



# Evaluation of physicochemical characteristics and digestibility of an extrudate with common bean for pigs

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## ABSTRACT

**Objective.** To evaluate the physicochemical properties, *In vitro* dry matter digestibility, and apparent nitrogen digestibility in fecal matter and urine of pigs of an extruded feed using common bean (*Phaseolus vulgaris* L.) as a soybean meal substitute. **Materials and methods.** The standardization of the extrusion process was performed for two formulations of bean flour (Pinto Saltillo), cornflour (Cafime), and soybean meal at different proportions: 20/67/13% and 30/60/10%, respectively. Samples were processed in a single screw extruder at 120-150°C and a moisture content of 18-22%. The evaluated physicochemical properties were expansion index, bulk density, water absorption index, water solubility index, hardness, water activity, and color parameters:  $L^*$ ,  $a^*$ , and  $b^*$ . The *In vitro* dry matter digestibility and apparent nitrogen digestibility in fecal matter and urine of pigs were evaluated using the optimal treatment previously obtained by seeking to decrease processing costs. **Results.** Optimal conditions for the 20% bean flour formulation were: Temperature of 124.4°C and moisture content of 18.59%. Extrusion temperature decreased bulk density, hardness, and water activity, but increased  $L^*$  and expansion index. Moisture content decreased bulk density, water activity, hardness, and  $a^*$ . *In vitro* dry matter digestibility was higher for the extruded bean diet compared to the control diet (92.33% vs. 85.33%). **Conclusions.** Results indicated that bean flour is a viable option for animal consumption in terms of nutritional value and digestibility.

**Keywords:** Monogastric livestock; nitrogen content; physicochemical properties; extrusion; standardization (Source: *USDA*).

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## RESUMEN

**Objetivo.** Evaluar las propiedades fisicoquímicas, digestibilidad *In vitro* y digestibilidad aparente de nitrógeno en materia fecal y orina de cerdos de un alimento extrudido usando frijol (*Phaseolus vulgaris* L.) como sustituto de harina de soya. **Materiales y métodos.** Se estandarizó el proceso de extrusión para dos formulaciones de harina de frijol (Pinto Saltillo), harina de maíz (Cafime) y harina de soya a diferentes proporciones: 20/67/13% y 30/60/10%, respectivamente. Las muestras se procesaron en un extrusor monotornillo a 120-150°C y 18-22% de contenido de humedad. Las propiedades fisicoquímicas evaluadas fueron: índice de expansión, densidad aparente, índice de absorción de agua, índice de solubilidad en agua, dureza, actividad de agua y parámetros de color:  $L^*$ ,  $a^*$  y  $b^*$ . La digestibilidad *In vitro* y digestibilidad aparente de nitrógeno en materia fecal y orina de cerdos se evaluaron usando el tratamiento óptimo previamente obtenido buscando disminuir costos de procesamiento. **Resultados.** Las condiciones óptimas para la formulación de 20% de harina de frijol fueron 124.4°C y 18.59% de contenido de humedad. La temperatura de extrusión redujo la densidad aparente, dureza y actividad de agua, pero incrementó  $L^*$  y el índice de expansión. El contenido de humedad redujo la densidad aparente, actividad de agua, dureza y  $a^*$ . La digestibilidad *In vitro* de materia seca fue mayor para la dieta de frijol extrudido en comparación a la dieta control (92.33% vs. 85.33%). **Conclusiones.** Los resultados indican que la harina de frijol es una opción viable para el consumo animal en términos de valor nutricional y digestibilidad.

**Palabras clave:** Ganado monogástrico; contenido de nitrógeno; propiedades fisicoquímicas; extrusión; estandarización (Fuente: *USDA*).

## INTRODUCTION

Swine production demands high-quality ingredients, a wide margin of food and health safety, and meticulous husbandry (1). The chemical composition of the ingredients of the formulation is one of the most decisive elements that determine the efficiency in the use of feed. However, feed structure and shape are also important for the optimum use of nutrients (2). In such a sense, the use of alternative sources in pigs feeding is a very suitable strategy since it allows obtaining viable production systems that contribute to environmental conservation, and that do not compete directly with the human diet (3). Legumes such as common beans (*Phaseolus vulgaris* L.) are a good source of proteins, minerals (iron and phosphorus), vitamins, and energy (4). After harvesting, sieve machines are used for screening, cleaning, and packing of beans, as a result, 5-10% of the total crop is substandard, broken, and/or undersized (also known as "granza"), which are not usually used for human consumption, and, in some places, they are even considered an industrial by-product and an economic loss (5). On the other hand, its utilization in animal feed as a raw state is limited, due to its antinutritional factors content, such as phytohemagglutinins and lectins. However, the use of this bean (*Phaseolus vulgaris* L.) in swine diets could represent a good alternative protein source since it offers an economic advantage

over the use of other common protein sources, such as soybean meal (SBM), which makes bean "granza" an attractive alternative. In addition, extrusion is a feasible processing alternative for "granza" utilization due to its capability to develop high nutritional quality products, since it minimizes the degradation of nutrients and adds acceptability features while offering advantages, such as low energy consumption and short-processing time. The detrimental effect of antinutritional factors, such as trypsin inhibitors in beans, can be reduced by up to 80-90% due to thermal deactivation (6).

The nutritional value of a ration, food, or nutrient can be expressed through its digestibility coefficient, which is the proportion of the food that is not excreted, and that is assumed to be absorbed in the digestive tract. The digestibility coefficient is closely related to the nutritional value of food. The amount and type of excretion of fecal material in pigs depend on several factors, such as age, environment, breed, and the nature of the diet. Therefore, it is essential to study the use of nutrients from diets to design balanced formulations for pigs feeding (7). The objective of this research was to evaluate the physicochemical properties, *In vitro* dry matter digestibility, and apparent nitrogen digestibility in fecal matter and urine of pigs of an extruded feed using common bean (*Phaseolus vulgaris* L.) as a soybean meal substitute.

## MATERIAL AND METHODS

**Study development.** The first stage consisted of the standardization of the balanced food with the inclusion of substandard beans in the diet. Two different formulas using cornflour (*Zea mays*, Cafime variety, CF), substandard bean flour (*Phaseolus vulgaris* L., Pinto Saltillo variety; BF), and soybean meal (*Glycine max* L., SBM); were prepared using the following proportions: (BF/CF/SBM) 20/67/13% and 30/60/10%, respectively. The formulation was balanced to approximately 13% of crude protein content to satisfy the nutritional requirements of <6 months pigs (8). A control diet was composed of ground corn, SBM, and wheat bran (Table 1).

**Table 1.** Ingredients of experimental diets.

Ingredients	Component (g/100g)		
	Control diet	Extruded Diet (20/67/13% CF/BF/SBM)	Extruded diet (30/60/10% CF/BF/SBM)
Ground corn	72.5	64.0	57.0
Extruded bean meal	-	20.5	30.0
Soybean meal	20.5	12.5	10.0
Wheat bran	4.0	-	-
Vitamin-mineral mix	3.0	3.0	3.0

**Extrusion.** All ingredients were milled separately (Lab Mill 3600, Perten Instruments AB, Sweden) at 1680 rpm and passed through a 40-mesh sieve (0.420 mm). The moisture content of all treatments was adjusted following the experimental design outlined in Tables 2 and 3, using atomization of distilled water on each sample, and was hand-mixed for 15 min. Water was allowed to diffuse for at least 12 h and samples were kept in sealed polyurethane bags at 4°C before processing. Cooking-extrusion was performed in a simple screw Brabender extruder (Model E 19/25 D, Instruments Inc., South Hackensack, NJ) with a screw compression ratio of 1:1, length/diameter ratio (L/D) of 20:1 and exit die diameter of 6 mm. The screw speed was 150 rpm to avoid clogging. The temperature profile in the extruder was 90, 100, and 110°C in the three first heating zones, respectively, and the

final heating zone varied following experimental design (Tables 2 and 3). The feeding hopper of the extruder was set at a constant rate of 60 rpm. After processing, extrudates were dried in a convection oven (Fabbe model 170) at 60°C for 4 h, following Höglund et al (9) method, and cooled down at room temperature, and placed in plastic bags for storage at 4°C until further analysis.

**Table 2.** Factors and levels of variation of experimental design for two factors ( $\alpha=1.41421$ ).

Factors	Variation levels				
	- $\alpha$	-1	0	+1	+ $\alpha$
Temperature (°C)	120	125	135	145.6	150
Moisture (%)	18	18.6	20	21.4	22

**Experimental design.** A rotatable central composite design with the temperature at the exit die ( $X_1$ ) and moisture content ( $X_2$ ) as independent variables was performed. A set of eleven treatments was generated for each diet: 20/67/13% and 30/60/10% (CF/BF/SBM respectively, Table 3). Response variables were BD, EI, WAI, WSI, H,  $a_w$ , and color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ). The experimental design was performed using Design-Expert 7.0.0® (State-Ease Inc., Minneapolis, MN, USA).

**Table 3.** Central composite design for the extrusion process.

Treatment	Independent variables			
	Coded		Decoded	
	$X_1$	$X_2$	Temperature [°C]	Moisture [%]
1	1.414	0	135	20
2	0	1.414	125	18.6
3	1	-1	150	20
4	0	0	135	22
5	0	0	135	20
6	-1	1	135	20
7	-1	-1	135	18
7	-1	-1	120	20
9	0	-1.414	145.6	18.6
10	1	1	145.6	21.4
11	0	0	125	21.4

**Physicochemical properties.** All extruded samples were analyzed for the following physicochemical properties:

Expansion index (EI) and bulk density (BD) were obtained following Oke et al (10) method (Equations 1 and 2, respectively).

$$E = D/d \quad (1)$$

In which D = extrudate average diameter, and d = inner diameter of extruder outlet. The diameter (d) and the longitude (l) of 10 randomly selected samples were measured. Later, each extrudate was weighed (Pm) to determine the density using Eq. 2. The results were expressed in g/cm<sup>3</sup>.

$$BD = Pm / (\pi(d/2)^2 l) \quad (2)$$

Water absorption index (WAI) and water solubility index (WSI) were determined following the method proposed by Qing-Bo et al (11). Hardness was evaluated using a Universal Texture Analyzer TA-XT2 (Texture Technologies Corp., Scarsdale, NY/Stable MicroSystems, Haslemere, Surrey, UK) (12). Water activity ( $a_w$ ) was determined using an electronic hygrometer Hygrolab (Rotronic AG, Bassersdorf, Switzerland). The color was determined using a colorimeter Hunter lab (MiniScan Hunter Lab, model 45/0L, Hunter Associates Lab., Inc., Reston, Virginia). Values of L (clarity),  $a^*$  (red-green chromaticity), and  $b^*$  (yellow/blue chromaticity) were obtained based on the CIEL\*a\*b\* system (13).

**Chemical composition.** AOAC (14) procedures were used to assess moisture content (925.09B), crude protein (CP, 992.23), crude fat (920.39C), and ash content (923.03) of the raw materials, optimal extruded diet, and a commercial diet which was used as control. Digestible energy (DE) and metabolizable energy (ME) were calculated according to the Nutrient Requirement of Swine (8).

**Numerical optimization.** Superimposition of surface response was performed for each treatment. Data analysis was performed using multiple quadratic regressions (Equation 3). Experimental data was adjusted to selected models and regression coefficients were obtained. Statistical significances of the regressions terms were examined by analysis of variance (ANOVA) for each response ( $p < 0.05$ ).

$$Y = B_0 + B_1 X_1 + B_2 X_2 + B_{11} X_1^2 + B_{22} X_2^2 + B_{12} X_1 X_2 \quad (3)$$

In which Y is the response,  $X_1$  temperature,  $X_2$  moisture content, and  $B_0$ ,  $B_1$ ,  $B_2$ ,  $B_{11}$ ,  $B_{22}$  y  $B_{12}$  are the regression coefficients.

### ***In vitro* dry matter digestibility (IVDMD) and apparent nitrogen digestibility in fecal matter and urine.**

In the second stage of the research, the *in vitro* dry matter digestibility and apparent nitrogen digestibility in fecal matter and urine of pigs fed with the optimal treatment previously obtained were analyzed following the Mireles et al procedure (15). To evaluate apparent nitrogen digestibility, eight 3-month-old ( $30 \pm 5$  kg) York-Landrace castrated male pigs were individually housed and fed using both diets: control diet and the optimal extruded diet (20/67/13% CF/BF/SBM) for twelve days (seven days of adaptation and five days of excrement and urine recollection). The feces were collected daily by the method of total collection at 8:00 a.m. (15). The food was supplied twice a day at 8:00 and 16:00. Throughout the experiment, the pigs had free access to water (16). From each animal, a representative sample of 100 g of fresh excreta/d was collected, which was stored at  $-20^\circ\text{C}$ . The excreta were dried at  $55^\circ\text{C}$  for 48 h and milled (Lab Mill 3600, Perten Instruments AB, Sweden) at 1680 rpm and passed through a 40-mesh sieve (0.420 mm). The following analyses were performed in the stool samples: Dry matter, #930.15 and total nitrogen by Kjeldahl method #990.03) according to the AOAC methods (14). To calculate apparent nitrogen digestibility in fecal matter and urine, Caicedo et al (17) and O'Quinn et al (18) methods were used (Equation 4).

$$FAD (\%) = (N_c - N_e) / N_c \quad (4)$$

In which FAD = apparent nitrogen digestibility of a nutrient in the diet,  $N_c$  = consumed nitrogen, and  $N_e$  = excreted nitrogen.

## **RESULTS**

**Physicochemical properties.** The regression coefficients of physicochemical properties and parameters obtained from color analyses of all treatments are shown in Table 4.

Tables 5 and 6 show a comparison of physicochemical properties among treatments at different processing conditions for both formulations.

**Table 4.** Analyses of variance of the extrusion temperature, moisture content, and bean concentration.

Response	R <sup>2</sup>	CV	SD	SEM	F Value	p-Value
BD	0.62	5.59	0.059	0.024	3.04	0.0365
EI	0.58	3.35	0.038	0.016	2.49	0.0696
WAI	0.50	4.03	0.041	0.017	1.78	0.1687
WSI	0.52	5.36	0.270	0.110	1.97	0.1325
H	0.54	26.08	19.800	8.080	2.04	0.1221
aw	0.25	16.12	0.064	0.026	0.54	0.7885
L*	0.60	6.18	0.980	0.400	2.44	0.0737
a*	0.40	1.00	0.320	0.130	1.07	0.4286
b*	0.25	11.66	1.250	0.510	0.48	0.8371

CV= coefficient of variation, SD= standard deviation, SEM= standard error of the mean, BD = bulk density, EI = expansion index, WAI = water absorption index, WSI = water solubility index, H = hardness, aw = water activity, L\* = luminosity, a\* = red/green chromaticity and b\* = yellow/blue chromaticity.

**Table 5.** Determination of physicochemical properties of extruded treatments at different extrusion temperatures, moisture content, and bean concentration.

Treatments	BD (kg/cm <sup>3</sup> )	EI (-)	WAI (g/g)	WSI (%)	H (N)	A <sub>w</sub> (-)
<b>20/67/13% (BF/CF/SBM)</b>						
1	0.98 ± 0.046 <sup>cdef</sup>	1.18 ± 0.032 <sup>bc</sup>	1.06 ± 0.121 <sup>ab</sup>	4.79 ± 0.672 <sup>ab</sup>	53.91 ± 6.349 <sup>fg</sup>	0.38 ± 0.004 <sup>b</sup>
2	1.02 ± 0.088 <sup>f</sup>	1.18 ± 0.038 <sup>b</sup>	0.96 ± 0.051 <sup>ab</sup>	4.66 ± 0.082 <sup>ab</sup>	67.64 ± 9.455 <sup>j</sup>	0.35 ± 0.002 <sup>kl</sup>
3	0.97 ± 0.052 <sup>bcde</sup>	1.2 ± 0.033 <sup>bc</sup>	1.02 ± 0.029 <sup>b</sup>	4.84 ± 0.083 <sup>b</sup>	46.23 ± 5.780 <sup>hg</sup>	0.34 ± 0.002 <sup>kl</sup>
4	1.02 ± 0.082 <sup>cdef</sup>	1.16 ± 0.041 <sup>bc</sup>	1.03 ± 0.068 <sup>ab</sup>	5.25 ± 0.218 <sup>b</sup>	68.88 ± 6.713 <sup>hg</sup>	0.34 ± 0.003 <sup>j</sup>
5	1.18 ± 0.077 <sup>bcd</sup>	1.07 ± 0.023 <sup>def</sup>	1.05 ± 0.098 <sup>b</sup>	5.20 ± 1.013 <sup>ab</sup>	98.97 ± 6.568 <sup>ij</sup>	0.35 ± 0.001 <sup>ij</sup>
6	1.06 ± 0.028 <sup>ef</sup>	1.18 ± 0.081 <sup>bc</sup>	0.93 ± 0.027 <sup>ab</sup>	4.70 ± 0.048 <sup>ab</sup>	59.64 ± 7.636 <sup>hi</sup>	0.34 ± 0.004 <sup>g</sup>
7	1.05 ± 0.095 <sup>g</sup>	1.17 ± 0.039 <sup>a</sup>	0.99 ± 0.088 <sup>ab</sup>	5.08 ± 0.181 <sup>ab</sup>	61.88 ± 8.553 <sup>e</sup>	0.47 ± 0.007 <sup>l</sup>
8	1.12 ± 0.068 <sup>def</sup>	1.14 ± 0.026 <sup>bc</sup>	0.93 ± 0.038 <sup>ab</sup>	5.18 ± 0.409 <sup>b</sup>	47.13 ± 7.291 <sup>ef</sup>	0.35 ± 0.001 <sup>j</sup>
9	0.88 ± 0.100 <sup>def</sup>	1.26 ± 0.036 <sup>bcd</sup>	1.04 ± 0.107 <sup>ab</sup>	4.76 ± 0.615 <sup>ab</sup>	72.08 ± 8.224 <sup>ef</sup>	0.33 ± 0.005 <sup>ij</sup>
10	1.06 ± 0.086 <sup>ab</sup>	1.18 ± 0.079 <sup>f</sup>	1.01 ± 0.019 <sup>ab</sup>	4.65 ± 0.892 <sup>ab</sup>	58.99 ± 10.066 <sup>bc</sup>	0.35 ± 0.005 <sup>jk</sup>
11	1.06 ± 0.081 <sup>cdef</sup>	1.16 ± 0.038 <sup>bcd</sup>	1.01 ± 0.047 <sup>ab</sup>	5.68 ± 0.864 <sup>a</sup>	94.98 ± 7.296 <sup>c</sup>	0.34 ± 0.003 <sup>kl</sup>
<b>30/60/10% (BF/CF/SBM)</b>						
1	1.14 ± 0.104 <sup>bc</sup>	1.08 ± 0.054 <sup>ef</sup>	1.04 ± 0.089 <sup>ab</sup>	5.32 ± 0.805 <sup>ab</sup>	105.74 ± 13.50 <sup>b</sup>	0.48 ± 0.003 <sup>b</sup>
2	1.02 ± 0.130 <sup>def</sup>	1.14 ± 0.061 <sup>cd</sup>	1.05 ± 0.030 <sup>ab</sup>	4.95 ± 0.117 <sup>ab</sup>	78.81 ± 11.902 <sup>d</sup>	0.48 ± 0.002 <sup>b</sup>
3	1.07 ± 0.149 <sup>def</sup>	1.15 ± 0.081 <sup>bcd</sup>	1.04 ± 0.142 <sup>a</sup>	4.75 ± 0.229 <sup>ab</sup>	82.28 ± 4.582 <sup>d</sup>	0.52 ± 0.002 <sup>a</sup>
4	1.04 ± 0.079 <sup>bcdef</sup>	1.13 ± 0.034 <sup>cde</sup>	1.09 ± 0.008 <sup>ab</sup>	4.84 ± 0.046 <sup>ab</sup>	68.47 ± 6.618 <sup>ef</sup>	0.44 ± 0.003 <sup>d</sup>
5	1.12 ± 0.091 <sup>bcd</sup>	1.11 ± 0.060 <sup>def</sup>	1.04 ± 0.040 <sup>ab</sup>	4.68 ± 0.136 <sup>b</sup>	105.80 ± 11.43 <sup>b7</sup>	0.45 ± 0.002 <sup>c</sup>
6	1.04 ± 0.107 <sup>cdef</sup>	1.15 ± 0.058 <sup>bcd</sup>	1.02 ± 0.044 <sup>ab</sup>	4.85 ± 0.191 <sup>ab</sup>	59.14 ± 5.551 <sup>hg</sup>	0.41 ± 0.001 <sup>f</sup>
7	1.03 ± 0.162 <sup>ef</sup>	1.18 ± 0.102 <sup>bc</sup>	1.04 ± 0.088 <sup>ab</sup>	4.76 ± 0.181 <sup>ab</sup>	47.13 ± 6.568 <sup>ij</sup>	0.36 ± 0.003 <sup>i</sup>
8	1.01 ± 0.089 <sup>ef</sup>	1.17 ± 0.040 <sup>bc</sup>	0.96 ± 0.038 <sup>ab</sup>	4.66 ± 0.409 <sup>b</sup>	59.09 ± 5.222 <sup>hg</sup>	0.34 ± 0.001 <sup>jk</sup>
9	1.08 ± 0.171 <sup>bcde</sup>	1.13 ± 0.071 <sup>cde</sup>	1.06 ± 0.041 <sup>ab</sup>	4.73 ± 0.200 <sup>ab</sup>	115.24 ± 15.116 <sup>a</sup>	0.42 ± 0.005 <sup>e</sup>
10	1.24 ± 0.140 <sup>a</sup>	1.06 ± 0.060 <sup>f</sup>	1.07 ± 0.116 <sup>ab</sup>	4.82 ± 0.031 <sup>ab</sup>	103.71 ± 10.721 <sup>b</sup>	0.37 ± 0.002 <sup>h</sup>
11	1.12 ± 0.080 <sup>bcd</sup>	1.10 ± 0.057 <sup>def</sup>	1.10 ± 0.111 <sup>a</sup>	5.62 ± 0.756 <sup>ab</sup>	114.77 ± 7.716 <sup>a</sup>	0.44 ± 0.001 <sup>c</sup>

± SD = standard deviation, BF = bean flour (%). Different letters on the same column indicate significant difference (p < 0.05). BD = bulk density, EI = expansion index, WAI = water absorption index, WSI = water solubility index, H = hardness, a<sub>w</sub> = water activity

**Table 6.** Determination of color parameters of extruded treatments at different extrusion temperatures, moisture contents, and bean concentrations.

Treatments	L*	a*	b*
<b>20/67/13% (BF/CF/SBM)</b>			
1	15.41 ± 0.086 <sup>bcde</sup>	32.08 ± 0.207 <sup>abc</sup>	13.16 ± 1.309 <sup>cd</sup>
2	15.56 ± 0.600 <sup>h</sup>	31.94 ± 0.262 <sup>a</sup>	11.55 ± 0.815 <sup>cde</sup>
3	15.47 ± 0.535 <sup>bcde</sup>	31.82 ± 0.072 <sup>bcdef</sup>	10.24 ± 0.255 <sup>def</sup>
4	14.5 ± 0.472 <sup>cdef</sup>	32.07 ± 0.145 <sup>abcde</sup>	12.21 ± 0.650 <sup>cde</sup>
5	14.81 ± 0.573 <sup>ab</sup>	32 ± 0.050 <sup>bcdef</sup>	12.09 ± 0.600 <sup>ab</sup>
6	16.9 ± 0.362 <sup>fgh</sup>	31.62 ± 0.185 <sup>abcde</sup>	10.75 ± 0.293 <sup>a</sup>
7	16.54 ± 0.423 <sup>h</sup>	31.91 ± 0.180 <sup>fg</sup>	12.9 ± 0.345 <sup>efg</sup>
8	16.53 ± 0.482 <sup>def</sup>	31.63 ± 0.193 <sup>bcdef</sup>	11.75 ± 0.145 <sup>bcd</sup>
9	15.25 ± 0.340 <sup>h</sup>	31.36 ± 0.174 <sup>abcde</sup>	10.09 ± 0.347 <sup>bc</sup>
10	16.52 ± 0.205 <sup>h</sup>	31.71 ± 0.145 <sup>ab</sup>	10.54 ± 0.359 <sup>bcd</sup>
11	15.35 ± 0.614 <sup>h</sup>	31.56 ± 0.032 <sup>defg</sup>	10.19 ± 0.249 <sup>efg</sup>
<b>30/60/10% (BF/CF/SBM)</b>			
1	18.94 ± 1.045 <sup>a</sup>	31.18 ± 0.343 <sup>cdefg</sup>	9.75 ± 0.379 <sup>fgh</sup>
2	15.77 ± 0.080 <sup>efg</sup>	31.62 ± 0.118 <sup>abcde</sup>	10.17 ± 0.416 <sup>efg</sup>
3	15.26 ± 0.269 <sup>gh</sup>	31.86 ± 0.205 <sup>bcdef</sup>	8.89 ± 0.475 <sup>hi</sup>
4	14.61 ± 0.704 <sup>fgh</sup>	32.05 ± 0.233 <sup>abcde</sup>	10.36 ± 0.131 <sup>fgh</sup>
5	17.28 ± 0.370 <sup>ab</sup>	31.42 ± 0.121 <sup>efg</sup>	10.02 ± 0.350 <sup>ghi</sup>
6	16.97 ± 0.197 <sup>abcd</sup>	31.76 ± 0.202 <sup>cdefg</sup>	10.6 ± 0.477 <sup>efg</sup>
7	15.62 ± 0.373 <sup>gh</sup>	31.71 ± 0.145 <sup>bcdef</sup>	10.57 ± 0.153 <sup>efg</sup>
8	17.66 ± 0.530 <sup>abc</sup>	31.01 ± 0.259 <sup>g</sup>	9.7 ± 0.300 <sup>ghi</sup>
9	13.54 ± 0.327 <sup>i</sup>	32.26 ± 0.210 <sup>ab</sup>	10.48 ± 0.437 <sup>gh</sup>
10	15.39 ± 0.477 <sup>efg</sup>	31.67 ± 0.073 <sup>bcdef</sup>	9.11 ± 0.055 <sup>i</sup>
11	15.99 ± 0.513 <sup>def</sup>	32.24 ± 0.284 <sup>abcd</sup>	10.03 ± 0.128 <sup>ghi</sup>

± SD = standard deviation BF = bean flour (%). Different letter on the same column indicate significant difference ( $p < 0.05$ ), L\* = luminosity, a\* = red/green chromaticity and b\* = yellow/blue chromaticity.

**Numerical optimization.** It was performed by the superimposition of response surfaces for each evaluated treatment. Obtained optimal processing conditions for both formulations were: Temperature of 124.4°C and moisture content of 18.59% with the following responses: WAI 1.022 g/g, WSI 5.07% and H 78.51 N. Only 20/67/13% CF/BF/SBM diet was fully evaluated since preliminary tests showed high content of antinutritional factors for the 30/60/10% CF/BF/SBM diet, possibly as a result of undercooking and the higher content of bean flour.

**Chemical composition.** The chemical compositions of ingredients, optimal extruded feed, and a commercial feed are shown in Table 7. The protein content of bean flour (22%) was within other reported values (4). The optimal extruded feed contained the required amount of protein for swine at the growing stage (15-18%). The low-fat content present in the

commercial feed could be explained due to the low-fat content of soybean meal, one of its main ingredients.

**Table 7.** Chemical composition of ingredients, optimal extrudate feed (20/67/13% CF/BF/SBM), and control feed (g/100 g DM).

Sample	Crude protein	Ether extract	Crude fiber	Ash	NFE
Corn flour	9.7 ±0.76	3.5 ±0.13	6.0 ±0.53	1.5 ±0.08	85.3 ±0.93
Bean flour	24.3 ±0.26	1.4 ±0.03	4.9 ±0.32	3.9 ±0.22	70.4 ±0.52
Soybean meal	51.2 ±3.72	2.3 ±0.14	4.6 ±0.11	6.0 ±0.07	40.5 ±3.85
Commercial feed	14.8 ±0.76	1.9 ±0.04	3.9 ±0.21	3.9 ±0.38	79.4 ±1.38
Extrudate feed	17.5 ±0.38	4.0 ±0.83	7.8 ±0.07	1.4 ±0.11	75.1 ±1.27

±SD = standard deviation, NFE = nitrogen free extract, DM = dry matter

**In vitro dry matter digestibility (IVDMD) and apparent fecal digestibility in fecal matter and urine.** *In vitro* dry matter digestibility (IVDMD) was higher for the extruded bean diet in comparison to the commercial diet (92.33 vs. 85.33, SEM 0.929,  $p < 0.001$ ). The latter may be explained since heat treatment destroys or modifies certain antinutritional properties of the common bean, which could limit the rate of enzymatic degradation in the assay. On the other hand, apparent nitrogen digestibility in the fecal matter was higher for the commercial diet but lower in apparent nitrogen digestibility in urine, compared to the extruded bean diet (Table 8).

**Table 8.** *In vitro* dry matter digestibility (DMD) and apparent nitrogen digestibility in fecal matter and urine for growing swine.

Diet	<i>In vitro</i> DMD (%)	Apparent Nitrogen Digestibility in Fecal matter (%)	Apparent Nitrogen Digestibility in Urine (%)
Control diet	85.33 ±0.488 <sup>b</sup>	90.05 ±0.713 <sup>a</sup>	98.630 ±0.993 <sup>a</sup>
Optimal extruded diet (20/67/13 CF/BF/SBM)	92.33 ±0.342 <sup>a</sup>	84.87 ±1.570 <sup>b</sup>	99.371 ±1.871 <sup>b</sup>
SEM	0.929	1.048	0.074
p-Value	0.0065	0.0011	0.0000

\*SEM: standard error of the mean; Different letters on the same column indicate a significant difference ( $p \leq 0.05$ ).

The SBM has a high content of degradable protein, therefore, it is safe to assume that the commercial diet used in these experiments (72.5% of SBM) will also have a higher degradability (90.05%). The inclusion of 20.5% of extruded common bean in the diet showed a slightly lower degradability (84.87%), although it is not yet possible to assess if this reduction is due to the nature of the substrate or a possible reduction in degradability due to thermal damage to the protein complexes (e.g. Maillard reactions) during its preparation.

## DISCUSSION

Regarding the regression coefficients of the physicochemical properties (Table 4), T in its linear term and MC in its quadratic term had a negative significant effect ( $p < 0.05$ ) on BD,

possibly because high temperature and moisture content generate more steam during the extrusion processing leading to a cellular wall rupture and a more expanded and less dense final product, which is also related to EI and the positive significant effect ( $p < 0.05$ ) of MC in its quadratic term; a higher amount of steam will generate a more voluptuous extrudate (3). However, for EI, T, and MC interaction presented a negative significant effect ( $p < 0.05$ ). Oke et al (10) concluded that decreased MC increases drag force and therefore exerts more pressure at the die resulting in a greater expansion of the final product. For WSI, only MC in its quadratic term had a negative significant effect ( $p < 0.05$ ); lower MC could have increased the drag force at the exit die, resulting in more gelatinized starch. For H, both T and MC quadratic terms showed negative significant effects ( $p < 0.05$ ); the decrease in H with an increase of T might be due to higher expansions at elevated temperatures (12). In addition, an increase in MC decreases lateral expansion due to the plasticization of melt (19). T quadratic term presented a positive significant effect ( $p < 0.05$ ) on  $L^*$ , this may be due to the formation of brown pigments through non-enzymatic Maillard reactions between proteins and reducing sugars that occur during the extrusion processing. MC quadratic term presented a negative significant effect ( $p < 0.05$ ) on  $a^*$ , indicating that at high MC red coloration decreases, which contradicts the obtained results of Do Carmo et al (20).

Physicochemical properties of extruded products have a direct impact on their acceptability and are an indicator of their nutritional quality. Changes in functional properties such as EI and BD of the extruded products occur due to the structural transformations of starch (21). MC quadratic term showed a positive significant effect on EI, which agrees with other reports. The presence of fibers and proteins in the formula tends to generate products with low EI due to the interaction of these components with starch, furthermore, fiber can rupture cell walls, avoiding air bubble formation (2). BD obtained results agree with Sobuñola et al (22), who found that increasing temperature significantly decreases BD, indicating that EI and BD depend on extrusion temperature (23) since BD is directly related to the expansion produced during extrusion.

Products with high BD indicate a more uniform and continuous protein matrix, resulting in a denser extrudate with parallel layers, without

Changes of color during extrusion may indicate the severity or intensity of the process since it relates to chemical and nutritional changes. Temperature showed an adequate effect on  $L^*$ , which could be attributed to the homogeneity provoked by the extrusion process.  $L^*$  obtained values ranged between 13.38 and 18.94; such low values could be related to processing conditions since they enhance reactions among reducing amino acids and sugars, which translates into the formation of colorful compounds.  $a^*$  values oscillate between 31.01 and 32.32, indicating that all treatments were in the red color classification. All  $b^*$  values were positive, indicating a tendency to the yellow region. Color in feeding products might influence the acceptability or rejection of the consumer. During cooking and extrusion processing, some darkening may occur due to Maillard reactions, which might contribute positively to flavor and smell, as occurs with caramel, coffee, bread, and cereals (32).

Regarding chemical composition, the protein content of BF (22%) agrees with other reports (4), thus securing swine proper nutrition, since the optimal extruded treatment contains the necessary protein content (15-18%) for swine in the growing stage. Due to its amino acid composition, BF represents a feasible alternative for swine feed that could substitute soy protein as a protein source, which would reduce production costs.

Ingested nitrogen is essential for both amino acids synthesis and nitrogen bases in nucleic acids. The nitrogen digestibility and its assimilation by swine vary to a great extent among the main components of the formulation: from 85% in concentrates (wheat grains and soymeal) to 50-60% in fibrous food (DDGS, soy husk, alfalfa flour, or beet pulp). Nitrogen digestibility is related to the bonding with the cellular wall, which may vary according to the foliar resource (15). The daily production of excreted is a function of the type and live weight of each animal species, the consumed food, temperature, and humidity of the environment, and the amount of water used in confined productions (33). One of the main factors to evaluate feed and forages energetic content is their digestibility. Thus, *in vivo* essays are required to evaluate digestibility using animals in specific production periods and under controlled conditions. Such essays, although not routinely performed, still are the reference methodology. *In vitro* digestibility techniques are fast, low-cost, and produce data that can be correlated to *in vivo* results, at least from

airbags nor swallowing after hydration (24). Increasing MC from 18 to 22% produced a decrease in EI of yam starch-based pasta (22). Filli et al (25) reported that alimentary materials with low MC tend to be more viscous and the pressure difference might lead to a less expanded product.

EI is related to bulk density (26). Regression coefficients indicate that temperature and MC have a positive correlation to EI. Some of the factors that affect EI during extrusion processing are the type of extruder, screw speed and screw configuration, moisture content, residence time, pressure, temperature profile, and feeding speed (27).

Water Absorption Index (WAI) and Water Solubility Index (WSI) are related to the functional properties of starch. WAI is used as an indicator of molecular component degradation and depends on the availability of the hydrophilic groups and the gelling capacity of macromolecules (28). WSI is associated with the presence of soluble starch molecules, which is closely related to dextrinization (23). The negative effect of MC on WSI could be related to the water presence during extrusion and the increase of gelatinized starch along with the decrease of denaturalized proteins, due to the lower shear force generated by the diminish of viscosity in the mixture. Changes in WSI are attributed to the increase of the surface area of extrudates and the fragmentation of starch chains by increasing the thermal energies of the molecules. Chains formed during processing are smaller and tend to become more soluble in water; also, low values of starch gelatinization are responsible for low WSI (3). This indicates that at low MC, the effect of friction predominates; at high MC the thermal effect predominates. Agyekum and Nyachoti (29) reported that the low solubility of animal diets negatively affects their digestion process. Obtained results agree with other reports (30), where extruded corn presented a higher susceptibility to enzymes and its water solubility is similar to conventional corn.

Extrudates texture is a physical characteristic highly related to the formula composition, temperature, and moisture content. Results obtained from this research showed that increasing T and MC decreases H. Vukmirović et al (31) recommend thermomechanical processing for swine consumption diets since it facilitates digestion and aids to avoid gastrointestinal diseases that could jeopardize swine's health.

the standpoint of organic matter digestibility and digestible energy in pigs (19). In livestock production, nitrogen losses occur through urine and fecal matter due to a lack of retention. A pig excretes more than half of the ingested nitrogen through fecal matter and urine on average (30). However, endogenous nitrogen and microbial nitrogen in the fecal matter are losses that should be minimized.

In conclusion, the chemical composition of the non-extruded and extruded optimal formula indicated that protein content was 18% higher compared to a commercial diet for swine in the growth stage. Optimal processing conditions

were found at 125°C and 18.6% MC with a BF content of 20%. Results showed that BF inclusion in swine feed is a feasible option since the functional characteristics of the evaluated extrudates are similar to existing commercial feeds. Dry matter and crude protein digestibility were high in both commercial and extruded diets, suggesting that the latter can be used in animal feeding, although further research is needed in this field.

### Conflicts of interest

No potential conflict of interest was reported by the authors.

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