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Evaluation of the Addition of Humicola Grisea Cellulase to Broiler Chicken Rations for a 21-Day Period

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Abstract: This study aimed to evaluate the addition of liquid cellulose, produced by Humicola grisea, in 21-day-old broiler chickens' diets. The treatments comprised control rations of corn and soybean meal and rations to which 500 mL/t and 1000 mL/t of cellulase were added. A total of 180 male broiler chickens were used, distributed in a completely randomized design, with three treatments and six replicates. Broiler chicken performance was monitored during the period from 1 to 21 days old. Significant effects were detected for digestibility only between four and seven days old, when a reduced dry matter nitrogen intake was recorded, and for nitrogen digestibility in the broilers fed cellulase-supplemented rations at a dose of 1000 m/L. Among the analyzed digestive organs, only the biometrics of the large intestine were affected significantly at seven days old. The absolute weights of the liver and pancreas and the activities of amylase, alkaline phosphatase, and transaminases were not affected significantly, indicating that cellulase did not affect the metabolism of these organs. No significant effect was detected in the serum for electrolytes, total protein, or alkaline phosphatase. So, the addition of liquid cellulase produced by Humicola grisea did not affect performance and metabolism in 21-day-old broiler chickens.

Keywords: aviculture; enzyme; metabolism; organ biometrics; performance

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1. Introduction

Poultry cannot digest approximately a quarter of the diet they receive, because they are devoid of the enzymes necessary to degrade certain feed components [1,2]. Lignocellulose biomass is a complex mixture of carbohydrate and non-carbohydrate compounds. Cellulose is the dominant component (40–60% of the total biomass weight), followed by hemicellulose (20–40%), and lignin (20–30%). It also contains proteins, lipids, pectin, minerals, and soluble sugars [3,4].

The basic diet for broiler chickens is composed of corn and soybean meal. Variations in the quality of soybean meal used as the main protein ingredient are related to the type and level of fibre, since the higher levels of hulls in soybean meal result in higher levels of cellulose fibre, hemicellulose and pectin. However, these feed constituents, known as non-starch polysaccharides (NSP), are indigestible to broilers. Corn contains approximately 0.9% soluble NSP and 6 to 8% insoluble NSP, whereas soybean meal contains approximately 6% soluble NSP and 18 to 21% insoluble NSP [5,6].

Microorganisms are excellent enzyme producers because they have the ability to give rise to more active and stable enzymes when compared to plant enzymes and animals. These enzymes have high yield and are easy to modify and optimize due to their biochemical diversity and susceptibility to gene manipulation [7,8].

Exogenous fibrolytic enzymes have been studied for the possibility of improving grain nutritional quality by degrading structural polysaccharides and reducing nutritional levels in rations, leading to economic advantages. These enzymes are produced by fungi, bacteria, yeast, marine algae and others, but the principal commercial source is filamentous fungi [9]. Humicola grisea var. thermoidea is one of these producers of fibrolytic enzymes [10].

Exogenous carbohydrases are used in rations for broiler chickens, and may be supplemented alone or as enzymatic complexes, producing better results in digestibility and weight gain. In addition, enzyme supplementation contributes to the best use of low-cost ingredients for animal feeding and reduces digesta viscosity, benefiting the action of endogenous enzymes on specific substrates [11].

The cellulases are a group of enzymes that hydrolyse cellulose or β-(1,4)-glucan. Individual recombinant cellulases can be as effective as complex mixtures of glycoside hydrolases in attenuating the detrimental effects of soluble polysaccharides in broilers [12].

Therefore, due to their characteristics, exogenous enzymes are actively studied with the purpose of improving the nutritional quality of grains used in animal feed by acting on the degradation of structural polysaccharides (non-starch polysaccharides—PNAs). In addition to the nutritional advantage, this strategy reduces the costs of animal feeding.

Several studies have shown positive responses in terms of nutrient digestibility and the performance of poultry supplemented with complex mixtures of glycoside hydrolases [13]. However, the effective use of enzymes to hydrolyse NSP has been the subject of much debate. It appears that there is no consensus on which particular enzyme or enzymes result in the greatest benefits. This is in part owing to the complexity of the potential substrates, which vary according to cereal and diet formulation [14].

Due to this, the search for different sources of enzyme production is relevant. It is expected that the effects of exogenous enzymes will be more relevant in the initial growth phase of broiler chickens. Furthermore, little is known about the use of cellulase produced by *Humicola grisea* in chicken production performance. Therefore, this study aimed to evaluate the effects of adding cellulase produced by *Humicola grisea* on the performance, blood parameters, biometrics of digestive organs and digestibility of broiler chickens up to 21 days of age on a diet of corn and soybean meal. From these investigations, it will be possible to verify whether there will be changes with regard to improving the performance of the chickens, better use of the feed, better development of uniform growth, and positive or negative interferences in the animals' organism, in order to make a more accurate decision regarding the inclusion of the enzyme in an adequate quantity, while respecting the bird's metabolism and the consumer's need for food with better nutritional quality without risks to their health.

2. Materials and Methods

2.1. Broilers Farming

The chicken experiment was conducted at Goiano Federal Institute (Instituto Federal Goiano—IF Goiano), Rio Verde campus, in the aviary and laboratories of Animal Nutrition and Animal Biochemistry and Metabolism. The experiment was approved by the Ethics Committee (protocol 008/14). A total of 180 one-day-old Cobb male broiler chicks were used. The chickens were housed in a barn that was properly prepared under ideal initial management conditions. The chickens were randomly distributed into the treatments.

The experiment followed a completely randomized design, with three treatments, six replicates, and 10 chickens per pen (0.90 m × 0.60 m × 0.40 m), which was considered the experimental unit. The chickens were subjected to a continuous light program (natural + artificial light), in which 100-watt light bulbs were used overnight. The rations and water were provided ad libitum for the period from one to 21 days old.

Feeders, water troughs, a 100-watt incandescent light bulb as a source of artificial heat for the experimental period from one to 14 days old, and fans for air renewal to provide the chickens with controlled thermal comfort were present in all of the cages. The environmental variables of temperature and humidity were recorded throughout

the period, two times each day (at 9 a.m. and 6 p.m.), using maximum and minimum thermometers and a thermo-hygrometer, respectively.

Temperature and humidity values that occurred simultaneously were measured daily using a thermo-hygrometer. The mean maximum and minimum temperatures were 30.9 and 24.6 °C, respectively, while the mean maximum and minimum humidity values were 48 and 43%.

The treatments consisted of (i) the control treatment: diet based on corn, soybean meal, soybean oil, and mineral and vitamin premix (basal ration); (ii) Treatment 2: basal ration treated with liquid cellulase at 500 mL/t; and (iii) Treatment 3: basal ration treated with liquid cellulase at 1000 mL/t. The diets for the production phases (pre-starter and starter) were formulated according to the recommendations [15] (Table 1). Enzyme doses were determined and used according to the commercial dosage and enzyme activities.

Table 1. Percentage and calculated composition of the experimental rations for two rearing phases.

Ingredient, %	Pre-Starter (1–7 Days)	Starter (8–21 Days)
Corn	57.5865	60.0401
Soybean meal	36.9273	34.0639
Soybean oil	1.2906	2.1716
Limestone	0.8081	0.8531
L-Lysine HCl	0.3525	0.3014
Salt	0.4470	0.4250
Dicalcium phosphate	1.9123	1.5581
DL-Methionine	0.3594	0.3055
Vitamin premix [1]	0.0800	0.0800
Mineral premix [2]	0.1000	0.1000
L-Threonine	0.1363	0.1012
Total	100.00	100.00
Calculated composition		
Crude protein, %	22.400	21.2000
Metabolizable energy, Mcal/kg	2.9600	3.0500
Digestible lysine, %	1.3240	1.2170
Digestible methionine, %	0.6625	0.5962
Digestible meth + cyst, %	0.9530	0.8760
Calcium, %	0.9200	0.8410
Sodium, %	0.2200	0.2100
Available phosphorus, %	0.4700	0.4010
Digestible threonine, %	0.8610	0.7910
Digestible tryptophan, %	0.2478	0.2322

[1] Vitamin and mineral supplementation in starter rations (per kg of product): iron 30.0000 g/kg, zinc 40.0083 g/kg, selenium 225.0000 mg/kg, copper 75.0000 g/kg, manganese 45.0000 g/kg, iodine 500.0000 mg/kg, cobalt 3.0000 mg/kg, magnesium 80.0000 mg/kg, vitamin A 5.3784 IU/Kg, vitamin D3 2.2744 IU/Kg, vitamin E 44.9894 IU/Kg, vitamin K 2239.37 mg/Kg, vitamin B1 1168.31 mg/Kg, vitamin B2 3585.60 mg/Kg, vitamin B6 1788.62 mg/Kg, vitamin B12 6723.00 mcg/Kg, folic acid 894.159 mg/Kg, nicotinic acid 22.402 g/Kg, pantothenic acid 8968.48 mg/Kg, biotin 89.64 mg/Kg, antioxidant 527.7 mg/Kg, nicarbazin 50 mg/Kg, and narasin 50.00 mg/Kg. [2] Mineral supplementation in rations starter period (per kg of product): iron 30.0000 g/kg, zinc 40.0083 g/kg, selenium 225.0000 mg/kg, copper 75.0000 g/kg, manganese 45.0000 g/kg, iodine 500.0000 mg/kg, cobalt 3.0000 mg/kg, magnesium 80.0000 mg/kg, vitamin A 4,196,448.00 IU/Kg, vitamin D3 1,293,904.80 IU/Kg, vitamin E 14,085,954.36 IU/Kg, vitamin K 1747.25 mg/Kg, vitamin B1 911.5614 mg/Kg, vitamin B2 2797.63 mg/Kg, vitamin B6 1395.55 mg/Kg, folic acid 697.6595 mg/Kg, nicotinic acid 17.4789 g/Kg, pantothenic acid 6997.58 mg/Kg, biotin 69.9408 mg/Kg, antioxidant 508.2839 mg/Kg, and salinomycin 66 mg/Kg.

In Table 1, the percentage and calculated composition of the experimental rations for two rearing phases are presented.

2.2. Origin of the Tested Enzyme

The cellulase was produced at the Laboratory of Enzymology of the Institute of Biological Sciences, Federal University of Goiás (Universidade Federal de Goiás (UFG)), in the municipality of Goiânia, Goiás state (GO), Brazil.

The sample of Humicola grisea that was used to produce the enzyme for the present study was isolated from compost at the Federal University of Viçosa (Universidade Federal de Viçosa (UFV)), in Minas Gerais state, Brazil. The microorganism was cultured in oat agar medium with 4.0% Quaker oat flour (*w*/*v*) and 1.5% agar (*w*/*v*), which had been autoclaved at 120 °C for 20 min. The filter paper activity (FPase) was 3.798 U/mL.

The culture was maintained for four days at 42 °C, and subsequently, the temperature environment for another three days. The plates were stored at 4 °C.

For the production of the enzyme, ten culture discs (5 mm), containing spores of the *H. grisea* removed from seven-day culture plates were inoculated in Erlenmeyer flasks of 1.0 L, containing 250 mL of induction medium (carbon source 5 g/L; extract of yeast 3 g/L; ammonium sulfate 1.4 g/L; $CaC1_{2.6}H_2O$ 0.3 g/L; magnesium sulfate 0.3 g/L: trace elements $CuSO_4$ and $FeSO_4$). Commercial cellulose was used as a source of carbon. The flasks were incubated in a rotary shaker (Controlled Environment Incubator Shaker Brunswick Scientific Co Inc., Wooster, OH, USA) at 42 °C and speed of 120 rpm, for 72 h. The FPASE enzyme activity obtained was 3.798 U/mL.

2.3. Performance Evaluation

The average weight of the chickens from each pen and the diets at 1, 7, 14, and 21 days old were evaluated. These performance variables were assessed: weight gain, ration consumption, and feed conversion index.

2.4. Metabolizability Apparent Analysis

Collections of excreta produced by the birds were carried out between the 4th and 7th day, and from the 14th and 17th of age, were carried out twice a day during the period of experiment. Excreta and diets were identified and stored under freezing and subsequently sent to the Animal Nutrition Laboratory of the IFGoiano, Rio Verde Campus, to determine dry matter (DM) levels and crude protein (CP) [16].

To prepare excreta samples for analysis, aliquots were collected, identified and subjected to pre-drying in a straight oven with forced ventilation (FANEM LTDA) at 55 ± 5 °C, and subsequently crushed in Wiley-type mills [16]. From the samples of experimental rations, the following were determined: dry matter of excreta in an oven with forced ventilation at temperatures of 55 °C ± 5 °C for 72 h; dry matter of experimental feed and excreta in an oven set at 105 °C for 12 h, with analyses carried out in duplicate; total nitrogen in feed experimental and excreta, using the micro-Kjeldahl method, and subsequently, crude protein values were calculated by multiplying the % N by 6.25; digestibility determined the difference between nutrients ingested and excreted divided by the nutrient; dry matter retention, obtained by the amount of dry matter ingested subtracted from the amount excreted in relation to weight gain; and crude protein retention, determined by the amount of protein gross intake subtracted from the amount excreted divided by weight gain [16].

The calculation of nutrient retention, taking into account nutrient balance and weight gain, was recorded over periods of 4–7 and 14–17 days.

2.5. Serum, Liver and Pancreas Biochemical Profile

Blood was collected from the birds, one from each experimental cage at 7, 14, and 21 days old, via cardiac puncture and placed in labelled tubes, which were then centrifuged at 6000 rpm for 10 min to obtain a serum. The serum was used for colorimetric determination of calcium (Ca) (mg/dL), phosphorus (P) (mmol/L), chlorine (Cl) (mmol/L), and potassium (K) mmol/L), and to determine alkaline phosphatase (AP) (IU/L) and protein (Prot) (g/dL) activity using commercial kits (Dolles®, Goiás, Brazil).

2.6. Intestinal Histomorphometry

At 7, 14, and 21 days old, one bird per pen was identified and transported to the slaughterhouse in the aviary of IF GOIANO, Rio Verde Campus, and slaughtered via cervical dislocation.

After slaughter, the viscera (liver, gizzard, proventriculus, and pancreas) comprising the gastrointestinal tract (GIT) were removed. Then, GIT length was determined, and the following tissues were measured and weighed: proventriculus plus the gizzard, pancreas, small intestine, large intestine, and liver (as the weight of the liver without the gallbladder). All of these weights were used to calculate the relative weight of each organ.

At necropsy, the liver and pancreas were removed, placed in properly identified containers, and quickly frozen using liquid nitrogen to stop enzymatic activity, then stored. This material was homogenized (1 g tissue and 9 mL water) and then centrifuged at 8000 rpm at 40 °C for 10 min. The supernatant was collected to determine amylase levels in the pancreas and the protein content and enzymatic activity of alkaline phosphatase and transaminases in the liver in triplicate, using commercial kits (Doles®, Goiás, Brazil). All procedures were performed in an ice bath with distilled water to prevent the loss of enzymatic activity.

2.7. Statistical Analysis

Statistical analysis of the data was performed via analysis of variance using the Statistical and Genetic Analysis System (Sistema de Análises Estatísticas e Genéticas (Saeg)), version 9.5 (Universidade Federal de Viçosa, 2007). Means were compared using the Tukey test at 5% probability.

3. Results

Table 2 shows the ration consumption, weight gain and feed conversion in pre-starter and starter stage broilers chickens fed cellulase-supplemented rations.

Table 2. Ration consumption, weight gain and feed conversion in in pre-starter (1–7 days), (1–14 days) and starter (1–21 days) stage broilers chickens fed cellulase-supplemented rations.

Rations	1–7 Days			1–14 Days			1–21 Days		
	RC (g)	WG (g)	FC	RC (g)	WG (g)	FC	RC (g)	WG (g)	FC
BD	123.56	103.68	1.20	390.51	254.33	1.54	687.96	392.53	1.75
BD + E500	125.60	101.38	1.24	390.68	270.13	1.44	649.45	399.06	1.64
BD + E1000	123.62	103.62	1.19	379.75	258.38	1.47	664.36	394.20	1.61
CV, %	6.46	8.02	9.84	6.27	5.96	8.60	6.09	8.34	9.25
p-value	0.670	0.928	0.128	0.464	0.054	0.741	0.065	0.320	0.634
SEM	0.072	0.092	0.114	0.107	0.991	0.110	0.091	0.097	0.101

BD: basal diet; BD + 500: basal diet + 500 mL/t cellulase; BD + 1000: basal diet + 1000 mL/t cellulase; coefficient of variation (CV); standard error of mean (SEM); ration consumption (RC); weight gain (WG); and feed conversion (FC).

For broiler chicken performance from 1 to 21 days old, the addition of cellulase had no effect on any of the parameters. Ration consumption, weight gain and feed conversion did not differ significantly between treatments ($p > 0.05$).

In the pre-starter phase, the parameters of excreted nitrogen (N), N balance, and N retention as a function of weight gain did not differ among the broiler chickens fed rations supplemented with cellulase at 500 mL/t or 1000 mL/t or the basal diet (Table 3).

Table 3. Dry matter digestibility coefficient, dry matter nitrogen intake, dry matter excreted nitrogen, nitrogen balance, nitrogen digestibility, and nitrogen retention in feces and rations collected from 4- to 7-day-old and 14- to 17-day-old broiler chickens.

Rations	DDMC (%)	DMNI (g)	DMEN (g)	NB (g)	ND (%)	RETEN (%)
			4 to 7 days			
BD	56.07	36.63 [a]	5.75	30.87	84.30 [a]	27.76
BD + E500	57.87	30.92 [b]	6.75	29.34	78.17 [b]	24.85

Table 3. *Cont.*

Rations	DDMC (%)	DMNI (g)	DMEN (g)	NB (g)	ND (%)	RETEN (%)
BD + E1000	56.00	35.35 [ab]	5.43	29.22	84.63 [ab]	27.40
CV%	7.94	10.54	7.49	14.57	6.09	7.89
p-value	0.190	0.037	0.107	0.186	0.037	0.220
SEM	0.147	0.178	0.179	0.137	0.033	0.174
			14 to 17 days			
BD	80.51	66.42	18.64	53.41	71.93	46.52
BD + E500	82.29	69.94	17.30	54.03	75.26	46.32
BD + E1000	79.98	67.48	18.12	55.85	73.15	45.62
CV%	8.95	7.38	13.40	7.22	3.16	6.97
p-value	0.287	0.527	0.215	0.281	0.417	0.082
SEM	0.221	0.158	0.176	0.151	0.025	0.152

[a,b] Means in the same column followed by different superscripted letters differ significantly according to the Tukey test ($p < 0.05$). BD: basal diet; BD + 500: basal diet + 500 mL/t cellulase; BD + 1000: basal diet + 1000 mL/t cellulase; coefficient of variation (CV); standard error of mean (SEM); dry matter digestibility coefficient (DDMC); dry matter nitrogen intake (DMNI); dry matter excreted nitrogen (DMEN); nitrogen balance (NB); nitrogen digestibility (ND); and N retention (RETEN).

A significant difference was observed in N intake and N digestibility when the enzyme was added at a dose of 1000 m/L, with lower values being observed in the presence of the enzyme ($p < 0.05$) (Table 3).

Table 4 shows the relative biometrics of organs during the pre-starter and starter phases.

Table 4. Biometrics of the digestive organs (relative weight) of broiler chickens at 7, 14 and 21 days old fed cellulase-supplemented rations.

Rations	GITM (cm)	GITW (%)	OESW (%)	PROGI (%)	PANC (%)	LIV (%)	SI (%)	LI (%)
7 days old								
BD	52.05	7.25	1.20	8.08	0.51	4.22	5.89	1.36 [b]
BD + E500	55.59	7.50	1.19	7.97	0.47	4.78	5.80	1.59 [a]
BD + E1000	57.35	7.48	1.18	7.95	0.48	4.22	5.91	1.57 [a]
CV, %	12.60	8.14	11.40	12.10	12.52	10.71	10.21	7.40
p-value	0.477	0.660	0.887	0.936	0.427	0.115	0.998	0.004
SEM	0.070	0.077	0.087	0.123	0.115	0.880	0.124	0.182
14 days old								
BD	26.70	5.42	0.89	4.53	0.34	2.69	4.38	1.13
BD + E500	26.57	5.46	0.91	4.53	0.31	2.53	4.37	1.08
BD + E1000	30.13	5.76	0.90	5.11	0.37	2.94	4.70	1.05
CV %	10.72	10.48	12.65	13.69	11.81	12.06	11.99	12.74
p-value	0.195	0.381	0.426	0.686	0.328	0.129	0.632	0.344
SEM	0.047	0.106	0.080	0.127	0.092	0.098	0.121	0.130
21 days old								
BD	15.82	4.36	0.62	3.76	0.26	2.47	3.56	0.80
BD + E500	16.18	4.27	0.63	3.87	0.25	2.38	3.40	0.86
BD + E1000	16.04	4.38	0.61	3.91	0.28	2.53	3.56	0.82
CV, %	10.38	9.00	11.52	9.86	10.82	9.83	11.63	7.12
p-value	0.870	0.917	0.803	0.703	0.206	0.609	0.303	0.323
SEM	0.059	0.083	0.076	0.094	0.110	0.122	0.093	0.117

In the table the letters a and b mean in the same column followed by different superscripted letters differ significantly according to the Tukey test ($p < 0.05$). BD: basal diet; BD + 500: basal diet + 500 mL/t cellulase; BD + 1000: basal diet + 1000 mL/t cellulase; coefficient of variation (CV); standard error of mean (SEM); gastrointestinal tract length (GITM); gastrointestinal tract weight (GITW); esophagus and crop weight (OESW); proventriculus and gizzard weight (PROGI); pancreas weight (PANC); liver weight (LIV); small intestine weight (SI); and large intestine weight (LI).

Adding the enzyme to the rations did not affect the parameters related to relative biometrics at 14 and 21 days old. GIT length and the weight of the GIT, esophagus and

crop weight, proventriculus and gizzard weight, pancreas, liver weight, and small intestine were all similar between treatments ($p > 0.05$) (Table 4).

On the other hand, cellulase supplementation affected the relative weight of the large intestine at 7 days old but had no effect on the parameters between 14 and 21 days old (Table 4). This is the result of better use of the fibre from the feed, which might have been affected by the conversion of cellulose into cellobiose, leading to more fermentation in the caecum.

According to the data presented in Table 5, there were no significant effects of adding cellulase to the rations on the absolute weight of the pancreas or amylase activity in the pancreas at 7, 14, and 21 days old.

Table 5. Absolute weight of the pancreas and amylase concentrations in the pancreas (amylase) of broiler chickens fed rations supplemented with cellulase at 7, 14, and 21 days old.

Rations	7 Days		14 Days		21 Days	
	Panc (g)	Amylase (IU/dL)	Panc (g)	Amylase (IU/dL)	Panc (g)	Amylase (IU/dL)
BD	0.88	794.38	1.53	793.36	2.40	780.28
BD + E500	0.80	804.59	1.45	800.95	2.23	809.74
BD + E1000	0.80	800.87	1.56	769.60	2.50	801.05
CV, %	9.34	2.30	11.81	8.36	10.82	4.58
p-value	0.391	0.071	0.298	0.187	0.226	0.254
SEM	0.115	0.021	0.092	0.060	0.110	0.030

BD: basal diet; BD + 500: basal diet + 500 mL/t cellulase; BD + 1000: basal diet + 1000 mL/t cellulase; coefficient of variation (CV); standard error of the mean (SEM); and pancreas (Panc).

Table 6 shows the results for the absolute weight of the liver and the total protein, alkaline phosphatase (AP), glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT) levels in the livers of the broilers at 7, 14, and 21 days old.

Table 6. Absolute weight of the liver, protein concentrations, and enzyme activities of alkaline phosphatase, glutamate-pyruvate transaminase, and glutamate-oxaloacetate transaminase in livers of broiler chickens in pre-starter and starter phases.

Rations	Liver (g)	Protein (mg/dL)	Phos (IU/L)	GPT (IU/L)	GOT (IU/L)
7 days old					
BD	7.20	2.14	212.10	30.96	262.04
BD + E500	7.91	2.95	205.49	31.29	258.30
BD + E1000	7.08	2.18	214.44	31.02	264.61
CV, %	10.02	9.326	9.326	11.36	9.22
p-value	0.128	0.081	0.388	0.055	0.384
SEM	0.112	0.100	0.057	0.210	0.119
14 days					
BD	12.01	2.62	218.06	25.65	299.98
BD + E500	11.55	2.58	206.32	25.13	294.18
BD + E1000	12.35	2.43	212.64	26.57	292.84
CV, %	8.18	11.75	10.04	7.16	7.19
p-value	0.141	0.549	0.170	0.198	0.177
SEM	0.130	0.219	0.133	0.092	0.074
21 days					
BD	22.26	2.14	209.00	30.96	262.04
BD + E500	21.06	2.21	208.78	31.29	266.54
BD + E1000	22.56	2.18	209.82	31.02	264.61
CV %	9.83	9.72	5.87	11.36	8.46
p-value	0.598	0.487	0.393	0.120	0.421
SEM	0.116	0.060	0.056	0.109	0.123

BD: basal diet; BD + 500: basal diet + 500 mL/t cellulase; BD + 1000: basal diet + 1000 mL/t cellulase; coefficient of variation (CV); standard error of mean (SEM); protein concentrations (Protein); alkaline phosphatase (Phos); glutamate-pyruvate transaminase (GPT); and glutamate-oxaloacetate transaminase (GOT).

There was no significant effect on the absolute weight of the liver, total protein, AP, GOT, and GPT in the livers of broiler chickens fed cellulase-supplemented rations at 7, 14, and 21 days old ($p > 0.05$) (Table 6).

Table 7 shows the determination of the serum biochemical profile in the pre-starter and starter phases.

Table 7. Levels of calcium, phosphorus, chlorine, potassium, protein, and alkaline phosphatase in the serum of broiler chickens fed cellulase-supplemented diets at 7, 14, and 21 days old.

Rations	Ca (mg/dL)	P (mmol/L)	Cl (mmol/L)	K (mmol/L)	Prot (mg/dL)	AP (IU/L)
7 days						
BD	10.85	5.57	81.92	6.55	3.55	213.13
BD + E500	10.76	5.05	85.42	5.86	3.41	204.12
BD + E1000	10.63	5.64	94.96	5.97	3.60	191.50
CV, %	3.090	9.35	12.51	8.28	10.28	7.37
p-value	0.215	0.126	0.136	0.067	0.147	0.071
SEM	0.039	0.065	0.107	0.084	0.065	0.066
14 days						
BD	9.36	5.50	86.57	7.71	2.54	260.08
BD + E500	9.61	5.42	85.92	7.52	2.66	266.77
BD + E1000	9.56	5.66	88.65	7.65	2.46	271.59
CV, %	4.68	3.98	9.45	7.03	9.35	5.31
p-value	>0.05	0.382	>0.05	>0.05	0.355	>0.05
SEM	0.029	0.061	0.104	0.107	0.085	0.055
21 days						
BD	10.57	5.53	74.47	8.19	3.22	206.57
BD + E500	10.85	5.61	72.95	8.07	3.29	203.14
BD + E1000	11.02	5.60	78.52	8.44	3.10	207.17
CV, %	2.25	9.09	10.13	5.05	11.20	6.51
p-value	0.197	0.109	0.323	0.318	0.226	0.078
SEM	0.051	0.082	0.118	0.074	0.064	0.0633

BD: basal diet; BD + 500: basal diet + 500 mL/t cellulase; BD + 1000: basal diet + 1000 mL/t cellulase; coefficient of variation (CV); standard error of mean (SEM); calcium (Ca); phosphorus (P); chlorine (Cl); potassium (K); protein (Prot); and alkaline phosphatase (AP).

The levels of Ca, P, Cl, K, Prot, and AP in the blood serum remained unaffected by supplementation with cellulase at 7, 14, and 21 days old ($p > 0.05$) (Table 7).

4. Discussion

In a previous study using protease, α-amylase, and cellulase in a corn and soybean meal-based diet, reference [17] detected no significant effect on performance parameters in 35-day-old chickens. They included a cellulase-based complex in a diet for broiler chickens and found no significant effect on weight gain. Ration consumption increased with enzyme supplementation, but there was no difference in final weight or feed conversion [18].

Reference [19] did not find an improvement in performance until 21 days old when they used exogenous enzyme supplementation in broiler diets based on corn and soybeans. In contrast, reference [20] found that the final bodyweight and weight gain of birds fed on barley-based diets supplemented with exogenous polysaccharidases were significantly higher than those of the control birds, which were not supplemented with microbial biocatalysts. The authors suggest that an improvement in feed intake was responsible for the better performances displayed by birds fed on diets containing the microbial cellulases.

These findings indicated inconsistent effects of cellulase supplementation in corn- and soybean-based diets on broiler performance (Table 2).

It is assumed that the enzyme cellulase produced by *H. grisea* in liquid form was not effective in broilers fed with corn and soybean. However, corn and soybean meal are ingredients of high nutritional value. Perhaps the use of more fibrous feed would have had better results on the performance of broilers from 1 to 21 days. Further studies should be

performed to test various doses and to verify whether the cellulase enzyme produced by *H. grisea* decreased activity in the GIT.

These results (Table 3) indicate that rations with enzymes did not improve N absorption by the broilers. Their immature digestive system in this phase reduces their ability to use the nutrients [21].

However, in the starter phase, the data showed no significant differences in N intake, excreted N, N balance, N digestibility, and N retention between the diets containing liquid cellulase (Table 3) at concentrations of 500 mL/t and 1000 mL/t ($p > 0.05$).

It is often difficult to evaluate the isolated effects of cellulase when added to broiler chicken rations because most studies use multi-enzyme complex to evaluate performance and digestibility.

According to [22], it is important to know the organ weights of broilers in the pre-starter phase to characterize their digestive development.

These data corroborate the findings of [23,24], who claim that the use of enzymes allows a portion of the insoluble fibre fraction to be solubilized, enabling an increased use of the energy content of the diets via cecal fermentation products, such as volatile fatty acids. Exogenous polysaccharidases may promote the proliferation of beneficial microflora in the final compartments of the monogastric GIT by increasing the quantity and quality of the substrates available for fermentation [25].

So, in this study, the weight of the large intestine was influenced by the increase in the amount of carbohydrates with the inclusion of enzyme cellulase, despite the fact that quantified nitrogen indicated lower protein absorption. The enzyme is related to non-starch polysaccharides and their fermentation in the large intestine [25].

The evolution of the development of organ weights observed in this experiment followed the expected trend. That is, a decreasing proportion was observed with increasing age, similar to the data reported [26].

There are few descriptions and studies of pancreatic diseases in birds, and amylase activity may increase as a result of pancreatic injury and other pathologies. References [27,28] reported that higher amylase activity in broilers at 14 days old coincides with the highest pancreas growth rate. Amylase activity decreases with age, and maximum pancreas development occurs in the second week of life. However, a contradictory pattern was found in the present study, in which higher amylase activity occurred in the broilers subjected to the treatments with cellulase supplementation at 21 days old, coinciding with the highest pancreas proportion.

Because the liver is a central organ that plays a key role in the metabolism of carbohydrates, lipids, and proteins, changes in the liver protein content may reveal general metabolic changes [29]. High enzyme levels typically indicate the degree of injury to the organ that produces the enzyme, rather than reduced liver function [30].

When the GOT and GPT results obtained for liver tissue were analyzed, these mean values (in IU/L) were found: 261.65 and 31.09, respectively, at seven days old; 295.66 and 25.78 at 14 days old; and 264.39 and 31.09 at 21 days old.

References [30,31] studied the effect of amylase supplementation on liver enzymes and protein concentrations. Similar values were obtained for GOT and AP. The recorded protein concentrations were similar to those found by [32]. The GPT values were lower than those determined in both studies.

The results suggested that there was no change in the evaluated enzymes and protein concentrations in the livers of broiler chickens fed rations supplemented with cellulase until 21 days old, which indicates that there were no metabolic changes in the liver.

However, the broilers fed rations supplemented with cellulase (500 mL/t) exhibited a 2:1 ratio for Ca:P, showing that the requirements of the birds were met by the rations, suggesting that rickets, a deficiency attributed to mild physiological hypocalcemia or compensatory hypercalcemia, did not occur under any of the treatments.

Reference [33] evaluated the calcium concentration in the blood of 21-day-old broiler chicks fed corn- and soybean-based rations with 20% and 23% protein and obtained mean

Ca levels of 8.31 and 8.23 mg/dL, respectively. The birds fed the ration supplemented with 1000 mL/t of cellulase exhibited a higher Ca concentration in the phase between 14 and 21 days old.

Reference [34] fed soybean and corn meal-based rations with different concentrations of P and Cl to broiler chickens and obtained mean Cl and P concentrations of 106.37 and 6.16 µmol/L, respectively.

Phos, an enzyme that is released mainly by osteoblasts inside the bone matrix during bone formation, diffuses readily into the blood, and is therefore used as an indicator of bone formation rate. There were no differences in the serum concentration of Phos in the broilers fed the experimental diets.

Reference [35] obtained lower values when broiler chicks were fed basic, purified diets with various concentrations of L-glutamic acid (L-Glu) and vitamin D, in which the maximum concentration of Phos obtained in the serum was 221.2 IU/L. Reference [35] demonstrated AP activity values of 1043 and 1324 IU/L during the second and third weeks of life, respectively, for broiler chickens fed commercial rations in the pre-starter phase. These values are much higher than those obtained in this experiment.

When the levels of Ca, P, and Phos were evaluated together, the levels of serum minerals and enzymes were found to be higher in the chickens in the second week of life, suggesting greater metabolic demand and adaptation in broilers fed rations supplemented with cellulase in the starter phase.

No changes were observed when the serum concentrations of Ca and protein were evaluated in broiler chickens fed diets supplemented with cellulase at 7, 14, and 21 days old. The normal serum concentration of total proteins in broilers varied from 3.0 to 6.0 g/dL, indicating normality during the pre-starter and starter phases. Low protein values (hypoproteinemia) are associated with liver and nutritional diseases. The values obtained for all of the phases of life were within normal levels, indicating that there were no changes in the liver [33].

There are few studies on the serum biochemical profile of broiler chickens fed cellulase-enriched diets, hindering discussion of the results, thus requiring new studies in this field. The results obtained in the present study could be used as a reference for the scientific community, as serum values are still unknown for rations supplemented with cellulase.

5. Conclusions

Supplementation of diets with cellulase from Humicola grisea did not affect the performance and metabolism of broiler chickens from 1 to 21 days old fed with corn and soybean meal. However, more studies involving this microorganism are necessary to understand the mode of action of the enzymes it produces and their potential benefits for animal production.

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References

1. Muszyński, S.; Dajnowska, A.; Arciszewski, M.B.; Rudyk, H.; Śliwa, J.; Krakowiak, D.; Piech, M.; Nowakowicz-Dębek, B.; Czech, A. Effect of Fermented Rapeseed Meal in Feeds for Growing Piglets on Bone Morphological Traits, Mechanical Properties, and Bone Metabolism. *Animals* **2023**, *13*, 1080. [CrossRef] [PubMed]
2. Ohja, B.K.; Singh, P.K.; Shrivastava, N. Enzymes in the animal feed industry. In *Enzymes in Food Biotechnology*; Academic Press: Cambridge, MA, USA, 2019; pp. 93–109.
3. Trbojević-Ivić, J. Microbial Cellulase in the Production of Second Generation Biofuels: State-of-the-Art and Beyond. In *Biomass and Bioenergy Solutions for Climate Change Mitigation and Sustainability*; IGI Global: Hershey, PA, USA, 2023; pp. 233–257.
4. Adegboye, M.F.; Ojuederie, O.B.; Talia, P.M.; Babalola, O.O. Bioprospecting of microbialstrainsfor biofuel production: Metabolic engineering, applications, and challenges. *Biotechnol. Biofuels* **2021**, *14*, 5. [CrossRef] [PubMed]
5. Plouhinec, L.; Neugnot, V.; Lafond, M.; Berrin, J.G. Carbohydrate-active enzymes in animal feed. *Biotechnol. Adv.* **2023**, *65*, 108145. [CrossRef] [PubMed]
6. Fortes, B.D.A.; Cafe, M.B.; Stringhini, J.H.; Brito, J.A.; Rezende, P.L.; Silva, R.D. Evaluating nutritional programs using carbohydrases and phytases in broiler diets. *Braz. Anim. Sci.* **2012**, *13*, 24–32.
7. Patel, A.K.; Dong, C.D.; Chen, C.W.; Pandey, A.; Singhania, R.R. Production, purification, and application of microbial enzymes. In *Biotechnology of Microbial Enzymes*; Academic Press: Cambridge, MA, USA, 2023; pp. 25–57.
8. Singh, P.; Kumar, S. Microbial enzyme in food biotechnology. In *Enzymes in Food Biotechnology*; Academic Press: Cambridge, MA, USA, 2019; pp. 19–28.
9. Sirohi, R.; Rai, A.K.; de Souza Vandenberghe, L.P.; Parameswaran, B. *Microbial Enzymes and Metabolites for Health and Well-Being*; CRC Press: Boca Raton, FL, USA, 2023.
10. Li, L.; Liu, C.; Qu, M.; Zhang, W.; Pan, K.; OuYang, K.; Zhao, X. Characteristics of a recombinant Lentinula edodes endoglucanase and its potential for application in silage of rape straw. *Int. J. Biol. Macromol.* **2019**, *139*, 49–56. [CrossRef]
11. Velázquez-De Lucio, B.S.; Hernández-Domínguez, E.M.; Villa-Garcia, M.; Diaz-Godinez, G.; Mandujano-Gonzalez, V.; Mendoza-Mendoza, B.; Alvarez-Cervantes, J. Exogenous enzymes as zootechnical additives in animal feed: A review. *Catalysts* **2021**, *11*, 851. [CrossRef]
12. Munyaka, P.M.; Nandha, N.K.; Kiarie, E.; Nyachoti, C.M.; Khafipour, E. Impact of combined β-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. *Poult. Sci.* **2016**, *95*, 528–540. [CrossRef]
13. Ramatsui, L.; Sithole, T.; Mzimkulu-Ncoyi, N.H.; Malgas, S.; Pletschke, B.I. The use of xylanases as additives to feeds: A mini-review of their effect on feed digestion and growth performance of monogastric animals. In *Microbial Bioprocesses*; Academic Press: Cambridge, MA, USA, 2023; pp. 83–105.
14. Masey O'neill, H.V.; Smith, J.A.; Bedford, M.R. Multicarbohydrase enzymes for non-ruminants. *Asian-Australas. J. Anim. Sci.* **2014**, *27*, 290–301. [CrossRef]
15. Rostagno, H.S.; Albino, L.F.T.; Donzele, J.L.; Gomes, P.C.; de Oliveira, R.F.; Lopes, D.C.; Ferreira, A.S.; Barreto, S.L.; Euclides, R.F. *Brazilian Tables for Poultry and Pigs: Feed Composition and Nutritional Requirements*; Federal University of Viçosa: Viçosa, MG, Brazil, 2011.
16. Silva, D.J.; Queiroz, A.C. *Feed Analysis (Chemical and Biological Methods)*, 2nd ed.; Federal University of Viçosa University Press: Viçosa, MG, Brazil, 2002; p. 165.
17. Pinheiro, C.C.; Rego, C.C.J.; Ramos, A.T.; Silva, R.K.B.; Warpechowski, B.M. Nutrient digestibility and performance of broiler chickens fed diets formulated with different levels of fiber and supplemented with exogenous enzymes. *Braz. Anim. Sci.* **2008**, *9*, 984–996.
18. Esonu, B.O.; Azubuike, J.C.; Emenalom, O.O.; Etuk, E.B.; Okoli, I.C.; Ukwu, H.; Nneji, C.S. Effect of enzyme supplementation on the performance of broiler finisher fed microdesmis puberula leaf meal. *Int. J. Poult. Sci.* **2004**, *3*, 112–114.
19. Zhu, H.L.; Hu, L.L.; Hou, Y.Q.; Zhang, J.; Ding, B.Y. The effects of enzyme supplementation on performance and digestive parameters of broilers fed corn-soybean diets. *Poult. Sci.* **2014**, *93*, 1704–1712. [CrossRef] [PubMed]
20. Guerreiro, C.I.P.D.; Ribeiro, T.; Ponte, P.I.P.; Lordelo, M.M.S.; Falcão, L.; Freire, J.P.B.; Ferreira, L.M.A.; Prates, J.A.M.; Fontes, C.M.G.A. Role of a family 11 carbohydrate-binding module in the function of a recombinant cellulase used to supplement a barley-based diet for broiler chickens. *Brit. Poult. Sci.* **2008**, *49*, 446–454. [CrossRef] [PubMed]
21. Qiu, K.; Chen, J.; Zhang, G.; Chang, W.; Zheng, A.; Cai, H.; Chen, Z. Effects of Dietary Crude Protein and Protease Levels on Performance, Immunity Capacity, and AA Digestibility of Broilers. *Agriculture* **2023**, *13*, 703. [CrossRef]
22. Stringhini, J.H.; Resende, A.D.; Café, M.B.; Leandro, N.S.M.; Andrade, M.A. Effect of initial weight and the ration supply period on broiler chicken performance. *Braz. J. Zootec.* **2003**, *32*, 353–360. [CrossRef]
23. Wiśniewska, Z.; Kołodziejski, P.; Pruszyńska-Oszmałek, E.; Konieczka, P.; Kinsner, M.; Górka, P.; Kaczmarek, S. Combination of emulsifier and xylanase in triticale-based broiler chickens diets. *Arch. Anim. Nutr.* **2023**, *77*, 187–204. [CrossRef]

24. Anderson, A.G.; Bedford, M.R.; Parsons, C.M. Effects of adaptation diet and exogenous enzymes on true metabolizable energy and cecal microbial ecology, short-chain fatty acid profile, and enzyme activity in roosters fed barley and rye diets. *Poult. Sci.* **2023**, *102*, 102768. [CrossRef]
25. Cysneiros, C.S.S.; Ferreira, R.N.; Oliveira, M.A.; Favoretto, A.O.; Arnhold, E.; Ulhoa, C.J. Production, characterization and evaluation of fibrolytic enzymes on digestibility of forage maize. *Braz. Anim. Sci.* **2013**, *14*, 426–435.
26. Marques, S.F.F.; Minafra, C.S.; Café, M.B.; Stringhini, J.H. Production and characterization of a trichoderma harzianum multi-enzyme complex and its application in broiler chicks diets. *Curr. Biotechnol.* **2016**, *4*, 1–8.
27. Capitelli, R.; Crosta, L. Overview of psittacine blood analysis and comparative retrospective study of clinical diagnosis, hematology and blood chemistry in selected psittacine species. *Clin. N. Am. Exot. Anim. Pract.* **2013**, *16*, 71–120. [CrossRef]
28. Sakamoto, M.I.; Faria, D.E.; Nakagi, V.S.; Negrão, J.A.; Araújo, R.B.; Souza, K.M.R.; Previero, T.C. Utilization of glutamine, associated with glutamic acid, in relation to development and enzymatic activity in broiler chickens. *Arq. Bras. Med. Veterinária Zootec.* **2011**, *63*, 962–972. [CrossRef]
29. Barbosa, A.A.; Müller, E.S.; Moraes, G.H.K.; Umigi, T.R.; Barreto, T.S.L.; Ferreira, M.R. Profile of aspartate aminotransferase and alanine aminotransferase and biometrics of Japanese quail. *Braz. J. Zootec.* **2010**, *39*, 308–312. [CrossRef]
30. Luo, C.; Wang, L.; Yuan, J. Supplemental enzymes and probiotics on the gut health of broilers fed with a newly harvested corn diet. *Poult. Sci.* **2023**, *102*, 102740. [CrossRef] [PubMed]
31. Minafra, C.S.; Marques, S.F.F.; Stringhini, J.H.; Ulhoa, C.J.; Rezende, C.S.M.; Santos, J.S.; Moraes, G.H.K. Serum biochemical profile of broiler chickens fed alpha-amylase from cryptococcus flavus and aspergillus niger HM2003. *Braz. J. Zootec.* **2010**, *39*, 2691–2696. [CrossRef]
32. Vieites, F.M.; Moraes, G.H.K.; Albino, L.F.T.; Rostagno, H.S.; Rodrigues, A.C.; Silva, F.Á.; Atencio, A. Electrolyte balance and crude protein levels in relation to the blood and bone parameters of 21-day-old broiler chicks. *Braz. J. Zootec.* **2004**, *33*, 1520–1530. [CrossRef]
33. Souza, B.B.; Bertechini, A.G.; Santos, C.D.; Lima, J.A.F.; Teixeira, A.S.; Freitas, R.T.F. Potassium balance and performance in broiler chickens supplemented with kcl in the summer. *Sci. Agrotechnol.* **2004**, *28*, 1160–1168.
34. Silva, F.A.; Morais, G.H.K.; Rodrigues, A.C.P.; Fonseca, C.C.; Oliveira, M.G.A.; Albino, L.F.T.; Rostagno, H.S.; Freitas, H.T. Serum chemical composition of broiler chicks fed purified diets. *Braz. J. Zootec.* **2002**, *31*, 1783–1788. [CrossRef]
35. Borsa, A.L.P.; Kohayagawa, M.E.; Boretti, M.E.; Kuibida, K. Serum levels of enzymes related to liver function in industrially reared, clinically healthy broiler chickens. *Arq. Bras. Med. Veterinária Zootec.* **2006**, *58*, 675–677. [CrossRef]