R. Bras. Zootec., 54:e20240100, 2025
https://doi.org/10.37496/rbz5420240100

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Effect of xylanase supplementation on diet digestibility and fermentation products of dogs fed whole and defatted corn germ

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Received: August 15, 2024
Accepted: May 16, 2025

How to cite: Sabchuk, T. T.; Souza, C. M. M.; Bastos, T. S.; Maiorka, A.; Félix, A. P. and Oliveira, S. G. 2025. Effect of xylanase supplementation on diet digestibility and fermentation products of dogs fed whole and defatted corn germ. Revista Brasileira de Zootecnia 54:e20240100. https://doi.org/10.37496/rbz5420240100

Editor:
Ines Andretta

ABSTRACT - The objective of this study was to evaluate the coefficients of total tract apparent digestibility (CTTAD) of nutrients, fecal characteristics, intestinal fermentation products, and diet palatability in dogs fed xylanase-supplemented diets with inclusion of whole or defatted corn germ in two experiments. Experiment I - six adult dogs were distributed in a 6 × 6 Latin square in a 3 × 2 factorial scheme with the experimental diets: control, without the addition of germ (CO−); CO plus xylanase (CO+); 200 g kg^{-1} inclusion of whole corn germ (WG−); WG plus xylanase (WG+); 200 g kg^{-1} inclusion of defatted corn germ (DG−); and DG plus xylanase (DG+). Experiment II - the palatability test was performed in pairs: CO vs. 200 g kg^{-1} WG, CO vs. 200 g kg^{-1} DG, and 200 g kg^{-1} WG vs. 200 g kg^{-1} DG. Xylanase addition, compared with enzyme-free, led to greater CTTAD of dry matter (0.807 × 0.787), crude protein (0.849 × 0.828), and gross energy (0.838 × 0.820) ($P<0.05$). An interaction showed that xylanase increased metabolizable energy in DG diet ($P<0.05$). Germ inclusion resulted in higher fecal dry matter compared with CO (407.4 × 377.3; $P<0.05$). The DG diet reduced propionic concentration and total short-chain fatty acids compared with WG and CO ($P<0.05$). There was a preference of WG over CO both in the first choice and intake ($P<0.05$). Xylanase supplementation improves nutrient digestibility and GE in dog fed diets containing whole or defatted corn germ, while not affecting fecal characteristics and intestinal fermentation products. The use of WG has a positive effect on diet palatability.

Keywords: arabinoxylans, co-products, enzymes, short-chain fatty acids

1. Introduction

Corn is widely used in animal feed, and due to its extensive production, there is a variety of generated residues that provide an opportunity to develop viable value-added ingredients. Corn germ is obtained by segregating the gluten, starch, and bran (Veljković et al., 2018); during the process, corn kernels are moistened, dried, pressed (Zhang et al., 2021), and subjected to solvent extraction. About 180 - 410 g kg^{-1} of the composition of corn germ is oil (Zhang et al., 2021), and the application of further fat extraction techniques has allowed for the use of defatted corn germ.

Corn germ effectively contributes to the supply of energy and amino acids in the diet. However, a potential risk when formulating with corn germ stems from their high concentration of non-starch polysaccharides (NSP). Arabinoxylans make up the majority of NSP in corn and its co-products (Petry and Patience, 2020). According to Gutierrez et al. (2013), these NSP are viscous and poorly

fermentable due to their insolubility, thus impairing nutrient digestion and absorption in the digestive tract and limiting their utilization and inclusion levels in diets.

The use of exogenous carbohydrases, such as xylanase, can help reduce the antinutritional effect of NSP by reducing the viscosity of the digesta, thereby preventing nutritional losses for dogs (Risolia et al., 2019; Sabchuk et al., 2021). Xylanases can also break down insoluble arabinoxylans, enabling the hydrolyzing action of endogenous enzymes upon previously unavailable substrates. Additionally, xylanases can promote a prebiotic action by producing xilo-oligosaccharides which are fermented by the intestinal microbiota, increasing the availability of short-chain fatty acids (SCFA), and modulating the microbiome to further benefit intestinal functionality (Sabchuk et al., 2021).

Industries have sought alternatives to reduce formulation costs, increasingly using industrial co-products. In this context, this study aimed to evaluate the coefficients of total tract apparent digestibility (CTTAD) of nutrients, diet palatability, fecal characteristics, and intestinal fermentation products in dogs fed diets with inclusion of whole or defatted corn germ, replacing corn, with or without the addition of exogenous xylanase.

2. Material and methods

All animal procedures were approved by the Ethics Committee for the Use of Animals of the Agricultural Sciences Sector of the Universidade Federal do Paraná, Curitiba, PR, Brazil (protocol no. 037/2014). The dogs underwent clinical and physical examinations before and after the respective experiments.

2.1. Nutrient digestibility, fecal characteristics, and intestinal fermentation products

2.1.1. Animals, facilities, and experimental design

Six adult beagle dogs (three males and three females), aged 2.0 ± 0.1 years on average and with an average body weight of 10.94 ± 0.74 kg, were used in this study for 60 days. During the period of adaptation to the diet (five days), the dogs had free access to an outdoor area of 1,137 m^2 for 4 h d^{-1} to allow voluntary exercise and socialization with other experimental dogs and people, under supervision of researchers. The dogs were also visited by the researchers at least four times a day. During the feces collection period (five days), all dogs were housed individually in concrete kennels (5 × 2 m), equipped with a bed and free access to fresh water, with bars on the side walls allowing limited visual interaction with neighboring dogs. Ambient temperature ranged from 17 to 29 °C with a 12-h light-dark cycle (light from 06:00 to 18:00 h).

The dogs were distributed in a 6 × 6 double Latin square design (six treatments × six periods), in a 3 × 2 factorial scheme (diets × enzyme). The diets were provided for 10-day periods for six periods, totaling 60 days, with six dogs in each period. All treatments were distributed in each period with an adaptation phase of five days and a total fecal collection phase of five days in each period.

2.1.2. Experimental diets

The experimental diets (Table 1) were formulated to meet the nutritional needs of adult dogs in maintenance according to the European Pet Food Industry Federation (FEDIAF, 2019). Six diets were manufactured: control, without the addition of germ (CO−); CO plus xylanase (CO+); 200 g kg^{-1} inclusion of whole corn germ (WG−); WG plus xylanase (WG+); 200 g kg^{-1} inclusion of defatted corn germ (DG−); and DG plus xylanase (DG+). Whole and defatted corn germ were added in replacement to corn. The xylanase utilized was Econase XT 25 (ABVista, Wiltshire, UK) obtained from *Trichodermareesei* strains, with a minimum activity of 32,000 birch xylanase units (BXU) per g of product. One BXU is the amount of enzyme that produces 1 nmol of reducing sugars from birch xylan, such as xylose, in 1 s at pH 5.3 and 50 °C.

After mixing the ingredients, the diets were ground in a hammer mill (Arthur H. Thomas Co., Philadelphia, PA, United States of America) equipped with 1.2 mm screens, homogenized, extruded in a single-screw extruder (Ferraz, E–130; Ribeirão Preto, SP, Brazil), and subsequently dried in a triple-deck dryer (100–110 °C). Poultry fat was applied onto the feed by coating at room temperature (approximately 30 °C), as described by Tortola et al. (2013), and the enzyme was added immediately after, by coating at a rate of 500 g/ton of feed (16,000 BXU kg of diet) resulting in complete adhesion of the enzyme powder to the extrudates. The homogenization of the feeds with oil was conducted for 15 min. The analyzed chemical composition of whole and defatted corn germs and the experimental diets is described in Table 2.

Table 1 - Ingredients of the experimental diets

Ingredient (g kg^{-1})	Control	200 g kg^{-1} WG	200 g kg^{-1} DG
Corn	605.9	415.9	400.9
Poultry byproduct meal	340.0	340.0	340.0
Whole corn germ (WG)	0.0	20.0	0.0
Defatted corn germ (DG)	0.0	0.0	20.0
Poultry oil	25.0	15.0	30.0
Soybean oil	10.0	10.0	10.0
Xylanase[1]	0.5	0.5	0.5
Potassium chloride	5.5	5.5	5.5
Sodium chloride	5.0	5.0	5.0
Vitamin and mineral premix[2]	4.0	4.0	4.0
Choline chloride	2.0	2.0	2.0
Calcium propionate	2.0	2.0	2.0
Citric acid	0.35	0.35	0.35
BHT	0.15	0.15	0.15
BHA	0.075	0.075	0.075

BHT - butylated hydroxytoluene; BHA - butylated hydroxyanisole.
[1] Econase-XT25 (AB Vista Wiltshire, UK) supplemented at 0.5 g kg^{-1}. Only in the diets containing xylanase. The same amount of an inert substance (kaolin) was added in diets without xylanase.
[2] Supplied per kg of product: vitamin A (retinol), 20,000 IU; vitamin D3 (cholecalciferol), 2,000 IU; vitamin E (alpha-tocopherol α), 48 mg; vitamin K3, 48 mg; vitamin B1, 4 mg; vitamin B2, 32 mg; pantothenic acid, 16 mg; niacin, 56 mg; choline, 800 mg; zinc as zinc oxide, 150 mg; iron as ferrous sulfate, 100 mg; copper as copper sulfate, 15 mg; iodine as potassium iodide, 1.5 mg; manganese as manganese oxide, 30 mg; selenium as sodium selenite, 0.2 mg; antioxidant, 240 mg.

Table 2 - Analyzed chemical composition (g kg^{-1} dry matter) of whole (WG) and defatted corn germ (DG) and experimental diets (with or without xylanase)

Item	Ingredient		Diet[2]					
					200 g kg^{-1}			
	WG	DG	CO–	CO+[1]	WG–	WG+	DG–	DG+
Dry matter	916.8	892.6	910.6	915.0	900.3	912.1	909.5	909.3
Crude protein	153.9	162.3	285.3	286.1	280.0	275.9	304.6	305.6
Ether extract	133.0	34.5	109.5	110.6	122.5	116.6	118.1	120.9
Mineral matter	41.5	64.2	88.0	89.2	78.3	87.7	100.8	105.4
Crude fiber	40.5	49.9	16.5	16.5	14.9	17.1	15.7	14.1
NDF	271.3	303.6	189.9	216.0	189.2	206.1	213.7	202.0
ADF	59.2	61.9	28.6	27.7	25.4	30.7	26.8	26.8
TDF	165.0	153.0	-	-	-	-	-	-
Insoluble fiber	152.0	143.0	-	-	-	-	-	-
Soluble fiber	14.0	10.0	-	-	-	-	-	-
Gross energy (kcal kg^{-1})	5,240	4,504	4,096	4,123	4,198	4,138	4,057	4,176

NDF - neutral detergent fiber; ADF - acid detergent fiber; TDF - total diet fiber.
[1] 16,000 xylanase units kg^{-1} of feed (Econase XT 25, AB Vista, Wiltshire, UK).
[2] CO–: control whithout the addition of germ; CO+: CO plus xylanase; WG–: 200 g kg^{-1} inclusion of whole corn germ; WG+: WG plus xylanase; DG–: 200 g kg^{-1} inclusion of defatted corn germ; DG+: DG plus xylanase.

2.1.3. Digestibility assay

The digestibility assay was conducted as per the recommendations of the Association of American Feed Control Officials (AAFCO, 2016), spanning six periods of 10 days each, including an adaptation phase (5 d) and a fecal collection phase (5 d), for a total of 60 d. The dogs were fed twice daily (at 8:00 and 16:00 h) with quantities sufficient to meet their metabolizable energy (ME) needs according to the equation described by the Nutrient Requirements of Dogs and Cats (NRC, 2006): ME (MJ/day) = 0.54 × Body weight$^{0.75}$. Water was provided *ad libitum* throughout the experimental period. Feces were collected at least twice daily, weighed, identified by period/animal, and frozen at −14 °C.

At the end of each five-day collection period, the feces were thawed, homogenized, and dried in a forced-air oven (320-SE, Fanem, São Paulo, Brazil) at 55 °C for 48 to 72 h or until constant weight. Feces and diet samples were then ground to 1 mm using a Willey hammer mill (Arthur H. Thomas Co., Philadelphia, PA, United States of America) and analyzed to determine the contents of dry matter (DM) by oven-drying at 105 °C for 12 h, ether extract in acid hydrolysis (EEHA, method number 954.02), and ash content (method number 942.05), following the methods of the Association of Official Analytical Chemists (AOAC, 1995). Nitrogen was analyzed (method number 954.01) and used to calculate crude protein (CP) as N × 6.25 (AOAC 1995). Organic matter (OM) was determined as ash - (100). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were conducted according to Van Soest et al. (1991). Total dietary fiber (TDF), insoluble fiber (IF), and soluble fiber (SF) were determined following Prosky et al. (1988). Additionally, the fractions of non-starch polysaccharides (NSP), both soluble and insoluble, present in WG, DG, and in the diets were analyzed according to the methodology described by Englyst et al. (1982) (Table 3). Gross energy (GE) was determined using a bomb calorimeter (Parr Instrument Co., Model 1261, Moline, IL, United States of America).

Table 3 - Chemical analyses (g kg^{-1}) of non-starch polyssacharides present in whole (WG) and defatted corn germ (DG) and experimental diets

Item	Ingredient				Diet					
	WG		DG		Control		200 g kg^{-1} WG		200 g kg^{-1} DG	
	S	I	S	I	S	I	S	I	S	I
Arabinose	2	38	2	36	1	12	0	14	0	13
Xylose	2	59	1	55	2	16	0	20	1	19
Mannose	1	0	1	0	1	0	1	0	1	0
Galactose	2	9	2	9	2	4	2	4	2	4
Glucose	1	44	1	43	1	15	0	17	2	15
Glycoronic acid	2	0	1	0	0	0	0	0	0	0
Galacturonic acid	4	0	3	0	0	0	0	0	0	0
Total	14	150	11	143	7	47	3	55	6	51

S - soluble; I - insoluble.

2.1.4. Fecal characteristics and intestinal fermentation products

At the end of each total fecal collection period, a fresh fecal sample was taken within 15 min after defecation and analyzed for fecal DM content (DMf), fecal consistency (based on a fecal score), pH, ammonia, sialic acid, SCFA, and branched-chain fatty acid (BCFA).

To determine DMf, a 2-g sample of feces was oven-dried at 105 °C for 48 h. Fecal consistency was assessed based on a fecal score, with scores ranging from 1 to 5 as follows: 1 = pasty and shapeless feces; 2 = soft and poorly formed feces; 3 = soft, formed, and moist feces; 4 = well-formed and consistent feces; 5 = well-formed, hard, and dry feces. This evaluation was consistently performed by the same researcher.

Fecal pH was measured using a digital pH meter (331, Politeste Instrumentos de Teste Ltda, São Paulo, SP, Brazil), using 3-g samples diluted in 30 mL of distilled water. Ammonia content (adjusted for DM) in feces was determined according to Brito et al. (2010), with the fecal ammonia concentration calculated as: ammonia-N (g kg^{-1}) = N × correction factor × 17 × (volume of acid - blank) / sample weight (g). For sialic acid content, feces were freeze-dried (Alpha 1-4 LO plus, Christ, Osterodeam Hans, NI, Germany), and the analysis was conducted following the method described by Jourdian et al. (1971).

For determination of SCFA (acetic, propionic, butyric, and valeric) and BCFA (isobutyric and isovaleric), 10 g of fecal sample were weighed and mixed with 30 mL of 16% formic acid. This mixture was homogenized and stored in a refrigerator at 4 °C for three to five days. Afterwards, the solutions were centrifuged at 5000 rpm for 15 min (2K15, Sigma, Osterodeam Hans, NI, Germany), and the supernatant was collected and subjected to new centrifugations; each sample underwent three centrifugations, and at the end of the last one, an aliquot of supernatant was transferred to a properly labeled Eppendorf tube and frozen. Later, the samples were thawed and centrifuged once more at 14000 rpm for 15 min (Rotanta 460 Robotic, Hettich, Tuttlingen, BW, Germany). Fecal SCFA were then analyzed by gas chromatography (SHIMADZU, model GC-2014, Kyoto, Japan), using a glass column (Agilent Technologies, HP INNOwax – 19091N, Santa Clara, CA, United States of America) with 30 m length and 0.32 mm width. Nitrogen was the carrier gas, with a flow rate of 3.18 mL/min. The working temperatures were 200 °C at injection, 240 °C in the column (at a rate of 20 °C/min), and 250 °C in the flame ionization detector.

2.1.5. Calculations and statistical analysis

The CTTAD of DM, OM, CP, EEHA, and GE were calculated according to AAFCO (2016) using the following equation:

$$CTTAD = ((g \text{ of nutrient ingested} - g \text{ of nutrient excreted}) / g \text{ of nutrient ingested}) \times 100.$$

Metabolizable energy was calculated according to AAFCO (2016) with the equation:

$$ME \text{ (kcal/g)} = \{kcal/g \text{ of ingested GE} - kcal/g \text{ of fecal GE} - [(g \text{ of ingested CP} - g \text{ of fecal CP}) \times 1.25 \text{ kcal/g}]\}/g \text{ of ingested feed.}$$

Variables were analyzed according to the following mathematical model:

$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij},$$

in which Y_{ij} = observation j of experimental unit subjected to treatments i, μ = general constant, β_i = effects of xylanase-supplemented diets with inclusion of whole or defatted corn germ, and ε_{ij} = random error associated to each observation.

All data were initially checked for normality (Shapiro-Wilk test) and subjected to ANOVA ($P<0.05$) using the GLM procedure of the SAS statistical package (Statistical Analysis System, version 9.4), including the effects of diets, enzyme, and the interaction between them. Mean comparisons were performed using the Tukey test ($P<0.05$). Fecal score was evaluated using the Kruskal-Wallis test ($P<0.05$) and the medians and their respective quartiles (1st and 3rd) were presented (SAS Inst. Inc., Cary, NC, United States of America). P-values lower than 0.05 were considered significant.

2.2. Palatability

2.2.1. Animals, experimental design, and palatability assay

The palatability assay was conducted with 16 adult beagle dogs, aged 2.0 ± 0.1 years and an average weight of 11.34 ± 0.84 kg. The animals were distributed in a completely randomized design, and the facilities and diet compositions were the same as in Experiment I, with the difference that the dogs were individually housed in kennels only for the duration of the test (about 30 min).

The test was performed using the two-bowl comparison method, resulting in the following test groups: CO− vs. 200 g kg^{-1} WG; CO− vs. 200 g kg^{-1} DG−; and 200 g kg^{-} WG− vs. 200 g kg^{-1} DG−. Both diets to be compared were offered for two consecutive days in two different bowls once a day (08:00 h) for 30 min or until one of the diets was completely consumed. The positions of the bowls were switched every day to avoid side conditioning. The quantity provided was 30% greater than the maintenance energy requirement for adult dogs described by the NRC (2006). Palatability was then determined by identifying the first choice, defined by recording the first bowl approached by the animal during the simultaneous offering of the diets, and quantifying the intake ratio (IR) between diets considering the quantity provided and the leftovers.

2.2.2. Calculations and statistical analysis

Intake ratio was calculated using the following equation: IR = Intake diet A or B [g] / total intake (A + B) [g].

All data were previously subjected to normality analysis (Shapiro-Wilk) using the GLM procedure of the SAS. Intake ratio means were analyzed using the Student's t test ($P<0.05$), and the first choice was analyzed using the Chi-squared test ($P<0.05$). P-values lower than 0.05 were considered significant.

3. Results

3.1. Nutrient digestibility, fecal characteristics, and intestinal fermentation products

Diets containing DG resulted in lower CTTAD of DM and GE, as well as ME ($P<0.05$; Table 4). Xylanase supplementation resulted in higher CTTAD of DM, CP, and GE compared with enzyme-free diets ($P<0.05$). There was an increase in ME when adding xylanase to DG diets, indicating an interaction among factors ($P<0.05$).

No difference was observed among groups for sialic acid, fecal score, pH, and ammonia contents (Table 5). The inclusion of either whole or defatted germ in the diets resulted in higher DMf compared with the control diet ($P<0.05$).

Table 4 - Coefficients of total tract apparent digestibility (CTTAD) and metabolizable energy (ME) of diets containing whole or defatted corn germ and supplemented with xylanase

Diet[1]	Xylanase[2]	CTTAD				ME
		DM	CP	GE	EEHA	
CO	−	0.796Ab	0.835b	0.830Ab	0.873B	3385.3Y
	+	0.805Aa	0.840a	0.837Aa	0.882B	3418.4Y
WG	−	0.791Ab	0.835b	0.824Ab	0.892A	3425.5XY
	+	0.819Aa	0.854a	0.846Aa	0.903A	3512.1X
DG	−	0.773Bb	0.815b	0.806Bb	0.896AB	3247.2Z
	+	0.796Ba	0.855a	0.832Ba	0.883AB	3448.7XY
SEM		0.301	0.346	0.291	0.277	15.807
P-value						
Diet (A)		<0.001	0.385	0.016	<0.001	0.016
Xylanase (B)		<0.001	<0.001	<0.001	0.602	<0.001
Interaction A × B		0.170	0.159	0.242	0.084	0.001

DM - dry matter; CP - crude protein; GE - gross energy; EEHA - ether extract in acid hydrolysis; ME - metabolizable energy; SEM - standard error of the mean.
[1] CO−: control whithout the addition of germ; CO+: CO plus xylanase; WG−: 200 g kg^{-1} inclusion of whole corn germ; WG+: WG plus xylanase; DG−: 200 g kg^{-1} inclusion of defatted corn germ; DG+: DG plus xylanase.
[2] 16,000 xylanase units kg^{-1} of feed (Econase XT 25, AB Vista, Wiltshire, UK).
A,B - Means followed by different uppercase letters differ significantly regarding the diet effect ($P<0.05$).
a,b - Means followed by different lowercase letters differ significantly regarding the xylanase effect ($P<0.05$).
X,Y,Z - Means followed by different uppercase letters differ significantly regarding the interaction effect ($P<0.05$).
n = 6/treatment.

Regarding intestinal fermentation products (Table 6), dogs fed the 200 g kg^{-1} DG diet showed a reduction in propionic acid concentration and total SCFA compared with the 200 g kg^{-1} WG and CO diets (P<0.05).

Table 5 - Fecal characteristics of dogs fed diets containing whole or defatted corn germ and supplemented with xylanase

Diet[1]	Xylanase[2]	DMf (g kg^{-1})	Score[3]	pH	Ammonia (g kg^{-1})	Sialic acid (µmol g^{-1})
CO	–	378.8B	3.39	7.08	0.95	0.679
	+	375.8B	3.53	7.10	0.92	0.736
WG	–	402.6A	3.63	7.11	0.86	0.669
	+	409.3A	3.68	6.97	0.99	0.704
DG	–	402.9A	3.56	6.94	1.02	0.625
	+	414.7A	3.70	6.94	0.98	0.772
SEM		0.396	-	0.041	0.003	0.026
P-value						
Diet (A)		<0.001	-	0.363	0.695	0.953
Xylanase (B)		0.442	-	0.648	0.788	0.153
Interaction A × B		0.657	-	0.704	0.564	0.678

DMf - fecal dry matter; SEM - standard error of the mean.
[1] CO–: control whithout the addition of germ; CO+: CO plus xylanase; WG–: 200 g kg^{-1} inclusion of whole corn germ; WG+: WG plus xylanase; DG–: 200 g kg^{-1} inclusion of defatted corn germ; DG+: DG plus xylanase.
[2] 16,000 xylanase units kg^{-1} of feed (Econase XT 25, AB Vista, Wiltshire, UK).
[3] Fecal score analyzed by Kruskal-Wallis (P<0.05).
A,B - Means followed by different uppercase letters differ significantly regarding the diet effect (P<0.05).
n = 6/treatment.

Table 6 - Intestinal fermentation products of dogs fed diets containing whole or defatted corn germ and supplemented with xylanase

Diet[1]	Xylanase[2]	Acetic	Propionic	Butyric	Valeric	Isobutyric	Isovaleric	Total SCFA
CO	–	30.82	14.33A	4.89	1.66	4.49	1.102	57.31A
	+	33.05	14.39A	4.88	1.63	4.60	1.11	59.67A
WG	–	32.30	13.39B	5.21	1.62	4.49	1.15	58.19B
	+	30.32	12.24B	4.25	1.52	4.37	1.09	53.81B
DF	–	27.62	10.43C	4.33	1.49	4.55	1.08	49.53C
	+	26.35	10.23C	5.04	1.82	4.35	1.16	48.97C
SEM		0.902	0.547	0.223	0.078	0.246	0.033	1.574
P-value								
Diet (A)		0.055	0.009	0.936	0.908	0.982	0.968	0.050
Xylanase (B)		0.845	0.671	0.852	0.694	0.888	0.863	0.779
Interaction A × B		0.578	0.874	0.353	0.537	0.971	0.748	0.667

SCFA - short chain fatty acids; SEM - standard error of the mean.
[1] CO–: control whithout the addition of germ; CO+: CO plus xylanase; WG–: 200 g kg^{-1} inclusion of whole corn germ; WG+: WG plus xylanase; DG–: 200 g kg^{-1} inclusion of defatted corn germ; DG+: DG plus xylanase.
[2] 16,000 xylanase units kg^{-1} of feed (Econase XT 25, AB Vista, Wiltshire, UK).
A,B - Means followed by different uppercase letters differ significantly regarding the diet effect (P<0.05).
n = 6/treatment.

3.2. Palatability

There was no difference in the first choice when comparing the CO vs. 200 g kg^{-1} WG diets (P>0.05; Table 7). On the other hand, dogs had a higher IR for the CO diet (P<0.05). Additionally, dogs preferred the 200 g kg^{-1} DG diet over the CO diet both in the first choice and intake (P<0.05). When comparing the diet 200 g kg^{-1} WG with the 200 g kg^{-1} DG, no differences were observed in the evaluated variables.

Table 7 - First choice and intake ratio of dogs fed diets containing whole or defatted corn germ

Diet A vs. B	n[1]	Intake ratio of diet A[2]
CO– vs. WG–	16	0.57 ± 0.04
CO– vs. DG–	11	0.34 ± 0.05*
WG– vs. DG–	16	0.48 ± 0.05

CO–: control whithout the addition of corn germ; WG–: 200 g kg^{-1} inclusion of whole corn germ; DG–: 200 g kg^{-1} inclusion of defatted corn germ.
* Significant value (P<0.05) for the number of visits for diet A by the chi-square test and Student's t test for intake ratio.
[1] Number of visits to the feeder with diet B obtained as 32 – n.
[2] Intake ratio = [g consumed of diet A or B/ g total consumed (A + B)].
n = 8/treatment.

4. Discussion

Co-products such as WG and DG can be alternative carbohydrate sources to corn and viable energy providers, although the high content of NSP such as arabinoxylans and other soluble and insoluble fibers must not be disregarded. Overall, corn germ has a lower digestibility compared with cereals. Fortes et al. (2010) compared various carbohydrate sources used in dog nutrition (sorghum, pearl millet, broken rice, corn germ, wheat bran, and rice bran) with the substitution of a reference diet for 300 g kg^{-1} of corn germ and reported lower nutrient digestibility of corn germ than sorghum and broken rice. Furthermore, the authors evaluated corn germs with lower protein and EE contents and higher fiber than those used in the present study, which also presented a reduced nutrient digestibility. In this study, only DG was lower than the CO diet; similar results were found in a study with dogs fed 200 g kg^{-1} defatted germ (Sabchuk et al., 2021) and in studies with varied NSP inclusion in dog diets (Silva et al., 2016; Sabchuk et al., 2017). Studies evaluating the use of corn germ for laying hens (Brunelli et al., 2010), pigs (Moreira et al., 2002; Lee et al., 2012; Weber et al., 2010), and broiler chickens (Brunelli et al., 2006) found negative effects above 200 g kg^{-1} dietary inclusion.

The composition of NSP dictates their utilization. When analyzing the levels of NDF (271.3 and 303.6 g kg^{-1}) and ADF (59.2 and 61.9 g kg^{-1}) in WG and DG, respectively, it was observed that both are higher than the values found in corn (112.0 g kg^{-1} NDF and 26.4 g kg^{-1} ADF; Rostagno et al., 2017). Therefore, the lower nutrient digestibility in the 200 g kg^{-1} DG diet can be explained by the elevated contents of arabinoxylans and other soluble or insoluble fibers in the ingredient, consequently impairing nutrient digestion and absorption in the tract by decreasing energy and increasing digesta transit rate and viscosity (Vanderhoof, 1998). Additionally, according to Brunelli et al. (2010), DG has a high concentration of phytic P that can interact with other nutrients and render them unavailable to the animal. This would explain the observed interaction between corn germ and xylanase, in which the dogs fed 200 g kg^{-1} DG diet without enzymatic supplementation had lower ME. Another possibility could be related to processing parameters applied to corn germ, as DG undergoes pelletizing compared with WG, which may have modified the starch structure and fostered the formation of resistant starch, reducing its digestion (Walter et al., 2005).

Dietary supplementation with exogenous enzymes or enzymatic complexes is a recommended practice to reduce the negative impact of NSP for dogs (Tortola et al., 2013; Pacheco et al., 2014; Machado et al., 2021; Sabchuk et al., 2021), allowing for a more cost-effective use of co-products and alternative raw materials. According to Bedford (2000), enzymatic supplementation not only tends to increase nutrient and energy availability but also reduces variation in the quality of nutrients in foods, improves intestinal functionality, and ameliorates the effect of antinutritional components, taking as an example the action of phytases and xylanases. As a result, the addition exogenous enzymes can also enhance the activity of endogenous enzymes (Tortola et al., 2013), further reducing the excretion of undigested nutrients (Bedford, 2000). Various studies have assessed different carbohydrate sources and exogenous enzymes in dog diets aimed at improving nutrient utilization (Twomey et al., 2003a; Twomey et al., 2003b; Félix et al., 2012; Sá et al., 2013; Pacheco et al., 2014; Silva et al., 2016; Villaverde et al., 2017; Machado et al., 2021; Sabchuk et al., 2021). However, several factors can influence the effectiveness of exogenous enzymes, such as dose, enzyme source, diet formulation strategies, ingredient variability,

interaction with other diet components, thermal processing, and intrinsic factors related to the animal (Bedford and Schulze, 1998), leading to a high variability between studies. Twomey et al. (2003a) compared diets based on rice, sorghum, and corn with or without the inclusion of an enzyme blend (1 mL/ton, xylanase, α-amylase, β-glucanase, hemicellulase, pectinase, and endoglucanase) and found no effect on nutrient digestibility. Likewise, Pacheco et al. (2014) evaluated diets with whole rice bran and the inclusion of an enzymatic complex (carbohydrases, phytase, and protease, 0.4 and 0.8 g kg^{-1} of diet), but the enzymes had no effect on nutrient digestibility. On the other hand, Félix et al. (2012) assessed the inclusion of an enzymatic complex (2 g kg^{-1}, galactosidase, β-glucanase, and xylanase; FraZymePeDry, Perstorp Química do Brasil Ltda, São Paulo, SP, Brazil) on diets based on soybean meal and poultry byproduct meal and observed greater nutrient digestibility.

In the current study, the evaluated xylanase (0.5 g kg^{-1}, Econase XT 25, AB Vista, Wiltshire, UK) led to improvements in digestibility regardless of the inclusion of corn germ in the diet. The analyses of dietary NSP shows similar contents of arabinoxylans (soluble and insoluble) for the CO (31 g kg^{-1}), 200 g kg^{-1} WG (34 g kg^{-1}), and 200 g kg^{-1} DG (33 g kg^{-1}) diets, thus indicating that all diets had reasonable amounts of substrate for the xylanase. Similar results were reported by Silva et al. (2016) using the same xylanase at the same concentration (0.5 g kg^{-1}) to a corn-based diet with up to 200 g kg^{-1} of distillers dried grain with solubles (DDGS), which is also a co-product of corn with a similar NSP profile to corn germ, primarily arabinose and xylose. In contrast, Risolia et al. (2019) evaluated diets with 200 g kg^{-1} DDGS and using xylanase (0.25 g kg^{-1}) and protease and did not observe any changes in digestibility. This lack of effect may be attributed to the use of a lower enzyme concentration compared with this study or that by Silva et al. (2016).

Diets with either WG or DG resulted in feces with higher DMf compared with the CO. This likely occurred because these diets contained more insoluble NSP, which, when increased, reduce the amount of water excreted in feces, leading to drier stools, which can be highly advantageous in formulating diets for dogs. This phenomenon aligns with observations made by Silva et al. (2016) and Sabchuk et al. (2021) in studies with dogs. However, fecal consistency, directly influenced by DMf, remained unchanged and within normal limits for the species (rated 4 on a scale of 1 to 5), characterized as well-formed and consistent. Furthermore, other fecal characteristic variables showed no differences regarding corn germ or enzyme inclusion.

The use of xylanase is not only advantageous in terms of extracting more energy from a highly insoluble and poorly fermentable fiber source, but is also effective in modulating the microbial community. Within this context, it is possible to formulate pet diets that address the specific nutritional needs of dogs, thereby promoting their overall health and welfare. This modulation favors the fermentation of corn-based arabinoxylan, potentially enhancing gastrointestinal functionality through the stimulatory effects of SCFA (Bach Knudsen et al., 2018). Additionally, by hydrolyzing arabinoxylans and making them available for fermentation, xylanase can partially improve substrate competition between the host and microbiota. The resulting products are vital for promoting the proliferation of non-pathogenic bacteria and maintaining intestinal eubiosis (Craig et al., 2020). However, reductions in total SCFA and propionic acid concentrations were observed in dogs fed the DG diet regardless of xylanase eend-products.

Finally, Lassiter and Edwards (1982) claimed that corn germ and gluten meal have low palatability, although corn germ boasts a better amino acid balance (with higher levels of lysine and tryptophan). Conversely, according to Freitas (1998) and Moreira et al. (2002), the DG meal exhibits good palatability in pigs. There are limited studies on palatability in dogs using this ingredient, but the literature indicates that the inclusion of DG in dog diets positively influences intake and first choice (Sabchuk et al., 2021), aligning with the findings of this study.

Notably, this study only examined one level of enzyme and corn germ inclusion in the diet, given the structural limitations of this study. Additional research could shed more light on the effects of varying inclusion levels.

5. Conclusions

Xylanase supplementation improves nutrient digestibility and ME in both whole and defatted corn germ dog diets, without altering fecal characteristics or intestinal fermentation. Additionally, defatted corn germ diets are more palatable for dogs, while whole corn germ inclusion does not affect nutrient digestibility.

Data availability

The data that support the results of this study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: Sabchuk, T. T.; Maiorka, A. and Oliveira, S. G. **Data curation:** Sabchuk, T. T. **Investigation**: Sabchuk, T. T.; Souza, C. M. M.; Bastos, T. S. and Félix, A. P. **Project administration:** Maiorka, A. and Oliveira, S. G. **Supervision:** Maiorka, A.; Félix, A. P. and Oliveira, S. G. **Writing – original draft:** Sabchuk, T. T. and Souza, C. M. M. **Writing – review & editing**: Sabchuk, T. T.; Souza, C. M. M.; Bastos, T. S.; Maiorka, A.; Félix, A. P. and Oliveira, S. G.

Conflict of interest

The authors declare no conflict of interest.

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