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Article

Impact of multicarbohydrase and phytase on apparent and standardized digestibility, energy, and nutrient balance in broilers fed sunflower meal

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Abstract

The apparent total tract digestibility (ATTD) (trial 1) and the apparent (AID) and standardized (SID) ileal digestibility of the amino acids (AAs) (trial 2) in sunflower meal (SM) were evaluated with the addition of exogenous multicarbohydrase (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, energy use, and the AID and SID of AA, in five replicate cages. Synergic effect was identified between MC and Phy on the ATTD of minerals and fibre. The same benefit occurred with the isolated inclusion of MC on the ATTD of dry matter, nitrogen, and energy of SM. The effects of enzyme inclusion on the AID and SID of AAs in SM, established by comparing the means, suggested a higher effect to the addition of MC + Phy combibation. Supplementation of MC or combination with MC and Phy was a viable alternative to increase the ATTD of nutrients and energy. The addition MC + Phy higher AID and SID of AA from SM.

Key words: amino acids, broilers, digestibility, enzymes, sunflower products

Résumé

La digestibilité apparente du tractus complet (ATTD — « apparent total tract digestibility »; expérience 1) et les digestibilités iléales apparentes (AID — « apparent ileal digestibility ») et normalisées (SID — « standardized ileal digestibility ») des acides aminés (AA) (expérience 2) dans le tourteau de tournesol (SM — « sunflower meal ») ont été évaluées avec l'ajout de multicarbohydrases (MC) et phytases (Phy) exogènes. Un total de 80 poulets à griller âgés de 28 jours ont été alloués dans un design expérimental entièrement aléatoire pour recevoir les traitements jusqu'à 35 jours d'âge. Un design factoriel 2 × 2 a été utilisé afin de déterminer les effets des enzymes sur l'ATTD des matières sèches, azote, calcium, phosphore, et fibres; l'utilisation d'énergie et les AID et SID des AA, en cinq cages réplicats. Un effet synergique a été trouvé entre MC et Phy sur l'ATTD des minéraux et des fibres. Le même avantage se trouvait avec l'inclusion isolée de MC sur l'ATTD des matières sèches, azote, et énergie du SM. Les effets de l'inclusion d'enzyme sur les AID et SID des AA dans le SM, déterminés par comparaison des moyennes, suggèrent une meilleure réponse à l'ajout de MC + Phy. Les suppléments de MC ou une combinaison de MC et Phy sont une option alternative viable pour augmenter l'ATTD des éléments nutritifs et l'énergie. L'ajout de MC + Phy augmentait davantage les AID et SID des AA provenant du SM. [Traduit par la Rédaction]

Mots-clés : acides aminés, poulets à griller, digestibilité, enzymes, produits du tournesol

Introduction

Sunflower meal (SM) is a by-product obtained after the extraction of oil from sunflower seeds and is currently used as a good source of crude protein in broiler nutrition (Bandegan et al. 2011; Sredanovic et al. 2012; Dadalt et al. 2016). SM contains approximately 33% crude protein and 17.64 MJ of energy (Rostagno et al. 2017) and can be used as a partial replacement for soybean meal and corn to minimize the cost of feed.

The main antinutritional factor in sunflower affecting broiler diets is its high content of non-starch polysaccharide (NSP) and phytates (Woyengo and Nyachoti 2011). Intestinal transit time and nutrient use can be negatively affected as the bird increases its NSP intake due to increased

intestinal viscosity (Kiarie et al. 2016; Gallardo et al. 2020). 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). According to Rostagno et al. (2017), SM is moderately rich in crude fibre and NDF, whose digestion can be favoured by exogenous enzyme supplementation—already considered a common practice to improve the use of plant ingredients with high fibre content and unavailable phosphorous (Slominski 2011; Woyengo and Nyachoti 2011; Gallardo et al. 2018; Lu et al. 2020).

As reported by Gallardo et al. (2020), the negative effects of phytic acid, which limits the availability of phosphorous, can be minimized with the use of phytase (Phy), whereas the negative effects of fibre on digestion can be reduced by supplementing with carbohydrases. Previous studies have shown an improvement in nutrient use, energy, and digestibility of amino acids (AAs) with the use of carbohydrates and Phy in the diets of swine and broilers (Kong and Adeola 2011; Dadalt et al. 2017; Gallardo et al. 2020; Trindade et al. 2020).

Thus, we evaluated the nutritional and energy balance and the digestibility of the AAs in SM, in the presence or absence of multicarbohydrases (MC) and Phy in broilers from 28 to 35 days of age.

Materials and methods

All the research methods and procedures were approved by the ethics committee for the use of animals at 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 according to animal welfare. The methodology was described in detail by Gallardo et al. (2020).

SM and enzymes

The SM 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 SM was similar to that described by Rostagno et al. (2017).

The enzymes used were an MC blend described as Endopower Beta; certificate analysis indicated minimum guaranteed enzyme activities: galactosidase 35 units/g, galactomannanase 110 units/g, xylanase 1500 units/g, beta-glucanase 1100 units/g (GNC Bioferm Inc., Saskatoon, SK, Canada). Phy described as Genophos, analysed by colorimetric method, ISO 30024, "Animal Feeding Stuffs—Determination of Phytase Activity: 13 790.39 FTU/g", CBO, Valinhos, Brazil. Additional technical information was provided by Uniquimica, São Paulo, Brazil.

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

Nutrient component (%)	Sunflower meal
Dry matter	90.96
Crude protein	33.24
Gross energy (MJ/kg)	17.75
Fat	4.87
Calcium	0.60
Phosphorous	1.01
Neutral detergent fibre	32.09
Essential amino acids (%)	
Arginine	2.06
Histidine	0.59
Isoleucine	1.03
Leucine	1.56
Lysine	0.78
Methionine	0.44
Phenylalanine	1.29
Threonine	0.87
Tryptophan	1.02
Valine	1.26
Non-essential amino acids (%)	
Alanine	1.60
Aspartic acid	3.20
Cystine	0.60
Glutamine	6.68
Glycine	2.05
Proline	1.35
Serine	1.50
Tyrosine	0.87

Diets and experimental design

The apparent total tract digestibility (ATTD) (trial 1) and the apparent (AID) and standardized (SID) ileal digestibility of the AAs (trial 2) of SM 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 the experiments were conducted in a completely randomized design in a 2 × 2 factorial arrangement of treatments (5 replicate cages and 16 birds per treatment). The factors were MC (0 and 200 mg/kg) and Phy (0 and 50 mg/kg). Basal diets used for an additional group of 24 birds kept in 6 cages were used to determine the enzyme effects, alone or in combination. A basal corn diet (BD_1) was used for ATTD determination, and a cornstarch basal diet (BD_2) containing 5% casein was used to estimate endogenous losses and the SID of AAs, as described in Gallardo et al. (2020). The test diets were made by mixing BD and SM 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), the birds received diet mash, where excreta were collected, and from day 34 to day 35 (trial 2), the birds received a new pelleted diet until slaughter at the end of the experimen-

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

Ingredients	Total collection[a] Basal diet (BD_1)	Ileal collection diet[b] 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[c]	5.0	5.0
Kaolin[d]	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 phosphorous	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

[a]Corn-based diet.
[b]Cornstarch and casein diet.
[c]Vitamin–mineral premix per kg of feed: vitamin A 9000 IU; vitamin D_3 1600 IU; vitamin E 14 IU; vitamin K_3 15 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 μg; niacin 0 03 mg; folic acid 0.3 mg; biotine 0.05 mg; Se 0.25 mg; Cu 9 mg; Fe 30 mg; I 1 mg; Zn 60 mg; and Mn 60 mg.
[d]Kaolin: mineral kaolinite, used as inert ingredient to adjust the formulation of diet.

tal period, when the ileal content was collected. Chromium oxide III (Cr_2O_3) was added at 0.3% to all the diets as an indigestible marker. In trials 1 and 2, all the 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).

In trial 1, to assess the balance of nutrients and the apparent metabolizable energy (EMA) in the SM, samples of excreta were collected twice a day (08:00 and 17:00) for five consecutive days. To determine the digestibility coefficients of the AAs 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 usedreviewed:

$$\text{Relative organ weight} = (\text{organ weight}/\text{postfasting weight}) \times 100$$

Sample analyses and data processing

The excreted and ileal samples were freeze-dried for 72 h at −40 °C (LH 0401, Terroni, São Carlos, Brazil) as described by Gallardo et al. (2017), showing the procedures of the diets, SM, excreta, and ileal digesta samples that were finely milled and analysed, according to the Association of Official Analytical Chemists (AOAC 2005) for determinations of dry matter (DM), gross energy (GE), nitrogen (N), calcium (Ca), phosphorous (P), and neutral detergent fibre (NDF). Test diets and ileal digesta samples were processed and analysed to determine the digestibility coefficients of AAs 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, NJ, USA). Cystine was expressed as cysteine. All the analyses were performed in duplicate.

Calculations

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

$$\text{Digestibility of nutrients } (\%) = 100 \times [(\text{NI} - \text{NO}_{\text{excreta}})/\text{NI}]$$

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

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

$$\text{DA} = (\text{DD} - (\text{DB} \times \text{DN}))/\text{DSM}$$

where DA is the retention of a nutrient (%) in an assay feedstuff (SM), DD is the digestibility of a nutrient (%) in the SM-containing diet, DB is the digestibility of a nutrient (%) in the corn-based diet, DN is the contribution of a nutrient (decimal percentage) from corn to the assay diet, and DRB is the contribution of a nutrient (decimal percentage) from SM in the SM-based diet.

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

$$\text{AME of SM (kcal/kg)} = [(\text{Gross energy retention for SM, \%}) \times (\text{Gross energy content in SM, kcal/kg})]/100$$

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

$$\text{AID (\%)} = 100 - \left[100 \times \left(\text{AA}_{\text{digesta}} \times \text{Cr}_2\text{O}_{3\text{diet}}\right) / \left(\text{AA}_{\text{diet}} \times \text{Cr}_2\text{O}_{3\text{digesta}}\right)\right]$$

where AA_{diet} and $AA_{digesta}$ are the AA content (mg/kg of DM) in the diet and digesta, respectively, and $Cr_2O_{3diet\ and}$ $Cr_2O_{3digesta}$ are the indigestible marker content (mg/kg of 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):

$$\text{AA}_{\text{EL}}\,(\text{g/kg}) = \text{AA}_{\text{digesta}} \times \left(\text{Cr}_2\text{O}_{3\text{diet}}/\text{Cr}_2\text{O}_{3\text{digesta}}\right)$$

where AA_{EL} = average endogenous AA loss (g/kg of DM).

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

$$\text{SID (\%)} = [\text{AID}_{\text{AA}} + (\text{AA}_{\text{EL}}/\text{AA}_{\text{diet}})] \times 100$$

Statistical analysis

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

$$Y_{ij} = \mu + a_i = b_j + (a_i \times b_j) + e_{ij}$$

where Y_{ij} = variable response of broilers fed with MC and Phy; μ = overall mean; a_i = MC effect; b_j = Phy effect; ($a_i \times b_j$) = interaction between MC and Phy; e_{ij} = error contribution with average 0 and variance $\sigma 2$, $I = 1, \ldots, a$, and $j = 1, \ldots, b$. Significance was accepted at $P < 0.05$.

Results

The effects of the treatments on nutrient balance and AME in broilers from 28 to 33 days of age, fed SM supplemented with MC and Phy, are shown in Table 3, and the AA content used to determine AID and SID in Tables 4 and 5. There was an interaction effect between MC and Phy on the AID of Ca ($P = 0.03$), P ($P = 0.01$), and NDF ($P = 0.01$). Isolated inclusion of carbohydrase indicated favourable effects ($P < 0.05$) on the AID of DM and N as well as energy use. In relation to Phy, there were no effects ($P > 0.05$).

The coefficients of apparent and standardized digestibility of the AAs 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 AAs. The average values of apparent and standardized digestibility of the 17 AAs of SM were as follows: 87.36% and 95.93% without enzyme, 90.88% and 96.23% with MC, 89.92% and 95.98% with Phy, and 89.62% and 96.56% 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

The chemical composition of the diets was different from those found according to the Nutrient Requirements for Poultry (), except for the Ca (0.60%) and P (1.01%); however, they were close to those found by Rostagno et al. (2017) for CP (33.24%), GE (17.75 MJ/kg), Ca (0.60%), and P (1.01%), as well as the AA profile of SM. Variations in AA composition and digestibility are related to the intrinsic characteristics of each ingredient, type of crop, processing method, protein fractions, as well as differences in the AA profile (Dadalt et al. 2016). Despite the differences in chemical composition, as stated in the literature, studies 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 indicated an increase in AA 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).

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 favouring of digestion by the action of carbohydrases could be associated with a reduction of intestinal viscosity and loss of nutrients as nitrogen for the environment, which would be a complementary benefit of enzyme dietary supplementation (Zijlstra et al. 2004; Gallardo et al. 2020).

In a study on broilers, Gallardo et al. (2017) reported that NSP and phytates are harmful antinutritional compounds in poultry feed. According to Egli et al. (2002), each kilogram of SM is composed of, on average, 4–64 g phytates and 276 g of NSP (Dusterhoft et al. 1997). The negative effects of NSP and phytates are complex formation with other minerals, reduction of the absorption of nutrients, encapsulation of nutrients, the reduction of the energy density of the feed and increased intestinal viscosity. Therefore, increased nutrient and energy retention is associated with total or partial degradation of these antinutritional compounds. 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 showing better absorption of minerals.

Research focused on the use of the enzymes xylanase and glucanase in broilers increases the efficiency of digestibility and availability of SM. According to Sorensen (1996) and Fafiolu et al. (2015), the presence of xylanase and glucanase in SM-based broiler diets increased the use of nutrients and energy, in comparison with a diet without enzyme supplementation. There are few studies in the literature evaluating

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

	Phy[b] 0		Phy 50			P^{d}		
Item	MC[a] 0	MC 200	MC 0	MC 200	SEM[c]	MC	Phy	MC × Phy
Dry matter	768.4	791.1	763.4	780.2	0.369	0.001	0.061	0.483
Nitrogen	756.2	767.3	744.6	763.8	0.467	0.005	0.098	0.365
AME (MJ/kg)	8.43	9.09	8.38	8.90	29.414	0.001	0.216	0.492
Calcium	715.1	773.5	683.8	799.1	1.183	0.001	0.806	0.028
Phosphorous	372.2	573.5	469.3	760.0	0.559	0.001	0.001	0.001
NDF (%)	478.2	488.2	443.5	532.4	0.517	0.001	0.415	0.001
ME/GE	435.4	469.3	431.7	456.9	0.636	0.001	0.214	0.492

Note: $N = 5$ replicate cages, as dry matter basis. AME, apparent metabolizable energy; NDF, neutral detergent fibre.
[a]MC, multicarbohydrase level (0 and 200 mg/kg of diet), (200 mg/kg of MC contains 700 U of alpha-galactosidase, 2200 U of galactomannanase, 30 000 U of xylanase, and 22 000 U of beta-glucanase per kilogram of diet).
[b]Phy, phytase level (0 and 50 mg/kg of diet).
[c]SEM, standard error of mean.
[d]Effect of MC, multicarbohydrase, Phy, phytase, and interaction MC × Phy, respectively.

Table 4. Apparent ileal digestibility of the amino acids of sunflower meal, combined or not with enzymes, for broilers at 35 days old.

	Phy[b] 0		Fitase 50			P^{d}		
Item*	MC[a] 0	MC 200	MC 0	MC 200	SEM[c]	MC	Phy	MC × Phy
Essential amino acids (%)								
Arginine	92.48	94.65	94.34	94.84	1.010	0.039	0.010	0.086
Histidine	93.12	95.06	94.92	94.71	0.804	0.050	0.028	0.009
Isoleucine	83.69	87.28	88.15	85.85	1.708	0.050	0.412	0.001
Leucine	88.13	92.05	91.82	91.26	1.171	0.014	0.006	0.001
Lysine	87.10	91.23	88.18	86.90	1.792	0.095	0.061	0.004
Methionine	93.57	94.13	95.21	93.03	1.478	0.685	0.239	0.050
Phenilalanine	92.31	94.15	94.21	94.82	1.025	0.104	0.002	0.027
Threonine	85.00	89.69	89.00	89.11	1.742	0.043	0.007	0.010
Tryptophan	83.94	86.17	82.47	87.65	0.829	0.076	0.004	0.031
Valine	89.17	91.97	91.69	91.33	1.450	0.167	0.078	0.027
Non-essential amino acids (%)								
Alanine	89.81	93.10	92.34	92.22	1.310	0.188	0.018	0.012
Aspartic acid	86.61	91.18	90.47	90.51	1.527	0.033	0.004	0.004
Glutamine	91.98	93.68	93.95	93.41	1.149	0.118	0.277	0.045
Glicine	84.24	86.15	87.25	84.67	1.563	0.289	0.636	0.005
Proline	77.09	86.63	78.11	78.25	1.879	0.001	0.001	0.001
Serine	77.13	84.85	84.25	83.17	2.129	0.012	0.003	0.001
Tyrosine	89.73	93.02	92.28	91.85	1.522	0.326	0.050	0.015

Note: $N = 5$ replicates cages.
*As dry matter basis.
[a]MC, multicarbohydrase level (0 and 200 mg/kg of diet) (200 mg/kg of MC contains 700 U of alpha-galactosidase, 2200 U of galactomannanase, 30 000 U of xylanase, and 22 000 U of beta-glucanase per kilogram of diet).
[b]Phy, phytase level (0 and 50 mg/kg of diet).
[c]SEM, standard error of mean.
[d]Effect of MC, multicarbohydrase, Phy, phytase, and interaction MC × Phy, respectively.

the effect of carbohydrate and Phy addition on the digestibility of SM in broilers.

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 combination of enzymes increased ($P < 0.05$) the AID of SM nutrients and AAs. The mechanism of releasing encapsulated nutrients by breaking the cell walls of SM may explain the positive effect of enzymes such as Phy and carbohydrases by decreasing the intestinal viscosity, stabilizing mucus production by decreasing water absorption, and allowing endogenous enzymes to interact with their respective substrates (Slominski 2011).

The pronounced effect of the use of exogenous enzymes on calcium and phosphorous retention ratifies the increase in availability with the hydrolysis of the phytate–mineral complex (Dadalt et al. 2017). Therefore, effects on phosphorous and calcium were expected, as noted by Gallardo et al. (2020) and Trindade Neto et al. (2020), when supplementing test diets for poultry and piglets, respectively, with Phy and carbo-

Table 5. Standardized digestible coefficient of amino acids in sunflower meal, combined or not with enzymes, in broilers at 35 days old.

	Phy[b] 0		Phy 50			P[d]		
Item*	MC[a] 0	MC 200	MC 0	MC 200	SEM[c]	MC	Phy	MC × Phy
Essential amino acids (%)								
Arginine	96.07	97.80	97.54	96.12	1.043	0.084	0.028	0.228
Histidine	96.82	97.79	97.84	96.60	0.802	0.097	0.125	0.110
Isoleucine	96.19	96.88	98.16	98.79	1.523	0.234	0.527	0.453
Leucine	95.72	96.72	96.14	95.96	1.478	0.118	0.023	0.119
Lysine	95.22	97.89	97.90	97.24	1.609	0.055	0.052	0.126
Methionine	98.86	98.98	98.41	98.17	1.363	0.922	0.318	0.770
Phenilalanine	96.90	97.86	98.21	97.68	1.024	0.105	0.006	0.122
Threonine	97.63	97.30	97.10	97.06	1.547	0.112	0.060	0.248
Tryptophan	87.02	89.32	82.59	93.74	0.829	0.067	0.001	0.004
Valine	96.66	98.24	97.49	98.99	1.146	0.034	0.401	0.044
Non-essential amino acids (%)								
Alanine	95.01	97.10	97.65	96.01	1.341	0.139	0.035	0.108
Aspartic acid	95.79	96.70	96.38	97.24	1.520	0.029	0.012	0.039
Glutamine	98.74	98.19	98.70	98.60	0.997	0.083	0.492	0.551
Glicine	89.87	90.57	92.01	93.86	1.623	0.335	0.338	0.048
Proline	92.27	94.05	92.81	95.63	1.440	0.761	0.040	0.007
Serine	96.35	96.59	97.28	97.43	1.424	0.012	0.100	0.005
Tyrosine	96.61	96.85	97.46	97.68	1.159	0.030	0.223	0.010

Note: $N = 5$ replicates cages.
*As dry matter basis.
[a]MC, multicarbohydrase level (0 and 200 mg/kg of diet), (200 mg/kg of MC contains 700 U of alpha-galactosidase, 2200 U of galactomannanase, 30 000 U of xylanase, and 22 000 U of beta-glucanase per kilogram of diet).
[b]Phy, phytase level (0 and 50 mg/kg of diet).
[c]SEM, standard error of mean.
[d]Effect of MC, multicarbohydrase, Phy, phytase, and interaction MC × Phy, respectively.

Table 6. Relative weight of organs of broilers at 35 days of age, fed with sunflower meal supplement or not, multicarbohydrase, and phytase.

	Phy[b] 0		Phy 50			P[d]		
Item	MC[a] 0	MC 200	MC 0	MC 200	SEM[c]	MC	Phy	MC × Phy
Initial BW (g)[e]	45	45	45	45	—	—	—	—
Final BW (g)	1500	1585	1548	1544	56.247	0.640	0.092	0.313
Liver/BW[f]	30.0	39.4	31.4	32.9	0.001	0.406	0.095	0.099
Pancreas/BW[f]	1.5	1.6	1.6	1.6	7.280	0.234	0.234	0.096

Note: $N = 5$ replicates cages.
[a]MC, multicarbohydrase level (0 and 200 mg/kg of diet), (200 mg/kg of MC contains 700 U of alpha-galactosidase, 2200 U of galactomannanase, 30 000 U of xylanase, and 22 000 U of beta-glucanase per kilogram of diet).
[b]Phy, phytase level (0 and 50 mg/kg of diet).
[c]SEM, standard error of mean.
[d]Effect of MC, multicarbohydrase, Phy, phytase, and interaction MC × Phy, respectively.
[e]BW, body weight.
[f]Relative to body weight.

hydrases. In this sense, the use of Phy can positively impact animal performance by increasing the release and absorption of phosphorous 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 phosphorous but also allows calcium and other minerals, energy, and nitrogen to participate in the same complex (Emiola et al. 2009; Gallardo et al. 2018). A study on the digestibility of SM in broilers supplemented with an enzyme complex of cellulase, beta-glucanase, xylanase, and Phy showed a significant ($P < 0.05$) improvement in the apparent coefficients of calcium and phosphorous and the use of energy, as was observed in the present research (Taverani et al. 2010).

Therefore, the combined action of enzymes can provide greater use of nutrients and increase animal performance (Cowieson et al. 2017; Dadalt et al. 2017). However, the antinutritional factors of SM limit the action of endogenous enzymes, increasing AA losses and impairing AA digestibility (Bao et al. 2013). As reported by Li et al. (1996), NPS-degrading enzymes can hydrolyse cell wall compounds, reducing vis-

cosity in the intestine and increasing the digestibility of AAs.

Regarding AA digestibility, the combination MC + Phy provided better results for the apparent and standardized coefficients, which may be associated with greater hydrolysis of NPS. The increase in standardized digestibility with the inclusion of enzymes stood out in comparison with SM without enzyme and, for some AAs, also in comparison with the isolated inclusion of MC or Phy. The positive effects on AA digestibility found in the present study are in line with the findings of Ravindran et al. (1999) and Gallardo et al. (2018, 2020). The increase in AA digestibility depends, in addition to enzyme activity, on substrate availability, which allows the use of these and other nutrients of the test ingredient (Emiola et al. 2009). Generally, foods with a higher amount of NPS respond better to carbohydrate supplementation (Adeola and Cowieson 2011), as suggested by the chemical composition of SM, as to the more pronounced action of MC compared with Phy on the digestibility of AA. When comparing the supply of some fibrous feeds for birds, Borges et al. (2005) found that fibre content and type promoted increases in endogenous secretions, underestimating AA digestibility. 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 both fibre and phytate increase the production of intestinal mucin and indirectly increase losses of endogenous AAs, which may affect the digestibility of AAs (Adeola and Cowieson 2011; Woyengo and Nyachoti 2011; Sredanovic et al. 2012). Therefore, the effect of the interaction of MC and Phy on the SID of AAs could be associated with decreased mucin production, due to the reduction of the NSP of SM. 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 intent was to evaluate possible responses of these organs to the SM digestion process in the presence or absence of MC and Phy, since such information is scarce. Broilers fed with SM supplemented with exogenous enzymes showed higher 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 fibre degradation and reduction of the secretory activity of these organs (Veldman and Vahl 1994). 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. 2004). However, Gallardo et al. (2017, 2018) found no effects of Phy and carbohydrates on the weight of these organs in broiler chicks.

Conclusions

Supplementation with MC or a combination of MC and Phy was a viable alternative to increase the ATTD of nutrients and energy use in broilers fed SM. The addition of MC and Phy resulted in higher apparent and standardized digestibility of AAs from SM.

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