Soybean meal apparent digestibility for *Litopenaeus vannamei*, including a critique of methodology[1]

S. Divakaran[1], Mario Velasco[2], Eric Beyer[1], Ian Forster[1], Albert G. J. Tacon[1]

[1]Oceanic Institute 41-202 Kalanianaole Hwy. Waimanalo, Hawaii 96795. USA
Telephone: +1-808-259-3122, Fasimile: +1-808-259-5971. iforster@oceanicinstitute.org
[2]Empacadora Nacional C.A. (ENACA), Guayaquil, Ecuador

ABSTRACT: Most trials conducted to examine the apparent digestibility coefficients of nutrients in an ingredient or a diet use arbitrary levels of inclusion of the ingredients and indigestible marker. The suitability of the levels typically used has not been critically examined, however, and it is not known whether inclusion levels affect the results obtained. This trial was undertaken to ascertain the apparent digestibility coefficients for dry matter, crude protein, and gross energy of dehulled, solvent extracted, toasted soybean meal (SBM) by combining a control diet (containing 23.7% SBM) with SBM at two levels (15 and 30 %; diets contained 23.7, 34.9 and 46,3% SBM) and two levels of chromic oxide (0.5 and 1.0 %) in the Pacific white shrimp *Litopenaeus vannamei*. Four replicate groups of shrimp were fed by hand three times daily and the feces were collected by siphoning from the tank onto a fine mesh screen. The first fecal strands were discarded to reduce the effects of coprophagy and intake of material other than feed. To minimize leaching of nutrients, fresh fecal strands were collected as soon as they were observed. The apparent digestibility coefficients (ADC) for dry matter, crude protein, and gross energy were calculated for the diets and these data were used to derive the apparent digestibility coefficients for the SBM at the two levels of substitution.
The dietary inclusion levels of the chromic oxide and SBM acted together to significantly ($p<0.05$) affect the ADCs obtained for SBM. Over all the treatments, the calculated ADC for SBM dry matter varied from 61.2 to 84.7%; for crude protein from 89.5 to 102.2%; and, for energy from 78.7 to 100.1%. The ADCs were generally higher among the diets that contained chromic oxide at 1.0 % than at 0.5 %, but the magnitude of the difference was different for the two levels of SBM substitution.

KEY WORDS: Soybean meal, shrimp, digestibility, amino acids, chromic oxide

INTRODUCTION

In recent years there has been increasing interest to identify and develop alternate protein sources for use within aquafeeds (Tacon *et al.* 1998). Originally, the impetus for this has been the often variable nature of the price and supply of fish meal of suitably high quality for the aquafeed industry. Recently, however, the concern raised about the negative impact of fish meal production on global fish stocks has heightened this interest (Naylor *et al.* 2000). Among the ingredients that are being investigated as alternatives to fish meal, soybean meal (SBM) is one of the most promising (Lim *et al.* 1998; Hardy, 1999) because of its security of supply, price and protein/amino acid composition. There have been a number of attempts to demonstrate the usefulness of this ingredient in aquafeeds, and these have been successful to a degree, so that this ingredient is routinely added to commercial formulations for many species. In the case of Pacific white shrimp, *Litopenaeus vannamei*, earlier work has demonstrated the suitability of SBM as an ingredient in feeds for this species. For example, Lim and Dominy (1990; 1992) found that SBM (both solvent-extracted and full-fat extruded SBM) could effectively replace up to 42% of fish meal protein in practical feeds for *L. vannamei*.

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There are several aspects that determine the nutritional quality of an ingredient as a nutrient source, specifically: its acceptability/palatability, level of the nutrient, presence of anti-nutritional components, physical suitability for inclusion into feeds, and availability of the nutrient (i.e., its digestibility). Studies conducted to ascertain the nutrients digestibility coefficients are most commonly conducted by feeding a diet containing fixed levels of a nutrient source and an indigestible marker, typically chromic oxide, to an animal and comparing the level of the nutrient and marker in the resulting feces (Cho and Slinger, 1979). It is assumed in this kind of experiment, that the digestibility of the nutrient is not affected by the inclusion level of the ingredient in the diet. It is also assumed that the level of chromic oxide has no effect on the apparent digestibility coefficients obtained (Shiau and Liang, 1995). This study examines the apparent digestibility of dehulled, defattted, toasted soybean meal by Pacific white shrimp at different levels of inclusion of soybean meal and chromic oxide.

MATERIALS AND METHODS

Experimental setup and conditions

Six circular flat-bottomed tanks (dia. 1.5 m x ht. 0.75 m) were used to hold the experimental animals in this trial. Each tank was partitioned by a plastic sheet into four equal units of 0.42 m^2 each (0.40 m water depth, 180 l volume). There was no water flow between the partitions, and each partition was considered to be an experimental unit (EU) (total of 24 EU). Water was obtained from a saltwater well and was filtered before being supplied to each EU at the rate of 90 l/h. During the course of the experiment, water temperature ranged from 25.5 to 26.5 °C, salinity from 32 to 34 ‰ and pH from 7.3 to 7.5, respectively, while dissolved oxygen concentration was above 5.0 mg/l, and total ammonia nitrogen was below 0.01 mg/l. Photoperiod was provided by natural lighting.

Animals

Shrimp, *L. vannamei*, were obtained from the Oceanic Institute stock shrimp. Initial stocking density was 15 shrimp/EU (33 shrimp/m^2). Initial shrimp body weight was 8.0-10.0 g/animal. Prior to the trial, the shrimp were maintained on a commercial feed.

Diets

Six dry, pelleted diets were formulated to contain dehulled, solvent extracted, toasted soybean meal (SBM) (IFN5-04-612) at three levels (23.7, 34.9 and 46.3%), and chromic oxide (an inert marker) at two levels (0.5 and 1.0%). The 23.7% SBM diets were the reference control diets, and the 34.9 and 46.3% SBM diets were formulated by substitution of 0, 15, and 30 % of SBM, respectively, to the entire control diet. The diets were prepared (Table 1) by mixing dry ingredients for 15 min (Model S-1, Davis Mixer Co., Bonner Springs, Kansas). Fluid ingredients were then added and mixed for an additional 30 min. Mixed ingredients were then steam-pelleted with a pellet mill (Model CL-5, California Pellet Mill Co., San Francisco, California) at a temperature of 90 ± 5°C through a 2.4 x 38.1 mm die. Pellets were oven-dried (convection oven, Despatch Oven Co., Minneapolis, Minnesota) at 95°C under constant wind draft for 8 min, bagged, and stored in a cool room until used.

Table 1. Ingredient composition of diets used to examine apparent digestibility coefficients of soybean meal by shrimp.

Ingredient	Diet (g/100 g as-fed basis)					
	Referenc e 1% Cr[1]	15% SBM[2] 1% Cr	30% SBM[2] 1% Cr	Reference 0.5% Cr	15% SBM[2] 0.5% Cr	30% SBM[2] 0.5%Cr
Wheat flour, Kilauea[3]	31.00	26.35	21.70	31.50	26.78	22.06
Menhaden fish meal[4]	30.00	25.50	21.00	30.00	25.50	21.00
Soybean meal (SBM)[5]	23.69	34.99	46.29	23.69	35.06	46.43
Squid meal[6]	4.00	3.40	2.80	4.00	3.40	2.80
Menhaden fish solubles[4]	4.00	3.40	2.80	4.00	3.40	2.80
Menhaden fish oil[4]	2.50	2.12	1.75	2.50	2.12	1.75
Sodium phosphate dibasic[7]	2.00	1.70	1.40	2.00	1.70	1.40
Lecithin[8]	1.00	0.85	0.70	1.00	0.85	0.70
Mineral mixture[9]	0.33	0.28	0.23	0.33	0.28	0.23
Vitamin mixture[9]	0.33	0.28	0.23	0.33	0.28	0.23
Vitamin C[10]	0.15	0.13	0.10	0.15	0.13	0.10
Chromic oxide[11]	1.00	1.00	1.00	0.50	0.50	0.50

[1] Chromic oxide levels in diets
[2] Percent substitution of soybean meal into reference diet
[3] Hawaiian Flour Mills, Honolulu, Hawaii
[4] Zapata Protein Inc., Reedville, Virginia
[5] Land O'Lakes Inc., Seattle, Washington, Trypsin inhibitors 20.0 g/kg SBM.
[6] Fukui & Co., Ltd., Tokyo, Japan
[7] I.C.N. Biomedicals Inc., Aurora, Ohio, USA
[8] National Lecithin Inc., Carlstad, New Jersey, USA
[9]Thomas Products Inc., Madera, California, USA. Mineral mixture composition (g/kg diet): cobalt carbonate (8.7), copper sulfate (39.7), ethylenediamine dihydroiodide (2.5), iron sulfate (384.1), manganous oxide (13.0), mineral oil (10.0), silicon dioxide (460.9), sodium selenite (20.0), zinc oxide (61.1).
Vitamin mixture composition (g/kg): Vit. A acetate (6.99), cholecalciferol (4.55), Vit. E 50% adsorbate (242.43), menadione sodium bisulfate (18.36), riboflavin (37.88), calcium d-pantothenate (32.94), choline chloride on silica (279.28), niacin (91.37), cyanocobalamine (3.03), folic acid (6.38), thiamine mononitrate (49.43), pyridoxine hydrochloride (18.40), biotin (30.30), inositol (90.91), wheat flour (77.74), mineral oil (10.00)
[10] L-Ascorbyl-2-Polyphosphate (25% active). Hoffman-LaRoche Inc., Nutley, New Jersey, USA
[11] Aldrich Chemical Company Inc., Milwaukee, Wisconsin, USA

Feeding protocol

Each of the six diets was assigned to one tank with each partition (EU) as a replicate. A 10-day period of adjustment to the new conditions and diets was implemented before the start of fecal collection. All groups were fed by hand to slight excess three times daily (08:15, 09:30 and 13:15 hr), seven days per week. Before each feeding, EUs were cleaned by siphoning out uneaten diet, feces, molts, and dead shrimp.

Sampling protocol

Continuous feces collection was begun within 2 h after the second and third feeding. Feces were removed by siphon and separated from the remaining feed by a mesh screen. Fecal samples were rinsed with distilled water, pooled and homogenized from each EU, frozen (-20 °C) and lyophilized.

Chemical Analyses:

Proximate analyses of diets (Table 2) and fecal samples were carried out in duplicate using AOAC (1990) methods. Gross energy was measured with a Parr bomb calorimeter (Parr Instrument Co., Moline, Il). The chromic oxide levels of diets and feces were measured spectrophotometrically (Model DU 70, Beckman Instruments, Inc., Fullerton, California) after perchloric acid oxidation, using the procedures of McGinnis and Kasting (1964). Soybean meal trypsin inhibitor level of diets was determined by the method of Mol and Meijer (1991).

On the last day of the experiment, the hepatopancreas was randomly collected from 8 shrimp from each treatment (two from each group) for *in vitro* digestibility determinations.

Table 2. Chemical analysis of diets used to calculate apparent digestibility coefficients of soybean meal by shrimp (values are means of two determinations).

Ingredient	Diet (g/100 diet as fed basis)					
	Reference 1% Cr[1]	15% SBM[2] 1% Cr	30% SBM[2] 1% Cr	Reference 0.5% Cr	15% SBM[2] 0.5% Cr	30% SBM[2] 0.5%Cr
Dry matter	4.09	5.26	4.90	5.49	4.94	6.18
Crude protein	42.87	43.46	44.52	43.67	44.52	44.26
Crude lipid	7.70	6.58	5.70	7.83	7.18	5.82
Ash	9.58	8.77	8.70	8.64	8.59	8.04
Gross energy (kJ/g diet)	18.8	18.6	18.5	18.9	18.8	18.5

[1] Chromic oxide levels in diet
[2] Percent soybean meal substitution of reference diet

Calculation and Statistics

The apparent digestibility coefficient of dry matter (ADCdm), crude protein (ADCcp) and gross energy (ADCge) in each diet was calculated from the following equation (Forster, 1999):
ADCdiet = 100 – (Nf/Nd x Crd/Crf) x 100;
where: Nf and Nd refer to the level of each component (DM, CP or GE) in the feces and diet, respectively; and, Crf and Crd refer to the chromic oxide content of the feces and diet, respectively.
The ADC of SBM was calculated from the following equation:
ADCsbm = ((a+b) x ADCcom – a x ADCref)/b;
where: a and b refer to the proportion of each component (DM, CP or GE) in the reference diet (ref) (diets 1 and 4) and the reference and SBM combined diet (com) (diets 2, 3, 5 and 6), respectively, and are calculated from the following equations:
a = Nr x (1-i),
b = Ns x i; where Nr and Ns refer to the percentage of each component (DM, CP or GE) in the reference diet and in the SBM, respectively, and i refers to the proportion of SBM in the diet.
The ADCdm, ADCcp, and ADCge data of the diets and of the SBM were subjected to two-way ANOVA procedures (SigmaStat 2.03, SPSS Inc., Chicago, IL), with dietary chromic oxide and SBM inclusion levels as the factors. Differences between treatment means were considered significant at the 5% error level.

RESULTS AND DISCUSSION

The apparent digestibility coefficients of crude protein by shrimp of the soybean meal in this trial varied from 91 to 102.2% and of gross energy from 78.6 to 100%. There were significant ($p<0.05$) differences in the ADCdm, ADCcp, and ADCge among the diets in this trial (Table 3). The ADCdm, ADCcp, and ADCge of the SBM, were also significantly ($p<0.05$) affected by the level of inclusion of SBM and chromic oxide (Table 4), with the values obtained from diets containing 1.0% chromic oxide being significantly ($p<0.05$) higher than those containing 0.5%. The calculated values for apparent digestibility coefficients of SBM were significantly ($p<0.05$) affected by the dietary levels of both SBM and chromic acid inclusion levels acting together on the ADCs for SBM (Figures 1, 2 and 3). Dietary SBM level did not consistently affect the ADC values calculated for this ingredient, but the observed effect was different for different levels of chromic oxide. Examining the results for the 0.5% chromic oxide diets only, the ADCcp and ADCge of SBM were significantly ($p<0.05$) higher for the diet containing 30 % SBM substitution (46.3% of diet) than for the diet with only 15% SBM substitution (35.0% of diet). The opposite effect was found for the diets containing 1.0% chromic oxide. In this case, the ADCcp and ADCge of SBM were significantly ($p<0.05$) lower for the 30 % substitution level diet than for the 15% substitution level diet. These interactive effects of SBM and chromic oxide are indicative of possible physical interactions between SBM and chromic acid in the diets and indicate a limitation of this type of analysis. One of the assumptions of determining digestibility coefficients using an inert marker, such as chromic oxide, is that the marker does not interfere with the nutrients under investigation, either by affecting the rate of passage or the actual absorption of the nutrients in the digestive tract, and that the marker and the nutrients pass through the digestive tract at the same rate. The results of this trial indicate that these assumptions may be invalid.
In spite of these apparent interactions, however, the results of this trial demonstrate the high degree of apparent digestibility of crude protein and energy of dehulled, defatted, toasted soybean meal and they provide further evidence that this protein source is suitable for use in feeds for Pacific white shrimp. Akiyama *et al*. (1989), using a chromic oxide level of 1% and an ingredient inclusion level of 88%, reported that the apparent protein digestibility coefficients for soybean meal (type not specified) and

soy protein (from ICN Labs) for *L. vannamei* were 89.9 and 96.4%, respectively. The apparent protein digestibility coefficient of soybean protein (49.5 % crude protein, 5.3 % crude lipid; from Productos Pesqueros de la Paz, Mexico) by *L. vannamei* (using a chromic oxide level of 1%) was reported to be 91.0% by an *in vivo* method (Ezquerra *et al.* 1997; 15% replacement of control diet) and 85.9% by an *in vitro* determination (Ezquerra *et al.* 1998).

Table 3. Apparent digestibility coefficients of dry matter, crude protein, and gross energy of diets containing three levels of soybean meal substitution of a control diet, and two levels of chromic oxide determined *in vivo* with *Litopenaeus vannamei*. Values are means of four observations. The probability and power values generated by a two-way analysis of variance are included at the bottom of the table.

Diet		Apparent digestibility coefficient		
SBM[1]	Cr[2]	DM[3]	CP[4]	GE[5]
0	1.0	78.8	88.1	83.8
15	1.0	79.5	90.4	86.4
30	1.0	79.6	90.9	86.1
0	0.5	82.9	89.8	88.0
15	0.5	79.6	89.8	86.4
30	0.5	80.6	91.3	87.2
SEM[6]		0.576	0.329	0.538
Probability[7]	SBM	0.109	<0.001	0.330
	Cr	**0.002**	**0.086**	**<0.001**
	Cr X SBM[8]	**0.007**	**0.008**	**0.003**
Power[9]		**80.5%**	**77.5%**	**89.4%**

[1] Soybean meal substitution level of reference diet (%)
[2] Chromic oxide level in diet (%)
[3] Apparent digestibility coefficient of dry matter
[4] Apparent digestibility coefficient of crude protein
[5] Apparent digestibility coefficient of gross energy
[6] Standard error of the means
[7] Significance level of each factor in two-way analysis of variance
[8] Chromic oxide by Soybean meal interaction
[9] Statistical power of test of Cr X SBM interaction (alpha = 0.05)

Table 4. Apparent digestibility coefficients of dry matter, crude protein, and gross energy of soybean meal derived from digestibility coefficients of diets containing two levels of soybean meal and two levels of chromic oxide. Values are means of four observations. The probability and power values generated by a two-way analysis of variance are included at the bottom of the table.

Diet			Apparent digestibility coefficient		
SBM[1]	Cr[2]		DM[3]	CP[4]	GE[5]
15	1.0		84.7	102.2	100.1
30	1.0		82.3	96.9	91.1
15	0.5		61.2	89.5	78.7
30	0.5		75.8	94.5	85.6
SEM[6]			2.52	1.01	1.08
Probability[7]		SBM	0.032	0.908	0.360
		Cr	<0.001	<0.001	<0.001
		Cr X SBM[8]	0.005	<0.001	<0.001
Power[9]			85.5%	99.7%	100.0%

[1] Soybean meal substitution level of reference diet (%)
[2] Chromic oxide level in diet (%)
[3] Apparent digestibility coefficient of dry matter
[4] Apparent digestibility coefficient of crude protein
[5] Apparent digestibility coefficient of gross energy
[6] Standard error of the means
[7] Significance level of each factor from two-way analysis of variance
[8] Chromic oxide by soybean meal interaction
[9] Statistical power of test of Cr X SBM interaction (alpha = 0.05).

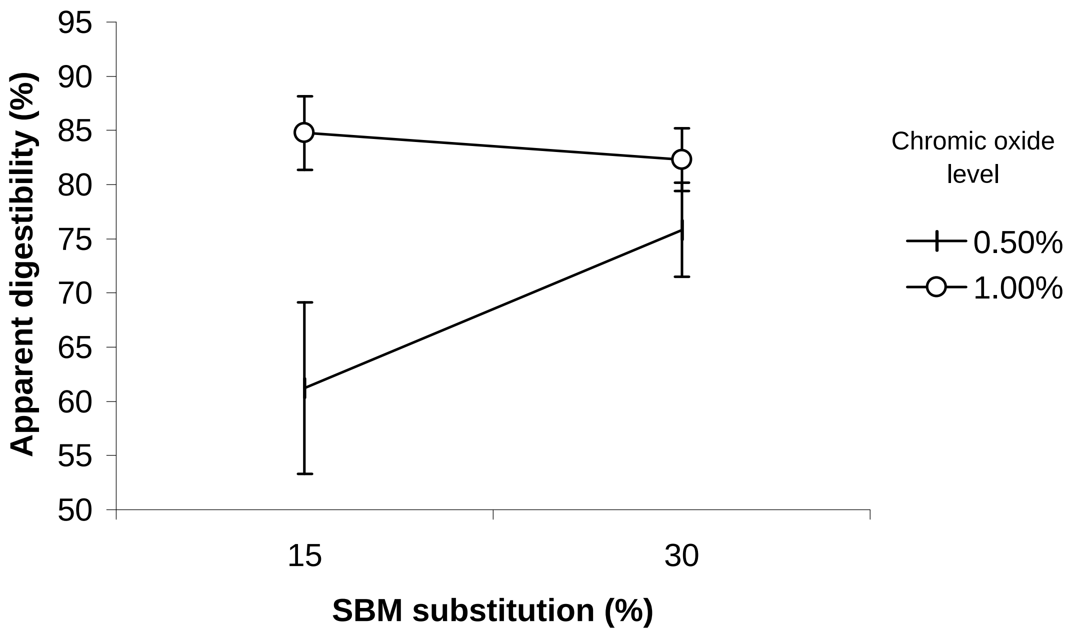

Figure 1. Observed apparent digestibility for dry matter of soybean meal calculated at two levels of substitution of a control diet and containing two levels of chromic oxide. Values are means of four observations. Error bars indicate one standard deviation.

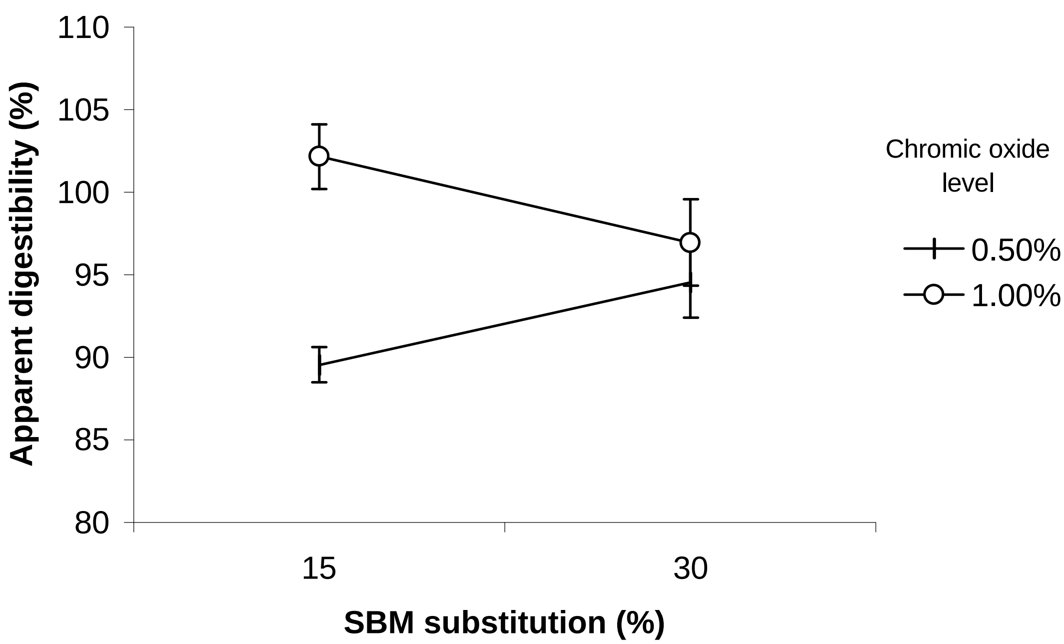

Figure 2. Observed apparent digestibility for crude protein of soybean meal calculated at two levels of substitution of a control diet and containing two levels of chromic oxide. Values are means of four observations. Error bars indicate one standard deviation.

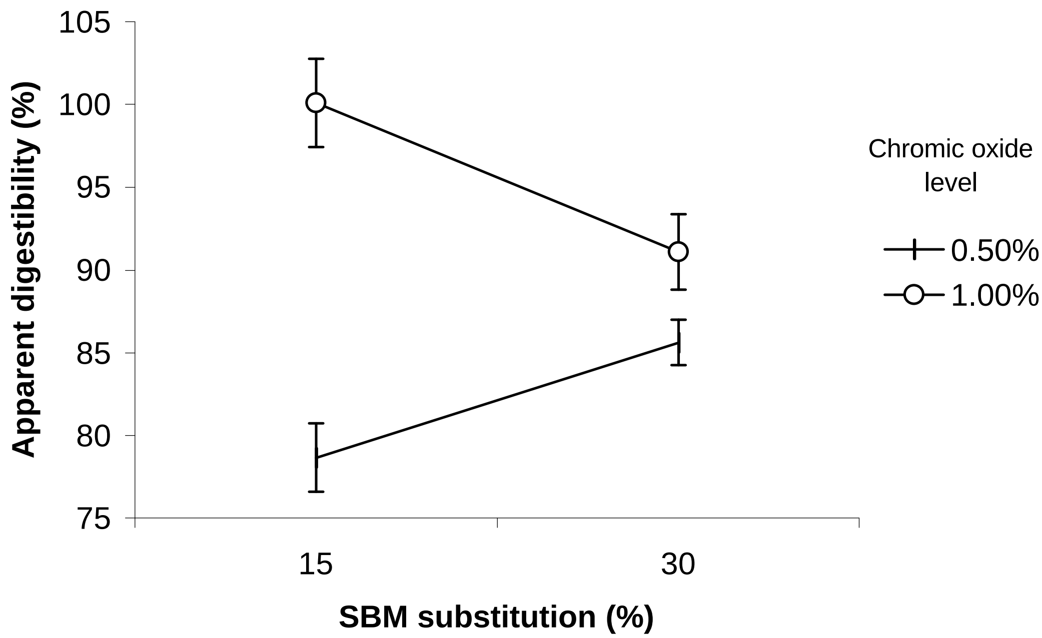

Figure 3. Apparent digestibility for energy of soybean meal calculated at two levels of substitution of a control diet and containing two levels of chromic oxide. Values are means of four observations. Error bars indicate one standard deviation.

CONCLUSIONS AND RECOMMENDATIONS

The results of this trial indicate that dehulled, defatted, toasted soybean meal is a source of highly available protein and energy. This work also indicates that there are limitations to the methodology commonly used to estimate digestibility coefficients. It is evident that the marker (chromic oxide) and the ingredient (soybean meal) interact with each other to affect the calculated digestibility coefficients. Possibly, the level of soybean meal influences the rate of passage of the chromic oxide through the digestive tract. This is an important finding, as most researchers uncritically use only one type and level of marker and one level of test ingredient in their work. In view of this variability, it is important that further work be done to ascertain the correct values for digestibility coefficients for ingredients in shrimp feeds. It is important that digestibility coefficients for an ingredient be ascertained either over a range of inclusion levels, or at least at an inclusion level likely to correspond to that of that ingredient in a practical feed. The findings of this trial also highlight the importance of identifying suitable alternatives to chromic oxide as an inert marker for digestibility trials in shrimp. This work is underway.

Further work that may provide information of use to feed manufacturers is the availability of the specific essential amino acids in ingredients, such as soybean meal, that are being considered as alternatives to fish meal. This information would allow the formulation of diets balanced for these nutrients.

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