

## Effects of soybean and canola meals as dietary protein sources on the production performance and meat quality of shrimp

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### Abstract

Vegetable dietary protein sources are cheaper alternatives to fishmeal in shrimp diets. This study assessed the impact of fermented and non-fermented soybean and canola meals on growth performance, *in vivo* and *in vitro* digestibility, digestive enzyme activities and meat quality of *Litopenaeus vannamei*. A total of 513 shrimps were randomly assigned to treatments (control and two experimental diets). Animals on the control diet (CD) were fed a commercial feed concentrate. For the other treatments, the fishmeal protein source was totally replaced by a mixture of soybean and canola meal (50:50), either unfermented (sc) or fermented (fsc). Results showed that fermentation significantly increased ( $P < 0.05$ ) the hydrolysis degree (HD) of sc meals by 12 % compared to that of their unfermented counterparts. Also, it was found that canola meal had higher HD compared to soybean meal, both before and after fermentation. The use of sc diet decreased protein digestibility in shrimps ( $P < 0.05$ ), although there was no significant variation in the activity of total proteases and chymotrypsin. Production performance traits of shrimps fed diets with fsc were comparable to those of shrimps on the control diet. The increase in shear force of shrimps fed fsc was accompanied by an increase in the water holding capacity (WHC) by 8-9 % ( $P < 0.05$ ). Sensory attributes were similar across diets. In summary, this study showed it is possible to fully replace fish meal with fsc in shrimp diets without impairing production performance traits, dietary protein digestibility, and shrimp meat quality.

**Keywords:** soybean; canola; fermentation; shrimps; yield; quality.

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## Study contribution

Vegetable protein sources are cheaper alternatives to fishmeal for shrimp feeding. However, there is little information on the effects of these ingredients on production performance and shrimp quality traits. This study evaluates the technical feasibility of fully replacing fishmeal with soybean and canola meal (fermented or not) in shrimp feeding. The results demonstrated that fermented soybean and canola meal had no effect on animal production performance or shrimp meat quality attributes. Therefore, it represents an alternative to reduce the dependence on the costly and less sustainable fishmeal. The unfermented soybean and canola meal is not recommended for shrimp feeding as it increases mortality.

## Introduction

The global supply of seafood products has increased due to growing demand.<sup>(1)</sup> Therefore, aquaculture has a steady growth to meet the demand for human consumption.<sup>(2)</sup> Fishmeal is usually the main protein source in shrimp diets due to its high nutritional value.<sup>(3,4)</sup> However, its use represents environmental (overfishing), economic (variations in prices and availability of raw material) and quality (variations in nutritional value and adulteration) difficulties.<sup>(5-7)</sup>

The use of high-quality proteins in shrimp diets improves their digestibility, growth, and health, as well as shrimp quality.<sup>(8)</sup> Shrimp quality attributes are associated with the perception of flavor, aroma, color, and firmness by consumers. Among these, firmness is the most important attribute for consumers.<sup>(8-10)</sup> It may vary depending on factors such as the farming system,<sup>(11)</sup> post-harvest handling (type of storage, preparation method),<sup>(12)</sup> and feeding.

Brauer et al.<sup>(13)</sup> reported that muscle lipid and protein content changes when shrimp are fed different protein sources (sardine meal, squid meal or a commercial formula). Along the same lines, Maldonado<sup>(14)</sup> evaluated the effect of dietary vegetable flours (soybean, wheat, and potato flours) on firmness-related attributes of *L. vannamei*, such as shear force and collagen type. In that research, there was no variation in shear force across diets, as shown by the similar electrophoresis patterns of type v collagen extracted from shrimp muscle. Other studies have shown that fishmeal can be substituted by plant-based proteins in shrimp diets,<sup>(15)</sup> provided that palatability and digestibility and amino acid balance are adequate. In this regard, soybean and canola meals are good options to replace fishmeal due to their nutritional attributes (containing almost all essential amino acids) and low cost compared to fishmeal.<sup>(16-18)</sup>

Soybean and canola meals have been tested in the feeding of different productive species (trout, salmon, other teleost fish and crustaceans) with positive results.<sup>(10, 19, 20)</sup> Moreover, it has been observed that the fermentation of these vegetable meals improves their nutritional value, increasing protein bioavailability and degrading anti-nutritional factors, which are abundant in these ingredients.<sup>(15)</sup> However, there is little information on the effects of vegetable protein sources (fermented or not) on digestibility, production performance, and quality of shrimp. Therefore, this study evaluated the effect of dietary fermented and non-fermented soybean and canola meals on growth performance, *in vivo* and *in vitro* digestibility, digestive enzyme activities, and meat quality of *L. vannamei*.

**Table 1.** Formulation (g/100 g) of the experimental diets

	Soybean & canola (SC)	Fermented SC (FSC)
Soybean protein concentrate	27	27
sc meal (50:50)	46	–
fsc meal (50:50)	–	46
Fish silage	5	5
L-lysine	1	1
Taurine	0.16	0.16
Fish oil	8	8
Corn starch	5	5
Vitamin/mineral premix	3	3
Carboxi-metil-celulose	1.5	1.50
Celite	1	1
Filler	2.34	2.34
Total	100	100

## Materials and methods

### Ethical statement

This research project was reviewed and approved by the Institutional Committee for Experimental Animal Care and Use (SICUAÉ) of the graduate program on Animal Health and Production, Faculty of Veterinary Medicine, National Autonomous University of Mexico (approval number SICUAÉ.MC-2020/2-2).

### Study design

A randomized design experiment of 3 treatments (control and two experimental diets) with 3 replicates was conducted. A total of 513 shrimps were randomly assigned to each of the 9 tank groups (replicates) of the three diets (171 shrimp per treatment). Animals on the control diet were fed a commercial feed concentrate (Api-shrimp®, Malta Cleyton). For the other treatments, fishmeal was fully replaced by a mixture of soybean and canola meal (50:50), either unfermented (sc) or fermented (fsc). The formulation of the sc and fsc diets is presented in [Table 1](#).

### Shrimps

A total of 513 Pacific white shrimps (*L. vannamei*; mean weight  $7 \pm 0.25$  g) supplied by the Unidad Multidisciplinaria de Docencia e Investigación de Sisal, Mexico, were used. The animals were reared in 500 L tanks (57 shrimp each), using a recirculating aquaculture system (RAS) of seawater. The feeding period was 60 days. The daily ratio was 10% of the biomass of each pond, with a periodic adjustment (15 days) according to biometrics. Feeding was provided 3 times a day. During the feeding period, the water was maintained at  $28 \pm 1$  °C and had a salinity of  $35.0 \pm 0.5$  psu, and a dissolved oxygen of  $6.5 \pm 1.0$  mg/mL. The recirculated seawater was filtered through cartridges with pore diameters of 20 and 5 µm

and subjected to uv light treatment. Feces were collected from each tank during the feeding period to test *in vivo* digestibility.

At the end of the feeding trial (60 d), shrimp recovered from each tank were counted and weighed (Ohaus balance at 0.01 g) to determine growth traits and mortality rate. Subsequently, shrimps were slaughtered by heat shock, and their cephalothoraxes were manually removed. Next, 90 shrimps were randomly selected from each experimental diet. Of these, two-thirds ( $n = 60$ ) were used to determine quality attributes and the remaining 30 for sensory evaluation. To avoid bias associated with spoilage and endogenous enzyme activity, samples were frozen at  $-18\text{ }^{\circ}\text{C}$  until analysis (approximately one month). Before analysis, samples were thawed at  $2\text{--}4\text{ }^{\circ}\text{C}$  for 24 hours.

### Feedstuff and diet formulation

Commercial soybean and canola meals were milled to particle size  $< 500\text{ }\mu\text{m}$  and then fermented using a modified method of Yabaya et al.<sup>(21)</sup> soybean meal (2 kg), 60.5 mg of commercial dry yeast, *Saccharomyces cerevisiae*, with a cell density of  $3 \times 10^6$  cells/g (Fermipan®Magidely®Mexport) and 1.1 L of distilled water (50 % humidity) were homogenized and incubated at  $40\text{ }^{\circ}\text{C}$  for 48 h. The yeast-fermented soybean meal was then dried at  $70\text{ }^{\circ}\text{C}$  and reground ( $< 500\text{ }\mu\text{m}$ ). The same process was carried out for fermented canola meal. Fish silage was added to the formulation to maintain diet palatability. For that purpose, 10 kg of frozen fish heads cut into small pieces and ground to a homogeneous paste were used. Subsequently, 100 mL of hydrochloric acid (HCl) was added and homogenized manually ensuring good acid contact.<sup>(22)</sup>

All diets were isoproteic, with 36 % crude protein (CP). Essential fatty acids (20:5n-3 and 22:6n-3) were added via fish products (silage and fish oil) and carbohydrates were chosen for their digestibility (corn starch).<sup>(23)</sup> The stability of the cold extruded feed (pellet) was ensured by adding carboxymethyl cellulose (CMC) to the formulations. Celite was used as a marker, including for the digestibility test. Chemical analyses of the diets were performed for dry matter (AOAC 934.01), ash (AOAC 942.05), lipids (AOAC 920.39), protein (AOAC 984.13), crude fiber (AOAC 962.09), and nitrogen free extract NFE (Tables 2 and 3). Before starting the experiment, the shrimps were acclimatized to the dietary treatments for 10 d before we started collecting their feces. The control diet consisted of a concentrated commercial feed (Api-shrimp®, Malta Cleyton), with fish meal, krill and squid as the protein source.

### In vitro digestibility

The pH-Stat method, which measures the degree of hydrolysis (HD), was used to test the *in vitro* digestibility of the ingredients, as described by Adler-Nissen.<sup>(24)</sup> *L. vannamei* fed with commercial diet (36 % protein) were slaughtered and dissected to obtain midgut glands, which were frozen in liquid nitrogen ( $-40\text{ }^{\circ}\text{C}$ ) until use. The midgut glands were individually homogenized (IKA T18 basic, ultra-turrax), and centrifuged at 13 300 rpm for 30 min at  $4\text{ }^{\circ}\text{C}$ , to obtain an enzymatic extract for the hydrolysis reaction. The degree of hydrolysis was measured in triplicate for the four ingredients. Samples were sieved through a  $250\text{ }\mu\text{m}$  mesh and homogenized

**Table 2.** Chemical composition of the experimental diets

Variables (% dry matter)	Soybean & canola (SC)	Fermented SC (FSC)	Control diet (CD)
Moisture	2.8	3.2	3.6
Crude protein	36.9	37.02	36.7
Ether extract	8.4	8.7	6.4
Ash	15.1	13.1	7.2
Crude fiber	7.9	7.2	5.01
Nitrogen free extract	26.7	27.8	41.3

to 0.966 mg in 5 mL of distilled water and the hd was measured.<sup>(25)</sup> The hd for protein<sup>(26)</sup> used 0.1 N NaOH for 5 min at 28 °C to maintain pH at 8.0 and was determined through the following formula:

$$HD \% = (B \times Nb \times 1.5/M (S/100)/8) 100$$

Where: B = mL of 0.1 N NaOH used to control pH; Nb = normal NaOH with a calibration factor of 1.5 at pH 8 and 28 °C; M = g of mixture; S = protein concentration (%); 8 = total peptide bond content (meqv g<sup>-1</sup>) for casein.<sup>(24)</sup> Total protein concentration was measured in a 5 µL sample at 1/10 dilution, mixed with 125 µL of reagent, incubated 5 min at room temperature and read at 595 nm.<sup>(27)</sup>

### In vivo digestibility

Feces were collected with the aid of siphon samplers from each tank one hour after feeding. Feces were washed with distilled water and dried at 60 °C. *In vivo* digestibility was measured by the acid insoluble ash method,<sup>(28)</sup> with celite as a marker.<sup>(29)</sup> For nitrogen and ether extract determination, we used an elemental analyzer (Thermo Quest AS 2000 Falsea TM1112) operated with a 10-mg sample of ingredients and muscle. Digestibility calculations were carried out as in the previous case:

$$\begin{aligned} \text{Apparent digestibility coefficient of dry matter (DM)} &= \\ &100 \times (1 - (\% \text{celite in feed} / \% \text{celite in feces})) \\ \text{Apparent digestibility coefficient of crude protein (CP)} &= \\ &100 \times (1 - (\% \text{celite in feed} / \% \text{celite in feces}) / (\% \text{CP in feed} / \% \text{CP in feces})) \\ \text{Apparent digestibility coefficient of ether extract (EE)} &= \\ &100 \times (1 - (\% \text{celite in feed} / \% \text{celite in feces}) / (\% \text{EE in feed} / \% \text{EE in feces})) \end{aligned}$$

### Digestive enzyme activity

Midgut glands from intermolt stage C or D<sub>0</sub> shrimp were dissected and frozen in liquid nitrogen and stored at -40 °C until analysis. Homogenate was performed individually by adding 500 mL of distilled water (IKA T18 basic homogenizer, ultra-turrax), and then centrifuged at 12 000 rpm for 20 min at 4 °C. Midgut gland-soluble protein concentration was measured with a Bio-Rad-500-0006 kit and read in a spectrophotometer at 595 nm;<sup>(27)</sup> enzyme activities were expressed as specific

activity (U/mg protein). The total protease activity was measured following the method of Jiang et al.<sup>(30)</sup> Collagen impregnated with 2.4 % azo dye (Azocoll, Sigma A4341, USA) was used as substrate diluted in 10 mM sodium phosphate buffer, pH 7 at 25 °C, with a 1:10 ratio. The rate of hydrolysis was measured, as an increase in absorbance, using a spectrophotometer (Spectrophotometer Genesys 10s uv, Thermo scientific, USA) at 520 nm at the end point, U/mg protein. The determination of trypsin activity followed Geiger and Fritz<sup>(31)</sup> methodology, while that of chymotrypsin was performed as described by Geiger,<sup>(32)</sup> both with modifications for development on the microplate reader. To determine trypsin activity, 100-mM BAPNA (n-benzoyl-L-arginine-p-nitro-anilide; Sigma B7632) was used as a substrate in 0.1 M TRIS buffer, pH 8 at 40 °C. The rate of hydrolysis was measured, using a spectrophotometer (BioTek Synergy HT Microplate Reader) at 405 nm for 2 min, with an extinction coefficient the  $\epsilon_{405} = 1.02 \text{ L}(\text{mol}^{-1}) \text{ cm}^{-1}$ .<sup>(32)</sup> Chymotrypsin activity was measured by the same method, but with another substrate, succinyl-alanine-2-proline-phenyl-p-nitro-anilide (SAPPNA), and an extinction coefficient of  $\epsilon_{405} = 1.02 \text{ L}(\text{mol}^{-1}) \text{ cm}^{-1}$ .

### *Shear force (SF)*

Shear force was determined using the second abdominal segment of each shrimp. A sample of 24 shrimps from each treatment was frozen in liquid nitrogen (-40 °C) (to stop any further deterioration process by endogenous enzyme activity), until processing. The meat was weighed and placed in transverse position in a Brookfield ct3® texturometer, set at 8 g force, 9.9 mm deformation and 2.5 mm/s speed, with a craft knife (TA-CKA). The results were expressed in newtons (N) as the mean of three replicates.

### *Determination of instrumental color variables*

Shrimp were cooked in boiling water for 4 min and then immersed in cold water (4 °C) for 5 min before the shells were manually removed. Color measurements were performed on the cooked shrimp (three replicates per sample) using a Hunter-Lab® MiniScan EZ-4500L portable spectrophotometer (Hunter Associates Laboratory). The instrument had an aperture size of 25 mm and was adjusted with CIELAB color scale, D65 illuminant, observer at 10° and specular component excluded.

The spectrophotometer was calibrated before measurements and after one hour of operation or 100 readings (whichever came first). In this way, color coordinates ( $L^*$ ,  $a^*$  and  $b^*$  values, corresponding to lightness, redness and yellowness, respectively), as well as hue angle ( $h^*$ ) and saturation ( $C^*$ ) were obtained. We also calculated the total color differences ( $\Delta E_{ab} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$ ) between shrimp fed vegetable protein sources and those fed the control diet. This calculation allowed estimation of whether instrumental differences in color were visually detectable by consumers.

### Water holding capacity (WHC)

The WHC of shrimp was determined by centrifugation, following the method of Díaz.<sup>(33)</sup> For this purpose, two shrimp segments of approximately 10 g each were used as replicates. The shrimps were dried with disposable paper towels before weighing and recording the initial weight. It was then centrifuged at 16 000 rpm for 30 min at 4 °C. Subsequently, the supernatant was discarded, and the shrimps were removed from the centrifuge tubes with forceps and dried with disposable paper towels before recording their final weight. The WHC of each sample was calculated as the mean percentage of the initial weight retained, using the following equation:

$$\text{WHC} = \left(1 - \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}}\right) \times 100$$

### pH value

The pH of the shrimps was measured with the aid of a pH meter (Starter 3100, OHAUS®). Two replicates of 10 g per sample were homogenized in 20 mL of distilled water at 25 °C for about 30 s, using a commercial blender (4655 classica, Oster®). Subsequently, the probe of the pH meter was introduced into the sample until the pH value stabilized.

### Total volatile basic nitrogen (TVB-N)

TVB-N was determined, in 35 shrimps per treatment, as described by Botta et al.<sup>(34)</sup> with some modifications. Briefly, duplicates of 15-g shrimp each, shelled, were mixed with 45 mL of 5 % trichloroacetic acid. This mixture was introduced into a distillation unit (Büchi® K-350 unit) and distilled with a steam flow and 3 % boric acid. The distilled solution was collected and titrated with 0.1 N HCl to neutral. TVB-N was calculated using the following equation:

$$\text{TVB-N} \left( \frac{\text{mg}}{100\text{g}} \right) = \frac{\text{Volumen}_1 - \text{Volumen}_0 \times 0.14 \times 2 \times 100}{25}$$

### Sensory evaluation

We used a panel of 30 consumers to conduct the sensory evaluation of cooked shrimp. The shrimps were cooked as described above for color measurements. Two abdominal segments per treatment were served at the same time to the panelists. Samples were assigned random identification numbers before sensory evaluation. The panelists were asked to rate the aroma, flavor, color, texture, and overall acceptability of the shrimp samples. For this purpose, a seven-point hedonic scale was used, ranging from 1 (I dislike it very much) to 7 (I like it very much). Scores >5 (I like it slightly) were considered positive ratings.

### Statistical analysis

The degree of hydrolysis, ADC values, digestive enzyme activity, weight gain, survival data and quality attributes were analyzed by one-way analysis of variance (ANOVA), with the following model:  $Y_{ij} = \mu + T_i + \epsilon_j$ ; whereby:

$Y_{ij}$  is the response variable, which is any observation for each i-level of a factor (diet) and the replicate (j) within each i-level,

$\mu$  is the mean,

$T_i$  is the effect of treatment i-level (diet), and

$\epsilon_j$  is the random error

When significant differences were observed, means were discriminated using a Tukey HSD multiple range test. To confirm the homogeneity of variance and normality, we used the Levene's and the Shapiro-Wilk tests, respectively. For sensory evaluation, a Chi-Square test was performed to evaluate whether there was an association between dietary protein source and sensory attributes. All statistical analyses were performed using the STATISTICA software package (Dell Software). Differences were considered significant at  $P < 0.05$ .

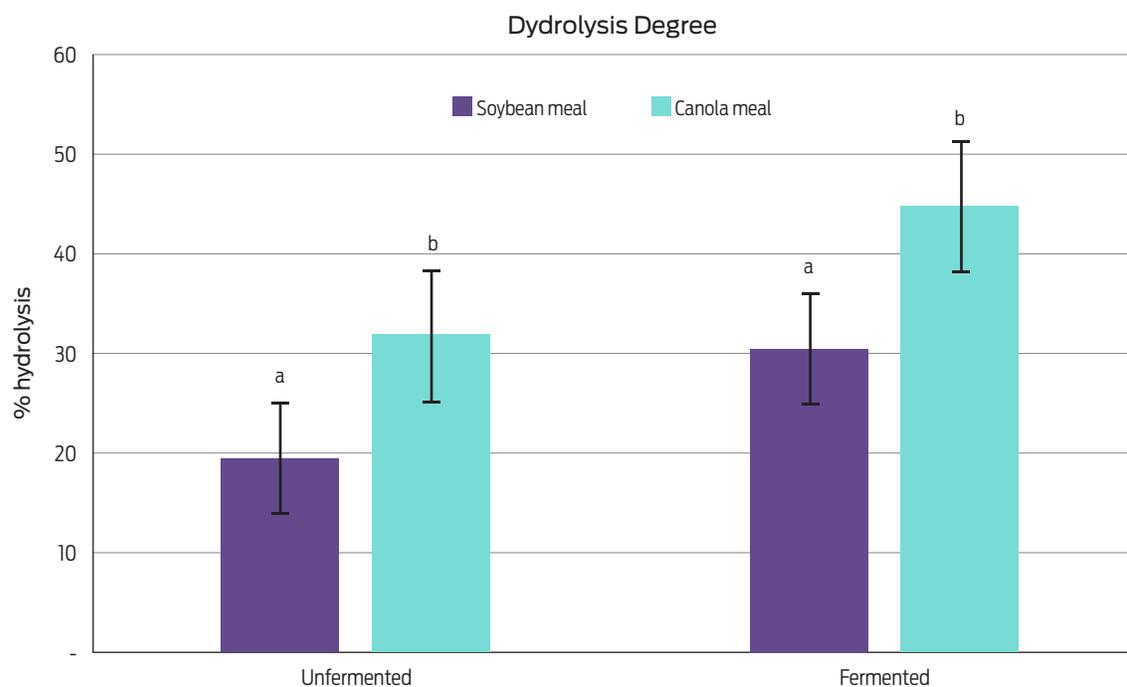
## Results and discussion

### In vivo and in vitro digestibility

The evaluation of *in vitro* and *in vivo* digestibility of feed is an ideal method for determining the nutritional quality of feedstuffs.<sup>(25)</sup> In this study, we observed that fermentation significantly increased ( $P < 0.05$ ) the HD of soybean and canola meals by 12 % compared to that of their unfermented counterparts (Figure 1). These findings agree with previous research documenting the positive impact of fermentation on shrimp feed digestibility.<sup>(25)</sup> This effect is attributed to the action of *S. cerevisiae* enzymes (i.e., phosphatases and phytases) that reduce antinutritional factors during the fermentation process,<sup>(19, 35)</sup> increases the content of free amino acids in the feed<sup>(36)</sup> and rises small peptides (< 15 kDa) through the cleavage of long-chain proteins, making protein hydrolysis more efficient.<sup>(19)</sup>

The study of ingredient digestibility is a relevant prerequisite for evaluating their potential in developing nutritious shrimp feeds.<sup>(37-39)</sup> We found that canola meal had higher *in vitro* HD compared to soybean meal, both before and after fermentation. Hence, it seems canola meal is more promising as a dietary protein source in shrimp feeding as compared to soybean meal, which is known for its high content of antinutritional factors and low digestibility.<sup>(14, 40, 41)</sup> However, the combination of soybean and canola meal is a good option, due to its content in sulfur amino acids (methionine, cysteine and taurine), which are determinants for the growth and survival of organisms.<sup>(29)</sup>

Apparently, the inclusion of vegetable protein sources did not affect the palatability of feed as there was no difference in apparent dry matter digestibility coefficients across treatments (Table 3;  $P = 0.2190$ ). Moreover, the apparent digestibility coefficient for ether extract was higher in shrimp fed with plant meals, unfermented or not, compared to the control diet. However, the apparent digestibility coefficient for crude protein of shrimps fed unfermented vegetable meals was lower as



**Figure 1.** Means and standard deviations of hydrolysis degree of dietary fermented and unfermented soybean and canola meals used in the experiment (n=36). <sup>a,b</sup> Means with different superscript in the same row are significantly different ( $P < 0.05$ ).

compared to that of shrimp fed either fsc or cd ( $P > 0.05$ ). These findings further confirmed the need to ferment plant meals to achieve digestibility coefficients comparable to those of fishmeal. The low apparent digestibility coefficient for crude protein of shrimps fed sc correlated with the low values for trypsin and chymotrypsin activities observed in shrimp fed this diet. Taken together, our results showed the use of non-fermented soybean and canola meals decreased dietary protein digestibility in shrimp, and thus, are not appropriate for shrimp feeding.

The activity of digestive enzymes is an indicator of the proper functioning of the shrimp digestive system, whose adequate activity favors the absorption of nutrients necessary for growth.<sup>(42)</sup> In this study, the activity of total proteases and chymotrypsin was similar across diets (Table 4). However, trypsin activity was higher in shrimps fed fsc compared to those on the unfermented and control diets. It is possible that this increased activity was related to a reduction of antinutritional factors (phytic acid and trypsin inhibiting factor) and to the prehydrolysis of the soybean and canola meals during fermentation.<sup>(43)</sup> Ayala-Borboa<sup>(44)</sup> mentions that the prehydrolysis of feed releases some metabolites, such as zinc and phosphorus, which are cofactors of trypsin and promote its proteolytic activity. Considering that *S. cerevisiae* has a complete enzyme package (with several types of lipases, proteases and carbohydrases),<sup>(16)</sup> it was expected that the fermentation of soybean and canola meals would favor the digestive process of the shrimp, increasing the apparent crude protein digestibility coefficients in the organisms fed the diets with fermented vegetable meals, equaling the coefficient of the control diet.

Summarizing results so far, we observed that the fermentation of the ingredients favored the physiological parameters (*in vivo* and *in vitro* digestibility and

**Table 3.** Means and standard deviations of apparent digestibility coefficients (ADC) of dry matter, crude protein, and ether extract in *L. vannamei* fed experimental diets (n = 30)

ADC (%)	Soybean & canola (SC)	Fermented SC (FSC)	Control diet (CD)	P-value
Dry matter	38.4 ± 4.2	40.2 ± 2.2	42.6 ± 2.2	0.2190
Protein	83.7 ± 6.2 <sup>a</sup>	93 ± 0.7 <sup>b</sup>	94.7 ± 0.4 <sup>b</sup>	< 0.0001
Ether extract	78 ± 5.8 <sup>b</sup>	71.2 ± 7.1 <sup>b</sup>	56 ± 7.9 <sup>a</sup>	< 0.0001

<sup>a,b</sup> Means with different superscripts in the same row are significantly different (P < 0.05).

**Table 4.** Means and standard deviations of total protease, trypsin and chymotrypsin enzyme activities of *L. vannamei* fed the experimental diets (n = 30)

Enzyme activity (mU/mg protein)	Soybean & Canola (SC)	Fermented SC (FSC)	Control diet (CD)	P-value
Total proteases	36.3 ± 5.4	33.0 ± 9.1	32.6 ± 4.5	0.6100
Trypsin	0.9 ± 1.2 <sup>a</sup>	3.0 ± 2.5 <sup>b</sup>	1.8 ± 1.5 <sup>a</sup>	0.0300
Chymotrypsin	16.4 ± 6.5	20.7 ± 9.7	15.9 ± 3.7	0.3000

<sup>a,b</sup> Means with different superscripts in the same row are significantly different (P < 0.05).

enzymatic activity) of the shrimp. Since the growth of these organisms is highly dependent on protein metabolism,<sup>(45)</sup> we would expect better production performance traits of the shrimp, as well as a better quality of the final product.

### Production performance

Production performance traits of shrimp fed diets with fermented soybean+canola meal as the major protein source was comparable to that of shrimp on a fish meal based commercial concentrate (Table 5). Conversely, unfermented sc caused a drastic increase in mortality. Overall, these findings are consistent with digestibility results and further confirmed that the fermentation of sc is a pre-requisite for its successful inclusion on shrimp diet as the major protein.<sup>(43, 46, 47)</sup> Hence, this study showed it is possible to fully replace fish meal with fermented soybean + canola meal without impairing feed consumption, dietary protein digestibility and animal production performance.

### Shrimp quality traits

The increase in shear force of shrimp fed fermented vegetable protein was accompanied by an increase in whc by 8-9 % (Table 6). This behavior was expected considering whc and shear force are positively correlated.<sup>(48, 49)</sup> Still, whc values observed here are within the range reported in *L. vannamei*.<sup>(33, 49)</sup> Dietary vegetable protein also increased the tvb-N content of shrimp (over 70 %) as compared to the commercial concentrate. Accumulation of tvb-N in shrimp is attributed to the presence of free amino acids in muscular tissue.<sup>(50)</sup> Perhaps, feeding vegetable proteins induced a higher concentration of free amino acids in shrimp muscle, favoring the formation of tvb-N.<sup>(29, 51)</sup> It is important to note, however, that tvb-N content was well

**Table 5.** Effect of dietary protein source on production performance (mean  $\pm$  standard deviation) of *L. vannamei* during the 60-d feeding trial

Performance traits	Experimental diets			
	Soybean & canola (SC)	Fermented SC (FSC)	Control diet (CD)	P-value
n	342	342	171	
Initial weight (g)	7.5 $\pm$ 0.3	7.3 $\pm$ 0.2	7.3 $\pm$ 0.3	0.42
Average weight gain (g/week)	1.1 $\pm$ 0.2	1.0 $\pm$ 0.1	0.9 $\pm$ 0.1	0.32
Final weight (g)	16.2 $\pm$ 1.3 <sup>ab</sup>	15.6 $\pm$ 0.4 <sup>ab</sup>	14.7 $\pm$ 0.4 <sup>a</sup>	0.02
Survival rate (%)	75.7 $\pm$ 1.4 <sup>b</sup>	87.1 $\pm$ 3.1 <sup>a</sup>	92.9 $\pm$ 3.5 <sup>a</sup>	0.03

<sup>a,b</sup> Means with different superscripts in the same row are significantly different ( $P < 0.05$ ). Average weight gain: (final weight– initial weight)/days. Survival rate: (final shrimp number– initial shrimp number)/100.

**Table 6.** Means  $\pm$  standard errors of shrimp quality attributes across experimental diets and total color differences ( $\Delta E_{ab}$ ) among treatments

Quality traits <sup>1</sup>	n	Experimental diets			
		Soybean & canola (SC)	Fermented SC (FSC)	Control diet (CD)	P-value
Shear force (N)	24	20.9 $\pm$ 1.9 <sup>ab</sup>	23.7 $\pm$ 1.0 <sup>b</sup>	16.2 $\pm$ 1.4 <sup>a</sup>	0.0077
WHC (%)	30	87.9 $\pm$ 2.2 <sup>b</sup>	88.8 $\pm$ 3.2 <sup>b</sup>	79.6 $\pm$ 1.8 <sup>a</sup>	0.0266
pH	30	7.4 $\pm$ 0.5	7.5 $\pm$ 0.1	7.5 $\pm$ 0.5	0.0565
TVB-N (mgN/100 g)	90	16.9 $\pm$ 0.3 <sup>a</sup>	16.7 $\pm$ 0.3 <sup>a</sup>	9.7 $\pm$ 0.4 <sup>b</sup>	<0.0001
L*	30	49.6 $\pm$ 1.6	49.6 $\pm$ 1.5	49.3 $\pm$ 1.6	0.8978
a*	30	26.7 $\pm$ 3.7 <sup>a</sup>	27.0 $\pm$ 1.3 <sup>a</sup>	30.7 $\pm$ 0.8 <sup>b</sup>	0.0078
b*	30	24.9 $\pm$ 0.5	25.0 $\pm$ 0.46	24.8 $\pm$ 0.3	0.3764
c*	30	36.6 $\pm$ 0.5 <sup>a</sup>	36.8 $\pm$ 0.4 <sup>a</sup>	39.4 $\pm$ 0.8 <sup>b</sup>	0.0067
h*	30	46.76 $\pm$ 0.67 <sup>a</sup>	47.12 $\pm$ 1.56 <sup>a</sup>	51.12 $\pm$ 0.40 <sup>b</sup>	0.0198
$\Delta E_{ab}$		SC vs CD	FSC vs CD	SC vs FSC	
		2.7	0.7	0.7	

<sup>1</sup> WHC: water holding capacity, TVB-N: total volatile basic nitrogen, L\*: lightness, a\*: redness, b\*: yellowness, c\*: Chroma, h\*: hue.  $\Delta E_{ab}$ : total color difference between treatments.

<sup>a,b</sup> Means with different superscripts in the same row are significantly different ( $P < 0.05$ ).

below permissible limits (30 mgN/100 g).<sup>(52)</sup> Hence, this disadvantage should not be relevant, considering shrimp is usually stored and distributed frozen, which inhibits autolytic and microbial enzyme activity on amino acids.<sup>(53-55)</sup>

Cooked shrimp color is another relevant attribute for consumers.<sup>(56)</sup> Usually, consumers associate the redness of cooked shrimp meat with higher quality.<sup>(57, 58)</sup> Dietary protein source caused some changes in chromatic coordinates (a\*), color appearance (h\*) and color saturation (c\*). In that sense, previous research has suggested that consumers tend to prefer cooked shrimp that is orange to intense orange,<sup>(59)</sup> an appearance that is associated with the range of h\* values observed here (close to 50). Therefore, the inclusion of the vegetable protein sources under study in shrimp diets did not cause color impairment in cooked shrimp. This hypothesis was further confirmed by the small total color difference ( $\Delta E_{ab}$ ) between

**Table 7.** Percentage of positive ratings<sup>1</sup> ( $\geq 5$ ) for aroma, flavor, color, texture, and overall acceptability of shrimp fed different protein sources

	Experimental diets			$\chi^2$	P-value
	Soybean & canola (SC)	Fermented SC (FSC)	Control diet (CD)		
n	30	30	30		
Aroma	80	76.6	63.3	2.1	0.34
Color	73.3	83.3	63.3	3.1	0.21
Flavor	57.1	60.7	58.6	0.1	0.95
Texture	66.6	76.6	53.3	3.6	0.16
Overall acceptability	68.9	74.2	62.06	0.7	0.70

<sup>1</sup> Ratings based on a 7-point hedonic scale (= 1 dislike it very much through 7 = I like it very much).

shrimp fed fsc and cd, as well as by the rather uniform proportion of positive ratings given by consumer panelists to shrimp fed any experimental diet (Table 7). The panelists did not detect differences in aroma between treatments. These findings agree with a report by Erickson et al.<sup>(12)</sup> showing a trained panel did not detect differences in the aroma of shrimp stored fresh (8 h on glassed ice), refrigerated (5 °C), or frozen (-18 °C). Furthermore, our study showed that the taste of shrimp fed with fermented plant meals and the control diet were similar (five on average). The flavor of shrimp is related its content of glutamate (or glutamic acid), which gives the characteristic flavor to seafood.<sup>(60)</sup> Therefore, we believe that determining the glutamate content of shrimp could helpful to complement the sensory evaluation.

## Conclusions

Taken together, results indicate it is possible to use fermented soybean + canola meal as the major protein source, in *L. vannamei* feeding, without compromising production performance, shrimp quality attributes or consumer acceptance. Hence, it represents an opportunity to reduce the dependence on costly animal ingredients, such as fish meal, for shrimp production. Despite quality attributes and consumer acceptance of shrimp fed unfermented soybean + canola meal was comparable to those of shrimp fed the commercial diet, its use as a major protein source is not recommended since it appears to impair productivity.

## Data availability

All relevant data of this research are within the paper. The datasets generated and/or analyzed during this study are available from the corresponding author on reasonable request.

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## Conflicts of interest

Authors María Salud Rubio-Lozano and Enrique Jesús Delgado-Suárez are members of the editorial board of Veterinaria México OA. Following the current journal's policies in this regard, they only participated as authors during the editorial process of this submission. Moreover, all authors agreed to publish the peer review process along with the article.

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