentello, & Place, 2014).

Navita 3011 (N-3011) is a kind of genetically unique, patented soybeans developed by Schillinger Genetics (Schillinger Genetics, West Des Moines, IA, USA). It is a kind of nongenetically modified soybean cultivars, which contains higher levels of protein and amino acids for animal feed compared with the typical soybeans. Some researches have been conducted to evaluate the potential of nongenetically modified soybean cultivars as protein sources in practical diets for Pacific white shrimp (Fang, Yu, Buentello, Zeng, & Davis, 2016; Zhou, Davis, & Buentello, 2015). However, there was only one supplemental level of dietary fishmeal used in the experimental diets, and the immune responses of the shrimp to the replacement of fishmeal by the nongenetically modified soybean protein were not reported. And the available data on the apparent digestibility coefficients of this product are limited. Therefore, the aim of this study was to evaluate the effects of replacement of fishmeal by graded levels of N-3011 on digestibility, growth performance, immune response and disease resistance of the Pacific white shrimp *L. vannamei*.

2 | MATERIALS AND METHODS

2.1 | Digestibility trial

The formulation and proximate composition of the experimental diets are shown in Tables 1 and 2. The test diet was consisted of a 70:30 mixture of the reference diet to test feedstuff with 1 g/kg of Y_2O_3 as a marker. Four tanks were used per diet, and forty shrimps per tank were used. The experimental shrimps (body weight: 5.09 ± 0.01 g) were acclimatized to the experimental condition including the experimental diets for 1 week. After that, the uneaten feed was removed and the faeces were collected by siphon 1 hr after each feeding. The faeces were collected over 1 week until adequate sample was obtained. The samples were stored in -20°C and dried in a laboratory vacuum dryer for further analysis.

TABLE 1 Formulation and proximate composition of the reference diet on a dry matter basis (g/kg diet)

Ingredients	Content (g/kg)
Fishmeal[a]	340.0
Wheat flour[a]	450.4
Soybean meal[a]	140.0
Lecithin	35.0
Fish oil	28.0
Vitamin premix[b]	3.0
Mineral premix[c]	1.5
Vitamin C	0.6
Vitamin E	0.3
Antioxidant	0.2
Yttrium oxide	1.0
Total	1,000
Proximate analysis (g/kg)	
Crude protein	383.4
Crude lipids	86.9
Gross energy (KJ/g)	17.8

Notes. [a]These ingredients were supplied by Qingdao Great Seven Bio-Tech, Co., Ltd. (Qingdao, China). Fishmeal: crude protein 737 g/kg, crude lipid 77 g/kg; wheat flour: crude protein 178 g/kg, crude lipid 13 g/kg; soybean meal: crude protein 551.5 g/kg, crude lipid 17.7 g/kg. [b]Vitamin premix (IU or g/kg diet): thiamine, 0.5; riboflavin, 0.7; pyridoxine HCl, 0.6; vitamin B12, 0.002; vitamin K3, 0.5; vitamin A, 450 000 IU; vitamin D3, 150 000 IU; vitamin E, 5; niacin acid, 3.5 g; folic acid, 0.15; biotin, 0.060; inositol, 8. [c]Mineral premix (g/kg diet): $MgSO_4 \bullet H_2O$, 25; $CuSO_4 \bullet 5H_2O$, 2; $FeSO_4 \bullet H_2O$, 2; $ZnSO_4 \bullet H_2O$, 10; $MnSO_4 \bullet H_2O$, 3; $CoCl_2 \bullet 6H_2O$, 0.08; $Ca(IO_3)_2$, 0.1; Na_2SeO_3, 0.01.

TABLE 2 Digestibility experimental diet formula (g/kg)

Diet	RD	TD
Reference diet	1,000	700
N-3011[a]	0	300
Total	1,000	1,000
Proximate analysis (g/kg)		
Crude protein	383.4	444.1
Crude lipids	86.9	63.7
Gross energy (KJ/g)	17.79	19.97

Notes. RD: reference diet; TD: test diet.
[a]N-3011: crude protein 560.6 g/kg, crude lipid 5.7 g/kg (Schillinger Genetics, Inc., NV, USA).

2.2 | Growth trial

2.2.1 | Experimental diets

The formulation and proximate composition of the experimental diets are shown in Table 3. The six diets were formulated to be isolipidic (about 82 g/kg crude lipid) and isonitrogenous (about 385 g/kg crude protein), and be with 0%, 20%, 40%, 60%, 80% and 100%

TABLE 3 Formulation and proximate composition of the experimental diets on a dry matter basis (g/kg diet)

Ingredients (g/kg)	D0	D20	D40	D60	D80	D100
Fishmeal[a]	300	240	180	120	60	0
Shrimp shell meal[a]	50	50	50	50	50	50
Squid visceral meal[a]	50	50	50	50	50	50
N-3011[b]	0	70.2	140.4	210.6	280.8	351
Peanut meal[a]	70	70	70	70	70	70
High gluten flour[a]	200	200	200	200	200	200
Wheat starch[a]	162.4	143.2	123.5	104	84.7	65
Wheat gluten flour[a]	70	68.9	67.7	66.6	65.5	64.3
Methionine	1.5	2	2.5	3	3.5	4
Lysine	0	0.2	0.5	0.7	0.9	1.2
Cysteine	1.1	0.8	0.6	0.4	0.2	0
Fish oil	24	28.8	33.6	38.4	43.2	48
Soybean oil	4.1	3.6	3.1	2.6	2.1	1.6
Soybean lecithin	15	15	15	15	15	15
Moult hormone	1	1	1	1	1	1
Cholesterol	2	2	2	2	2	2
Microcrystalline cellulose	0.4	5.8	11.6	17.2	22.6	28.4
Choline chloride	1	1	1	1	1	1
Mould inhibitor	1	1	1	1	1	1
Ethoxyquin	0.5	0.5	0.5	0.5	0.5	0.5
$Ca(H_2PO_4)_2$	15	15	15	15	15	15
L-ascorbyl-2-monophosphate	1	1	1	1	1	1
Vitamin premix[c]	15	15	15	15	15	15
Mineral premix[d]	15	15	15	15	15	15
Proximate analyses (g/kg diet)						
Crude protein	387.7	389.7	384.7	387.8	381.2	377.5
Crude lipid	81.8	79.1	81.0	83.5	80.1	83.5
Moisture	87.8	114.2	101.3	98.1	93.2	83.8
Ash	140.4	150.1	145.5	166.5	151.9	164.4

Notes. [a]Those ingredients were supplied by Qingdao Great Seven Bio-Tech, Co., Ltd. Fishmeal: steam dried fishmeal (COPENCA Group, Lima, Peru), crude protein 737 g/kg, crude lipid 77 g/kg; shrimp shell meal: crude protein 486 g/kg, crude lipid 36 g/kg; peanut meal: crude protein 592 g/kg, crude lipid 17 g/kg; squid visceral meal, crude protein 515 g/kg, crude lipid 107 g/kg; high gluten flour: crude protein 178 g/kg, crude lipid 13 g/kg; wheat gluten flour: crude protein 839 g/kg, crude lipid 6 g/kg; wheat starch: crude protein 0.04 g/kg, crude lipid 6 g/kg. [b]N-3011: crude protein 560.6 g/kg, crude lipid 5.7 g/kg (Schillinger Genetics, Inc., NV). [c]Vitamin premix (IU or g/kg diet): thiamine, 0.5; riboflavin, 0.7; pyridoxine HCl, 0.6; vitamin B12, 0.002; vitamin K3, 0.5; vitamin A, 450 000 IU; vitamin D3, 150 000 IU; vitamin E, 5; niacin acid, 3.5; folic acid, 0.15; biotin, 0.060; inositol, 8. [d]Mineral premix (g/kg diet): $MgSO_4 \bullet H_2O$, 25; $CuSO_4 \bullet 5H_2O$, 2; $FeSO_4 \bullet H_2O$, 2; $ZnSO_4 \bullet H_2O$, 10; $MnSO_4 \bullet H_2O$, 3; $CoCl_2 \bullet 6H_2O$, 0.08; $Ca(IO_3)_2$, 0.1; Na_2SeO_3, 0.01.

replacement of fishmeal by N-3011 on a protein basis, respectively. They were named as D0, D20, D40, D60, D80 and D100, respectively.

2.2.2 | Experimental procedure

The Pacific white shrimps were obtained from a commercial farm in Zhanjiang, Guangdong Province, China. Prior to the initiation of this feeding trial, juvenile shrimps were acclimatized to the experimental condition and diets for 2 weeks. Then juvenile shrimps (initial weight: 0.38 ± 0.00 g) were randomly stocked into 36 tanks (250 L) with 40 shrimps per tank. Every 6 tanks of shrimps were fed one of the six experimental diets.

Each diet was hand-fed 4 times daily (07:00, 11:00, 16:00 and 21:00) to apparent satiation for 10 weeks. The shrimps were initially

fed 8% and 10% of the initial stocked weight in week 1 and week 2, respectively. From the week 3, the amount of the feeds offered to shrimp was adjusted weekly according to the daily checking of uneaten feed. Uneaten feed, faeces and moults were removed by siphoning the aquaria prior to the morning feeding. During the feeding trial, the water temperature was 22–30°C, dissolved oxygen was not <7.0 mg/L, pH was 7.8–8.2, salinity was 26.5–28.0 g/L, and the total ammonia nitrogen level was <0.03 mg/L.

2.2.3 | Sample collection

At the end of the feeding trial, all shrimps were not fed for 24 hr before sampling, and then, the total numbers and body weight of shrimps in each tank were counted and weighed to calculate survival, feed conversion ratio (FCR) and specific growth rate (SGR).

Six shrimps per tank were randomly selected and frozen at −20°C for determination of the whole-body composition. Another five shrimps per tank were randomly chosen for the immune parameter assays. For each shrimp, 1 ml of haemolymph was withdrawn from the ventral sinus with sterile syringes, and 2 ml of anticoagulant solution (30 mmol/L trisodium citrate, 10 mmol/L EDTA, 0.34 mmol/L sodium chloride, 0.12 mmol/L glucose, adjust pH to 7.55 and osmotic pressure to 780 m Osm/kg) was added. The haemolymph from the five shrimps per tank was pooled as a replicate to measure respiratory burst activity. The remaining haemolymph without anticoagulant solution was allowed to clot at 4°C for 12 hr. After being centrifuged at 8,000× *g* for 10 min at 4°C, the serum was collected and frozen at −80°C until assayed.

2.2.4 | The challenge test

At the termination of the feeding trial, three tanks of shrimp from each treatment were challenged with *Vibrio parahaemolyticus*. Fifteen shrimps were used per tank. The concentration of *V. parahaemolyticus* was adjusted to 5×10^6 CFU/ml. Shrimps were intraperitoneally injected with 50 μl of bacterial suspension, which corresponds to the LD50 of this bacterial suspension. Shrimps continued to be fed four times daily with the experimental diets, and mortality was recorded twice daily for 7 days. Cumulative mortality rate was calculated.

2.3 | Sample analysis

The proximate compositions of diets, feed ingredients, shrimp samples and faecal samples were analysed using standard methods of AOAC (1995). Samples of diets and shrimps were dried to a constant weight at 105°C to determine dry weight. Crude protein was calculated from the determination of the total nitrogen (N × 6.25) using the Kjeldahl method (2300-Autoanalyzer, FOSS, Denmark). Crude lipid was determined by gravimetric analysis following ether extraction of the lipids according to the Soxhlet method (36680-analyzer, BUCHI, Switzerland). Ash content was determined following the loss of mass after combustion of a sample in a muffle furnace at 550°C for 12 hr.

The amino acid compositions of the experimental diets were analysed by automatic amino acid analyser (Biochrom 30; GE Healthcare Co. Ltd, Cambridge, UK). Dietary energy contents were analysed by the adiabatic bomb calorimeter (PARR1281, USA). Yttrium was determined by the inductively coupled plasma atomic emission spectrophotometer (ICP-OES, VISTA-MPX, VARIAN, USA).

Respiratory burst activity, phenoloxidase activity, superoxide dismutase activity (SOD), lysozyme activity (LZM) and total nitric oxide synthase (T-NOS) activities were analysed as described previously (Guo et al., 2016).

2.4 | Calculations and statistical methods

The survival rate, growth, feed utilization and cumulative mortality rate were calculated by the following formulae:

Survival rate (SR, %) = 100 × (final amount of shrimps)/(initial amount of shrimps)

Specific growth rate (SGR, % day^{-1}) = 100 × (Ln final weight − Ln initial weight)/days

Feed conversion ratio (FCR) = dry feed fed/(final wet weight − initial wet weight)

Feeding rate (FR, % average body/weight/day) = 100 × feed fed/[days × (initial weight + final weight)/2]

Cumulative mortality rate (%) = 100 × (final death of shrimps)/(initial injected shrimps)

The apparent digestibility coefficient (ADC) of dry matter, protein, energy and amino acids were calculated according to Cho, Slinger, and Bayley (1982) as follows:

ADC of dry matter (%) = 100 − [(100 × Y_2O_3 in feed/Y_2O_3 in faeces) × 100]

ADC of nutrients or energy (%) = [1 − (dietary Y_2O_3/faecal Y_2O_3) × faecal nutrient or energy/dietary nutrient or energy)] × 100%

ADC of the test ingredients (N-3011) was calculated according to Bureau and Hua (2006) as follows:

$$ADC\ (\%) = ADC_{TD} + (ADC_{TD} - ADC_{RD}) \times (0.7 \times Nutr_{RD}/0.3 \times Nutr_{ING})$$

where ADC_{TD} is the apparent digestibility of the nutrients or energy in the test diet (TD), ADC_{RD} is the apparent digestibility of nutrients or energy in the reference diet (RD), $Nutr_{RD}$ is the nutrients or energy concentration in the RD, and $Nutr_{ING}$ is the nutrients or the energy concentration in the test ingredient.

Results are presented as mean ± *SE* (standard error of means). Data from each treatment were subjected to one-way analysis of variance (ANOVA) using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). When overall differences are significant ($p < 0.05$), Tukey's test was used to compare the mean values among the treatments.

TABLE 4 Apparent digestibility coefficients (ADC) of the ingredient (N-3011) and reference diet by shrimp

Diet/ingredient	ADC of dry matter (%)	ADC of crude protein (%)	ADC of energy (%)
Reference diet	72.87 ± 1.71	84.83 ± 0.49	85.26 ± 0.81
N-3011	90.13 ± 2.56	96.61 ± 3.49	96.10 ± 0.00

Note. Values are means ± *SE* of four replicates.

3 | RESULTS

3.1 | Digestibility trial

The ADCs of dry matter, dietary protein and energy for the reference diet and ingredient (N-3011) are presented in Table 4. The ADCs of dry matter, dietary protein and energy for the reference diet were 72.87%, 84.83% and 85.26%, respectively. Those ADCs for the N-3011 were 90.13%, 96.61% and 96.10%, respectively. The amino acid digestibility coefficients for N-3011 are presented in Table 5, which is ranged from 77.43% to 99.47%.

3.2 | Growth trial

3.2.1 | Survival and growth performance

The survival and growth performance of shrimp are presented in Table 6. Shrimp fed with D100 showed significantly lower final mean body weight and SGR than those fed with D0, D20 and D40 ($p < 0.05$). The D100 had the lowest survival, and there was no significant difference among the other all treatments. Meanwhile, the D100 had the highest feeding rate, and there was no significant difference among the other all treatments. There were no significant differences in feed conversion ratio among all the treatments.

TABLE 5 The amino acid digestibility coefficients of the N-3011

Amino acids	Amino acid digestibility (%)
Asp	94.60 ± 0.19
Thr	89.38 ± 0.02
Ser	91.75 ± 0.05
Glu	99.47 ± 0.49
Gly	88.21 ± 0.06
Ala	90.75 ± 0.05
Cys	88.80 ± 0.01
Val	90.77 ± 0.04
Met	77.43 ± 0.05
Ile	92.24 ± 0.04
Leu	93.83 ± 0.12
Tyr	93.86 ± 0.09
Phe	91.06 ± 0.04
Lys	94.89 ± 0.03
His	94.16 ± 0.03
Arg	96.02 ± 0.07

3.2.2 | Body compositions

The body compositions of shrimps are presented in Table 7. There were no significant differences in the contents of dry matter (248–263.6 g/kg), ash (92.4–103.5 g/kg), crude lipid (48.4–61.3 g/kg) and crude protein (711.1–740.6 g/kg) among all the treatments ($p > 0.05$).

3.2.3 | Immune responses

The parameters on immune responses are shown in Table 8. D80 had the significant highest activities of T-NOS (23.58 U/ml). The SOD activities significantly increased from D0 (173.83 U/ml) to D60 (198.39 U/ml). Compared to D0, D20 had the significant higher activities of PO and LZM. However, the significant highest activity of RB was found in D40 (0.57 O.D. 630 nm).

3.2.4 | The challenge test

As indicated in Table 9, statistical analysis showed no significant differences in the cumulative mortality among all the treatments after the challenge test ($p > 0.05$).

4 | DISCUSSION

4.1 | Digestibility and growth

Digestibility is an important factor to be considered in determining the utilization of a feedstuff. In the present study, the ADC of protein and energy for N-3011 (96.61% and 96.10%, respectively) was higher than that of FM reported by Qiu and Davis (2017) in FM1 (62.07% and 69.77%) and FM2 (65.78% and 71.3%) and Brunson, Romaire, and Reigh (1997) (80.81% and 71.55%). Meanwhile, the ADCs of dry matter, protein and energy for N-3011 were also higher than other soybean meal reported by Cruz-Suárez et al. (2009) (82.7%, 95.7% and 88.1%, respectively), Siccardi et al. (2006) (63.5%, 87.1% and 80.8%, respectively), Yang et al. (2009) (69.98%-71.2%, 88.95%-90.89% and 74.12%-82%, respectively) and Zhu, Davis, Roy, Samocha, and Lazo (2013) (68.89%-80.2%, 78.8%-93.5% and 65.4%-74.73%, respectively).

The higher ADC of protein is translated to higher amino acid digestibility in the present study. The amino acid digestibility coefficients for N-3011 ranged from 77.43% to 99.47% (Table 5), which were higher than those of good ingredients reported by Qiu and Davis (2017) in PepsoyGen SBM (71.4%–88.5%) and FM (54.39% and 90.28%), Yang et al. (2009) in fermented SBM (68.96%–89.27%) and Akiyama, Coelho, Lawrence, and Robinson (1989) in FM (78.4%–83.1%). These ADCs in the present study are more in line with those

TABLE 6 Effects of replacement of dietary fishmeal by N-3011 on survival and growth performance of shrimp

Diet	Final body weight (g)	Specific growth rate (% per day)	Survival (%)	Feeding rate (% average/BW/day)	Feed conversion ratio
D0	9.91 ± 0.29^{b}	4.40 ± 0.02^{b}	95.42 ± 3.32^{b}	4.09 ± 0.14^{a}	1.57 ± 0.16
D20	10.04 ± 0.57^{b}	4.42 ± 0.03^{b}	97.50 ± 1.29^{b}	3.85 ± 0.09^{a}	1.49 ± 0.11
D40	10.01 ± 0.41^{b}	4.42 ± 0.02^{b}	95.83 ± 1.39^{b}	4.16 ± 0.12^{ab}	1.60 ± 0.13
D60	9.67 ± 0.42^{ab}	4.37 ± 0.02^{ab}	96.25 ± 1.25^{b}	3.95 ± 0.04^{a}	1.52 ± 0.07
D80	9.63 ± 0.25^{ab}	4.35 ± 0.02^{ab}	93.75 ± 1.91^{ab}	4.31 ± 0.08^{ab}	1.65 ± 0.08
D100	9.14 ± 0.40^{a}	4.29 ± 0.03^{a}	81.67 ± 5.35^{a}	4.41 ± 0.15^{b}	1.65 ± 0.07
ANOVA					
F value	4.244	5.159	4.279	4.230	0.633
p value	0.005	0.002	0.005	0.001	0.616

Note. Values are means ± *SE* of six replicates, and values within the same row with different letters are significantly different ($p < 0.05$).

TABLE 7 Effects of replacement of dietary fishmeal by N-3011 on body composition of shrimp (in g/kg of dry weight basis)

Diet	Dry matter	Ash	Crude lipid	Crude protein
D0	263.6 ± 2.7	99.6 ± 1.1	61.3 ± 5.3	736.0 ± 6.8
D20	258.6 ± 6.2	103.5 ± 1.4	54.6 ± 4.6	740.6 ± 2.0
D40	248.0 ± 8.6	92.4 ± 4.7	48.4 ± 4.6	725.9 ± 3.2
D60	259.1 ± 5.0	99.4 ± 2.5	51.5 ± 1.9	717.1 ± 3.2
D80	262.0 ± 0.8	99.2 ± 1.9	54.1 ± 4.7	722.0 ± 5.8
D100	261.0 ± 4.3	92.6 ± 4.9	50.2 ± 0.5	711.1 ± 3.6
ANOVA				
F value	1.404	0.544	1.486	3.019
p value	0.268	0.708	0.234	0.251

Note. Values are means ± *SE* of six replicates, and values within the same row with different letters are significantly different ($p < 0.05$).

in the previous studies, in which the nongenetically modified soybean meals were used (Fang et al., 2016; Zhou et al., 2015). We can also determine that N-3011 is more digestible than FM for shrimp.

The N-3011 has a low crude fibre content (25.8 g/kg), low acid detergent fibre (33.5 g/kg) and high protein content (560.6 g/kg). In general, the typical SBM had 36.4 g/kg of crude fibre, 73.7 g/kg of acid detergent fibre and 489.1 g/kg of crude protein. Meanwhile, N-3011 is a meal product using hexane extraction of dehulled soybeans and further sizing of the toasted meal before processing into meal. Appropriate heat treatment of SBM can reduce or eliminate some ANFs (e.g., protease inhibitors, lectins and glycinin) to enhance bioavailability of plant proteins (Francis, Makkar, & Becker, 2001). Combined with above factors, it is confirmed that the N-3011 had better nutrient availability than the typical SBM. It may be a suitable protein source for replacing FM in shrimp diets.

In general, high level of FM replacement by SBM reduced the palatability of diet, which also in turn causes reduced growth performance (Hilton & Slinger, 1986). A significant increasing trend in feeding rate of shrimp in the group of D100 was observed in the present study. This could be explained by increasing feeding rate to obtain much energy due to the loss of dietary digestible energy with the increasing SBM level (Cheng et al., 2010; Kokou, Rigos, Henry, Kentouri, & Alexis, 2012). This result is different from some previous studies, which showed that the feeding rate significantly decreased with the increasing dietary SBM (Boonyaratpalin, Suraneiranat, & Tunpibal, 1998; Chen et al., 2011; Espe et al., 2006; Kaushik, Coves, Dutto, & Blanc, 2004; Pratoomyot, Bendiksen, Bell, & Tocher, 2010), but similar to those findings (Cheng et al., 2010; Gomes, Corraze, & Kaushik, 1993). The present study indicated that the appropriate inclusion of N-3011 in shrimp diet will not depress the diet palatability.

SBM product has been successfully utilized as a replacement for FM in practical diets for the Pacific white shrimp. Samocha et al. (2004) reported that coextruded soybean poultry by-product meal with egg supplement can be included up to 100% as a replacement for FM in the diet for Pacific white shrimp without causing negative effects on growth performance. In addition, Amaya et al. (2007) have proved that fish meal can be completely replaced using 39.6% SBM with 4.8% corn gluten meal in practical shrimp diet without compromising production and economic performance. Moreover, Alvarez et al. (2007) demonstrated that substituting 76.5 ± 2% soybean meal for fishmeal is feasible and can benefit the growth performance of juvenile white shrimp.

In this study, the better digestibility, high protein content and appropriate handling method may be in turn responded by good growth performance in high plant protein diet. The reduction of FM in the experimental diets progressively increasing replacement with N-3011 did not affect the survival of the shrimps in diet from D0 to D80. Nevertheless,

TABLE 8 Effects of replacement of dietary fishmeal by N-3011 on nonspecific immune parameters of shrimp

Diet	T-NOS activity (U/ml)	SOD activity (U/ml)	PO activity (O.D. 490 nm)	LZM activity (U/ml)	RB activity (O.D. 630 nm)
D0	18.55 ± 0.33^{ab}	173.83 ± 2.49^{a}	0.51 ± 0.02^{a}	0.060 ± 0.001^{a}	0.30 ± 0.02^{a}
D20	20.84 ± 0.17^{bc}	174.03 ± 5.17^{a}	0.67 ± 0.04^{b}	0.089 ± 0.003^{b}	0.47 ± 0.02^{ab}
D40	18.29 ± 0.39^{ab}	183.24 ± 2.02^{ab}	0.50 ± 0.02^{a}	0.051 ± 0.007^{a}	0.57 ± 0.02^{c}
D60	18.37 ± 0.44^{ab}	198.39 ± 3.08^{b}	0.52 ± 0.02^{a}	0.057 ± 0.004^{a}	0.52 ± 0.03^{ab}
D80	23.58 ± 1.32^{c}	189.68 ± 4.73^{ab}	0.63 ± 0.03^{ab}	0.067 ± 0.003^{ab}	0.54 ± 0.02^{ab}
D100	15.97 ± 0.46^{a}	191.74 ± 4.76^{ab}	0.57 ± 0.04^{ab}	0.062 ± 0.008^{ab}	0.45 ± 0.01^{b}
ANOVA					
F value	16.837	4.463	4.983	4.963	19.124
p value	<0.001	0.018	0.002	0.019	<0.001

Notes. Values are means ± *SE* of six replicates, and values within the same row with different letters are significantly different ($p < 0.05$).
LZM: lysozyme; PO: phenoloxidase; RB: respiratory burst; SOD: superoxide dismutase: T-NOS: total nitric oxide synthase.

TABLE 9 Effects of replacement of dietary fishmeal by N-3011 on the cumulative mortality of shrimp after challenge test (%)

Diet	The cumulative mortality (7th)
D0	75.55 ± 8.89
D20	77.78 ± 4.45
D40	77.78 ± 12.37
D60	84.44 ± 8.01
D80	75.56 ± 4.44
D100	84.45 ± 2.22
ANOVA	
F value	0.642
p value	0.733

Note. Values are means ± *SE* of three replicates, and values within the same line with different letters are significantly different ($p < 0.05$).

when substitution level was 100%, the survival was significantly lower compared to the diet of D0-D60. Meanwhile, shrimp fed with D100 also showed significantly lower final mean body weight and SGR than those fed with D0, D20 and D40. It is suggested that the content of N-3011 in practical diet for shrimp should not be higher than 80%.

4.2 | Immune response and disease resistance

Shrimps lack the specific immune mechanism and thus depend on a nonspecific immune mechanism to resist infections (Hertrampf & Mishra, 2006). Blood parameters are being increasing used as indicators of physiological condition of shrimp (Kader et al., 2012). To varying degrees, these indicators showed the upswing trend with the increasing substitution levels in this present study. Activity of T-NOS, SOD and respiratory burst showed the highest activity in Diets D80, D60 and D40, respectively. Both the Dietary inclusion of SBM containing some of immunologically active globular proteins, glycinin and β-conglycinin, could induce non-infectious subacute enteritis in the distal intestines (Baeverfjord & Krogdahl, 1996). Disruption of the mucosal integrity caused by the hypersensitivity reactions, inflammatory response and immune-stimulating effects resulted in those increasing indicators of nonspecific immune capacity (Burrells, Williams, Southgate, & Crampton, 1999; Sitjà-Bobadilla et al., 2005). Similar results reported in fish using soybean (Gabrielsen & Austreng, 1998; Krogdahl, Bakke-McKellep, Roed, & Baeverfjord, 2000; Rumsey, Siwicki, Anderson, & Bowser, 1994) also showed increased nonspecific immune responses.

Nevertheless, when substitution level was 100%, in the present study, the lowest T-NOS and RB activities were also observed. This result may indicate that an immunological tolerance had been induced. The shrimp could be immune-suppressed as were fed with excess of SBM, which is also consistent with the poor growth performance and low survival rate. In the same way, the depression of the humoural of cellular immune response by the inclusion of high plant protein at 300–400 g/kg has been reported in rohu *Labeo rohita* (Sharma, Saha, & Saha, 2014) and common carp *Cyprinus carpio* (Sharma et al., 2014; Suprayudi et al., 2015). Burrells et al. (1999) also reported that the high concentration of the dietary soybean protein in diet depressed the nonspecific immune capacity of rainbow trout.

Nutrition has long been considered as a key factor in host defence against pathogen (Kaushik et al., 1995). Bacterial, stress or virus challenge test was often used as a final indictor of aquatic animal health status after nutrition trial (Lim, Yildirim-Aksoy, Li, Welker, & Klesius, 2009). Fish antioxidative status is strongly related to immune system, contributing to enhance resistance towards different stressors. Although nonspecific immune response was suppressed in high concentration SBM dietary (D100), statistical analysis showed no significant differences in the cumulative mortality among all the treatments after the challenge test in the present study. It suggested that there is no negative effect of replacement of fishmeal by N-3011 on shrimp's health. In the same way, Krogdahl et al. (2000) suggested that systemic stimulation by SBM diet could provide the aquatic animal with some protection against disease. Siwicki, Anderson, and Rumsey (1994) reported that various plant protein immunostimulants in rainbow trout diet can elevate immune parameters and conferred improved resistance to *A. salmonicida* challenge. Dawood,

Koshio, Ishikawa, and Yokoyama (2015) also reported that amberjack fed a diet containing 30% of SBM showed higher tolerance in low-salinity stress challenge than other groups. Nevertheless, Krogdahl et al. (2000) reported that the systemic health of salmon fed SBM diet could have been compromised and weakened and this also led to high mortality rates in high SBM group.

5 | CONCLUSION

In conclusion, results indicated that selectively bred NMSBM could increase the nutrition values resulting in better biological performance for shrimp. The present study demonstrated that replacement of up to 80% of fishmeal protein by N-3011 did not have significant negative effects on shrimp based on the digestibility, growth performance, feed utilization, body compositions, immune response and disease resistance.

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