



## Nutritional evaluation of inactivated whole soy and protease enzyme for growing and finishing pigs

[Avaliação nutricional de soja integral desativada e enzima protease para suínos em crescimento e terminação]

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### ABSTRACT

The inactivated whole soy (IWS) was studied in pigs to determine the energy value through a metabolism trial and evaluate the effect of IWS and protease on performance, carcass traits, and economic viability. Metabolism with pigs (53.80±4.15kg) to determine digestibility coefficients of dry matter (DM), organic matter (OM), and crude protein (CP), digestible energy (DE), apparent metabolizable energy (AME), and nitrogen-corrected AME (AMEn). For the performance trial, 60 immunocastrated male pigs and 60 female pigs (30.09±1.46kg) were used in a 2 (gender) x 2 (with and without IWS) x 2 (protease) factorial arrangement. The DM, OM, and CP metabolizability coefficients of IWS were 83.77, 84.43, and 89.18%, respectively. The DE, AME, and AMEn values were 4904±117, 4805±273, and 4656±255kcal/kg, respectively. In growth phase I, enzyme provided an increase in average daily feed intake. In the economic viability, diet with IWS and without the enzyme had the lowest cost per kilogram of weight gained and provided the highest economic efficiency index and net revenue. The IWS has a high energy value and when used in diets for growing and finishing pigs provides satisfactory performance and better economic efficiency.

Keywords: anti-nutritional factors, metabolism, performance, swine production, protein feed

### RESUMO

A soja integral desativada (SID) foi estudada em suínos para determinar o valor energético, por meio de um ensaio de metabolismo, e avaliar o efeito da SID e da protease no desempenho, nas características da carcaça e na viabilidade econômica. Foi realizado um ensaio de metabolismo com suínos (53,80±4,15kg) para determinar os coeficientes de digestibilidade da matéria seca (MS), da matéria orgânica (MO) e da proteína bruta (PB), a energia digestível (ED), a energia metabolizável aparente (EMA) e a EMA corrigida pelo nitrogênio (EMAn). Para o ensaio de desempenho, foram utilizados 60 suínos machos imunocastrados e 60 suínos fêmeas (30,09 ± 1,46kg), em arranjo fatorial 2 (sexo) x 2 (com e sem SID) x 2 (protease). Os coeficientes de metabolizabilidade da MS, MO e PB do SID foram de 83,77, 84,43 e 89,18%, respectivamente. Os valores de ED, AME, e AMEn foram 4904 ± 117, 4805±273, e 4656±255kcal/kg, respectivamente. Para o desempenho na fase de crescimento I, a enzima proporcionou um aumento no consumo médio diário de ração. Em termos de viabilidade econômica, a dieta com SID e sem a enzima teve o menor custo por quilograma de peso ganho e proporcionou o maior índice de eficiência econômica e receita líquida. A SID possui um alto valor de energia e, quando usada em dietas para suínos em crescimento e terminação, proporciona um desempenho satisfatório e uma melhor eficiência econômica.

Palavras-chave: fatores antinutricionais, metabolismo, desempenho, produção de suínos, ração proteica

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## INTRODUCTION

Profitability in pig farming depends on the availability of ingredients at compatible prices paid per kilogram of finished pig. Thus, feeding constitutes a determining factor for the success of this activity.

Soy is one of the most cultivated grains in the world, with a total production of 398.21 million tons. Brazil is the main producer of the crop, with 156 million tons, representing 39.2% of global production (Production..., 2024). Some of the derivatives of the processing of this grain are soybean oil and soybean meal, the latter being a conventional ingredient in animal feed. However, processed whole soy may be superior to soybean meal due to the high energy value provided by the presence of oil, which may represent economic advantages in pig farming (Ludke *et al.*, 2007).

Bioavailability of nutrients in the soybeans increases when there are not the anti-nutritional factors, and this can be done by thermally processing the grains, since pressure cooking is made to inactivate these factors without changing the energy and protein value of whole soybeans (Jahanian and Rasouli, 2016). Inactivation is a simpler process compared with defatting, to obtain soybean meal.

In addition to improving soybean utilization, soybean processing inhibits the anti-nutritional factors present in soybeans, such as protease inhibitors, lectins, allergenic proteins and saponins. These compounds, when present, interfere with the absorption of nutrients, causing disturbances during animal growth (Yasothai, 2016).

According to Toledo *et al.* (2011), IWS has potential use in feed because, in addition to containing good amounts of essential amino acids, it has high levels of metabolizable energy thanks to the presence of the oil. As mentioned by those authors, the metabolizable energy value in non-hulled and hulled IWS for piglets is 4,111 and 3,768kcal/kg, respectively.

The addition of exogenous enzymes to the feed improves the utilization of the ingredients that

compose it, increasing the digestibility and consequent absorption of nutrients (Opalinski *et al.*, 2011). Protease inhibitors interfere with protein digestion, resulting in decreased animal growth.

The hypothesis of the present study is that the inclusion of IWS in swine diets reduces the cost of feed per gain by replacing partially soybean meal, corn, and the supplementary oil, and by using protease enzyme, considering its nutritional advantages, to not increase this cost while at the same time maintain animal performance. Therefore, the objective was to evaluate the nutritional value of IWS in a metabolism trial and to evaluate the effect of adding IWS to pig diets with and without protease on performance, carcass traits, and economic viability.

## MATERIAL AND METHODS

The study was approved by the institutional committee on the use of animals (CEUA, approval no. 018/2018), whose authorization was valid until December 31, 2021. The experiments were carried out in Concórdia - SC, Brazil (27°18'48"71" S, 51°59'34"07" W).

Two experiments were conducted: one metabolism trial, using the total collection method; and one performance experiment involving female and male growing and finishing pigs. The males used in the experiments were immunocastrated with injectable vaccines. The first dose of the vaccine in the males used in the metabolism trial was applied when the animals were 61 days old, and the second dose at 75 days old. In the performance trial, the doses were applied at 28 and 14 days before slaughter, corresponding to the finishing phase.

These experiments evaluated inactivated whole soy (IWS) (Tab. 1). It was processed at the company Cooperalfa whose whole grain partially dehulled had been subjected to inactivation using a sealed reactor with steam injection, heating at 108 °C, under a pressure of 1.0 kgf/cm<sup>2</sup> and vacuum for 18 min, to inactivate the antinutritional factors.

Table 1. Chemical composition and amino acid content of the inactivated whole soy (IWS) used in the experiment

Nutritional composition of IWS	%	Amino acids in IWS	%
Dry matter	89.4	Arginine	2.432
Crude protein	40.07	Histidine	0.909
Gross energy (kcal/kg)	6119	Isoleucine	1.533
Ash	4.94	Leucine	2.621
Ether extract	25.96	Lysine	2.186
Crude fiber	5.64	Methionine	0.397
Protein solubility	88.46	Cystine	0.549
Urea activity	0.02	Methionine + cystine	0.946
-	-	Phenylalanine	1.798
-	-	Tyrosine	1.533
-	-	Phenylalanine + tyrosine	3.019
-	-	Threonine	1.420
-	-	Tryptophan	0.549
-	-	Valine	1.580

A metabolism trial was carried out to determine the digestibility of dry matter (DM), organic matter (OM), and crude protein (CP), as well as the digestible energy (DE), apparent metabolizable energy (AME), and nitrogen-corrected AME (AMEn) values of IWS by the total fecal and urine collection method, using the ferric oxide (Fe<sub>2</sub>O<sub>3</sub>) marker. The trial involved 16 immunocastrated male pigs weighing 53.80 ± 4.15 kg, with a mean age of 104 ± 0.2 days from the cross between MS115 - Pietrain (62.5%), Large White (18.75%), and Duroc (18.75%) sires and F1 (Landrace × Large White) dams. The experimental period consisted of seven days for adaptation and five days of collection. The

design (initial weight) with eight replicates, one animal per experimental unit, and two treatments: 1) Control diet; and 2) Test diet, i.e., control diet with 30% replaced with IWS.

The animals were housed in a climate-controlled room with an average temperature of 20 °C, where they were individually allocated to metabolic cages in the model described by Pekas (1968).

Control diet (Table 2) was formulated based on maize and soybean meal and supplemented with minerals and vitamins, meeting the minimum recommendations for the growth phase (30 to 50 kg) according to Postano *et al.* (2017).

Ingredient	(%)	Calculated composition	(%)
Maize grain	76.291	Crude protein (%)	15.762
Soybean meal	20.314	Metabolizable energy (kcal/kg)	3239
Dicalcium phosphate	1.456	Ether extract (%)	3.485
Calcium limestone	0.775	Crude fiber (%)	3.191
Common salt	0.474	Av. phosphorus (%)	0.310
Min.-vit. premix <sup>1</sup>	0.300	Digestible methionine (%)	0.291
L-lysine HCL	0.303	Digestible methionine + cystine (%)	0.560
DL-methionine	0.043	Digestible lysine (%)	0.995
L-threonine	0.042	Sodium (%)	0.200
Total	100.000	Calcium (%)	0.700

<sup>1</sup>Folic acid (min.) 114.00mg/kg; Pantothenic acid (min.) 3,013.50mg/kg; Biotin (min.) 30.13mg/kg; Copper (min.) 28.35g/kg; Choline (min.) 16.67g/kg; Ethoxyquin (min.) 250.00mg/kg; Iron (min.) 30.15g/kg; Iodine (min.) 276.70mg/kg; Manganese (min.) 10.10 g/kg; Mineral matter (max.) 480.00g/kg; Niacin (min.) 5,596.90g/kg; Selenium (min.) 67.84 mg/kg; Vitamin A (min.) 2,250.00, IU/kg; Vitamin B1 (min.) 338.00 mg/kg. Vitamin B12 (min.) 5,625.00 mcg/kg; Vitamin B2 (min.) 942.00 mg/kg; Vitamin B6 (min.) 374.85 mg/kg; Vitamin D3 (min.) 450,000.00 IU/kg; Vitamin E (min.) 5,000.00 IU/kg; Vitamin K3 (min.) 300.00 mg/kg; Zinc (min.) 26.85 g/kg; Calcium (min.) 140.00 g/kg; Calcium (max.) 150.00 g/kg.

Water was available *ad libitum*. The amount of feed provided to the animals was defined based on intake during the adaptation phase and calculated relative to their metabolic weight. The DM, CP, mineral matter (MM), and gross energy (GE) contents of feces and feed samples were determined. Urine was analyzed for nitrogen content and GE. For the analysis of GE, the urine samples were initially oven dried. Dry matter, MM, and CP were analyzed by the method described by Detmann *et al.* (2021), and GE using a bomb calorimeter (model IKA 200). The DE, AME, and AMEn values of IWS were calculated using equations by Matterson (1965).

Sixty immunocastrated male pigs and 60 female pigs from the cross between MS115 - Pietran (62.5%), Large White (18.75%), and Duroc (18.75%) sires and F1 (Landrace × Large White) dams were used sequentially in the growth I ( $30.09 \pm 1.46$  kg and age  $75 \pm 1.4$  days), growth II ( $52.68 \pm 2.74$  kg and age  $100 \pm 1.4$  days), and finishing ( $77.80 \pm 5.15$  kg and age of  $128 \pm 1.4$  days) phases, which make up the total period. The diets were formulated to meet the nutritional requirements for each phase (Rostagno *et al.*, 2017). Equal diets were used for both sexes, considering the requirement of the females based on the recommendations for regular-performance sows with high genetic potential (Table 3). The experiment was laid out in a randomized block design (initial weight within sex). Four treatments were tested in 15 replicates per sex, with each replicate consisting of one animal. The animals were housed in two rooms, each of which contained 60 concrete pens with partially

slatted floors, with a total area of 2.5m<sup>2</sup> per pen. Treatments consisted of a 2 × 2 factorial arrangement represented by 1) Control: diet based on maize and soybean meal; 2) Control + Enzyme: control diet with the inclusion of the enzyme protease (EC 3.4.21.62); 3) IWS: diet including sequential levels of IWS; and 4) IWS + Enzyme: diet with sequential levels of IWS and protease (EC 3.4.21.62). The enzyme recommended usage rate: 0,05 per ton to provided 4000U/kg of feed. The nutritional matrix with the contribution of the enzyme used was recommended by the manufacturer (Table 4).

The enzyme used is produced by genetic modification of *Bacillus licheniformis*. Water and feed were available *ad libitum*. Orts were weighed every 14 days to calculate daily feed intake. The animals were also weighed every 14 days throughout the experimental period to monitor weight gain.

At the end of the performance trial, an *in vivo* evaluation was performed by ultrasound, using an Aloka SSD instrument, with measurements taken between the 10th and 13th ribs on the *longissimus dorsi* muscle to obtain images of the loin-eye area, backfat thickness (BFT), and length and depth of the muscle. The values were generated using Biotronics software (BioSoft Toolbox<sup>®</sup> II for pigs). The ultrasound data were then entered in an equation to predict the percentage of lean meat (PLM, %), using the values of BFT, loin depth (LD), and hot carcass weight (HCW) (Guidoni, 2000):

$$PLM \% = 65.92 - (0.685 * BFT) + (0.094 * LD) - (0.026 * HCW)$$

Ending the performance trial, the animals were weighed, fasted for 12 h and weighed again. Then, they were sent to a commercial slaughterhouse with Federal Inspection System (SIF 1). After 18 h of fasting, the pigs were stunned by electronarcosis, bled, shaved,

eviscerated, and weighed again and their carcasses were cooled at an average temperature of 4 °C for 24 h for further evaluation. Carcass dressing percentage was calculated by the formula below:

$$DP = [(HOT CARCASS WEIGHT * 100) / FASTING WEIGHT]$$

After weighing, the carcasses were sawn along the midline. The measurements performed on the left half carcass were in accordance with the Brazilian Carcass Assessment Method described

for Brazilian Association of Pig Breeders (1973). Backfat thickness was determined as an average from two measurements, taken at the first and last ribs, using a caliper.

Nutritional evaluation...

Table 3. Centesimal and calculated composition (as-is) of experimental diets for pigs in growth phase I (30 to 50 kg), growth phase II (70 to 50 kg), and finishing phase (70 to 90 kg)

Ingredient	Experimental diet (%)											
	Phase 1 (30 to 50 kg)				Phase 2 (50 to 70 kg)				Phase 3 (70 to 90 kg)			
	Control <sup>3</sup>	Control + enzyme <sup>4</sup>	IWS without enzyme <sup>5</sup>	IWS + enzyme <sup>6</sup>	Control <sup>3</sup>	Control + enzyme <sup>4</sup>	IWS without enzyme <sup>5</sup>	IWS + enzyme <sup>6</sup>	Control <sup>3</sup>	Control + enzyme <sup>4</sup>	IWS without enzyme <sup>5</sup>	IWS + enzyme <sup>6</sup>
Maize grain	65.588	68.263	55.447	57.339	69.022	71.698	60.928	62.813	73.266	75.955	66.034	67.918
Soybean meal	30.381	27.728	16.484	14.025	27.681	25.028	13.512	11.052	23.865	21.206	11.416	8.956
IWS	0.000	0.000	20.000	20.000	0.000	0.000	17.500	17.500	0.000	0.000	15.000	15.000
Wheat bran	0.000	0.000	5.531	6.038	0.000	0.000	5.839	6.357	0.000	0.000	5.483	6.003
Soybean oil	1.586	1.484	0.000	0.000	1.286	1.184	0.000	0.000	1.015	0.914	0.000	0.000
Limestone	0.959	0.962	0.960	0.968	0.879	0.882	0.859	0.871	0.775	0.790	0.739	0.752
Phosphate	0.249	0.269	0.151	0.158	0.036	0.056	0.000	0.000	0.000	0.000	0.000	0.000
Common salt	0.434	0.435	0.426	0.426	0.400	0.400	0.393	0.393	0.373	0.373	0.373	0.373
Adsorbent	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Min. vit. premix <sup>1</sup>	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
L-lysine HCl	0.140	0.172	0.236	0.262	0.100	0.132	0.241	0.267	0.117	0.149	0.246	0.272
DL-methionine	0.071	0.078	0.125	0.129	0.038	0.045	0.102	0.106	0.036	0.043	0.093	0.097
L-threonine	0.041	0.053	0.090	0.099	0.015	0.027	0.083	0.092	0.016	0.028	0.078	0.087
Choline chloride	0.035	0.035	0.035	0.035	0.027	0.027	0.027	0.027	0.021	0.021	0.021	0.021
Phytase <sup>2</sup>	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Protease	0.000	0.005	0.000	0.005	0.000	0.005	0.000	0.005	0.000	0.005	0.000	0.005
Enramax 8%	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006
Cost BRL kg/feed	2.045	2.033	2.030	2.026	1.998	1.987	1.980	1.970	1.955	1.943	1.939	1.930
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition												
CP (%)	17.95	17.35	18.91	18.41	16.90	16.29	17.13	16.62	15.489	14.883	15.613	15.110
ME (kcal/kg)	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300	3300
Ether extract (%)	4.19	4.16	6.67	6.73	3.99	3.95	6.29	6.35	3.82	3.79	5.89	5.95
Crude fiber (%)	2.93	2.84	3.27	3.22	2.85	2.76	3.14	3.09	2.72	2.63	2.99	2.94
Av. phosphorus (%)	0.328	0.328	0.328	0.328	0.285	0.285	0.295	0.294	0.273	0.270	0.289	0.288
Methionine+ cystine	0.629	0.626	0.700	0.698	0.572	0.569	0.633	0.631	0.533	0.531	0.586	0.585
Dig. lysine (%)	0.968	0.968	0.968	0.968	0.875	0.875	0.875	0.875	0.885	0.879	0.993	0.988
Sodium (%)	0.185	0.185	0.185	0.185	0.171	0.171	0.171	0.171	0.160	0.160	0.163	0.162
Calcium (%)	0.663	0.663	0.663	0.663	0.577	0.577	0.577	0.577	0.520	0.520	0.520	0.520
Dig. threonine (%)	0.736	0.731	0.828	0.824	0.671	0.666	0.749	0.746	0.616	0.611	0.683	0.679
Dig. tryptophan (%)	0.223	0.221	0.252	0.253	0.207	0.206	0.223	0.223	0.185	0.184	0.198	0.198

IWS - inactivated whole soy; BRL - Brazilian Real; CP- crude protein; ME - metabolizable energy. <sup>1</sup>Folic acid (min.) 114.00 mg/kg; Pantothenic acid (min.) 3,013.50 mg/kg; Biotin (min.) 30.13 mg/kg; Copper (min.) 28.35 g/kg; Choline (min.) 16.67 g/kg; Ethoxyquin (min.) 250.00 mg/kg; Iron (min.) 30.15 g/kg; Iodine (min.) 276.70 mg/kg; Manganese (min.) 10.10 g/kg; Mineral matter (max.) 480.00 g/kg; Niacin (min.) 5,596.90 g/kg; Selenium (min.) 67.84 mg/kg; Vitamin A (min.) 2,250.00 IU/kg; Vitamin B1 (min.) 338.00 mg/kg; Vitamin B12 (min.) 5,625.00 mcg/kg; Vitamin B2 (min.) 942.00 mg/kg; Vitamin B6 (min.) 374.85 mg/kg; Vitamin D3 (min.) 450,000.00 IU/kg; Vitamin E (min.) 5,000.00 IU/kg; Vitamin K3 (min.) 300.00 mg/kg; Zinc (min.) 26.85 g/kg; Calcium (min.) 140.00 g/kg; Calcium (max.) 150.00 g/kg; <sup>2</sup> Phytase (EC 3.1.3.26) *Buttiauxella spp.* <sup>3</sup>Control: diet based on maize and soybean meal; <sup>4</sup>Control + protease enzyme; <sup>5</sup> Diet with IWS and without enzyme protease; <sup>6</sup> Diet with IWS and with enzyme protease.

Table 4. Nutritional matrix of protease (EC 3.4.21.62) in the formulation of the experimental diets

Component	CP contribution/Amino acid (%)
Crude protein	0.36
Lysine	0.036
Threonine	0.023
Tryptophan	0.014
Valine	0.040
Cysteine	0.010
Isoleucine	0.033
Leucine	0.047
Methionine	0.005

Economic viability analysis was performed for each phase and for the total experimental period. Initially, the cost of feed per kilogram of weight gained (in Brazilian Real, BRL) was determined, as described by Bellaver and Snizek (1985). To calculate the feed cost, the price of each ingredient used in the experimental diets was considered as charged in the state of Santa Catarina, Brazil, in April 2021. Then, the Economic Efficiency Index (EEI) was calculated as suggested by Tavernari *et al.* (2009):

$$EEI = (LC / CT) * 100$$

in which LC = lowest diet cost/kg of weight gained, among the treatments; CT = cost of the treatment considered.

$$y_{ijkl} = \mu + b_{ij} + Se_j + So_k + E_l + SeSo_{jk} + SeE_{jl} + SoE_{kl} + SeSoE_{jkl} + e_{ijkl}$$

Where:  $y_{ijkl}$ , is the value observed in the  $i$ -th block within the  $j$ -th gender,  $k$ -th deactivated soybean level and  $l$ -th enzyme level;  $\mu$ , is a fixed factor that represents the general average of the observations of the experiment;  $b$  represents the block effect within gender;  $Se$ ,  $So$  and  $E$  represent the main effects of sex, deactivated soy and enzyme;  $SeSo$ ,  $SeE$ ,  $SoE$  and  $SeSoE$  represent the interactions of the factors included in the model and  $e_{ijkl}$  represents the unobservable random experimental error assumed to follow the normal probability distribution with zero mean and constant variance  $\sigma^2$ . The effect of the factors evaluated in the model was considered significant whenever the  $F$  test presented a descriptive level of probability lower than 5% ( $p \leq 0.05$ ). Occasional detailing of significant effects was obtained by Tukey's test for multiple comparison of means.

## RESULTS

The DM, OM, and CP digestibility coefficients of IWS were  $83.77\% \pm 2.02$ ,  $84.44\% \pm 2.08$ , and  $89.19\% \pm 5.94$ , respectively; and the DE, AME, and AMEn values were  $4904 \pm 117$ ,  $4805 \pm 273$ , and  $4656 \pm 255$  kcal/kg DM, respectively.

Gross revenue (BRL) in the period was calculated as the price of pigs (BRL/kg of live weight) multiplied by weight gain per animal in the period (kg). Net revenue (BRL) was calculated as gross revenue in the period (BRL) minus the feed cost (BRL). For the remuneration on carcasses, the value of BRL 6.91/kg adopted in April 2021 was used.

The experimental design for the performance experiment was in randomized blocks in a factorial arrangement [two sexes  $\times$  two levels of deactivated soybean (with and without)  $\times$  two levels of enzyme inclusion (with and without)].

The data were submitted to analysis of variance through the SAS GLM procedure (2012) according to the model below:

There was no interaction effect between the factors ( $P > 0.05$ ) on performance or carcass traits in each phase or in the total experimental period (Table 5).

When the main factors were evaluated, there was no effect ( $P > 0.05$ ) on performance in the growth II and finishing phases or in the total period. In growth phase I, protease had a significant effect on average daily feed intake ( $P = 0.04$ ), which was higher in the pigs fed diets containing the enzyme. Neither IWS nor protease affected ( $P > 0.05$ ) the evaluated carcass traits (Tab. 6). But for carcass evaluation, the gender had a significant effect with female carcasses presenting ( $69.87\% \pm 0.12$ ) higher carcass yield than immunocastrated male pigs ( $69.45\% \pm 0.11$ ).

When evaluating economic viability there was no significant effect ( $P > 0.05$ ) on any of parameters in the phases or in the total period, except in growth phase I (30 to 50 kg of LW), when there was an interaction effect between the factors ( $P = 0.05$ ) on feed cost per kilogram of weight gained, EEI, and net revenue.

*Nutritional evaluation...*

Table 5. Effect of inactivated whole soy (IWS) and exogenous protease on growth performance (mean ± SE) of pigs

IWS/Enzyme	ADWG (kg/d)	ADFI (kg/d)	FC (kg/kg)	ADWG (kg/d)	ADFI (kg/d)	FC (kg/kg)
Phase 1 (30 to 50 kg)			Phase 2 (50 to 70 kg)			
<b>IWS</b>						
With	0.740±0.01	1.608±0.01	2.138±0.02	0.984±0.01	2.345±0.03	2.387±0.02
Without	0.761±0.01	1.634±0.02	2.157±0.02	0.981±0.01	2.287±0.03	2.345±0.02
<b>Protease</b>						
With	0.739±0.01	1.657±0.01a	2.156±0.02	0.986±0.01	2.314±0.03	2.356±0.02
Without	0.762±0.01	1.585±0.02b	2.183±0.02	0.979±0.01	2.318±0.03	2.377±0.02
<b>Probability</b>						
IWS	0.108	0.285	0.357	0.848	0.154	0.239
Protease	0.066	0.004	0.504	0.715	0.871	0.521
IWSx Protease	0.137	0.915	0.060	0.433	0.881	0.247
Phase (70 to 90 kg)			Total period			
<b>IWS</b>						
With	1.090±0.01	3.189±0.06	2.929±0.03	0.942±0.01	2.393±0.03	2.540±0.01
Without	1.076±0.02	3.193±0.07	2.972±0.04	0.942±0.01	2.380±0.03	2.530±0.02
<b>Protease</b>						
With	1.087±0.02	3.186±0.07	2.934±0.04	0.939±0.01	2.370±0.03	2.523±0.02
Without	1.080±0.01	3.195±0.06	2.964±0.03	0.934±0.01	2.402±0.03	2.547±0.02
<b>Probability</b>						
IWS	0.464	0.975	0.367	0.994	0.856	0.878
Protease	0.667	0.857	0.419	0.763	0.332	0.284
IWS × Protease	0.639	0.616	0.167	0.902	0.595	0.285

Table 6. Effect of inactivated whole soy (IWS) and exogenous protease (mean ± SE) on pig carcass quality

IWS /Enzyme	Muscle area	Fat area	DP	BFT first rib	BFT last rib	Muscle/ Fat ratio
<b>IWS</b>						
With	44.58±0.01	19.08±0.06	69.56±0.01	24.52±0.05	15.90±0.05	2.44±0.08
Without	43.65±0.08	20.16±0.08	69.87±0.01	24.92±0.07	16.82±0.05	2.26±0.08
<b>Protease</b>						
With	43.37±0.08	19.60±0.06	69.72±0.01	24.31±0.07	16.20±0.05	2.33±0.08
Without	44.88±0.08	19.69±0.05	69.60±0.01	24.50±0.06	16.52±0.05	2.37±0.01
<b>Probability</b>						
IWS	0.544	0.363	0.121	0.824	0.159	0.326
Protease	0.148	0.667	0.389	0.638	0.549	0.829
IWS x Protease	0.259	0.993	0.620	0.110	0.982	0.449

SE - standard error; DP - dressing percentage; BFT - backfat thickness.

The effect of the IWS and enzyme interaction are in Table 7. Inactivated whole soy affected feed cost (BRL/kg weight gained) in the treatments without the enzyme (P = 0.0295) in growth phase I (30 to 50), with the diet containing IWS being

more expensive than without the ingredient. Nevertheless, the inclusion of IWS resulted in a higher EEI (P = 0.0195) and a higher net revenue (P = 0.0241).

Table 7. Economic indices of the decomposed IWS x Enzyme interaction effect in growth phase I (30 to 50 kg)

IWS/Enzyme	Without enzyme	With enzyme	Mean	ProbF
	Feed cost/kg weight gained (BRL/kg)			
With IWS	4.496±0.068	4.407±0.058	4.451±0.044	0.2514
Without IWS	4.308±0.074	4.455±0.075	4.380±0.053	0.1162
Mean	4.400±0.051	4.431±0.047	4.415±0.036	0.7606
ProbF	0.0295	0.5992		
Economic efficiency index (%)				
With IWS	79.01±1.15	80.49±1.02	79.75±0.77	0.2796
Without IWS	82.62±1.36	79.82±1.25	81.24±0.94	0.0885
Mean	80.84±0.92	80.16±0.80	80.50±0.61	0.6545
ProbF	0.0195	0.6690		
Net revenue (BRL)				
With IWS	255.05±3.01	259.80±2.82	257.47±2.07	0.0759
Without IWS	261.01±2.98	258.04±2.95	259.53±2.09	0.3667
Mean	258.03±2.14	258.94±2.03	258.49±1.47	0.5384
ProbF	0.0241	0.6973		

BRL – Brazilian Real.

## DISCUSSION

Toledo *et al.* (2011) tested hulled IWS for piglets in the nursery phase and determined digestibility coefficients of DM ( $DC_{DM}$ ) and CP ( $DC_{CP}$ ) of 94.20% and 92.18%, respectively, which are higher than those determined in the present experiment (83.77% and 89.18%). Oliveira Junior *et al.* (2017) reported lower results, with a  $DC_{CP}$  of 79.99% in IWS for growing pigs. In a study aimed at determining the digestibility coefficients of extruded semi-whole soybeans for piglets in the starter phase, Thomaz *et al.* (2012) observed a  $DC_{DM}$  of 88.87% and a  $DC_{CP}$  of 86.81%. These values are similar to the 88.1% ( $DC_{DM}$ ) and 90.0% ( $DC_{CP}$ ) registered by Rostagno *et al.* (2017) in soybean meal with 44% CP.

Mendes *et al.* (2004) evaluated the chemical composition and nutritional value of raw soybean subjected to different thermal processes for growing pigs and found the respective DE and apparent  $DC_{CP}$  and  $DC_{DM}$  values in the tested feedstuffs: 3583 kcal/kg, 90.8%, and 85.7% in soybean meal; 4065 kcal/kg, 86.1%, and 81.9% in extruded semi-whole soy; 3803 kcal/kg, 73.9%, and 74.4% in expanded whole soy; and 5272 kcal/kg, 95.2%, and 93.2% in micronized whole soy. Those authors observed that the extrusion of semi-whole soy and the

micronization of whole soy were efficient in inactivating anti-nutritional factors and improving their digestibility. The DE and apparent  $DC_{CP}$  and  $DC_{DM}$  values determined in IWS in the present study are within the range of results presented by the various cited authors, with variability due to processing.

Ludke *et al.* (2007) worked with different forms of soybean processing for growing pigs and found the following AMEn and ether extract (EE) values in soy inactivated by heating reactor and steam under pressure, soy inactivated by heating through dielectric loss, soy inactivated by dry extrusion, and semi-defatted extruded soy: 4,124kcal/kg and 18.52%; 4,199kcal/kg and 18.84%; 5,122kcal/kg and 18.55%; and 3,510kcal/kg and 8.74%, respectively. Considering the AMEn values calculated in the present study (4656kcal/kg and 25.96% EE), we can confirm that the inactivation process was carried out satisfactorily. In addition, these energy values are higher when compared with that of soybean meal with 44% CP, according to Rostagno *et al.* (2017). This is because IWS does not undergo the defatting process, having the advantage of requiring less or even no oil supplementation in the feed and thus increasing the economic profitability of pig production.

There was a significant increase in intake in growth phase I (30 to 50 kg), which was likely due to the enhancement of the enzyme, since the diet containing this additive was formulated by attributing a nutritional matrix to the enzyme in crude protein and digestible essential amino acids. The animal would compensate for the marginal level of these nutrients by increasing its intake, but the enzyme had an action, causing the absolute value of weight gain to increase by 3.0% and thus not affecting feed conversion. For Aranda-Aguirre *et al.* (2021), the mode of action and main effects of proteases used in diets for growing pigs are to improve the apparent total digestibility of dry matter, crude energy, crude protein and blood urea nitrogen.

Min *et al.* (2019) and Ruiz *et al.* (2008) reported different results, as they did not observe a significant effect on the intake of animals fed diets containing protease. However, the enzyme was added in the “on top” strategy, which consists of supplementing the enzyme over a standard formulation. The present study, in contrast, considered the nutritional value of the enzyme and its contribution to the digestible amino acids in the diet, corroborating the above explanation.

Nery *et al.* (2000) observed that the addition of enzymes (protease, amylase, and lipase) for piglets weighing 10 to 30 kg did not significantly influence weight gain or feed intake, but improved feed conversion, with protease supplementation. The authors argued that the better feed conversion was because there was an improvement in the digestibility of nutrients, particularly protein, which animals in this phase require in larger amounts, retaining more of it.

The inclusion of protease had no significant impact on the performance variables of the animals in the other phases, nor on carcass traits. The lack of significant effects on performance with the use of protease in diets for growing and finishing pigs may indicate that the effect of the enzyme compensated for the nutritional matrix used in the diets, improving nutrient digestibility. On the other hand, it is also possible that, even in the diets including the enzyme, the nutritional levels used were satisfactory to meet the requirements of the tested categories.

Amorim *et al.* (2011) found that the addition or non-addition of enzyme complex for pigs in the same phases did not affect the performance variables. Likewise, Payling *et al.* (2017) observed similar results using *Bacillus* spp. and the protease enzyme in diets for growing pigs, that is, no effect was detected on performance or carcass traits. The main of this study suggested mode of action of proteases is increasing the hydrolysis of proteins in the small intestine (Olukosi *et al.*, 2015), liberating peptides and amino acids for absorption and utilization, however, proteases use in pigs has inconsistent results. In the study carried out by Ramani *et al.* (2021) with enzyme supplementation efficacy on pig gut health, the authors stated that the ameliorating effect of enzyme supplementation on feed appears to be based on the substrate which is added.

When the inclusion of IWS was evaluated, no effect was observed on the performance or carcass variables. The diets with IWS had a higher EE content (6.67%) than those without the ingredient (4.16%). According to Maciel (2012), fats can improve the digestibility of diet nutrients, as they are potent inhibitors of gastric emptying. Nonetheless, the presence of IWS did not significantly increase performance or the fat content in the carcass of the animals. This was likely because the diets were isoenergetic, as the treatments containing IWS were formulated with the addition of 5.53% wheat bran to dilute the energy (because IWS has a higher energy content than soybean meal).

In this way, the fiber content in the diets containing IWS was increased from 2.93% to 5.2%, causing heat increment to grow by 0.34%. Thus, when the Net Energy for growth ( $Ne_g$ ) of these diets was estimated using the equation described by Rostagno *et al.* (2017), the results were 2466 kcal/kg (diet without IWS) and 2618 kcal/kg (diet with IWS), showing the proximity of both diets in GE content. Therefore, even with a higher fat content and a slight increase in  $Ne_g$ , these differences were not enough to induce changes in animal performance or carcass traits.

The result is like that described by Toledo *et al.* (2011), who did not observe an effect of IWS (with or without hull) on the performance of piglets weighing 6 to 15 kg. Ludke *et al.* (2007) also did not observe a significant difference after

including 8% whole soy processed in different forms, which had EE content of 18.64%, lower than that of the ingredient used in this study.

The muscle/fat ratio was similar between pigs fed the diets with and without IWS, demonstrating that despite increasing the fat content of the diet, this ingredient did not affect muscle or fat deposition in the animals. Rather, its use had a positive effect, given the appreciation of carcasses with a higher meat content.

For Sakomura and Rostagno (2016), to optimize the efficiency of utilization of a feedstuff, it is essential to know its energy value as well as its nutritional and economic importance when related to feed formulation.

With the addition of IWS, the cost per kilogram of feed decreased in all phases. Considering the economic parameters of cost of feed per kilogram of weight gained, economic efficiency, and net revenue, only in growth phase I (30 to 50kg) was there an interaction of factors, that is, the diets containing IWS showed significantly better values for these variables, but the same effect did not occur when the enzyme was present. Even though the price of this ingredient is a little higher (BRL 2.66/kg) than that of soybean meal (BRL 2.48/kg), its use did not imply an increase in feed cost, due to the lack of addition of soybean oil (BRL 6.4/kg), as well as the reduced inclusion of soybean meal and maize (BRL 1.63). In addition, to obtain these parameters, the cost was related to the intake and weight gain of the animals. Therefore, even with an increase in feed intake, the trend ( $P = 0.066$ ) of increase in weight gain was sufficient to improve the economic viability of using IWS.

These results agree with reports by Toledo *et al.* (2011), who evaluated two types of IWS at three inclusion percentages (4.5, 9.0, and 13.5%) in the diet of piglets. Those authors concluded that the two types of inactivated soy (with and without hulls) can be included in the diet of piglets up to the highest tested level, as there was a linear decrease in feed cost per kilogram of weight gained with increasing IWS inclusion. Those authors inferred that its use up to this percentage will depend on the price relationship between the ingredients.

The enzyme did not change the cost of the diets, regardless of the inclusion or non-inclusion of IWS, in any of the studied phases, demonstrating the possibility of its supplementation in swine diets. This may have been due to the nutritional values enhancement of the enzyme, which resulted in a reduction in the cost per kilogram of feed with its addition. In contrast, Biancalana *et al.* (2018) added a multienzyme complex (phytase, protease, xylanase,  $\beta$ -glucanase, cellulase, amylase, and pectinase) “on top” of the diet of finishing pigs and obtained a higher feed cost and an unviable economic index.

## CONCLUSION

The present study showed that inactivated whole soy has a high energy value (4656 kcal/kg) and its use in diets for growing-finishing pigs provides satisfactory performance. When added in conjunction with nutritional enhancement, the protease enzyme does not increase the feed cost of these animals.

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