Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

e-ISSN: 1806-9061 2024 / v.26 / n.2 / 001-010

http://dx.doi.org/10.1590/1806-9061-2023-1828

Original Article

Nutrient and Energy Balance, and Amino Acid Digestibility in Broilers Fed Canola Meal and an Exogenous Enzyme Combination

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■Keywords

Amino acid digestibility, broiler chicks, canola meal, multi-carbohydrases, phytase.

Submitted: 18/July/2023
Approved: 15/March/2024

ABSTRACT

The apparent total tract digestibility (ATTD; trial 1) and the apparent (AID) and standardized (SID) ileal digestibility of the amino acids (AA) (trial 2) in canola meal (CM) were evaluated with the addition of exogenous multi-carbohydrase (MC) and phytase (Phy). A total of 80 28-day-old broilers were allotted in a completely randomized design to receive treatments up to 35 days of age. A 2 × 2 factorial design was used to determine the enzyme effects on the ATTD of dry matter, nitrogen, calcium, phosphorus and fibre; as well as energy use and the AID and SID of AA, in five replicate cages. Supplementation with exogenous enzymes showed a positive interaction (p<0.05) between MC and Phy for nitrogen and energy. The isolated inclusion of Phy or MC showed a favorable effect (p<0.05) for dry matter, calcium, phosphorus and fiber. The enzyme inclusion on the AID and SID of AA in CM, established by comparing the means, suggested a better response to the addition of MC or Phy. Supplementation with Phy or MC was shown to be a viable alternative to increase the ATTD of nutrients and energy. The isolated inclusion of Phy or carbohydrate resulted in higher apparent and standardized digestibility of AA from CM.

INTRODUCTION

Canola meal (CM) is the secondary product of the extraction of seed oil by methods that commonly use the combination of mechanical pressure and chemical solvent extraction. This is the preferred and most effective method of extracting oil from oilseeds for most large-scale plants. CM is an important ingredient in the formulation of poultry feed; with soybean meal being considered the better and most used protein alternative in poultry feed, followed by CM. This protein source is rich in fiber and anti-nutritional factors (Canola Council of Canada, 2023), the digestibility of which is limited in non-ruminants. Therefore, it can only be included at moderate levels in poultry feed formulations. Compared with soybean meal, canola meal contains more anti-nutritional factors, such as glucosinolates and fibers (Mejicanos *et al.*, 2016). Glucosinolates and fiber in canola meal may reduce digestibility of energy and amino acids, and feed palatability (Maison *et al.*, 2015; Mosenthin *et al.*, 2016; Landero *et al.*, 2018). CM is rich in sulfur amino acids; however, it contains less metabolizable energy than soybean meal and crude fiber (Rostagno *et al.*, 2017). CM contains anti-nutritional factors such as glucosinolates (Mejicanos *et al.*, 2016). Although their content has been decreased in seed of modern canola cultivars (Landero *et al.*, 2018), glucosinolates in CM may reduce feed palatability, and the digestibility of energy and amino acids (AA) (Mosenthin *et al.*, 2016).

The possibility of using MC was considered as a means to reduce the negative effects of fiber on digestion, by breaking down the non-starch polysaccharides (NSP) of the cell wall; paired with Phy to attenuate the negative effects of phytate, increasing the availability of complexed phosphorus (Woyengo & Nyachoti, 2011). Intestinal transit time and nutrient use can be negatively affected when birds increase their NSP intake due to increased intestinal viscosity (Kiarie *et al.*, 2016; Gallardo *et al.*, 2020; Araujo *et al.*, 2021, 2022). On the other hand, studies have shown that exogenous enzymes can increase the digestibility of vegetable ingredients used as alternatives in the diets of poultry and swine. Therefore, dietary supplementation with enzymes can increase the efficiency of nutrient and energy utilization by non-ruminants, based on apparent or standardized digestibility assessments (Dadalt *et al.*, 2017; Gallardo *et al.*, 2020; Araujo *et al.*, 2021, 2022).

As reported by Araujo *et al.* (2021), (2022) and Gallardo *et al.* (2020), Phy reduces the negative effects of phytic acid, increases the release of P from phytate and multi-carbohydrases, and reduces the negative effects of fiber on digestion. Therefore, it was hypothesized that the use of nutrients from CM may be favored by the inclusion of the tested ingredients in the diet of broilers at 35 days of age. Previous studies have shown an improvement in nutrient use, energy, and digestibility of AA with the use of carbohydrates and Phy in the diets of swine and broilers (Dadalt *et al.*, 2017; Gallardo *et al.*, 2020; Trindade *et al.*, 2020; Araujo *et al.*, 2021, 2022).

Thus, we evaluated the nutritional and energy balance, and the digestibility of the AA in CM, with and without MC and Phy in broilers at 35 days of age.

MATERIAL AND METHODS

All research methods and procedures were approved by the ethics committee for the use of animals of the São Paulo State University "Júlio de Mesquita Filho", UNESP - School of Veterinary Medicine and Animal Science - FMVZ, Botucatu / SP. (Registration No. 0094/2018), Brazil, and were followed to ensure animal welfare. The methodology has been described in detail by Gallardo *et al.* (2020).

Canola meal and enzymes

The CM used for this study was obtained from Hi-Tech Feeds, Pelotas, Rio Grande do Sul, Brazil, and its chemical composition is shown in Table 1. The nutritional composition of the CM was similar to that described by Rostagno *et al.* (2017).

The enzymes used were a blend of MC (700 U α-galactosidase, 2,200 U galactomannanase, 3,000 U xylanase and 22,000 U β-glucanase kg / diet) from Endopower Beta, and Phy (500 FTU kg / diet) was sourced from Genophos, as reported in Gallardo *et al.* (2017). Exogenous enzyme activities were suggested by GNC Bioferm Inc. (Saskatoon, Saskatchewan, Canada), and technical information was provided by Uniquimica, São Paulo, Brazil.

Table 1 – Nutrient composition (as-fed basis) of canola meal used in the study.

Nutrient component (%)	Canola Meal
Dry matter	89.61
Crude protein	38.56
Gross energy (MJ/kg)	17.82
Calcium	0.92
Phosphorus	0.97
Neutral detergent fiber	26.55
Essential amino acids (%)	
Arginine	2.37
Histidine	1.05
Isoleucine	1.74
Leucine	2.43
Lysine	1.95
Methionine	0.62
Phenylalanine	1.26
Threonine	1.49
Tryptophan	1.64
Valine	1.78
Non-essential amino acids (%)	
Alanine	1.48
Aspartic acid	2.29
Cystine	0.70
Glutamine	5.74
Glycine	1.56
Proline	2.07
Serine	1.37
Tyrosine	1.03

Diets and experimental design

The apparent total tract digestibility (ATTD; Trial 1) and the apparent (AID) and standardized (SID) ileal digestibility of the AA (Trial 2) of CM were evaluated with the addition of exogenous MC and Phy. A total of 80 male broilers, at 28 days of age, were allotted in a completely randomized design to receive treatments up to 35 days of age. All experiments were conducted in a completely randomized design in a 2 x 2 factorial arrangement of treatments (5 replicate cages with 16 birds per treatment). The factors were MC (0 and 200 mg/kg) and Phy (0 and 50 mg/kg). Basal diets were used for an additional group of 24 birds kept in 6 cages, which was used to determine the enzyme effects, alone or in combination. A basal corn diet (BD_1) was used for ATTD determination, and a corn-starch basal diet (BD_2) containing 5% casein was used to estimate endogenous losses and SID of AA, as described by Gallardo *et al.* (2020). The test diets were made by mixing BD and CM in an 8:2 (wt/wt) ratio.

From 21 days of age, the birds were fed with experimental diets until the beginning of the experiment. In trial 1 (days 28 to 33), excreta were collected, and from day 34 to day 35 (trial 2), the birds received a new diet until slaughter at the end the experimental period, when the ileal content was collected. Chromium oxide III (Cr_2O_3) was added at 0.3% to all diets as an indigestible marker. In trials 1 and 2, all diets (Table 2) were supplemented with vitamins and minerals, meeting the nutritional requirements of broilers in the growth phase as recommended by Rostagno *et al.* (2017).

Table 2 – Composition of diets, as fed basis (g/kg diet).

Ingredients	Total collection	Ileal collection diet
	Basal diet (BD_1)	Basal diet (BD_2)
Yellow corn	850.1	-
Amido	-	502.7
Soybean oil	20.0	12.0
Choline chloride 60	0.2	0.2
Salt	2.5	3.2
Sodium bicarbonate	3.8	3.5
Limestone	8.1	6.9
Bicalcium phosphate	24.0	23.7
Cellulose	50.0	50.0
Dextrose	-	300.0
Casein	-	50.0
Chromic oxide	-	3.0
Vitamin-mineral premix[3]	5.0	5.0
Kaolin[4]	36.3	39.8
Total	1000	1000
Calculated composition		
Dry matter	888.0	929.3
Crude protein	67.0	42.1
Calcium	9.2	8.6
Available phosphorus	4.0	3.4
Metabolizable energy (MJ/kg)	12.77	12.98
Sodium	2.2	2.2
Choline (mg/kg)	2000	1900
Linoleic acid	26.8	6.3

[1]Corn based diet; [2]Cornstarch and casein diet; [3]Vitamin-mineral premix per kg of feed: vitamin A 9 000 UI; vitamin D3 1 600 UI; vitamin E 14 UI; vitamin K3 1 5 mg; vitamin B_1 1 mg; vitamin B_2 4 mg; pantothenic acid 8 28 mg; vitamin B_6 18 mg; vitamin B_{12} 12 mµg; niacin 0 03 mg; folic acid 0 3 mg; biotin 0 05 mg; Se 0 25 mg; Cu 9 mg; Fe 30 mg; I 1 mg; Zn 60 mg; Mn 60mg. [4]Kaolin: mineral kaolinite, used as inert ingredient to adjust the formulation of diet.

In trial 1, to assess the balance of nutrients and apparent metabolizable energy (EMA) in the CM, samples of excreta were collected twice a day (8 am and 5 pm) for 5 consecutive days. To determine the digestibility coefficients of the AA in trial 2, all birds were weighed and slaughtered for ileal collection in the last 10 cm, before the 2 cm proximal to the ileocecal junction. Prior to analysis, excreta were stored in a freezer at –20 °C, and at the end of the experiment, they were homogenized and freeze-dried.

At 35 days of age, five birds per treatment were collected, and liver and pancreas weights were taken to determine the relative weights of these organs in relation to the post-fasting weight, expressed as a percentage. The following equation was used:

Relative organ weight = (organ weight/post-fasting weight) × 100.

Sample analyses and data processing

The excreted and ileal samples were freeze-dried for 72 h at –40ºC (LH 0401, Terroni, São Carlos, BR) as described by Gallardo *et al.* (2017). The diets, CM, excreta and ileal digesta samples were finely milled and analyzed, according to Association of Official Analytical Chemists (AOAC, 2005) for determinations of dry matter (DM), gross energy (GE), nitrogen (N), calcium (Ca), phosphorus (P) and neutral detergent fiber (NDF). Test diets and ileal digesta samples were processed and analyzed to determine the digestibility coefficients of AA as described by Gallardo *et al.* (2017). Tryptophan was determined by the colorimetric method of Spies (1967), using a standard curve of pure tryptophan (Merck, Germany), and detected at 590 nm with a spectrophotometer (DU-640 UV/Vis; Beckman Coulter, Basking Ridge, New Jersey, (USA). Cystine was expressed as cysteine. All analyses were performed in duplicate.

Calculations

As reported by Dadalt *et al.* (2017), all formulas related to apparent nutrient digestibility and amino acid digestibility (AID and SID) were as follows:

Digestibility of nutrients (%) = $100 \times [(NI - NO_{excreta})/NI]$,

where NI is the nutrient intake (g), and $NO_{excreta}$ is the nutrient output in excreta (g).

The retention of nutrients in CM was determined by the difference method (Fan & Sauer, 1995), with the corn-based diet used as the BD, using the following equation:

$DA = (DD - (DB \times DN)) / DCM$

where DA is the retention of a nutrient (%) in a feedstuff assay (CM),

DD is the digestibility of a nutrient (%) in the CM-containing diet,

DB is the digestibility of a nutrient (%) in the corn-based diet, DN is the contribution of a nutrient (decimal percentage) to the assay diet from corn and,

DRB is the contribution of a nutrient (decimal percentage) in the CM-based diet from CM.

The apparent metabolizable energy (AME) content of CM was calculated according to the following equation (Woyengo *et al.*, 2010):

AME of CM (kcal/kg) = [(Gross energy retention for CM, %) × (Gross energy content in CM, kcal / kg)]/100

The AID and SID (%) of amido acids were calculated using the following formula (Nyachoti *et al.*, 1997):

$AID (\%) = 100 - [100 \times (AA_{digesta} \times Cr_2O_{3diet}) / (AA_{diet} \times Cr_2O_{3digesta})$

Where AA_{diet} and $AA_{digesta}$ are the amino acid content (mg/kg of DM) in the diet and digesta, respectively, and $Cr_2O_{3diet\ and}$ $Cr_2O_{3digesta}$ are the indigestible marker contents (mg/kg de DM) in the diet and digesta, respectively.

Apparent ileal AA digestibilities were standardized using average values for basal endogenous AA losses calculated using the following formula (Nyachoti *et al.*, 1997):

AA_{EL} (g/kg) = $AA_{digesta} \times (Cr_2O_{3diet}/Cr_2O_{3digesta})$

Where AA_{EL} = average endogenous amino acid loss (g/kg of DM).

The SID of AA was calculated according to the following equation. as described by Opapeju *et al.* (2006):

$SID (\%) = [AID_{AA} + (AA_{EL}/AA_{diet})] \times 100$

Statistical analysis

The GLM procedure of SAS (Statistical Analysis System, 2014, version 9.4) was used to determine the main effects of, and the interaction between, MC and Phy. The homogeneity of variances was evaluated by the Shapiro-Wilk test (UNIVARIATE procedure). The statistical model used was:

$Yij = \mu + ai = bj + (ai \times bj) + eij,$

where Yij = variable response of broilers fed with MC and phy; μ = overall mean; ai = MC effect; bj = Phy effect; (ai × bj) = interaction between MC and Phy; eij = error contribution with average 0 and variance $\sigma 2$, I = 1 … a, and j = 1 … b. Significance was accepted at $p<0.05$.

RESULTS

The effects of the CM diets supplemented with MC and Phy on nutrient balance and AME in broilers from 28 to 33 days of age are shown in Table 3, and the amino acids contents used to determine AID and SID are shown in Table 4 and 5.

Table 3 – Apparent nutritional balance of canola meal, combined or not with enzymes, for broilers at 35 days of age(g/kg).

Item	Phy[2] 0		Phy 50			*p*-valor[4]		
	MC[1] 0	MC 200	MC 0	MC 200	SEM[3]	MC	Phy	MCxPhy
Dry matter	777.4	792.4	786.1	782.2	0.002	0.002	0.013	0.214
Nitrogen	749.2	758.6	769.3	779.8	0.004	0.008	0.509	0.013
AME (MJ/Kg)	13.90	13.98	14.7	14.99	0.027	0.001	0.100	0.001
Calcium	745.1	751.8	786.5	778.1	0.044	0.448	0.043	0.878
Phosphorus	568.2	625.3	601.5	615.0	0.054	0.293	0.049	0.112
NDF (%)	365.2	405.5	411.2	404.4	0.006	0.001	0.020	0.043
ME/GE	686.4	690.7	691.01	729.9	0.001	0.001	0.029	0.001

N = 5 replicates cages. As dry matter basis. AME: apparent metabolizable energy; NDF: Neutral detergent fiber.

[1]MC, multi-carboidrase level (0 and 200 mg/kg of diet), (200 mg/kg of MC contains 700 U of α-galactosidase, 2200 U of galactomannanase, 30 000 U of xylanase and 22 000 U of β-glucanase/kg of diet). [2]Phy, Phytase level (0 and 50 mg/kg of diet). [3]SEM: standard error of mean. [4]Effect of MC - Multi-carboidrase, Phy - Phytase, and interaction MC x Phy, respectively.

The ATTD coefficients and apparent energy use of broilers fed with CM supplemented with exogenous enzymes characterize a positive interaction ($p<0.05$) between MC and Phy for nitrogen and energy. The isolated inclusion of Phy or carbohydrate indicated a favorable effect ($p<0.05$) on the digestibility of dry matter, calcium, phosphorus and fiber.

The coefficients of apparent and standardized digestibility of the AA in SM, supplemented or not with MC and Phy, are shown in Tables 4 and 5, respectively. The general results indicate that the isolated or combined supplementation of MC and Phy positively influenced ($p<0.05$) the AID and SID coefficients of the SM AA. The marked effect ($p<0.05$) of the MC and Phy combination on the AID and SID of flaxseed meal was also evident for histidine, methionine, serine, glutamine, arginine, isoleucine, lysine, phenylalanine, threonine, aspartic acid, glycine and proline amino acids. The average values of apparent and standardized digestibility of the 17 AA of FM were as follows:

Table 4 – Apparent ileal digestibility of the amino acids of canola meal, combined or not with enzymes, for broilers at 35 days of age*.

Item	Phy[2] 0		Phy 50		SEM[3]	*p*-valor[4]		
	MC[1] 0	MC 200	MC 0	MC 200		MC	Phy	MCxPhy
Essential AA (%)								
Arginine	90.14	94.13	93.97	90.64	1.636	0.787	0.001	0.962
Histidine	85.93	94.30	94.04	89.29	1.038	0.001	<0.001	0.004
Isoleucine	84.82	86.94	84.29	87.17	0.811	<0.001	0.301	0.687
Leucine	89.86	92.17	90.73	90.41	1.173	0.076	0.023	0.409
Lysine	86.68	90.78	87.41	89.40	1.251	<0.001	0.050	0.571
Methionine	92.48	95.91	96.51	95.97	1.290	0.023	0.003	0.003
Phenylalanine	89.59	92.58	92.96	89.46	1.453	0.701	0.001	0.856
Threonine	77.59	87.81	86.73	80.80	1.592	0.008	<0.001	0.153
Tryptophan	79.40	85.54	81.33	83.60	1.181	0.002	0.192	0.843
Valine	85.92	90.53	88.95	87.00	1.035	0.011	<0.001	0.587
Non-essential amino acid (%)								
Alanine	83.90	93.94	93.54	84.03	1.610	0.721	<0.001	0.852
Aspartic acid	89.13	90.01	89.66	89.47	1.358	0.583	0.389	0.990
Glutamine	90.11	93.84	93.40	92.15	0.974	0.011	<0.001	0.085
Glycine	80.54	89.39	88.63	82.26	1.552	0.092	<0.001	0.497
Proline	84.76	82.02	81.49	85.81	1.233	0.172	<0.001	0.636
Serine	74.18	84.28	84.04	81.39	1.423	<0.001	<0.001	<0.001
Tyrosine	88.12	91.43	89.82	89.43	1.193	0.015	0.003	0.782

N = 5 replicates cages. *As dry matter basis.

[1]MC, multi-carboidrase level (0 and 200 mg/kg of diet), (200 mg/kg of MC contains 700 U of α-galactosidase, 2200 U of galactomannanase, 30 000 U of xylanase and 22 000 U of β-glucanase/kg of diet). [2]Phy, Phytase level (0 and 50 mg/kg of diet). [3]SEM: standard error of mean. [4]Effect of MC - Multi-carboidrase, Phy - Phytase, and interaction MC x Phy, respectively.

Table 5 – Standardized digestible coefficient of amino acids in canola meal, combined or not with enzymes, in broilers at 35 days of age*.

Item	Phy[2] 0		Phy 50		SEM[3]	*p*-value[4]		
	MC[1] 0	MC 200	MC 0	MC 200		MC	Phy	MCxPhy
Essential AA (%)								
Arginine	94.42	96.72	96.57	94.57	1.646	0.835	0.010	0.999
Histidine	91.94	97.01	96.87	93.98	1.471	0.116	<0.001	0.170
Isoleucine	92.46	95.10	93.34	94.86	1.316	0.003	0.362	0.583
Leucine	94.16	95.83	94.66	94.67	1.171	0.127	0.131	0.536
Lysine	91.81	95.30	92.44	94.30	1.252	0.001	0.164	0.739
Methionine	98.35	96.01	97.91	99.59	0.640	0.011	0.011	0.011
Phenylalanine	93.36	95.28	95.52	93.10	1.452	0.705	0.004	0.993
Threonine	88.58	94.22	93.28	90.68	1.183	0.011	<0.001	0.289
Tryptophan	85.22	90.75	81.43	94.54	1.181	0.004	0.001	0.872
Valine	92.36	95.26	94.04	93.24	1.040	0.038	0.001	0.720
Non-essential amino acid (%)								
Alanine	90.78	96.77	96.54	90.91	1.610	0.813	<0.001	0.944
Aspartic acid	94.23	94.93	94.78	95.41	1.960	0.460	0.963	0.565
Glutamine	95.51	97.79	97.51	97.40	0.973	0.024	0.014	0.050
Glycine	87.33	93.40	92.85	89.10	1.580	0.125	<0.001	0.411
Proline	89.94	89.32	88.35	91.15	1.088	0.039	0.003	0.808
Serine	88.90	95.34	95.37	95.30	1.544	0.001	0.001	0.001
Tyrosine	92.05	93.91	93.26	93.31	1.653	0.216	0.240	0.690

N = 5 replicates cages. *As dry matter basis.

[1]MC, multi-carboidrase level (0 and 200 mg/kg of diet), (200 mg/kg of MC contains 700 U of α-galactosidase, 2200 U of galactomannanase, 30 000 U of xylanase and 22 000 U of β-glucanase/kg of diet). [2]Phy, Phytase level (0 and 50 mg/kg of diet). [3]SEM: standard error of mean. [4]Effect of MC - Multi-carboidrase, Phy - Phytase, and interaction MC x Phy, respectively.

85.93% and 91.85% without enzyme, 90.78% and 95.28% with MC, 89.66% and 94.66% with Phy, and 89.29% and 95.10% with MC + Phy.

Liver and pancreas weights relative to body weight are shown in Table 6. Regarding the effects of the inclusion of enzymes on the body weight of birds and the relative weights of the liver and pancreas, the differences were not significant (p>0.05).

DISCUSSION

In the AID of the ingredients by the total collection of excreta, better responses were observed with the addition of exogenous enzymes, compared to not including the same enzymes. The greater efficiencies occurred with the addition of Phy alone in CM. In comparison to the enzyme-free treatment, the addition of Phy in CM led to an increase in digestibility of 1.1% of dry matter, 5.3% of calcium, 5.5% of phosphorus, and 11.2% in NDF.

In a study on broilers, Gallardo *et al.* (2018; 2020) reported that phytates and NSP are harmful anti-nutritional compounds in broiler feed. The negative effects of phytates and NSP are complex formation with other minerals, reduction of nutrient absorption, nutrient encapsulation, the reduction of the energy density of the feed, and improved intestinal health. The favoring of digestion by the action of carbohydrases could be associated with the reduction of intestinal viscosity and loss of nutrients (Gallardo *et al.*, 2020), while also reducing nitrogen emission to the environment, which would be a complementary benefit of enzyme dietary supplementation (Sun & Kim, 2019). Therefore, increased nutrient and energy retention is associated with total or partial degradation of these anti-nutritional compounds. Thus, the highest numerical values observed for the inclusion of exogenous enzymes shown in Table 3 indicate the important effect of carbohydrases and phytase through their likely action on cell walls and nutrient decapsulation, favoring the effectiveness of phytase, as reported by Gallardo *et al.* (2017). In this case, the dietary addition of exogenous enzymes increases the digestibility and use of nutrients in plant ingredients, by reducing the viscosity of the digesta, and thus showing better absorption of minerals. The composition of amino acid and digestibility are related to the intrinsic characteristics of each ingredient, processing methods, protein fractions, crop types, and also differences in amino acid profiles (Dadalt *et al.*, 2016). Despite the differences in chemical composition established in the literature, studies have reported positive effects on the digestibility of different plant by-products when supplementing exogenous enzymes in the diets of broiler chickens (Barekatain *et al.*, 2014; Liu *et al.*, 2015; Amerah *et al.*, 2017). Some studies have indicated an increase in amino acid digestibility, apparent nitrogen balance, and energy of plant ingredients in broilers and swine that received exogenous enzymes (Gallardo *et al.*, 2017, 2018, 2020; Trindade *et al.*, 2020; Araujo *et al.*, 2021, 2022).

The enzymes carbohydrase and Phy have specific mechanisms of action; therefore, their positive effects were due to the availability of a substrate for each enzyme. The action of carbohydrases ruptures cell walls, increasing nutrient digestibility (Gallardo *et al.*, 2020). The action of carbohydrases favoring digestion could be associated with a reduction of intestinal viscosity and loss of nutrients, as well as a reduction of nitrogen emission to the environment; which would be a complementary benefit of enzyme dietary supplementation (Zijlstra *et al.*, 2004; Gallardo *et al.*, 2020).

The positive effect on the balance of nutrients and energy use may be directly associated with the supplementation of the enzymes Phy and carbohydrases. Our results showed that the enzymes increased (p<0.05) the AID of CM nutrients and AA. The mechanism of releasing encapsulated nutrients by breaking the cell walls of CM may explain the positive

Table 6 – Relative weight of organs of broilers at 35 days of age, fed with canola meal supplement or not multi-carbohydrase and phytase.

Item	Phy[2] 0		Phy 50		SEM[3]	*p*-value[4]		
	MC[1] 0	MC 200	MC 0	MC 200		MC	Phy	MCxPhy
Initial BW (g)[5]	45	45	45	45	-	-	-	-
Final BW (g)	1561	1645	1566	1694	50.07	0.678	0.624	0.607
Liver/BW[5]	29.9	33.5	34.5	32.9	1.167	0.283	0.451	0.951
Pancreas/BW[5]	1.7	1.7	1.7	1.7	0.002	0.836	0.836	0.836

N = 5 replicates cages.

[1]MC, multi-carboidrase level (0 and 200 mg/kg of diet), (200 mg/kg of MC contains 700 U of α-galactosidase, 2200 U of galactomannanase, 30 000 U of xylanase and 22 000 U of β-glucanase/kg of diet). [2]Phy, Phytase level (0 and 50 mg/kg of diet). [3]SEM: standard error of mean. [4]Effect of MC - Multi-carboidrase, Phy - Phytase, and interaction MC x Phy, respectively. [5]BW: Body weight.

effect of enzymes such as Phy and carbohydrases, which decrease intestinal viscosity, stabilize mucus production by decreasing water absorption, and allow endogenous enzymes to interact with their respective substrates (Slominski, 2011).

The pronounced effect of the use of exogenous enzymes on calcium and phosphorus retention ratifies the increase in availability with the hydrolysis of the phytate–mineral complex (Dadalt *et al.*, 2017). Therefore, effects on phosphorus and calcium were expected, as reported by Araujo *et al.* (2021), Gallardo *et al.* (2020) and Trindade Neto *et al.* (2020) when supplementing test diets for poultry and piglets, respectively, with Phy and carbohydrases. In this sense, the use of Phy can positively impact animal performance by increasing the release and absorption of phosphorus and the use of energy (Wu *et al.*, 2015). Furthermore, given its high capacity to bind nutrients, it must be considered that Phy also reduces mineral availability in the gastrointestinal tract. Thus, the inclusion of Phy not only contributes to the release of phytic phosphorus, but also allows calcium and other minerals, energy, and nitrogen to participate in the same complex (Emiola *et al.*, 2009; Gallardo *et al.*, 2018). The supplementation with Phy acted in the cellular dissociation of the fiber, leading phytase to be favored for the dephosphorylation of the P-phytate complex, and thus reflecting on apparent increase in the digestibility of the CM.

This prominent effect of Phy in combination with carbohydrases suggests an increase in amino acid digestibility, which provided better results for the apparent and standardized coefficients and may be associated with greater hydrolysis of NPS in canola, as seen other vegetable ingredients used as a dietary ingredient for poultry and pigs (Gallardo *et al.*, 2017, 2018; 2020; Trindade Neto *et al.*, 2020; Araujo *et al.*, 2021). The increase in standardized digestibility with the inclusion of enzymes stood out in comparison with CM without enzyme. Our results corroborated the findings of Ravindran *et al.* (1999), Gallardo *et al.* (2017, 2018, 2020), and Trindade Neto *et al.* (2020), who also reported positive effects on amino acid digestibility with CM. In addition to enzyme activity, the increase in amino acid digestibility depends on substrate availability, which allows the use of these and other nutrients of the tested ingredient (Emiola *et al.*, 2009). However, the inclusion of exogenous enzymes could reduce the endogenous losses of AA, improving the digestibility of nitrogen and AA. On the other hand, studies have shown that phytate increases the production of intestinal mucin and indirectly increases losses of endogenous AA, which may affect AA digestibility (Adeola & Cowieson, 2011; Woyengo & Nyachoti, 2011; Sredanovic *et al.*, 2012). Therefore, the effect of exogenous enzymes on the SID of AA could be associated with decreased mucin production, due to the reduction of the NSP of CM. According to Nian *et al.* (2011), mucin should also be considered a main source of endogenous carbohydrates in the digesta.

In the complementary assessment of liver and pancreas weights relative to body weight, the goal was to evaluate possible responses of these organs to the CM digestion process in the presence or absence of MC and Phy, since such information is scarce. Broilers fed with CM supplemented with exogenous enzymes showed numerically greater or equal liver and pancreas weights than the birds of the control group, which can be attributed to the positive effects of enzymes on nutrient absorption via fiber degradation and reduction of the secretory activity of these organs (Veldman & Vahl, 1994). Studies have found no effects of Phy and carbohydrates, alone or combination, on the weight of these organs in broiler chicks (Gallardo *et al.*, 2017; 2018; Araujo *et al.*, 2021). In addition, the presence of exogenous enzymes in the diet could be related to the increased availability and absorption of nutrients, decreasing endogenous enzymatic activity (Wu *et al.*, 2015).

CONCLUSIONS

Supplementation with Phy or MC was a viable alternative to increase the ATTD of nutrients and energy use in broilers fed CM. Isolated inclusion of Phy or carbohydrate indicated resulted in higher apparent and standardized digestibility of AA from CM.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest regarding the publication of the manuscript and the dissemination of the results obtained.

ACKNOWLEDGEMENTS

The authors thank the São Paulo State Research Foundation (FAPESP) for funding this research (2016/07352-8). They also thank Uniquimica (São Paulo, Brazil) for providing supplemental enzymes. This study was also partly financed by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001.

REFERENCES

Adeola O, Cowieson AJ. Opportunities and challenges in using exogenous enzyme to improve non-ruminant animal production. Journal Animal Science 2011;89(10):3189-218. https://doi.org/10.2527/jas.2010-3715

Amerah AM, Romero LF, Awati A, *et al.* Effect of exogenous xylanase, amylase, and protease as single or combined activities on nutrient digestibility and growth performance of broilers fed corn/soy diets. Poultry Science 2017;96(4):807-16. https://doi.org/10.3382/ps/pew297

Araujo RGAC, Gallardo C, Sartori JR, *et al.* Apparent and standardized digestibility in broilers fed flaxseed meal with multi-carbohydrase and phytase at 35 days of age. Canadian Journal of Animal Science 2021;102(1):124-32. https://doi.org/10.1139/cjas-2021-0051

AOAC - Association of Official Analytical Collaboration. Official methods of analysis. 18th ed. Washington; 2005.

Barekatain MR, Noblet J, Wu SB, *et al.* Effect of sorghum distillers dried grains with solubles and microbial enzymes on metabolizable and net energy values of broiler diets. Poultry Science 2014;93(11):1-9. https://doi.org/10.3382/ps.2013-03766

Canola Council of Canada. Canola grower´s manual. Canola meal: premium protein for dairy, livestock and fish diets [cited 2023 Dez 28]. Available from: https://www.canolacouncil.org/about-canola/meal

Dadalt JC, Gallardo C, Polycarpo GV, *et al.* Ileal amino acid digestibility of broken rice fed to postweaned piglets with or without multicarbohydrase and phytase supplementation. Asian Australasian Journal Animal Science 2016;29(10):1483-9. https://doi.org/10.5713/ajas.15.0855

Dadalt JC, Gallardo C, Polycarpo GV, *et al.* Ileal amino acid digestibility in micronized full fat soybean meal and textured soy flour fed to piglets with or without multicarbohydrase and phytase supplementation. Animal Feed Science and Technology 2017;229:106-16. https://doi.org/10.1016/j.anifeedsci.2017.05.006

Emiola IA, Opapeju FO, Slominski BA, *et al.* Growth performance and nutrient digestibility in pigs fed wheat distillers dried grains with solubles-based diets supplemented with a multicarbohydrase enzyme. Journal Animal Science 2009;87(7):2315-22. https://doi.org/10.2527/jas.2008-1195

Fan MZ, Sauer WC. Determination of apparent ileal amino acid digestibility in barley and canola meal for pigs with the direct, difference, and regression methods. Journal Animal Science 1995;73(8):2364-74. https://doi.org/10.2527/1995.7382364x

Gallardo C, Dadalt JC, Kiarie E, *et al.* Effects of multi-carbohydrase and phytase on standardized ileal digestibility of amino acids and apparent metabolizable energy in canola meal fed to broiler chicks. Poultry Science 2017;96(9):3305-13. https://doi.org/10.3382/ps/pex141

Gallardo C, Dadalt JC, Trindade Neto MA. Nitrogen retention, energy, and amino acid digestibility of wheat bran, without or with multicarbohydrase and phytase supplementation, fed to broiler chickens. Journal Animal Science 2018;96(6):2371-9. https://doi.org/10.1093/jas/sky062

Gallardo C, Dadalt JC, Trindade Neto MA. Carbohydrases and phytase with rice bran, effects on amino acids digestibility and energy use in broiler chickens. Animal 2020;14(3):482-90. https://doi.org/10.1017/S1751731119002131

Kiarie E, Walsh MC, Nyachoti CM. Performance, digestive function and mucosal responses to selected feed additives for pigs. Journal of Animal Science 2016;94(3):169-80. https://doi.org/10.2527/jas.2015-9835

Landero JL, Wang LF, Beltranena E, *et al.* Feed preference of weaned pigs fed diets containing soybean meal, Brassica napus canola meal, or Brassica juncea canola meal. Journal of Animal Science 2018;96(2):600-11. https://doi.org/10.1093/jas/skx052.

Liu SY, Selle PH, Cowieson AJ. Strategies to enhance the performance of pigs and poultry on sorghum-based diets. Animal Feed Science and Technology 2015;181:1-14. https://doi.org/10.1016/j.anifeedsci.2013.01.008

Maison T, Liu Y, Stein HH. Digestibility of energy and detergent fiber and digestible and metabolizable energy values in canola meal, 00-rapeseed meal, and 00-rapeseed expellers fed to growing pigs. Journal of Animal Science 2015;93(2):652-60. https://doi.org/10.2527/jas.2014-7792

Mejicanos G, Sanjayan N, Kim IH, *et al.* Recent advances in canola meal utilization in swine nutrition. Journal of Animal Science and Technology 2016;58(7):1-10. https://doi.org/10.1186/s40781-016-0085-5

Mosenthin R, Messerschmidt U, Sauer N, *et al.* Effect of the desolventizing/toasting process on chemical composition and protein quality of rapeseed meal. Journal of Animal Science Biotechnology 2016;7:1–12. https://doi.org/10.1186/s40104-016-0095-7

Nyachoti CM, Lange CF, Schulze H. Estimating endogenous amino acid flows at the terminal ileum and true ileal amino acid digestibilities in feedstuffs for growing pigs using the homoarginine method. Journal of Animal Science 1997;75(12):3206-13. https://doi.org/10.2527/1997.75123206x

Nian F, Guo YM, Ru YJ, *et al.* Effect of exogenous xylanase supplementation on the performance, net energy and gut microflora of broiler chickens fed wheat-based diets. Asian Australasian Journal of Animal Science 2011;24(3):400-6. https://doi.org/10.5713/ajas.2011.10273

Opapeju FOA, Golian CM, Nyachoti LD. Amino acid digestibility in dry extruded-expelled soybean meal fed to pigs and poultry. Journal of Animal Science 2006;84(5):1130-7. https://doi.org/10.2527/2006.8451130x

Ravindran V, Selle PH, Bryden WL. Effects of phytase supplementation, individually and in combination, with glycanase, on the nutritive value of wheat and barley. Poultry Science 1999;78(11):1588-95. https://doi.org/10.1093/ps/78.11.1588

Rostagno HS, Albino LFT, Hannas MI, *et al.* Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4th ed. Viçosa, MG: Universidade Federal de Viçosa; 2017.

SAS - Statistical Analysis System. User's huide. Version 9.4. 3rd ed. Cary (NC): SAS Institute; 2014.

Slominski BA. Recent advances in research on enzymes for poultry diets. Poultry Science 2011;90(9):2013-23. https://doi.org/10.3382/ps.2011-01372

Sredanovic SA, Levic JD, Jovanovic RD, *et al.* The nutritive value of poultry diets containing sunflower meal supplemented by enzymes. Acta Periodica Technologica 2012;43(43):79-91. https://doi.org/10.2298/APT1243079S

Spies JR. Determination of tryptophan in proteins. Analytical Chemistry 1967;39(12):1412-6. https://doi.org/10.1021/ac60256a004

Sun HY, Kim IH. Effects of multi-enzyme on production performance, egg quality, nutriente digestibility, and excreta noxious gas emission of early phase Hy-line brown hens. Poultry Science 2019;98(10):4889-95. https://doi.org/10.3382/ps/pez237

Trindade Neto MA, Dadalt JC, Gallardo C. Nutrient and energy balance, and amino acid digestibility in weaned piglets fed wheat bran and an exogenous enzyme combination. Animal 2020;14(3):1-9. https://doi.org/10.1017/S1751731119002052

Veldman A, Vahl H. Xylanase in broiler diets with differences in characteristics and content of wheat. British Poultry Science 1994;35(4):537-50. https://doi.org/10.1080/00071669408417719

Woyengo TA, Kiarie E, Nyachoti CM. Energy and amino acid utilization in expeller-extracted canola meal fed to growing pigs. Journal of Animal Science 2010;88(4):1433-41. https://doi.org/10.2527/jas.2009-2223

Woyengo TA, Nyachoti CM. Review: Supplementation of phytase and carbohydrases to diets for poultry. Canadian Journal of Animal Science 2011;91(2):177-92. https://doi.org/10.4141/cjas1008

Wu D, Wu SB, Choct M, *et al.* Comparison of phytases on energy utilization of a nutritionally marginal wheat-soybean meal broiler diet. Poultry Science 2015;94(11):2670-6. https://doi.org/10.3382/ps/pev222

Zijlstra RT, Li S, Owusu-Asiedu A, *et al.* Effect of carbohydrase supplementation of wheat- and canola-meal-based diets on growth performance and nutrient digestibility in group-housed weaned pigs. Canadian Journal of Animal Science 2004;84 (4):689-95. https://doi.org/10.4141/A03-127.