

# **Research** Article

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# Energy Partitioning by *Litopenaeus vannamei* (Boone, 1931) Given Plant Protein Diets

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### Abstract

The nutritive value of soybean meal (SBMd), feed pea meal (FPMd) and canola meal (CNAd) was evaluated for the shrimp L. vannamei. The apparent digestive coefficient (ADC) of dry matter (DM) varied from 80-86% for diets (p<0.05) and 64-87% for ingredients (p<0.05). ADC protein varied in a narrow range but still differed amongst diets (p<0.05); CNA provided the least ADC protein. ADC energy followed the same pattern as ADC protein but the range of variation was higher (87-92%) than with protein. All parameters derived from oxygen consumption were measured to determine an energy budget calculated on a 24 h basis, with animals weighing 6 g on average and given practical diets. SBMd and FPMd yielded recovered energy (RE) of 0.9 and 0.7kJ, respectively, in comparison to CNAd, which yielded a value as low as 0.4 kJ RE. The latter thus represents a poor diet alternative as compared to soy or pea meal diets in terms of energy use for growth. Substrates were provided to shrimp at a high level and trypsin was able to hydrolyze all protein sources similarly. The result of this process was protein with tertiary structures. Such structures could be attacked in peptide chain regions where radical -COOH is present and at Arg and Lys linkages. A priori, the results of hydrolysis would release the same amount of amino acid. Therefore, any difference in ADC will be derived from the presence of inhibitor or from excess nitrogen-free extract. This could explain the lower ADC protein value observed with CNA. Diets fed to shrimp led to an energy budget where a large part of the energy was derived from catabolism of amino acids. Such budgets typically reflect a better output from SBM and FPM as compared to CAN. Therefore, we conclude that ADC is a useful tool for evaluating vegetable-derived protein in the formulation of shrimp diets.

Keywords: L. vannamei; Plant proteins; Digestibility; Metabolism

# Introduction

World aquaculture shrimp production in 2004 was 6.1 mt [1], of which 2.5 mt (41%) was obtained through shrimp farming. Farmed shrimp are generally fed manufactured feeds that contain approximately 25% fish meal. Protein ingredients selected to substitute fish meals, either partially or completely, include terrestrial plant and animal byproducts that are readily available on world markets [2]. The quantity and quality of dietary protein are primary factors influencing shrimp growth, nitrogen loading of the culture system and feed costs. Considerable research has been conducted to evaluate the suitability of various vegetable-derived feed ingredients as alternative protein sources for fish meal [3]. Plant-based ingredients are tested with increasing frequency in formulated feeds for shrimp. Research has revealed the presence of anti-nutritional factors in soy bean meal (SBM), although these factors may be eliminated through heat treatment (extrusion), a process that also improves the digestibility of soybean protein [5]. Some studies have focused on the use of soybean meal for shrimp [6-8]. However, other plant sources such as peas, Pisum sativum [9,10], and canola low in glucosinolate and erucid acid [11-13] are also available and regarded as potential sources of protein for shrimp diets.

Measuring the apparent digestive coefficient (ADC) of nutrients in an ingredient or a feed provides a very practical estimate, though sometimes overestimated, of nutrient availability [14,15]. An advantage of this method over testing single ingredients is that test ingredients could be more acceptable to animals when supplied in combination with other ingredients and may lead to a normal level of feed intake [16]. Nutrient digestibility can be measured far more readily than nutrient availability. This parameter is widely reported in discussions [17] of the options for alternative compounded feeds. The digestibility of ingredients and diets has been described by many authors [3,16,18-21] and differences between diets may be explained by the amount of indigestible energy, poorly digestible ingredients in the formulation [2,3] and/or leaching [14,15]. Leaching prior to feces collection was observed with P. setiferus [19] and led to occasional overestimation of digestibility. Careful monitoring of the residence time of fecal material in water and collection of the feces within 30 m after ingestion limited the problem. Furthermore, the peritrophic membrane of the fecal strands is likely to prevent strand collapse and reduced nutrient loss, to some extent [22]. Loss of soluble nutrients from feces was described in fish by De la Noüe et al. [23]. Given the difficulties of undertaking field studies, it was found that an in vitro method allowed the collection of information on nutrient digestibility and the determination of the ADC of ingredients [21]. Results of a study undertaken by Forster [20] with L. vannamei yielded low statistical variability that confirmed the in vitro model as being acceptable for shrimp. There is also need to evaluate feed utilization by shrimp to predict waste outputs for different species; the use of a bioenergetics approach has been demonstrated to be effective in fish [24,25]. In contrast, crustacean species, in particular penaeid shrimp, provide only

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limited information [26,27]. In this study on *L. vannamei* juveniles, we evaluated the nutritive value of soy bean meal (SBM), feed pea meal (FPM) and canola (CNA) as candidate ingredients in practical diets. Energy partitioning by shrimp fed these protein sources was studied to develop feeds with minimal fishmeal content and waste products.

# Materials and Methods

At a local research facility, all shrimp were received from CENIA-CUA and derived from a process of selection reaching the  $F^{10}$  generation. We studied domesticated animals adapted to their environment and fed with commercial feed including 40% CP and ~23% carbohydrates.

The experimental tanks had the following dimensions: diameter, 1.5 m; height, 0.5 m; volume, 883 L. Effects of dietary treatments were analyzed with regard to feed intake, ADC dry matter, energy and protein.

# Diets and ingredients

The diets were formulated on an as-fed basis and are presented in Table 1. The diets were prepared by mixing dry ingredients for 10 min in a bowl, then liquid ingredients were poured and mixed for an additional 10 min. Mixed ingredients were then steam-pelleted at  $90 \pm 2^{\circ}$ C through a 2.5 x 3 mm die. Pellets were oven-dried at  $73^{\circ}$ C under constant air for 45 min, bagged, and stored in a cool room at 24°C. Two percent Celite<sup>®</sup>NF (acid-washed standard Super-Cel<sup>®</sup>NF, Celite Corporation, Lompoc, CA, USA) was included in the experimental diets as an inert indicator. Chemical contents of the experimental diets were obtained and are given in Table 2.

# Analytical procedure

Chemical analyses were carried out on ingredients, diets and juvenile shrimp carcasses. Protein levels were calculated from the determi-

	ref.diet	SBMd	FPMd	CNAd
fish meal <sup>a</sup>	33	23.1	23.1	23.1
squid meal	3.0	2.1	2.1	2.1
soybean meal		30		
pea feed meal <sup>e</sup>			30	
canola meal <sup>f</sup>				30
soybean meal	17	11.9	11.9	11.9
wheat gluten <sup>b</sup>	12	8.4	8.4	8.4
wheat flour	26.2	17.7	17.7	17.7
fish oil	4	2.8	2.8	2.8
soybean lecithin	2	1.4	1.4	1.4
cholesterol	0.5	0.35	0.35	0.35
vit. & min. premix <sup>c</sup>	0.25	0.25	0.25	0.25
celited	2	2	2	2

<sup>a</sup> Peruvian fishmeal; <sup>b</sup> Vital wheat gluten (Flour & Starch Union); Defatted soybean meal and solvent-extracted regular SBM, properly cooked and used in poultry feeds. Pea meal flour produced with selected varieties of yellow peas picked up in France from Sopropeche; the flour was obtained using a physical process combining de-hulling, micro-grinding and extrusion with low amounts of residual anti-trypsin factor (<600UI). Canola meal purchased from Canada. <sup>c</sup> Vitamin and mineral premix includes (IU/kg or g/kg or mg/kg of premix): Vit. A, 10000 IU/g; B<sub>1</sub>, 30 mg/kg; B<sub>2</sub>, 15 mg/kg; DL Ca pantothenate, 50 mg/kg; B<sub>6</sub>, 35 mg/kg; B<sub>12</sub>, 40 mcg/kg; Ascorbic, 150 mg/kg; K<sub>3</sub>, 3 mg/kg; D<sub>3</sub>,3500 IU/g; E, 150 IU/g ; niacin, 100 mg/kg; folic acid, 4 mg/kg; biotin, 1000 mcg/kg; Mn, 40 mg/kg; Zn, 40 mg/kg; Cu, 25 mg/kg; Fe, 100 mg/kg; Se, 0.3 mg/kg; I, 0.35 mg/kg. d Celite. Acid-washed standard super-cel ®NF. Celite corporation, Lompoc, CA, USA. <sup>e</sup> Pea feed meal (Sopropeche , Boulogne-Sur-Mer, Cedex, France) fCanola meal (Bunge Canada, Altona Manitoba, Canada).

Table 1: Diet composition (% as fed) of reference and tests SBMd, FPMd and CNAd.

	ref.d.	SBMd	FPMd	CNAd
protein (Nx6.25)	43	45	39	44
LYS	2.7	2.7	2.7	2.9
ARG	2.1	2.2	2.3	2.3
HIS	1.0	1.0	1.0	1.1
LEU	2.9	3.0	2.9	3.1
ILE	1.8	1.9	1.8	1.9
VAL	2.1	2.2	2.2	2.3
THR	1.5	1.6	1.6	1.7
MET	1.0	1.0	0.9	1.1
PHE	1.6	1.7	1.7	1.7
TRP	0.5	0.6	0.4	0.5
crude fat	10	8	7	9
carbohydrateª	25	29	38	26
DE (kJ/g DM)	18	19	18	16
crude fiber	3	1	1	3
moisture	8.4	7.5	6.8	7.8
ash	9	9	8	9
% DM loss⁵	10ª	12°	10 <sup>a</sup>	12 <sup>⊳</sup>

<sup>a</sup> carbohydrate (by difference).

<sup>b</sup> stability of the diets in dry matter (DM) after 1 h-immersion in seawater (Aquacop 1978). Values were means of four replicates. Means within columns with the same letter were not significantly different at p<0.05.</li>
 <sup>c</sup> Reference diet abbreviated as ref. d

Table 2: Analytical composition of experimental diets (% as fed).

nation of total nitrogen with a LECO auto-analyzer, and the calculation was based on N x 6.25 (A.O.C.S, No. Aa 5-91). Dietary crude fat content was determined gravimetrically following lipid extraction using the Soxhlet method (A.O.A.C, No. 920.39), and amino acid determination was performed with an automatic AA analyzer ([4,28], No.988.15). Feed and animal energy content were measured using an adiabatic bomb calorimeter. The procedures for analyzing moisture (A.O.A.C, No. 934.01), crude fiber (A.O.A.C, No. 962.09) and ash (A.O.A.C, No. 942.05) were followed accordingly. Feed collets (obtained by cold extrusion) were tested for water stability, determined after 1 h in seawater [29].

# In vivo digestibility experiment

The technique was previously described [22] and celite was the marker thoroughly mixed with other ingredients; practical feeds and feces were analyzed for marker using Acid-insoluble ash (AIA) content in diets and excreta was determined as in Atkinson et al. [30].

Water temperature was 26.7-30.4°C, salinity was 34-36 gl<sup>-1</sup>, and dissolved oxygen concentration was  $6.0 \pm 0.8$  mg l<sup>-1</sup>. Each tank was stocked with 33 animals ( $11.4 \pm 0.9$  g av. wt.). Treatments were randomly assigned to 16 tanks (500 L), with each treatment having four replicates. The shrimp were allowed to acclimatize to the dietary treatment for 10 days before fecal collection commenced. Shrimp were fed three times daily at 08:00, 12:00, and 21:00 h. Two hours after feeding, the rearing tanks were cleaned out to remove uneaten feed and fecal residues. Fecal matter was siphoned from each tank two times a day (10:00 and 14:00 h), and samples were gently rinsed with distilled water to eliminate excess salts. Daily fecal samples from each tank were pooled together before analysis.

ADCs in experimental diets were calculated according to the following formulae:

 $ADC_{DM}$  of diet (%) = 100x[1-(% marker in diet/% marker in feces)] (1)

The ratios in feces and feed based on respective marker concentrations provided an estimate for  $ADC_{DM}$ .

ADC<sub>nutrient</sub> of diet (%) = 100x[1-(% marker in diet/ % marker in feces)]x(nutrient concentration in feces/nutrient conc. in diet)] (2)

 $ADC_{energy}$  of diet (%) = 100x[1-(% marker in diet/% marker in feces)]x(energy concentration in feces/energy conc. in diet)] (3)

ADCs for ingredients were obtained with a combination of a reference diet and test diet; the composition of each is given in table 1. To calculate the ADC of ingredients, the following formula was used according to Forster [20]:

$$ADC_{ingredient} = [(70\%*Nutr_{basal}+Nutr_{ingredient}*30\%)*AD_{test}-(70\%*Nutr_{basal}*AD_{basal})]/Nutr_{ingredient}*30\%$$
(4)

The reference diet substitution method has been applied for other species such as abalone and described by Sales & Britz [16].

## Respirometry trial and energy budget

Shrimp with  $4.4\pm0.4$  g average weight (av. wt) were allowed to acclimatize to the dietary treatment for 30 days prior to the beginning of the experiment. The technique described earlier [27] was applied to *L. vannamei*. Twelve shrimp per treatment were randomly taken (5.8±0.4 g av. wt.) for respirometry trials and energy budget calculations.

Water temperature was constant at 28°C, salinity was 34–36 g l<sup>-1</sup> and dissolved oxygen concentration was 6.0±0.8 mg l<sup>-1</sup>. The information from the feeding trial was used to build an energy budget following the established nomenclature [31] and was based on energy partitioning, taking into account the following steps: intake (IE), digestible energy (DE), metabolizable energy (ME), urinary and branchial excretion (UE+ZE) and basal metabolism (HeE). The feed administered and the remaining feed was determined in each metabolic chamber. The values of the digestible energy were obtained based on the daily consumption of feed/shrimp. Two chambers without animals were used as controls for each ten chambers stocked with shrimp. A metabolic chamber with continuous flow rate [32] allowed us to measure oxygen consumption individually for 12 shrimp (5.8±0.4 g) on each diet. Dissolved oxygen at the entrance and exit of respirometric chambers was measured using an oxygen micro-electrode (OXY 315i) and calculated according to:

 $O_2 \text{ mg mn}^{-1} = [O_2 \text{ tank (mg } L^{-1})-O_2 \text{ control (mg } L^{-1})]^*\text{flow rate (L mn}^{-1}).$ 

Oxygen consumption transformed with the oxycalorific coefficient 13.6kJ g<sup>-1</sup>O<sub>2</sub> [24] yielded a value for heat production (kJ). Urinary and branchial excretion (UE+ZE) was calculated from the following equation: UE+ZE=0.08\*(RE+HeE+HiE+HxE) from Bureau et al. [33], without taking into account the last item for molting (HxE) due to the short duration of the study.

## Statistical methods

All analyses were performed with the Statistical Analysis Software Program [34]. Apparent digestive coefficients were subjected to an analysis of variance, and Duncan's multiple range test provided the ranking.

# Results

The reference diet was readily digested by juveniles. The fishmeal content was replaced by plant protein sources in the tested diets without significantly affecting the results; ADC for dry matter, protein and energy of the diets examined is presented in Table 3. Diets differed with regard to dry matter, with the lowest value observed in CNAd (80%). CNAd also showed the lowest  $ADC_{protein}$  (94%). Despite a narrow range of variation,  $ADC_{protein}$  differed amongst diets (p<0.05).  $ADC_{energy}$  followed the same pattern as  $ADC_{protein}$  but the range of variation (87-92%) was higher than with protein.

ADC dry matter, protein and energy for the ingredients are presented in Table 4. The canola meal showed the lowest  $ADC_{DM}$  (64%),  $ADC_{protein}$  (89%), and  $ADC_{energy}$  (75%) amongst all ingredients (p<0.05). There was no change between SBM and FPM in  $ADC_{DM}$ ,  $ADC_{protein}$  and  $ADC_{energy}$  (p>0.05). Final calculated values for DE were obtained with the coefficient previously determined by Cuzon & Guillaume [35], and fitted with experimental data from the relevant test diet.

The amino acid profiles for the reference diet and the three test diets were similar (Table 2).

Animals (3 – 5 g) fed experimental diets on a weekly basis were used to obtain the energy budget, the results of which are given in Table 5. SBMd and FPMd provided higher DE values (1.56 and 1.43kJ shrimp<sup>-1</sup> day<sup>-1</sup>, respectively) than CNAd (1.04 kJ shrimp-1day<sup>-1</sup>). Energy used for maintenance metabolism (HeE) did not change at the dietary protein levels used for this experiment. The estimated value in kJ shrimp<sup>-1</sup>day<sup>-1</sup> from UE+ZE for CNAd was only 0.06 while values for HiE (0.3) and HeE (0.25-0.32) were very similar for SBMd, FPMd and CNAd. Consequently, practical diets including canola meal did not provide sufficient energy (0.4 kJ shrimp-1day-1) for growth as compared to soya or feed pea and produced RE in the range of 0.7–0.9. RE was similar for both soy and pea meal diets, suggesting that *L. vannamei* can utilize dietary energy with the same efficiency from these sources, contrary to observations made with diets produced using CNA.

### Discussion

Among dietary ingredients, soy bean meal is one of the most stu-

Diets	ref. diet	SBM	FPM	CNA
ADC	86.2 ± 2.5a	85 ± 1.2ab	86 ± 1.5a	80 ± 2.3c
ADC	$95.5 \pm 0.9^{a}$	$95.8 \pm 0.7^{a}$	95.8 ± 0.5a	94.2 ± 1.0b
ADC	92.4 ± 1.7a	91.4 ± 1.0a	92.8 ± 0.7a	87.5 ± 1.8b

Values are means of four replicates $\pm$ S.D. Means within columns with the same letter are not significantly different at P<0.05

Table 3: Shrimp diet's ADC (±S.D.) for dry matter, protein and energy.

Ingredient	SBM	FPM	CNA
ADC	80.0ÆÁ.8a	87.0Á±Á5.0a	64.0Á±Á8.2b
ADC	96.7ÆÁ.7a	97.9Á±Á3.7a	89.4Á±Á4.8b
ADC	88.9ÆÁ.4a	93.9Á±Á2.6a	75.5Á±Á6.4b

Values are means of four replicates  $\pm$  S.D. Means within columns with the same letter are not significantly different at P<0.05.

Table 4: Ingredient ADC (±S.D.) for dry matter, protein and energy.

	DE <sup>(1)</sup>	UE+ZE <sup>(2)</sup>	HiE <sup>(3)</sup>	HeE <sup>(4)</sup>	RE <sup>(5)</sup>	$\begin{array}{l} \text{HiE mg O}_2 \\ \text{g}^{-1}\text{h}^{-1} \end{array}$	HeE mg O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup>
SBM	1.6	0.13	0.3	0.25	0.9	1.62	0.15
FPM	1.4	0.11	0.3	0.32	0.7	1.27	0.20
CNA	1.0	0.06	0.3	0.30	0.4	1.47	0.18

<sup>(1)</sup>DE: digestible energy values from ADC experiment.

<sup>(2)</sup>UE+ZE: urinary and gill excretion (Bureau et al., 2000).

<sup>(3)</sup>HiE: heat increment of feeding on the basis of one meal per day.

 $^{\rm (4)}{\rm HeE}:$  maintenance based on value measured at  $t_{\rm o}.$ 

<sup>(5)</sup>RE: recovered energy.

 Table 5: Impact of feed composition on energy budget (kJ shrimp<sup>-1</sup>day<sup>-1</sup>) for individual animals placed in a metabolic chamber for 24 h.

died in the context of shrimp nutrition and feeding [7,18,36,37]. To determine suitability of alternate dietary ingredients, particularly from vegetable sources such as pea meal, sorghum and canola meal, the determination of nutrient digestibility is important priority. Nutrient digestibility controls energy partitioning by the animal; furthermore, protein is important for growth and energy [38]. In the present study,  $\mathrm{ADC}_{_{\mathrm{DM}}}$  with de-hulled, micro-ground, extruded P. sativum meal (FPM) reached 87% (p<0.05) and indicated the level of carbohydrate digestion.

FPM-fed tiger shrimp showed an 80% ADCDM that is comparable to values reported by Smith et al. [36] and in P. monodon [9]. With 73% ADC<sub>DM</sub> for SBM at two substitution levels, L. vannamei displayed values that varied from 61-85% [7]. These measurements contrasted with the values generally obtained at 30% inclusion; CNA yielded the lowest value, 64% (p<0.05). ADC $_{\rm energy}$  in this trial reached 88.9% with SBM at an inclusion level of 30%. Thus, SBM values were within the range previously observed for L. vannamei (79-100%), even though shrimp were fed at two levels of fishmeal substitution [7]. At this level of inclusion, protein digestibility of ingredients was found to be high, around 90% in all trials. ADC  $_{\rm protein}$  of an ingredient or feed provides a practical value that sometimes could be considered as overestimated from the viewpoint of nutrient availability [15]. P. stylirostris extruded feces that leached an amount of protein and carbohydrates estimated at 4 and 8%, respectively after 6 h immersion [39]. However, high ADC<sub>protein</sub> values in the present study were in accordance with those reported for SBM [18,19]. The effect of protein level on ADC needs to be investigated further for possible associative effects as described for quality protein sources such as SBM. SBM has been the focus of studies due to its digestibility [18] and amino acid profile in shrimp muscle; these factors make it an ideal candidate for feed formulation. High ADC<sub>protein</sub> values observed for CNA [13] and FPM [36] were similar to those observed here, with values falling between 91-94%.

In the evaluation of diets including an ingredient supplemented at levels of 30%, feed intake is a first concern. However, the formulation in the present study did not produce any real effect on ingestion, and the experimental diets formulated with plant-based ingredients showed no decrease in digestibility as compared to the reference diet, indicating that the vegetable components replacing fish meal in this study did not affect the digestive process of shrimp, as has been found in previous studies [37]. Energy content differed between SBMd and FPMd as compared to CNAd; this may be due to the higher fiber level in CNAd, unidentified anti-nutritional factors, or the way ingredients were prepared. It is interesting to note that the  $ADC_{protein}$  level differed significantly from one plant source to another; it will be important to determine the reasons for these variations. The main factors influencing the digestibility of proteins may be related to factors linked to the protein itself, including its quaternary or tertiary structure and the possible aggregation of H-linked sub-units as occurs in the case of keratin, with disulfide bridges that may affect protein solubility. A secondary structure with disulfide linkages could also have an effect, especially in the case of keratin. There is documented impact of heat denaturing on animal proteins such as blood meals; this phenomenon may be expected to have only moderate impact on plant proteins fed to rats [40]. Plant proteins such as SBM are less readily hydrolyzed as compared to their native form, which is more compact than the denatured protein. Finally the primary structure may have an effect due to the distribution of peptide linkages near the NH<sub>3</sub> or COOH terminal and to the frequency of amino acids such as diacids, or alternately to the frequency of basic amino acids (Lys and Arg), which are targets for proteases. Among extrinsic factors, we noted the presence of inhibitors such as anti-trypsins or tannins and phytic acid. Anti-nutritional factors were substantially reduced in plant concentrates or eliminated from meals by adequate selection programs [41]. In the case of concentrates, an apparent benefit for plant digestibility could be linked with technological improvement during processing (Maldonado pers.com.). The accessibility of proteins to enzymes will depend on cellulose membranes and the fineness of particles. There are other factors modifying transit such as raw starch, lipids or pharmacodynamic action. In addition to factor acting on digestion, other factors may modify absorption, such as the equilibrium between amino acids and the competition for transport systems, although these are likely to be of minor importance [42]. Some researchers have hypothesized the existence of a relationship between biological value (BV= proteic accretion/absorbed protein) and digestibility, but in practice, this is not found because BV is not additive. In summary, the digestibility of proteins will depend on many physical and nutritional factors that can vary in intensity from one ingredient to another as displayed in Table 6.

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Diets evaluated for digestibility were used to establish an energy budget and trace the energy derived from both protein and energy fractions (carbohydrate and lipid) in each diet. The estimated value of excretion with CNAd was only about 0.06kJ shrimp<sup>-1</sup>day<sup>-1</sup>. Heat increment (HiE) values and maintenance (HeE) with SBMd, FPMd and CNAd were similar. HeE did not change in the range of dietary protein levels selected for this experiment but HiE in juvenile L. stylirostris changed regardless of whether marine protein (0.14kJ) or plant sources (0.3kJ) were used [27]. Plant protein produced higher energy expenditure in P. japonicus [43]. This population did not respond similarly when fed soy protein concentrate; heat production as percentage of gross energy was high when compared to shrimp ingesting marine protein sources. Shrimp use energy for activity; this is one of the most difficult parameters to control in this type of study. When comparing two concentrates such as crab and soya, it could be expected that HiE should be reduced in crab, which is theoretically a better protein source. Among other bioenergetic parameters affecting the energy budget, recovered energy (RE) differed between treatments. In general, RE remained higher for shrimp receiving animal protein than for shrimp ingesting plant protein, as shown in P. japonicus with 20 and 9% for crab protein and soy protein, respectively [43]. These findings are in line with results from P. stylirostris [27] and L. vannamei (in the present study). RE gives an estimate of the energy channeled to weight gain, and while no data on growth are presented here, the trend indicates an equal potential for growth for each of the four diets. Ratios reported for reported digestible protein (DP) show an average DP/DE equal to 23 mg kJ-1 (22-25), coinciding with previous data [44]. Values above or below this would produce weight gain under optimum laboratory conditions with animals raised in clear seawater. Energy budgets have been reported [45,46] for penaeid larvae. These energy budgets can be limited in the presence of low reserves, which in turn necessitate frequent feeding that is rare among juveniles. Notably, reserves (lipid and glycogen) were not taken into account in the course of respirometry trials, contrasting with the approach of dynamic energy budget (DEB) analysis. According to this methodology, data are used to create a dynamic repartition of energy for growth or reproduction and to engineer a predictive model [47].

fineness (µ)	800	250	80
water capacity (%)	+++	50-400	++
cellulose (%)	3.4	1.4	13
tannins			++
trypsin inhibitor (mg/g)	<4		
trypsin inhibitor (UI)		600	
accessibility to proteases	+++	+++	++

Table 6: Impact of physical and biological factors on ADC of ingredients.

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Currently, SBM is one of the main alternatives to the use of fishmeal in shrimp diets, along with meals from sources such as lupin, sorghum, pea, canola, cotton and sunflower. In addition, there are a number of potential protein concentrates that may also be used including soya, wheat, and potato. Thus, there are additional options available for achieving feed formulations without fishmeal that have no negative impact on shrimp texture, nutrition or flavor.

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