












Nutritional functionality of the xylanolytic complex obtained from *Aspergillus japonicus* var. *aculeatus* UFMS 48.136 in swine diets

Funcionalidade nutricional do complexo xilanolítico obtida de *Aspergillus japonicus* var. *aculeatus* UFMS 48.136 em dietas para suínos

Fernanda Aparecida de Oliveira¹ , Charles Kiefer^{*1} , Karina Márcia Ribeiro de Souza Nascimento¹ , Giovana Cristina Giannesini¹ , Fabiana Fonseca Zanoelo¹ , Anderson Corassa² , Elis Regina de Moraes Garcia³ , Ulisses Simon da Silveira³ , Tânia Mara Baptista dos Santos³ 

1 Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brazil

2 Universidade Federal de Mato Grosso (UFMT), Sinop, Mato Grosso, Brazil

3 Universidade Estadual do Mato Grosso do Sul (UEMS), Aquidauana, Mato Grosso do Sul, Brazil

*corresponding author: charles.kiefer@ufms.br

Abstract: The objective of this study was to evaluate the nutritional functionality of the xylanolytic complex produced from *Aspergillus japonicus* var. *aculeatus* UFMS 48.136 isolated from the Cerrado/Pantanal biome in Mato Grosso do Sul, compared to commercial xylanase, in swine diets. Sixteen barrows were used, with an initial weight of 64.23 ± 10.5 kg, distributed in a randomized block experimental design, with four diets: control, formulated according to nutritional recommendations; negative control, formulated with a reduction of 100 Kcal / kg of metabolizable energy (ME); negative control + xylanase Cerrado / Pantanal; negative control + commercial xylanase; with four repetitions each. The xylanase supplementation provided higher ($P < 0.05$) values of digestible energy (DE), ME, and higher ($P < 0.05$) digestibility of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in relation to the negative control diet, but without differing ($P > 0.05$) from the control diet. The inclusion of xylanases increased ($P < 0.05$) in the coefficients of digestibility (CD) and metabolism of crude energy (CE), DM, OM, CP, EE, NDF, and ADF. There was no difference ($P > 0.05$) in digestibility and CD values between Cerrado/Pantanal and commercial xylanase. The inclusion of xylanases made it possible to reduce 100 Kcal of ME per kilogram of diet. Cerrado/Pantanal xylanases therefore have the same nutritional efficiency as commercial xylanases.

Keywords: additives; carbohydrases; digestibility; energy; enzymes.

Resumo: Realizou-se este estudo com o objetivo de avaliar a funcionalidade nutricional do complexo xilanolítico produzido a partir de fungos da linhagem *Aspergillus japonicus* var. *aculeatus* UFMS 48.136, oriundo do bioma Cerrado/Pantanal sul mato-grossense em comparação à xilanase comercial, em dietas de suínos. Foram utilizados dezesseis suínos machos, com peso inicial de $64,23 \pm 10,5$ kg, distribuídos em delineamento experimental de blocos ao acaso, com quatro dietas: controle, formulado de acordo com as recomendações nutricionais; controle negativo, formulado com redução de 100 Kcal /

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kg de energia metabolizável (EM); controle negativo + xilanase Cerrado/Pantanal; controle negativo + xilanase comercial; com quatro repetições cada. A suplementação das xilanasas proporcionou maiores ($P < 0,05$) valores de energia digestível (ED), metabolizável (EM) e maiores ($P < 0,05$) digestibilidade da matéria seca (MS), matéria orgânica (MO), proteína bruta (PB), extrato etéreo (EE), fibra em detergente neutro (FDN) e fibra em detergente ácido (FDA) em relação à dieta controle negativo, mas sem diferir ($P > 0,05$) da dieta controle. A inclusão das xilanasas proporcionou aumento ($P < 0,05$) nos coeficientes de digestibilidade (CD) e metabolizabilidade da energia bruta (EB), MS, MO, PB, EE, FDN e FDA. Não foi constatada diferença ($P > 0,05$) nos valores de digestibilidade e de CD entre as xilanase Cerrado/Pantanal e Comercial. A inclusão das xilanasas possibilita a redução de 100 Kcal de EM por kg da dieta. A xilanase Cerrado/Pantanal possui a mesma eficiência nutricional em comparação com a xilanase comercial.

Palavras-chave: aditivos; carboidrases; digestibilidade; energia; enzimas.

1. Introduction

Pig diets may contain non-starch polysaccharides (NSPs) that cannot be digested by pigs because of their glycosidic bonds. Corn and soybean meal, which are considered good quality feed for pigs, contain 6.83 and 16.46% of NSPs, respectively ⁽¹⁾.

In addition to containing nutrients that are unavailable to animals, NSPs can impair nutrient absorption by increasing the viscosity of the digesta ⁽²⁾, reduce the diffusion rate of particles, and reduce enzyme-substrate contact ⁽³⁾. Microorganisms have developed various enzymes that degrade NSPs. These microorganisms transport nutrients through the plasma membrane and secrete exoenzymes that hydrolyze the macromolecules present in their substrates ⁽⁴⁾. Filamentous fungi are microorganisms that produce enzymes. They are easy to grow, their cultivation conditions are easily controlled, take up little space, and grow wherever a carbon source is available ^(4, 5).

There are several benefits to using enzymes such as xylanase in pig diets. Xylanase acts on NSPs ⁽⁶⁾ and hydrolyzes the structure of arabinoxylans ⁽⁷⁾, exposing starch and other stored nutrients to endogenous enzymes and microbial fermentation ^(8, 9). Xylanase reduces the amount of undigested substrate during ileal digestion, reducing the viscosity of the digesta and enabling substrate/enzyme contact. This alters the composition of the substrate accessible to the microbiota of the large intestine ⁽⁹⁾. Xylanase can indirectly improve protein accessibility ⁽⁹⁾, alter the microbiota in the large intestine ⁽¹⁰⁾, increase motility in the gastrointestinal tract, and reduce stool volume ⁽¹¹⁾. In addition to improving the intestinal microecology ⁽¹²⁾, xylanase also improves the intestinal physical barrier, immune barrier functions in piglets ⁽¹³⁾, and digesta pH ⁽¹⁴⁾.

Thus, xylanase supplementation can be considered a viable alternative for increasing digestibility ⁽¹⁵⁾, nutrient availability ⁽¹⁶⁾, nutritional value ⁽¹⁷⁾, and ME value of diets ⁽¹⁸⁾. They also improve animal performance ^(17, 19, 20). Therefore, this study aimed to evaluate the nutritional functionality of the xylanolytic complex produced by the fungal strain *Aspergillus japonicus* var. *aculeatus* UFMS 48.136, from the Cerrado/Pantanal biome in Mato Grosso do Sul, compared to commercial xylanase, in pig diets.

2. Material and methods

The experiments were conducted at the Federal University of Mato Grosso do Sul (UFMS). All procedures and practices for the use of animals were in accordance with the ethical principles of animal experimentation and approved by the UFMS Animal Use Ethics Committee under protocol number 1124/2020. Sixteen barrows were used, with an initial weight of 64.23 ± 10.5 kg, housed individually.

The enzymes used in this study were xylanases produced from the fungus *A. japonicus* UFMS 48.136, isolated from soils in the region of Mato Grosso do Sul, identified ⁽²¹⁾, maintained in the mycotek of UFMS/Campo Grande/MS, and a commercial xylanase (Natugrain). To obtain the xylanases from *A. japonicus*, the Sorgatto-Rizzatti (SR) liquid medium was used, following the methodology of Rizzatti et al. ⁽²²⁾ using wheat bran as a carbon source to induce the enzyme. After growing at 30 °C for 96 hours, the medium was filtered to obtain the xylanolytic complex.

The animals were distributed in a randomized block design, with four diets (control diet formulated according to the nutritional recommendations for the phase, negative control diet formulated with a reduction of 100 Kcal/kg of metabolizable energy, negative control diet + xylanase Cerrado/Pantanal UFMS, and negative control diet + commercial xylanase) and four replicates, each experimental unit consisting of one animal. The initial weight of the animals was recorded when the blocks were formed.

The experimental diets (Table 1) were prepared from corn and soybean meal supplemented with vitamins, amino acids, and minerals, and formulated according to previous recommendations ⁽¹⁾. Commercial xylanase (Natugrain) and the Cerrado/Pantanal UFMS xylanolytic complex were included in the diets in the same proportion of 100 g t⁻¹, following the manufacturer's recommendation to contain a minimum of 10,000 UX/g of dietary product. Enzymes were added to replace the inert material (kaolin). The animals had non-restricted access to food and water. The digestibility trial lasted eight days, with four days of adaptation to the diet and four days of feces collection.

Table 1 Centesimal and nutritional composition of experimental diets

Ingredients, %	Control	Negativ control	Cerrado/Pantanal xylanase	Commercial xylanase
Corn 7.86%	70.23	70.23	70.23	70.23
Soybean meal 46.5%	23.46	23.46	23.46	23.46
Inert (kaolin)	1.85	3.06	3.05	3.05
Dicalcium phosphate	1.47	1.47	1.47	1.47
Soybean oil	1.21	0.00	0.00	0.00
Limestone	0.64	0.64	0.64	0.64
Salt	0.46	0.46	0.46	0.46
L-Lysine HCl	0.35	0.35	0.35	0.35
L- Threonine	0.09	0.09	0.09	0.09

DL-Methionine	0.08	0.08	0.08	0.08
L-Tryptophan	0.01	0.01	0.01	0.01
Vitamin premix ¹	0.10	0.10	0.10	0.10
Mineral premix ²	0.05	0.05	0.05	0.05
Cerrado/Pantanal xylanase	0.00	0.00	0.01	0.00
Commercial xylanase	0.00	0.00	0.00	0.01
Nutritional composition calculate*				
Crude Protein, %	16.86	16.86	16.86	16.86
ME, Kcal/kg	3,230	3,130	3,130	3,130
Digestible lysine, %	1.006	1.006	1.006	1.006
Digestible Meth + cyst, %	0.563	0.563	0.563	0.563
Digestible threonine, %	0.634	0.634	0.634	0.634
Digestible tryptophan, %	0.181	0.181	0.181	0.181
Sodium, %	0.200	0.200	0.200	0.200
Digestible phosphorus, %	0.340	0.340	0.340	0.340
Calcium, %	0.722	0.722	0.722	0.722

¹ Content per kg of product: Vit. A, 6,000,000UI; Vit. D3, 1,000,000UI; Vit. E, 12,000UI; Vit. B1, 0.5g; Vit. B2, 2.6g; Vit. B6, 0.7g; panthothenic acid, 10g; Vit. K3, 1.5g; niacin, 22g; Vit. B12, 0.015g; folic acid, 0.2g; biotin, 0.05g; colin, 100g e excipient, 1,000g.

² Content per kg of product: iron, 100g; copper, 10g; cobalt, 0.2g; manganese, 30g; zinc, 100g; iodine, 1.0g; selenium, 0.3g and excipient, 1,000g.

* Values calculated based on the nutritional composition of the raw materials ⁽¹⁾.

The methodology adopted was an indigestibility indicator analysis and partial collection of feces. Titanium dioxide was used as an indigestibility indicator, according to the following formula:

Indigestibility factor (IF) in feces: $IF = \frac{[TiO_2] \text{ in the diet}}{[TiO_2] \text{ sample (feces)}}$, where $[TiO_2]$ concentration of titanium dioxide.

Metabolizability coefficient (CM): $CM = \frac{(\% \text{ of nutrient in diet}) - (\% \text{ of nutrient in feces} \times FI)}{(\% \text{ of nutrient in diet})}$.

Stool samples were collected daily at 8 am and 3 pm, weighed, placed in labeled plastic bags, and stored in a freezer. At the end of the experiment, the feces were thawed, pooled by repetition and homogenized, removing an aliquot of 700 g, which was kept in a forced ventilation oven for 72 hours at 55 °C, for drying. The samples were then weighed, ground, and packed for analysis.

The dry matter (DM), organic matter (OM), ether extract (EE), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of the diets and feces were determined according to a previously described methodology ⁽²³⁾. The gross energy (GE) of the diet and feces was determined using a calorimeter.

Digestible energy (DE) values were determined from crude energy (CE) concentrations in the diets and feces. Metabolizable energy (ME) values were estimated considering 50% protein retention ⁽²⁴⁾, where $ME = DE - \text{energy lost in urine (EU)}$; and $EU = \text{digestible protein (DP, g N of the diet)} \times (10 - 50\% \text{ retention})$, considering a loss of 9.17 Kcal/g of N in urine ⁽²⁵⁾. The nutrient digestibility coefficients were calculated according to the literature ⁽²⁶⁾.

During the experimental period, the temperature and relative humidity of the house were monitored daily using dry- and wet-bulb and black-globe thermometers. The recorded values were converted into the black-globe temperature and humidity index (BGTHI) to characterize the thermal environment in which the animals were kept. The maximum and minimum temperatures were 31.7 ± 1.25 °C and 24 ± 1.0 °C, respectively. The air temperature recorded inside the shed was 28.9 ± 1.0 °C, the relative humidity was $90.9 \pm 5.4\%$, the black globe temperature was 28.8 ± 1.4 °C and the BGTHI was 79.9 ± 1.54 .

The data obtained were subjected to analysis of variance using the GLM procedure. The differences between the means of the diets were compared using an orthogonal contrast test. The contrasts tested were as follows: 1) control diet versus negative control, 2) control diet versus diets containing xylanases, 3) negative control diet versus diets containing xylanases, 4) negative control diet versus Cerrado/Pantanal xylanase, 5) negative control diet versus commercial xylanase, and 6) Cerrado/Pantanal xylanase diet versus commercial xylanase. Analyses were performed using SAS statistical program at a 5% significance level.

3. Results

There were higher DE and ME values ($P < 0.05$) and higher MO and EE digestibility values ($P < 0.05$) in the control diet group compared to the negative control diet (Table 2). The digestibility of DM, CP, NDF and ADF were similar ($P > 0.05$) between the control and negative control diets. Supplementation with xylanases resulted in higher ($P < 0.05$) digestibility values for DM, CP, NDF, and ADF than the control diet. However, the control diet showed higher EE digestibility ($P < 0.05$) than diets containing xylanases. There was no significant difference ($P > 0.05$) in DE, ME, and MO digestibility between the control diet and diets containing xylanase.

Supplementation of the diets with xylanases resulted in higher ($P < 0.05$) DE and ME values, and higher ($P < 0.05$) digestibility of DM, DM, CP, EE, NDF, and ADF than the negative control diet. There were no significant differences ($P > 0.05$) in DE, ME, and digestibility of DM, MO, CP, EE, NFD, and ADF between the negative control diet and the diet containing commercial xylanase.

Supplementation with commercial xylanase resulted in a higher ME value ($P < 0.05$) than the control diet. There were no differences ($P > 0.05$) in the DE values and digestibility of DM, OM, CP, EE, NDF, and ADF between the negative control diet and the commercial xylanase diet. There were no significant differences ($P > 0.05$) in DE, ME, and digestibility of DM, MO, CP, EE, NFD, and ADF between the diets containing Cerrado/Pantanal xylanase and commercial xylanase.

The EE digestibility coefficient of the control diet was significantly higher ($P < 0.05$) than that of the negative control diet (Table 3). However, the digestibility coefficient and metabolizability of CE, and the digestibility coefficients of DM, DM, CP, NDF, and ADF were similar ($P > 0.05$) between the control and negative control diets.

Table 2 Digestibility of diets containing Cerrado/Pantanal xylanase and commercial xylanase for growing pigs

Diets ¹	Variables ²							
	DE, Kcal/kg	ME, Kcal/kg	DM dig, %	OM dig, %	CP dig, %	EE dig, %	NDF dig, %	ADF dig, %
C	3,267	3,115	70.29	76.99	17.55	11.46	19.18	4.55
NC	3,172	3,063	70.36	73.86	17.95	4.09	18.64	4.53
CPX	3,271	3,178	72.73	76.59	18.51	4.34	20.21	5.91
CX	3,254	3,133	72.51	76.22	18.43	4.39	20.13	5.64
CV, %	1.83	2.29	1.87	2.02	2.09	2.13	3.87	8.03
Contrasts values P> ³								
C x NC	0.015	0.042	0.932	0.003	0.090	<0.001	0.235	0.945
C x Xs	0.888	0.267	0.003	0.461	<0.001	<0.001	0.019	<0.001
NC x Xs	0.008	0.021	0.004	0.005	0.014	<0.001	<0.001	<0.001
NCxCPX	0.011	0.014	0.008	0.005	0.020	0.005	0.003	<0.001
NCxCX	0.031	0.110	0.014	0.017	0.042	<0.001	0.004	<0.001
CPX x CX	0.612	0.289	0.786	0.685	0.720	0.514	0.851	0.266

¹ Diets: C (control); NC (negative control); NC+CPX (negative control + Cerrado/Pantanal xylanase); NC+CX (negative control + commercial xylanase).

² Variables: DE (digestible energy); ME (metabolizable energy); DM (dry matter); OM (organic matter); CP dig (digestible crude protein); EE dig (digestible ether extract); NDF dig (digestible neutral detergent fiber); ADF dig (digestible acid detergent fiber).

³ Contrasts: C x NC (control x negative control); C x Xs (control x xylanases); NC x Xs (negative control x xylanases); PX x CX (Cerrado/Pantanal xylanase x commercial xylanase).

Supplementation with xylanases resulted in higher (P<0.05) digestibility and metabolizability coefficients for CE and digestibility coefficients for DM, CP, NDF, and ADF compared to the control diet. However, the control diet had a higher EE digestibility coefficient than diets containing xylanases (P < 0.05). There was no significant difference (P>0.05) in the MO coefficient between the control diet and diets containing xylanases.

Supplementation with xylanases in the diets resulted in higher (P<0.05) digestibility coefficients for CE, DM, OM, CP, EE, NDN, ADF, and CE metabolizability compared with the negative control diet. There were no significant differences (P>0.05) in the digestibility coefficients of CE, DM, MO, CP, EE, NFD, ADF, or CE metabolizability between the negative control diets and the xylanase Cerrado/Pantanal diet.

Table 3 Nutrient digestibility coefficient of diets containing Cerrado/Pantanal xylanase and commercial xylanase for growing pigs

Dieta ¹	Variables ²							
	CEDC	CEMC	DMDC	OMDC	CPDC	EEDC	NDFDC	ADFDC
C	81.67	77.87	81.47	82.14	81.80	84.04	66.51	45.33
NC	82.14	79.32	82.34	80.99	83.53	67.29	64.97	42.98
CPX	84.93	82.52	85.14	83.99	86.18	71.09	69.84	54.41
CX	84.22	81.10	84.73	83.58	85.90	71.94	70.91	51.90

CV, %	1.83	2.30	1.87	2.03	2.09	3.16	3.86	8.00
Contrasts values P> ³								
C x NC	0.602	0.194	0.351	0.253	0.110	<0.001	0.328	0.313
C x Xs	0.002	<0.001	<0.001	0.068	<0.001	<0.001	0.010	<0.001
NC x Xs	0.006	0.017	0.005	0.005	0.012	<0.001	<0.001	<0.001
NC x CPX	0.006	0.009	0.007	0.007	0.020	0.013	0.006	<0.001
NC x CX	0.032	0.116	0.018	0.017	0.034	0.003	0.001	<0.001
CPX x CX	0.433	0.203	0.655	0.680	0.787	0.533	0.493	0.283

¹ Diets: C (control); NC (negative control); NC+CPX (negative control + Cerrado/Pantanal xylanase); NC+CX (negative control + commercial xylanase).

² Variables: CEDC (crude energy digestibility coefficient); CEMC (crude energy metabolizability coefficient); DMDC (dry matter digestibility coefficient); OMDC (organic matter digestibility coefficient); CPDC (crude protein digestibility coefficient); EEDC (ether extract digestibility coefficient); NDFDC (neutral detergent fiber digestibility coefficient); ADFDC (acid detergent fiber digestibility coefficient).

³ Contrasts: C x NC (control x negative control); C x Xs (control x xylanases); NC x Xs (negative control x xylanases); and CPX x CX (Cerrado/Pantanal xylanase x commercial xylanase).

The CE metabolizability coefficient was higher ($P < 0.05$) between the control and commercial xylanase diets. However, there was no significant difference ($P > 0.05$) in the CE, DM, MO, CP, EE, NFD, and ADF coefficients between the negative control diet and the commercial xylanase diet. There were no differences ($P > 0.05$) in the digestibility coefficients of CE, DM, MO, CP, EE, NFD, ADF, or the metabolizability of CE between diets containing Cerrado/Pantanal xylanase and commercial xylanase.

4. Discussion

According to the results, we can see that the control diet had higher DE and ME values of 95 and 52 Kcal, respectively, compared to the negative control diet (Table 2). Although the difference in energy values was below that initially defined in the study methodology as 100 Kcal, there was no difference between the digestibility coefficients of the diet components, except for EE. The difference in EE digestibility coefficient can be explained by the difference in soybean oil concentrations between the diets.

When we analyzed the diets supplemented with xylanases, we observed that Cerrado/Pantanal xylanase provided increases of 99 and 115 Kcal in the DE and ME values, respectively, compared with the negative control diet. Similarly, commercial xylanase provided increases of 82 Kcal and 70 Kcal in the DE and ME values, respectively, compared to the negative control diet.

The results of the present study are consistent with the literature ⁽¹⁴⁾, indicating an increase in energy digestibility in diets containing soybean and wheat bran with the inclusion of xylanase for newly weaned piglets, and with the studies of Tiwari et al. ⁽²⁷⁾, which found better digestibility of crude energy in piglets fed corn-based diets with the inclusion of xylanase.

According to Kiarie et al. ⁽²⁸⁾, pigs fed diets containing corn, corn distiller grains with solubles, corn germ meal, and soybean meal with the addition of xylanase obtained a 4.5%

higher digestibility of BE compared to pigs fed a control diet. According to Petry et al. ⁽¹⁸⁾, in a study of piglets fed diets rich in fiber with xylanase supplementation, a 2.2% improvement in CE digestibility was observed.

Supplementation of the diets with xylanases resulted in significantly higher digestibility values and coefficients for all nutritional components evaluated. This fact justifies the improved results observed for the energy values of the diets supplemented with enzymes and proves the effectiveness of the enzymes under study.

In general, dietary fiber is related to a decrease in the digestibility of the fractions that produce energy. Xylanase can improve the digestibility coefficient of diets by hydrolyzing parts of the fibers. This response was observed in the present study because xylanases increase the digestibility of NDF and ADF. The average increases in the digestibility coefficients of NDF and ADF provided by xylanase supplementation were 8.3 and 23.7%, respectively. This result corroborates those obtained from previous literature ⁽¹⁴⁾. In a study conducted on newly weaned piglets, there was a 31% increase in the digestibility of NDF for diets containing soybean and wheat bran. Diets containing high levels of NSPs also increase the viscosity of the chyme ⁽²⁹⁾, which decreases the endogenous enzymatic activity of the diet and considerably reduces nutrient digestibility ⁽³⁰⁾.

Xylanases hydrolyze arabinoxylans and reduce the formation of arabinose polymers, reducing the physical barriers between the substrate and enzymes ⁽³¹⁾, and increasing the intestinal transit time and pH ⁽³²⁾, which makes it possible to increase the digestibility of nutrients ^(12,33) and the number of digestive enzymes produced by the body ⁽²⁹⁾. These changes in the availability of nutrients and the environment alter the intestinal microbiota ⁽¹⁰⁾ and its composition ⁽³⁴⁾, which in turn improves pig health.

Xylanase also improves the digestibility of CP and the ileal digestibility of amino acids ⁽³⁵⁾. The mechanism of action of the enzyme on amino acid digestibility is related to the efficiency of xylanase relative to the substrate, which enables the activity of endogenous enzymes on the substrate ⁽³¹⁾. This response was observed in the present study, in which xylanases resulted in an average increase in the digestibility coefficient of CP by 3%.

A response was observed by Weiland and Patience ⁽³⁶⁾, in which the inclusion of xylanase in gilt diets containing low and high fiber content tended to increase the digestibility of CP. This effect was also confirmed in a meta-analytical study by Lehnen ⁽³⁷⁾, who analyzed 21 articles and found that xylanase increased the ileal digestibility of essential and non-essential amino acids by 2–3%. This response may vary depending on the action of xylanase and the amount of NSPs in the diet.

Thus, it can be inferred that the inclusion of xylanases was effective in promoting an increase in the digestibility of energy in the diet and compensating for the reduction in energy concentration. It can also be inferred that Cerrado/Pantanal xylanase is as effective at digesting nutrients in the energy fraction of diets as the commercial xylanase.

5. Conclusion

Inclusion of the xylanase produced by *A. japonicus* var. *aculeatus* UFMS 48.136 was effective in increasing the digestibility of diets containing corn and soybean meal and in compensating for the reduction of 100 Kcal of metabolizable energy per kg of diet. The nutritional functionality of Cerrado/Pantanal xylanases in pig diets was also proven.

Declaration of conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: C. Kiefer, G. C. Giannesi, and F. F. Zanoelo. Data curation: C. Kiefer, K. M. R. S. Nascimento, G. C. Giannesi, F. F. Zanoelo, A. Corassa, and E. R. M. Garcia. Formal Analysis: C. Kiefer, K. M. R. S. Nascimento, G. C. Giannesi, F. F. Zanoelo, A. Corassa, and E. R. M. Garcia. Funding acquisition: C. Kiefer. Investigation: F. A. Oliveira, C. Kiefer, and G. C. Giannesi. Methodology: C. Kiefer, G. C. Giannesi, and F. F. Zanoelo. Project administration: C. Kiefer. Supervision: C. Kiefer. Writing-original draft: F. A. Oliveira. Writing-review & editing: C. Kiefer, K. M. R. S. Nascimento, G. C. Giannesi, F. F. Zanoelo, A. Corassa, E. R. M. Garcia, U. S. Silveira, and T. M. B. Santos.

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