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Effects of a multicarbohydrase complex and corn distillers dried grains on performance, nutrient digestibility, carcass yield and intestinal health in broilers

Complexo multicarboidrase e inclusão de grãos secos de destilaria de milho na dieta sobre o desempenho, digestibilidade de nutrientes, rendimento de carcaça e saúde intestinal de frangos de corte

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Highlights

Evaluation of multicarbohydrase complex and DDGS inclusion in broiler diets.
Enzyme supplementation improved nutrient digestibility.
DDGS inclusion reduced the intestinal absorption area.

Abstract

The aim of this study was to evaluate the effects of supplementing broiler diets containing different levels of corn distillers dried grains with solubles (DDGS) with a multicarbohydrase complex on productive performance, carcass yield, nutrient digestibility and intestinal health. A total of 2016 one-day-old chicks were randomly assigned to a 4 × 2 factorial scheme (4 levels of DDGS inclusion × with and without enzyme supplementation). Productive performance was assessed weekly until 42 days of age by weighing the birds and leftover feed. At 21 days, 96 birds were transferred to metabolism cages and allocated to eight treatments with six replicates each for a digestibility trial, using total excreta collection. DDGS inclusion in poultry diets negatively affected production performance and carcass and commercial cut yields regardless of enzyme supplementation ($p<0.05$). However, supplementation

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with the multicarbohydrase complex increased apparent metabolizable energy (AME), nitrogen-corrected apparent metabolizable energy (AMEn) and neutral detergent fiber digestibility in diets with up to 10% DDGS (p<0.05). The inclusion of DDGS led to a smaller absorption area in the jejunal and ileal mucosa (p<0.05) and decreased digestibility coefficients for dry matter, crude protein, and ether extract in broilers diets (p<0.05). Enzyme supplementation increased cecal acetic acid concentrations and improved dry matter digestibility, crude protein and acid detergent fiber (p<0.05). The variability of its nutritional composition means that the use of DDGS in poultry diets should be approached with caution. The results demonstrated that the DDGS source tested is unsuitable for starter broiler diets and, for use in grower and finisher phases, should not exceed 10% inclusion rate without concurrent multicarbohydrase complex supplementation. Therefore, to ensure the safe use of DDGS in broiler diets, significant improvements in the standardization and quality of this product are essential.
Key words: Corn. Enzymes. Intestinal quality. Non-starch polysaccharides. Short-chain fatty acids.

Resumo

O objetivo deste estudo foi avaliar o efeito da suplementação com complexo multicarboidrase em dietas preparadas com diferentes níveis de inclusão de grãos secos de destilaria de milho com solúveis (DDGS) sobre o desempenho produtivo, rendimento de carcaça, digestibilidade dos nutrientes e saúde intestinal em frangos de corte. Para tanto, 2016 pintos de um dia foram distribuídos em esquema fatorial 4 × 2 (4 níveis de inclusão de DDGS × com e sem complexo multicarboidrase). Para avaliar o desempenho produtivo, as aves e as sobras de ração foram pesadas semanalmente até os 42 dias. Aos 21 dias, 96 aves foram alocadas em gaiolas de metabolismo, distribuídas em 8 tratamentos com 6 repetições, para o ensaio de digestibilidade, utilizando o método de coleta total de excretas. A inclusão de DDGS em dietas para aves resultou em pior desempenho produtivo e rendimento de carcaça e cortes comerciais independentemente da suplementação com complexo multicarboidrase (p<0,05). A suplementação com complexo multicarboidrase em dietas com níveis de até 10% de DDGS resultou em aumento de AME e EMAn e na digestibilidade da fibra em detergente neutro (p<0,05). A inclusão de DDGS resultou em menor área de absorção da mucosa do jejuno e íleo (p<0,05) e reduziu o coeficiente de digestibilidade da matéria seca, proteína bruta e extrato etéreo nas dietas de frangos de corte (p<0,05). A suplementação enzimática aumentou a concentração de ácido acético no conteúdo cecal, melhorou a digestibilidade da matéria seca, proteína bruta e fibra em detergente ácido (p<0,05). A variação da composição nutricional do DDGS limita seu uso em dietas de aves. Os resultados demonstraram que a fonte de DDGS testada não pode ser incluída em dietas de frangos de corte iniciais e o uso nas fases de crescimento e terminação é recomendado com uma taxa máxima de inclusão dietética de 10% e associado ao complexo multicarboidrase. Portanto, para o uso mais seguro de DDGS em dietas para frangos de corte, é necessário desenvolver melhorias na padronização e qualidade deste produto.
Palavras-chave: Milho. Enzimas. Qualidade intestinal. Polissacarídeos não amiláceos. Ácidos graxos de cadeia curta.

Introduction

Nutrition represents the highest production cost in Brazilian poultry farming, accounting for 72% of total costs. Poultry diets are formulated using corn and soybean meal as the primary ingredients due to their high nutritional quality. However, these raw materials are subject to frequent market price fluctuations (Xu & Zhang, 2021), which directly impacts profitability.

In order to reduce feed costs and environmental impacts without compromising productivity, nutritionists are increasingly exploring alternative ingredients or industrial byproducts to partially or totally replace ingredients such as corn and soybean meal.

DDGS (distillers' dried grains with solubles) is a coproduct of corn-based ethanol production. Due to its high protein content, it can be used as a partial substitute for soybean meal in animal diets (Liu, 2011; Damasceno et al., 2020).

While corn is the primary raw material for ethanol production in the USA, Brazil continues to predominantly use sugarcane for this purpose. In 2023, the USA had 187 ethanol plants with a production capacity of 17.6 billion liters per year (U.S. Energy Information Administration [EIA], 2023), while in Brazil, 18 plants were in operation, producing 4.5 billion liters of corn ethanol (Ministério da Agricultura e Pecuária [MAPA], 2024).

The Brazilian corn ethanol continues to evolve, with the introduction of new techniques to improve the quality and standardization of by-products. Nevertheless, the nutritional potential of these co-products can vary significantly depending on the grain processing methods used in different ethanol plants (Lewandrowski et al., 2019).

In general, the concentration of non-fermentable nutrients, such as proteins, lipids and minerals in DDGS is approximately three times higher than that found in the original grain (Liu, 2011). Likewise, levels of non-starch polysaccharide (NSP) are also higher when compared to unprocessed grains (Swiatkiewicz et al., 2016).

NSPs are known for their low digestibility and anti-nutritional effects. Their presence in poultry diet increases digesta viscosity and leads to nutrient encapsulation, making them unavailable for digestion and absorption (Ward, 2021).

In addition to the presence of NSPs, other challenges associated with DDGS use in poultry diets include the presence of mycotoxins and the variability in nutrient composition. To overcome these limitations, some authors have highlighted the potential of exogenous enzymes as an important mechanism to enhance the nutritional value of DDGS-containing diets (Campasino et al., 2015; Dal Pont et al., 2022).

The use of exogenous enzymes in animal diets can improve intestinal morphology and quality, especially when high-fiber alternative ingredients are included in diets, since non-ruminant animals cannot efficiently take advantage of the nutrients present in foods rich in NSPs and phytate, due to the absence of specific enzymes to degrade these compounds. Enhanced fiber degradation reduces digesta viscosity, increases nutrient availability, and modulates the intestinal microbiota (Mathlouthi et al., 2002; Raza et al., 2019).

Carbohydrases such as xylanases contribute to the formation of short-chain xylans and xylo-oligosaccharides, which serve as prebiotics and can be used by *Lactobacillus* and *Bifidobacterium* (Morgan et al., 2019).

The cecal microbiota can use the fibrous content of the diet for fermentation and production of SCFAs, ammonia, carbon dioxide, and methane (Teng & Kim, 2018). Authors report that the presence of acetic, propanoic and butyric acid in the cecum of birds can promote the proliferation of beneficial bacteria and reduce the growth of pathogenic bacteria (Kheravii et al., 2018), in addition to stimulating the proliferation and differentiation of intestinal epithelial cells. SCFAs are also used as a source of energy for host tissues (Jha & Berrocoso, 2015).

This study aimed to assess the effects of supplementing broiler diets containing different levels of DDGS with a multicarbohydrase complex on productive performance, nutrient digestibility, carcass yield and intestinal health.

Material and Methods

All procedures involving animals and the collection of biological material were approved by the Committee for Ethical Conduct in the Use of Animals in Experimentation (protocol no. 15/2021).

The experiment was carried out at the Experimental Poultry Facility of the Federal University of Paraná – Palotina Campus. A total of 2,016 one-day-old male Ross AP95 chicks were used, obtained from 40-week-old breeders from the same flock. The birds were randomly distributed in a completely randomized factorial design, with 4 levels of DDGS inclusion (0, 10, 20 and 30%) and two enzyme treatments (with and without enzyme supplementation), totaling 8 treatments with 6 replicates of 42 birds per pen.

A commercial enzymatic complex containing carbohydrases (amylase, xylanase, β-glucanase and β-mannanase) and protease was added to the diets at a dose of 50g/ton, following the manufacturer's guidelines. This enzyme complex provided the following minimum guaranteed levels per kilo: amylase (120,000 U/g), xylanase (20,000 IU/g), β-glucanase (7,500 IU/g), β-mannanase (250 IU/g) and protease (1,400 U/g).

The nutritional program was divided into 3 phases: starter (1 - 18 days old), grower (19 – 35 days old) and finisher (36 – 42 days old). The experimental diets were formulated with corn and soybean meal to meet the nutritional requirements for each rearing phase, according to local agroindustry standards and considering the nutrient contribution (matrix) of DDGS. Bromatological and mycotoxin analyses of the DDGS used in the experiment yielded the following results: ether extract: 7.87%; crude fat: 8.58%; crude protein: 35.24%; mineral residue 2.33%; moisture: 9.70%; deoxynivalenol (ppm): not detected; fumonisin (ppm): 1.48; total aflatoxin (ppb): 4.77; zearalenone (ppb): not detected. The soybean and corn fertilizer and DDGS used were from the same batch.

All diets were supplemented with phytase (500 FTU/kg) using the following nutritional matrix: 0.15% P and 0.15% Ca. The composition and nutritional values of the diets for each rearing phase are presented in Tables 1, 2 and 3.

Table 1
Composition and nutritional levels of the diet in the starter phase

Ingredients (Kg/ton)	T1	T2	T3	T4	T5	T6	T7	T8
Maize	478.24	449.01	419.03	389.87	509.76	480.23	450.67	421.13
Soybean meal	442.50	376.00	309.80	243.20	429.00	362.70	296.40	229.80
DDGS	-	100.00	200.00	300.00	-	100.00	200.00	300.00
Soybean oil	44.80	38.90	33.00	27.00	26.42	20.50	14.65	8.70
DL-Methionine 99%	2.89	2.70	2.55	2.38	2.82	2.65	2.47	2.31
L-Lysine 54.6%	0.62	2.62	4.62	6.64	0.87	2.87	4.89	6.89
L-Threonine	0.40	0.47	0.55	0.63	0.38	0.45	0.52	0.60
L-Tryptophan 98%	-	-	-	0.13	-	-	-	0.13
Calcitic limestone	13.40	14.20	15.00	15.70	13.50	14.30	15.00	15.90
Dicalcium phosphate	9.20	7.80	6.70	5.40	9.20	7.90	6.60	5.40
Salt	3.70	3.80	3.90	3.90	3.80	3.80	3.90	3.90
Choline chloride	0.25	0.50	0.85	1.15	0.25	0.60	0.90	1.24
Vit/min premix[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Multicarbohydrase complex[2]	-	-	-	-	0.05	0.05	0.05	0.05
Nutritional Levels								
Crude Protein (%)	24.44	24.43	24.44	24.44	24.44	24.44	24.44	24.44
Ether Extract (%)	6.93	6.72	6.51	6.29	5.19	4.97	4.77	4.55
Crude fiber (%)	3.68	4.32	4.96	5.60	3.66	4.30	4.94	5.58
AME (Kcal/Kg)	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050
SID Lysine (%)	1.28	1.28	1.28	1.28	1.28	1.28	1.28	1.28
SID TSAA (%)	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
SID Threonine (%)	0.85	0.84	0.85	0.85	0.85	0.85	0.84	0.84
SID Tryptophan (%)	0.28	0.26	0.24	0.23	0.27	0.25	0.24	0.23
SID Arginine (%)	1.52	1.42	1.33	1.24	1.50	1.41	1.31	1.22
Calcium (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Available Phosphorus (%)	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Mineral Matter (%)	5.51	5.33	5.19	5.00	5.50	5.33	5.15	4.99

[1]Mineral vitamin premix: Vitamin A (2,750,000 IU/kg). vitamin D3 (750,000 IU/kg). vitamin E (10,000 IU/kg), vitamin K3 (500 mg/kg), vitamin B2 (1,750 mg/kg), vitamin B12 (3,750 mcg/kg), niacin (10 g/kg), calcium pantothenate (3,000 mg/kg), iron (15 g/kg), manganese (19.95 g/kg), zinc (19.75 g/kg), copper (2,000 mg/kg), iodine (251.8 mg/kg), selenium (74.25 mg/kg), vitamin B1 (750 mg/kg), vitamin B6 (1,000 mg/kg), folic acid (250 mg/kg), biotin (25 mg/kg), BHT (3,825 mg/kg), BHA (249 mg/kg), nicarbazine + narasin (25 g/kg), phytase (500 FTU/kg).
[2]Multicarbohydrase complex: beta mannanase (3.13 U/g), beta-glucanase (93.75 U/g), protease (17.5 U/g), amylase (1,500 U/g), xylanase (250 U/g).

Table 2
Composition and nutritional levels of the diet in the grower phase

Ingredients (Kg/ton)	T1	T2	T3	T4	T5	T6	T7	T8
Maize	554.70	525.14	495.57	466.14	586.30	556.58	527.61	497.73
Soybean meal	371.50	305.00	238.90	172.20	358.00	291.70	225.00	158.50
DDGS	-	100.00	200.00	300.00	-	100.00	200.00	300.00
Soybean oil	45.80	39.90	34.00	28.00	27.40	21.50	15.70	9.60
DL-Methionine 99%	2.14	1.97	1.79	1.62	2.10	1.90	1.72	1.60
L-Lysine 54.6%	0.43	2.43	4.40	6.45	0.70	2.68	4.67	6.70
L-Threonine	0.10	0.16	0.24	0.32	0.10	0.14	0.20	0.30
L-Tryptophan 98%	-	-	-	0.17	-	-	-	0.17
Calcitic limestone	11.50	12.30	13.00	13.90	11.50	12.50	13.00	14.00
Dicalcium phosphate	6.30	5.10	3.80	2.50	6.30	5.00	3.80	2.60
Salt	3.30	3.40	3.40	3.50	3.30	3.40	3.40	3.50
Choline chloride	0.23	0.60	0.90	1.20	0.30	0.60	0.90	1.30
Vit/min premix[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Multicarbohydrase complex[2]	-	-	-	-	0.05	0.05	0.05	0.05
Nutritional Levels								
Crude Protein (%)	21.70	21.69	21.70	21.70	21.71	21.70	21.69	21.70
Ether Extract (%)	7.17	6.96	6.75	6.53	5.42	5.21	5.01	4.78
Crude fiber (%)	3.41	4.06	4.70	5.34	3.40	4.04	4.68	5.32
AME (Kcal/Kg)	3,150	3,150	3,150	3,150	3,150	3,150	3,150	3,150
SID Lysine (%)	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
SID TSAA (%)	0.81	0.81	0.81	0.81	0.82	0.81	0.81	0.82
SID Threonine (%)	0.73	0.73	0.73	0.73	0.73	0.73	0.72	0.73
SID Tryptophan (%)	0.24	0.22	0.20	0.20	0.24	0.22	0.20	0.20
SID Arginine (%)	1.33	1.24	1.14	1.05	1.31	1.22	1.13	1.03
Calcium (%)	0.85	0.85	0.85	0.85	0.84	0.85	0.84	0.85
Available Phosphorus (%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Mineral Matter (%)	4.77	4.61	4.42	4.27	4.74	4.59	4.39	4.25

[1]Mineral vitamin premix: Vitamin A (2,200,000 IU/kg), vitamin D3 (600,000 IU/kg), vitamin E (8,000 IU/kg), vitamin K3 (400 mg/kg), vitamin B2 (1,400 mg/kg), vitamin B12 (3,000 mcg/kg), niacin (8 g/kg), calcium pantothenate (2,400 mg/kg), iron (15 g/kg), manganese (19.95 g/kg), zinc (19.75 g/kg), copper (2,000 mg/kg), iodine (251.8 mg/kg), selenium (74.25 mg/kg), vitamin B1 (600 mg/kg), vitamin B6 (800.5 mg/kg), folic acid (200 mg/kg), biotin (20 mg/kg), BHT (3,825 mg/kg), BHA (249 mg/kg), salinomycin (18 g/kg), phytase (500 FTU/kg).
[2]Multicarbohydrase complex: beta mannanase (3.13 U/g), beta-glucanase (93.75 U/g), protease (17.5 U/g), amylase (1,500 U/g), xylanase (250 U/g).

Table 3
Composition and nutritional levels of the diet in the finisher phase

Ingredients (Kg/ton)	T1	T2	T3	T4	T5	T6	T7	T8
Maize	665.93	636.55	607.37	578.03	697.77	668.24	639.40	608.77
Soybean meal	267.50	201.00	134.60	67.60	254.30	187.80	120.50	55.00
DDGS	-	100.00	200.00	300.00	-	100.00	200.00	300.00
Soybean oil	40.50	34.70	28.60	22.50	22.20	16.20	10.20	4.50
DL-Methionine 99%	1.88	1.71	1.55	1.40	1.80	1.64	1.47	1.30
L-Lysine 54.6%	2.21	4.22	6.24	8.30	2.45	4.47	6.48	8.40
L-Threonine	0.38	0.46	0.54	0.62	0.36	0.44	0.50	0.58
L-Tryptophan 98%	-	0.06	0.25	0.45	-	0.06	0.25	0.45
Calcitic limestone	10.00	10.50	11.00	12.00	9.70	10.50	11.20	12.00
Dicalcium phosphate	4.20	3.00	1.70	0.50	4.00	2.90	1.80	0.50
Salt	3.10	3.20	3.20	3.30	3.10	3.10	3.20	3.20
Choline chloride	0.30	0.60	0.95	1.30	0.32	0.65	1.00	1.30
Vit/min premix[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Multicarbohydrase complex[2]	-	-	-	-	0.05	0.05	0.05	0.05
Nutritional Levels								
Crude Protein (%)	17.99	17.99	18.00	18.00	18.00	18.00	17.98	18.02
Ether Extract (%)	6.84	6.64	6.41	6.18	5.11	4.89	4.67	4.48
Crude fiber (%)	3.02	3.66	4.31	4.95	3.01	3.65	4.29	4.93
AME (Kcal/Kg)	3,250	3,250	3,250	3,250	3,250	3,250	3,250	3,250
SID Lysine (%)	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
SID TSAA (%)	0.70	0.70	0.70	0.71	0.70	0.70	0.70	0.70
SID Threonine (%)	0.63	0.63	0.63	0.63	0.63	0.63	0.62	0.63
SID Tryptophan (%)	0.19	0.17	0.17	0.17	0.18	0.17	0.17	0.17
SID Arginine (%)	1.06	0.96	0.87	0.77	1.04	0.95	0.85	0.76
Calcium (%)	0.72	0.71	0.70	0.71	0.70	0.70	0.70	0.70
Available Phosphorus (%)	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Mineral Matter (%)	4.05	3.86	3.65	3.51	3.97	3.81	3.65	3.47

[1]Mineral vitamin premix: Vitamin A (1,760,000 IU/kg), vitamin D3 (480,000 IU/kg), vitamin E (6,400 IU/kg), vitamin K3 (320 mg/kg), vitamin B2 (1,120 mg/kg), vitamin B12 (2,400 mcg/kg), niacin (6.4 g/kg), calcium pantothenate (1,920 mg/kg), iron (15 g/kg), manganese (19.95 g/kg), zinc (19.75 g/kg), copper (2,000 mg/kg), iodine (251.8 mg/kg), selenium (74.25 mg/kg), vitamin B1 (480 mg/kg), vitamin B6 (640.5 mg/kg), folic acid (160 mg/kg), biotin (16 mg/kg), BHT (3,825 mg/kg), BHA (249 mg/kg), phytase (500 FTU/kg).
[2]Multicarbohydrase complex: beta mannanase (3.13 U/g), beta-glucanase (93.75 U/g), protease (17.5 U/g), amylase (1,500 U/g), xylanase (250 U/g).

The birds were housed in a climate-controlled facility equipped with exhaust fans, electric brooders for heating, and evaporative cooling pads, all controlled by an automated system. Humidity and temperature were maintained within the recommended comfort ranges for each age group according to the breeder management guide (Aviagen, 2018). Feed was provided *ad libitum* throughout the experimental period.

Metabolism assay

At 21 days of age, 96 birds were removed from the poultry house (2 birds per replicate) and relocated to metabolism cages, following the same experimental design used in boxes (8 treatments with 6 replicates, totaling 48 experimental units). A metabolism assay was conducted using the total excreta collection method in a temperature-controlled room.

The experimental period lasted 7 days, including 3 days of adaptation and 4 days of excreta collection. On days 24 and 27, the feed and leftovers were weighed and recorded to calculate feed intake during this period.

Excreta were collected once a day, using collection trays lined with black plastic. The daily samples were pooled by experimental unit in labeled plastic bags, and stored in a freezer at -20ºC until the last collection day, when they were weighed, homogenized and dried in a forced-air oven at 65ºC for 72 hours (moisture 1), followed by additional drying at 105oC for 4 hours (moisture 2).

The excreta and experimental diet samples were sent to the Laboratory of Bromatological Analysis (CBO Análises Laboratoriais, Valinhos – SP) to determine gross energy, crude protein, ether extract, neutral detergent fiber, acid detergent fiber, calcium and phosphorus.

Based on feed intake data, excreta production and laboratory results, the following digestibility coefficients were calculated: crude protein (DCCP), dry matter (DCDM), ether extract (DCEE), neutral detergent fiber (DCNDF), acid detergent fiber (DCADF), calcium (DCCa) and phosphorus (DCP). Apparent metabolizable energy (AME) and nitrogen-corrected apparent energy (AMEn) were also determined, using the equations proposed by Matterson et al. (1965).

Zootechnical performance

The birds were weighed weekly, along with leftover feed, to evaluate zootechnical performance (average weight, weight gain, feed intake and feed conversion). Feed conversion was corrected weekly for bird mortality, according to the methodology of Sakomura and Rostagno (2007).

Carcass and commercial cutting yield

At 43 days of age, carcass, breast, leg, wing, and abdominal fat yield was determined, using 8 birds per experimental unit (48 birds per treatment), with live weight ranging from -2% to +2% of the average box weight. The birds were identified and fasted for 6 hours before being stunned by electroshock and slaughtered by cutting the jugular vein. They were then scalded, plucked and eviscerated.

Carcass yield was calculated based on the hot eviscerated carcass weight (excluding the feet, head and abdominal fat), expressed as a percentage of individual live weight before slaughter. For commercial cut yields, the weight of the whole breast (with skin and bones), legs (drumstick and thigh with bones and skin), back and wings (with skin) was considered, expressed as a percentage of eviscerated carcass weight.

The abdominal fat around the cloaca, cloacal pouch, gizzard, proventriculus, and adjacent abdominal muscles was removed as described by Smith (1993), then weighed and calculated as a percentage of eviscerated carcass weight.

Intestinal morphometry

At the end of the metabolism assay, the birds were returned to their regular diet and at 28 days of age, the same birds (12 per treatment) were euthanized and samples of the jejunum and ileum were collected. The samples were fixed in buffered formaldehyde solution and then embedded in paraffin. Each fragment was sectioned semi-serially at 5 µm thickness and stained with hematoxylin-eosin (HE).

For morphometric analysis, the images were captured by a light microscope (10x objective), connected to a computerized image analyzer system (ImagePro-Plus - Version 5.2 - Cybernetic Media). Twenty villi and 20 crypts per sample were measured for villus height and crypt depth, respectively. These measurements were used to calculate the absorptive surface area of the intestinal mucosa, using the formula proposed by Kisielinski et al. (2002).

Absorption Area:

$$\frac{(LV \times AV) + (LV/2 + DC/2)2 - (LV/2)^2}{(LV/2 + DC/2)^2}$$

Where: LV: villus length, AV: villus height, DC: crypt depth.

Determination of short-chain fatty acids in cecal content

Short-chain fatty acids (SCFA) were determined at 28 days using the same birds (12 per treatment) that were sacrificed for intestinal morphometry. Cecal content was collected, diluted in NaOH (sodium hydroxide), homogenized and then centrifuged at 3,000 rpm for five minutes. One mL of the supernatant was collected, to which 0.2 mL of formic acid (P.A.) was added. This mixture was stored in a freezer at -18°C until analysis. SCFA concentration was determined by gas chromatography, using an 80/120 CarbopackTM B-DA*/4% Carbowaxâ 20M column.

Statistical analysis

The data were tabulated and analyzed using a 4 x 2 factorial scheme, with analysis of variance (ANOVA) performed via the General Linear Model procedure (PROC GLM) in the Statistical Analysis System (SAS, version 9.0). When significant effects were detected, means were compared using Tukey's test ($p<0.05$).

When significant interactions were observed between DDGS inclusion levels and multicarbohydrase complex addition, interaction effects were analyzed separately. Data related to DDGS inclusion levels (0, 10,

20 and 30%) were submitted to regression analysis (PROC REG) up to second-order polynomials (quadratic).

Residuals were tested for normality using the Shapiro-Wilk test and histogram inspection, and for homoscedasticity using the Brown-Forsythe test. Outliers were excluded from the dataset before statistical analyses if their residuals deviated by ± 2 standard deviations from the residual mean of the parameter (or until normality was achieved).

Results and Discussion

Performance analysis showed that the inclusion of 10% DDGS in the diet did not negatively affect live weight, weight gain or feed conversion when compared to the control group (0% DDGS) during the 1 - 7, 1 - 21 and 1 – 42-day periods (Table 4). However, diets containing 20% and 30% DDGS resulted in significantly poorer ($p<0.05$) performance outcomes across all these phases.

Table 4
Productive performance from 1 to 7, 1 to 21 and 1 to 42 days of broilers, regardless of multicarbohydrase complex supplementation in diets prepared with DDGS

	Live weight, g	Weight gain, g	Feed intake, g	Feed conversion
		1 to 7 days		
DDGS				
0	178.63[a]	132.40[a]	142.44	1.076[c]
10	175.79[a]	129.14[a]	146.73	1.138[bc]
20	168.71[b]	121.88[b]	147.34	1.210[b]
30	161.51[c]	115.39[c]	152.63	1.324[a]
Enzyme				
Multicarbohydrase	171.91	125.64	150.32[a]	1.203
Control diet	170.41	123.77	144.26[b]	1.171
CV, %	2.58	3.62	6.85	7.76
DDGS	<.0001	<.0001	0.1208	<.0001
Enzyme	0.2500	0.1580	0.0439	0.2329
DDGS x Enzyme	0.4113	0.4688	0.6614	0.9459
Regression test	Linear	Linear[1]	-	Linear[2]
		1 to 21 days		
DDGS				
0	963.12[a]	917.99[a]	1148.02	1.252[c]
10	929.75[a]	883.39[a]	1155.98	1.309[c]
20	845.95[b]	796.64[b]	1144.88	1.438[b]
30	774.15[c]	728.04[c]	1135.10	1.551[a]

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Enzyme				
Multicarbohydrase	876.51	830.00	1161.21	1.409[a]
Control diet	879.98	833.03	1132.08	1.369[b]
CV, %	5.52	5.99	5.30	4.41
DDGS	<.0001	<.0001	0.9103	<.0001
Enzyme	0.8059	0.8342	0.1090	0.0304
DDGS x Enzyme	0.8102	0.8600	0.8414	0.2481
Regression test	Linear	Linear[3]	-	Linear[4]
		1 to 42 days		
DDGS				
0	3090.89[a]	3044.96[a]	4620.60	1.518
10	3021.09[ab]	2974.92[ab]	4610.94	1.550
20	2918.78[b]	2872.84[b]	4631.38	1.612
30	2639.27[c]	2593.30[c]	4526.59	1.716
Enzyme				
Multicarbohydrase	2890.54[b]	2844.50[b]	4606.97	1.622
Control diet	2944.47[a]	2898.51[a]	4588.42	1.578
CV, %	3.18	3.22	4.00	2.02
DDGS	<.0001	<.0001	0.5343	<.0001
Enzyme	0.0506	0.0500	0.7318	<.0001
DDGS x Enzyme	0.2177	0.2181	0.8356	0.0187
Regression test	Quadratic	Quadratic[5]	-	-

[1]$\hat{Y}$= 133.44807-0.5829x, R^2: 0.98; [2]$\hat{Y}$= 1.07308+0.00687x, R^2: 0.97. [3]$\hat{Y}$= 930.004-6.566x, R^2: 0.97; [4]$\hat{Y}$= 1.23411+0.01023x, R^2: 0.97. [5]$\hat{Y}$= 3037.69+1.14156x-0.5237x^2, R^2: 0.96.

The effects of DDGS inclusion on performance variables were evaluated using regression analysis. Body weight and weight gain decreased linearly with increasing DDGS levels during the 1 - 7 and 1 – 21-day periods, regardless of multicarbohydrase complex supplementation. Consequently, feed conversion in these same periods exhibited a linear increase in these same periods, indicating a decline in feed efficiency as DDGS levels rose in both supplemented and non-supplemented groups (Table 4).

Supplementation with the multicarbohydrase complex increased feed intake during the 1 – 7-day period but led to a poorer feed conversion ratio ($p<0.05$) in the 1 – 21-day phase. Similarly, including the multicarbohydrase complex from 1 to 42 days resulted in lower ($p<0.05$) body weight and weight gain.

There was no significant interaction between DDGS inclusion and enzyme supplementation for body weight, weight gain and feed intake in any of the evaluated

periods (1 - 7 days, 1 - 21 days and 1 - 42 days). However, a significant interaction ($p<0.05$) between these factors was detected for feed conversion during the 1–42-day period.

This interaction (Table 5) indicated that at 30% DDGS inclusion, feed conversion was negatively affected ($p<0.05$) by the addition of the multicarbohydrase complex.

Table 5
Interaction between DDGS and enzyme inclusion for feed conversion from 1 to 42 days of broilers, regardless of multicarbohydrase complex supplementetion in diets prepared with DDGS

	DDGS, %					
Enzyme	0	10	20	30	Regression	p-value
	Feed conversion from 1 to 42 days					
Multicarbohydrase	1.528^{c}	1.555^{c}	1.638^{b}	1.767^{aA}	Quadratic^{1}	0.0015
Control diet	1.507^{c}	1.544^{bc}	1.587^{b}	1.674^{aB}	Quadratic^{2}	0.0401
p-value	0.2211	0.5458	0.0606	0.0003		

Means with different uppercase superscripts in the same column differ significantly ($p<0.05$). Means with different lowercase superscripts in the same row differ significantly ($p<0.05$).
$^{1}\hat{Y}= 1.5284078-0.00480966x+0.00030023x^{2}$, R^{2}: 0.87; $^{2}\hat{Y}= 1.509000+0.001708688x+0.0001241x^{2}$, R^{2}: 0.85.

For the 1-42-day period, regression analysis showed a quadratic effect ($p<0.05$) of DDGS inclusion on feed conversion. Optimal feed conversion was observed at 8.01% DDGS in the enzyme-supplemented group, whereas in the non-supplemented group, the maximum tolerable level without compromising feed conversion was 6.88% DDGS.

Live weight and weight gain during the 1 – 42-day period showed a quadratic response to DDGS inclusion in both supplemented and non-supplemented birds, differing from the linear decrease observed in earlier phases. The regression equation shows that 1.08% DDGS resulted in the best weight gain (Table 4).

Zootechnical performance suggests that while DDGS inclusion can affect broiler performance, only low levels (less than 10%) can be added to the diets without compromising final performance (1 to 42 days).

In the present study, multicarbohydrase complex supplementation did not improve animal performance, regardless of DDGS inclusion. Birds' digestive systems are largely developed by approximately 14 days of age, making them more sensitive to variations in diet quality (Batal & Parsons, 2002).

Some sources of DDGS exhibit considerable variability in nutritional value, which complicates its use in poultry diets due

to reduced nutrient utilization (Campasino et al., 2015). Furthermore, DDGS may contain high levels of soluble fiber, which increases digesta viscosity, forming gels that obstruct the action of hydrolytic enzymes, thereby reducing nutrient availability for digestion and absorption, which is reflected in poorer feed conversion ratios and lower weight gain (Min et al., 2015; Schone et al., 2017; Kim et al., 2018).

Carbohydrases are commonly added to poultry and pig feed to hydrolyze NSPs, increase nutrient digestibility, and improve energy use (Raza et al., 2019). However, the effectiveness of enzyme supplementation depends on the presence and concentration of the substrate in the diet, the age of the animals and the type of diet used (Cowieson et al., 2019). In addition, the nutritional quality of feed ingredients is crucial for successful enzymatic hydrolysis, ensuring that nutrients are released and available for digestion and absorption.

Responses to exogenous enzyme supplementation in diets are variable and difficult to predict, partly due to the heterogeneous chemical composition of the NSPs across ingredients, hindering efforts to generalize findings in the literature (Campasino et al., 2015). Therefore, for exogenous enzymes to enhance zootechnical performance and optimize production costs, a better understanding of the amount and composition of NSPs in feed ingredients is essential, since the enzymes act on specific substrates.

It is important to note that excess dietary fiber reduces nutrient absorption and consequently can decrease muscle protein deposition, thereby impairing performance (Valentim et al., 2020). In addition, during the thermal processing of corn to obtain ethanol, protein denaturation may occur, lower protein extractability and digestibility in DDGS (Böttger & Südekum, 2018).

An interaction between DDGS inclusion and enzyme supplementation was observed for relative carcass and wing weights. Multicarbohydrase complex supplementation improved carcass yield in diets without DDGS, but had no significant effect ($p>0.05$) when DDGS was added at any level (Table 6).

Table 6
Carcass yield and commercial cuts (relative weights) and interaction between DDGS and enzyme inclusion for relative carcass and wing weights at 43 days of age in broilers, regardless of multicarbohydrase complex supplementation in diets prepared with DDGS

	Carcass, %	Breast, %	Legs, %	Wings, %	Fat, %
DDGS					
0	78.21	37.32^{b}	32.56^{a}	9.67	1.29
10	77.23	38.11^{a}	32.10^{ab}	9.68	1.27
20	77.34	38.28^{a}	31.71^{b}	9.73	1.19
30	75.58	37.29^{b}	32.28^{ab}	10.25	1.24
Enzyme					
Multicarbohydrase	77.16	37.81	32.19	9.85	1.28
Control diet	77.01	37.69	32.13	9.82	1.21
CV, %	2.86	5.59	3.96	4.35	37.17
DDGS	<.0001	0.0137	0.0036	<.0001	0.6540
Enzyme	0.5880	0.6807	0.7048	0.5742	0.2284
DDGS x Enzyme	0.0388	0.1285	0.2227	0.0038	0.9403
Regression test	-	Quadratic[1]	Quadratic[2]	-	-
Interaction					
DDGS, %					
Enzyme	0	10	20	30	Regression	p-value
Carcass, %						
Multicarbohydrase	78.90^{Aa}	76.75^{abc}	77.48^{ab}	75.53^{bc}	Linear[3]	<.0001
Control diet	77.51^{aB}	77.71^{a}	77.19^{ab}	75.62^{b}	Linear[4]	0.0026
p-value	0.0239	0.0851	0.6417	0.8655		
Wings, %						
Multicarbohydrase	9.58^{bB}	9.54^{bB}	9.72^{b}	10.37^{a}	Quadratic[5]	<.0001
Control diet	9.77^{bA}	9.82^{bA}	9.74^{b}	10.13^{a}	Linear[6]	<.0001
p-value	0.0400	0.0144	0.8580	0.0638		

Means with different uppercase superscripts in the same column differ significantly ($p<0.05$). Means with different lowercase superscripts in the same row differ significantly ($p<0.05$).
[1]$\hat{Y}= 37.3022+0.12431x-0.0039x^2$, R^2: 0.81; [2] $\hat{Y}= 32.6208-0.0798x+0.00221x^2$, R^2: 0.85; [3]$\hat{Y}= 78.6917-0.1123x$, R^2: 0.74; [4]$\hat{Y}= 77.842-0.0627*x$, R^2: 0.71; [5]$\hat{Y}= 9.76989-0.0462x+0.00221x^2$, R^2: 0.94; [6]$\hat{Y}= 9.62793+0.01459x$, R^2: 0.51.

For wing yield, the interaction revealed that birds fed diets with 0 and 10% DDGS had poorer ($p<0.05$) performance when supplemented with the multicarbohydrase complex compared to the non-supplemented group.

Regression analysis of the interaction between DDGS inclusion and enzyme supplementation (Table 6) for relative carcass weight revealed a decreasing linear effect with increasing DDGS levels in both supplemented and non-supplemented birds, indicating that carcass yield declined as dietary DDGS increased, regardless of enzyme use.

For relative wing weight, a quadratic response to DDGS levels was observed in the enzyme-supplemented group, with the lowest wing yield occurring at a dietary inclusion of 10.45% DDGS. By contrast, in the non-supplemented group, wing yield increased linearly as a function of DDGS inclusion levels.

An isolated effect of DDGS inclusion was found for relative breast and leg weights (Table 6). DDGS inclusion of 15.94% resulted in the highest breast yield, regardless of enzyme supplementation. For relative leg weight, a quadratic effect was also observed, with the lowest yield recorded at 18.05% DDGS inclusion.

No isolated effect of multicarbohydrase complex supplementation was detected on carcass yield or the weights of commercial cuts. The relative abdominal fat weight was not influenced by DDGS inclusion or enzyme supplementation.

Carcass yield declined with DDGS inclusion in the diet, regardless of enzyme supplementation.

The multicarbohydrase complex did not affect the relative weights of carcass, breast, legs, wings or abdominal fat, even in diets without DDGS, indicating that the ability of enzymes to improve nutrient availability did not influence carcass yield or commercial cuts.

Selvaraj et al. (2017) emphasize that enzymes act on specific substrates. Therefore, to improve zootechnical performance and optimize production costs, a deeper understanding of the amount and composition of the different NAPs in dietary ingredients is essential.

Among the other variables analyzed in the digestibility assay, only DCCa (digestibility coefficient of calcium) and DCP (digestibility coefficient of phosphorus) were unaffected by the treatments. For all other parameters, isolated effects of increasing DDGS levels and multicarbohydrase complex supplementation were observed. Enzyme supplementation improved the digestibility coefficients of DM, CP and ADF compared to diets without supplementation (Table 7).

In this study, DDGS inclusion in the birds' diet reduced the digestibility of DM, CP and EE, regardless of multicarbohydrase complex supplementation. Birds fed diets containing the highest DDGS level (30%) exhibited poorer digestibility coefficients for DM, CP and EE ($p<0.05$) when compared to the control group (no DDGS). Conversely, higher DDGS inclusion levels improved ADF digestibility coefficients ($p<0.05$) (Table 7).

A significant interaction ($p<0.05$) was observed between DDGS inclusion levels and multicarbohydrase complex supplementation for AME, AMEn and DCNDF.

Table 8 shows that diets containing 20% DDGS supplemented with the multicarbohydrase complex improved ($p<0.05$) AME, AMEn and DCNDF, compared to the unsupplemented group. However, at the highest DDGS inclusion level (30%), enzyme supplementation had the opposite effect, decreasing these coefficients.

Birds not receiving enzyme supplementation showed a linear reduction in energy (AME and AMEn) as DDGS levels increased. However, enzyme supplementation enabled birds to use diets containing up to 10.45% and 10.17% of DDGS, respectively. The inclusion of the multicarbohydrase complex in diets with increasing DDGS levels resulted in a quadratic effect for AME, AMEn and DCNDF. The best use of AME and AMEn occurred with 10.45% and 10.17% DDGS to the diet, respectively.

For DCNDF, including 15.47% DDGS in enzyme-supplemented diets led to higher digestibility. However, in non-supplemented diets, 16.67% DDGS in the diet resulted in lower DCNDF.

On the other hand, for non-supplemented diets, AME and AMEn values decreased linearly with increasing DDGS, while DCNDF showed a quadratic effect, with 16.67% DDGS resulting in the poorest fiber digestibility.

Table 7
Values of apparent metabolizable energy (AME), corrected apparent (nAME), digestibility coefficients of dry matter (DCDM), crude protein (DCCP), ether extract (DCEE), neutral detergent fiber (DCNDF), acid detergent fiber (DCADF), calcium (DCCa) and phosphorus (DCP) evaluated between 21 and 28 days of broiler chickens, regardless of multicarbohydrase complex supplementation in diets prepared with DDGS

	AME	nAME	DCDM	DCCP	DCEE	DCNDF	DCADF	DCCa	DCP
DDGS									
0	3893.40	3655.68	84.45^{a}	78.65^{a}	92.16^{a}	64.21	60.66^{c}	65.61	73.57
10	3867.53	3636.03	83.05^{a}	76.17^{ab}	90.02^{ab}	58.22	67.38^{b}	64.25	72.11
20	3814.85	3574.11	81.86^{ab}	77.75^{ab}	89.12^{ab}	62.59	70.94^{ab}	65.74	76.62
30	3727.40	3502.78	79.71^{b}	73.37^{b}	88.35^{b}	60.58	73.67^{a}	69.42	74.39
Enzyme									
Multicarbohydrase	3820.23	3583.73	83.25^{a}	77.76^{a}	90.35	61.58	69.73^{a}	66.29	75.03
Control diet	3831.37	3600.57	81.28^{b}	75.20^{b}	89.47	61.22	66.60^{b}	66.23	73.26
CV, %	2.78	2.68	3.16	5.51	3.20	10.48	7.90	9.27	5.91
DDGS	0.0240	0.0015	0.0006	0.0211	0.0151	0.1388	<.0001	0.2135	0.1319
Enzyme	0.7183	0.5475	0.0121	0.0417	0.2953	0.8450	0.0508	0.9739	0.1736
DDGS x Enzyme	0.0223	0.0181	0.4758	0.7299	0.6830	0.0025	0.1504	0.2084	0.5565
Regression test	-	-	Linear[1]	Linear[2]	Linear[3]	-	Linear[4]	-	-

[1] $\hat{Y}= 84.5767-0.1539x$, R^2: 0.98; [2] $\hat{Y}= 78.6242-0.1427x$, R^2: 0.63; [3] $\hat{Y}= 91.7631-0.1234x$, R^2: 0.93; [4] $\hat{Y}= 61.7701+0.42613x$, R^2: 0.95.

Table 8
Interaction between DDGS and enzyme inclusion for AME, nAME and NDF digestibility coefficient values evaluated between 21 and 28 days of broilers, regardless of multicarbohydrase complex supplementation in diets prepared with DDGS

	DDGS, %					
Enzyme	0	10	20	30	Regression	p-value
			AME			
Multicarbohydrase	3852.43^{a}	3878.86^{a}	3883.16^{aA}	3666.46^{bB}	Quadratic[1]	0.0027
Control diet	3934.38^{a}	3856.21^{ab}	3746.55^{bB}	3788.35^{abA}	Linear[2]	0.0483
p-value	0.2208	0.7827	0.0488	0.0033		
			nAME			
Multicarbohydrase	3615.91^{a}	3641.40^{a}	3635.16^{aA}	3442.43^{bB}	Quadratic[3]	0.0027
Control diet	3695.45^{a}	3630.66^{ab}	3513.07^{bB}	3563.12^{abA}	Linear[4]	0.0304
p-value	0.1802	0.8866	0.0547	0.0013		
			DCNDF			
Multicarbohydrase	59.39^{a}	61.49^{a}	67.06^{aA}	58.39^{aB}	Quadratic[5]	0.0975
Control diet	69.02^{a}	54.96^{b}	58.13^{bB}	62.77^{abA}	Quadratic[6]	0.0100
p-value	0.0559	0.1790	0.0296	0.0208		

Means with different uppercase superscripts in the same column differ significantly ($p<0.05$). Means with different lowercase superscripts in the same row differ significantly ($p<0.05$).
[1] $\hat{Y}= 3842.4863+12.6986x-0.607825x^2$, R^2: 0.92; [2] $\hat{Y}= 3913.5295-5.4773x$, R^2: 0.74;
[3] $\hat{Y}= 3608.16742+11.10058x-0.545571x^2$, R^2: 0.95; [4] $\hat{Y}= 3677.76166-5.145833x$, R^2: 0.69;
[5] $\hat{Y}= 58.5095+0.83253333x-0.0269x^2$, R^2: 0.65; [6] $\hat{Y}= 68.228750-1.558375x+0.04675417x^2$, R^2: 0.81;

The digestibility coefficients found in the present study demonstrate the importance of assessing nutrient digestibility to optimize feed formulation and nutrient utilization. Improved digestibility helps prevent excess or lack of nutrients in the diet, which favors cost reduction and lower environmental nutrient excretion (Xu & Zhang, 2021).

The ability to increase intestinal viscosity is a key characteristic of soluble fiber, which reduces the contact time between enzymes and their substrate, thereby impairing nutrient digestibility and limiting energy use. Despite the high nutritional value of lipids in corn DDGS, energy availability is significantly influenced by NSP content (Świątkiewicz & Koreleski, 2006).

Another factor that may have reduced energy availability with DDGS inclusion is the density of the corn grain. Lower grain density is associated with reduced AME levels.

In Brazil, corn ethanol is produced from surplus grains, that is, those not designated for domestic consumption. This raises concerns regarding the nutritional quality and food safety of distillers' grains, since current regulations for contaminants and standardization apply only to corn itself, as established by Normative Instructions No. 60 of 12/22/2011 and No. 88, of 03/26/2021.

The improved nutrient digestibility observed with enzyme supplementation is attributed to the action of enzymes in degrading cell wall components, facilitating access for endogenous digestive enzymes (Pack et al., 1998).

The inclusion of DDGS in the diet increased the digestibility of ADF, regardless of enzyme complex supplementation. This is due to the concentration of fibrous components during ethanol production, which removes starch and part of the fat fraction and enriching the fiber content, especially the pericarp, which consists of cellulose (23%) and hemicellulose (67%) (El-Hack et al., 2015).

This result demonstrates the positive effect of multicarbohydrase complex supplementation on nutrient recovery that would not be naturally degraded by endogenous enzymes in non-ruminant animals.

There was no interaction ($p>0.05$) between DDGS inclusion and enzymatic supplementation for SCFA concentration in the cecum. Multicarbohydrase complex concentration, regardless of DDGS inclusion, resulted in a higher ($p<0.05$) concentration of acetic acid in the cecum when compared to the non-supplemented group. DDGS levels showed a quadratic effect on propanoic acid concentration, with the lowest concentration found at 18.08% DDGS inclusion (Table 9).

Table 9
Determination of short chain fatty acid concentration (mmol/kg) in the cecal content of broilers at 28 days of age, regardless of multicarbohydrase complex supplementation in diets prepared with DDGS

	Acetic acid, mmol/kg	Propanoic acid, mmol/kg	Butyric acid, mmol/kg
DDGS			
0	40.39	3.32	6.60
10	42.31	2.68	6.61
20	37.13	2.30	5.97
30	47.22	2.47	7.02
Enzyme			
Multicarbohydrase	45.23[a]	2.91	7.09
Control diet	38.30[b]	2.48	5.99
CV, %	38.61	50.45	46.22
DDGS	0.1852	0.0552	0.6920
Enzyme	0.0381	0.1329	0.0798
DDGS x Enzyme	0.7537	0.3871	0.9987
Regression test	-	Quadratic[1]	-

[1] $\hat{Y}= 3.6615625-0.14503x+0.00401145x^2$, R^2: 0.61.

The concentration and type of SCFA produced in the cecum are modified by the microbial activity of the bacteria that colonize the cecal environment (Yadav & Jha, 2019). The presence of acetic, propanoic and butyric acid in the cecum of birds can favor beneficial bacteria proliferation and reduce pathogenic bacteria growth (Kheravii et al., 2018).

In addition, SCFA stimulates the proliferation and differentiation of intestinal epithelial cells, potentially increasing villus length, and consequently, the absorptive surface area (De vadder et al., 2014). Acetic and propanoic acid act as an energy substrate for tissues (Yadav & Jha, 2019), while butyric acid supports enterocyte development and proliferation in the small intestine, also acting as an energy source for the host's metabolic activities (Jha & Berrocoso, 2015).

The cecal microbiota can ferment dietary fibrous content to produce SCFAs, ammonia, carbon dioxide, and methane (Teng & Kim, 2018). However, only small, soluble particles, along with digestive fluids and uric acid, can reach the cecum (Svihus et al., 2013). In the present study, 18.08% DDGS inclusion resulted in lower propanoic acid concentration in the cecum, indicating that higher dietary fiber does not necessarily enhance cecal fermentation and SCFA production.

Multicarbohydrase supplementation increased acetic acid concentration, regardless of DDGS inclusion, which may be attributed to the enzymatic degradation of NSPs into lower molecular weight compounds. These fragments can enter the cecum and be fermented by microbes, thereby increasing SCFA production, due to the increase in beneficial microbiota (Macambira et al., 2021).

Certain fibers, such as oligosaccharides, act as prebiotics when supplemented in poultry diets, stimulating beneficial intestinal microbiota proliferation and colonization (Zhang et al., 2014), thereby improving intestinal mucosal health and increasing SCFA production (Yadav & Jha, 2019).

Multicarbohydrase complex supplementation independently influenced the intestinal morphometry of the jejunum. In the ileal mucosa, enzyme supplementation reduced crypt depth when compared to the non-supplemented group, regardless of DDGS inclusion in the diet. Inclusion of 30% DDGS resulted in a smaller intestinal absorption area and a lower villus:crypt ratio ($p<0.05$), when compared to birds fed the control diet or a diet with 10% DDGS. On the other hand, the inclusion of 10% DDGS did not affect villus length when compared to the control group (0% DDGS), regardless of enzyme supplementation.

Regression analysis revealed a linear decrease ($p<0.05$) in villus length, villus:crypt ratio and jejunal absorption area with increasing DDGS levels in the diet.

No significant interaction was observed between DDGS levels and multicarbohydrase complex supplementation for ileal mucosal morphometry analysis. Enzyme supplementation reduced ($p<0.05$) crypt depth when compared to the non-supplemented group, regardless of DDGS inclusion (Table 10).

Table 10
Evaluation of the morphometry of the jejunal and ileal intestinal mucosa at 28 days of age in broilers, regardless of multicarbohydrase complex supplementation in diets prepared with DDGS

	Villus length, µm	Crypt depth, µm	Villus: crypt ratio	Absorption area, µm²	Villus length, µm	Crypt depth, µm	Villus: crypt ratio	Absorption area, µm²
	Jejunum				Ileum			
DDGS								
0	785.87[a]	142.10	5.60[a]	11.49[a]	501.83[a]	129.91	3.91[a]	7.85[a]
10	754.08[ab]	142.26	5.50[a]	11.39[a]	484.18[a]	135.42	3.59[a]	7.70[a]
20	697.38[bc]	141.35	4.99[ab]	10.36[ab]	456.87[ab]	126.14	3.64[ab]	7.14[ab]
30	660.45[c]	139.18	4.88[b]	9.88[b]	433.81[b]	132.23	3.30[b]	6.70[b]
Enzyme								
Multicarbohydrase	725.84	138.74	5.29	10.80	469.42	127.33[b]	3.71	7.41
Control diet	722.50	143.71	5.19	10.77	469.49	134.52[a]	3.52	7.30
CV, %	15.22	13.67	19.44	16.25	15.54	9.32	15.67	16.92
DDGS	0.0007	0.9419	0.0347	0.0040	0.0099	0.0690	0.0049	0.0066
Enzyme	0.8828	0.2109	0.6158	0.9226	0.9965	0.0050	0.1097	0.6912
DDGS x Enzyme	0.4856	0.3805	0.6768	0.6118	0.9056	0.5968	0.4111	0.7112
Regression test	Linear[1]	-	Linear[2]	Linear[3]	Linear[4]	-	Linear[5]	Linear[6]

[1] $\hat{Y}$= 789.254-4.3261x, R^2: 0.99; [2] $\hat{Y}$= 5.64383-0.0268x, R^2: 0.97; [3] $\hat{Y}$= 11.6559-0.0584x, R^2: 0.92; [4] $\hat{Y}$= 503.822-2.3133x, R^2: 0.94; [5] $\hat{Y}$= 3.87818-0.0179x, R^2: 0.84; [6] $\hat{Y}$ = 7.9445-0.04x.

DDGS inclusion significantly influenced villus length, villus:crypt ratio and absorption area. Diets with 30% DDGS inclusion resulted in lower values for all these variables, when compared to the diets containing 0 or 10% DDGS. The behavior of DDGS inclusion levels confirms this result, which showed a linear decrease in villus length, villus:crypt ratio and absorption area decrease as DDGS inclusion increased.

In the ileal mucosa, enzyme supplementation reduced villus length, villus:crypt ratio and, consequently, the absorption area as DDGS inclusion increased.

Mucosal morphology is an important indicator of gastrointestinal tract function. Villus length, crypt depth, and the villus:crypt ratio can be used to evaluate nutrient absorption efficiency and intestinal health in poultry (Apperson & Cherian, 2017).

This effect can be attributed to the higher content of insoluble fiber in the diet. High-fiber diets can increase digesta viscosity, which damages the intestinal villi, reducing villus length and impairing nutrient absorption (Mathlouthi et al., 2002; Latorre et al., 2017). The effect of dietary fiber on intestinal morphology depends on the physicochemical characteristics of the fibrous ingredient, its inclusion level in the diet, exposure time, animal age, and the intestinal segment evaluated.

Deeper crypts indicate faster tissue renewal in response to normal desquamation or inflammation caused by aggressive agents, pathogens, or toxins. Therefore, it can be inferred that the addition of enzyme complex to the diet mitigated fiber-induced damage in the ileal mucosa, resulting in shallower crypts.

Multicarbohydrase supplementation had no effect on the absorption area of the jejunal or ileal mucosa of birds, regardless of DDGS inclusion. This suggests that the improvement in nutrient digestibility (DM, CP and ADF) observed with enzyme supplementation may not be directly associated with a beneficial effect on intestinal mucosal components.

Conclusion

The inclusion of DDGS in poultry diets resulted in poorer performance, reduced carcass and commercial cut yields, and a smaller absorption area of the jejunum and ileum, regardless of multicarbohydrase complex supplementation.

Enzyme supplementation increased acetic acid concentration in the cecal content and improved dry matter digestibility, crude protein and acid detergent fiber. Additionally, its inclusion in diets with up to 10% DDGS enhanced EMA, nEMA and NDF.

The inclusion of DDGS, especially in broiler starter diets, should be approached with caution. Improvements in the standardization and quality control of DDGS are necessary to ensure its safer use in animal feed.

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