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Effects of dietary enzymolysis products of wheat gluten on the growth performance, serum biochemical, immune, and antioxidant status of broilers

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ABSTRACT

This study is aimed at determining the effects of dietary enzymolysis products of wheat gluten on the growth performance, organ weights, serum and intestinal antioxidant indices, serum biochemical indicators, and immunoglobulins of broiler chickens. The treatments consisted of a basal control diet, 2% of wheat gluten, and 1%, 2%, 3%, and 4% of enzymolysis products of wheat gluten. The inclusion of up to 4% of enzymolysis products of wheat gluten improved body weight and average daily gain. At 21 days, serum superoxide dismutase (T-SOD) had increased in the 2% of wheat gluten treatment ($P < .05$). At 42 days, both serum T-SOD and blood urea nitrogen had increased while uric acid had decreased ($P < .05$) in 2% enzymolysis products of wheat gluten treatment. These results showed that dietary inclusion of 2% enzymolysis products of wheat gluten can have beneficial effects on the growth performance and intestinal antioxidant activities of broiler chickens.

Abbreviations: ADG: average daily gain; ADFI: average daily feed intake; AKP: alkaline phosphatase; BUN: blood urea nitrogen; BW: body weight; F/G: feed efficiency; GSH-Px: glutathione peroxidase; MDA: malondialdehyde; T-AOC: total antioxidant capacity; T-SOD: superoxide dismutase; CAT: catalase

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Introduction

Up to 70% of the total production costs in the poultry industry are currently spend on feed, and the high prices present a significant challenge (Gorter et al., 2013; Taheripour, Hertel, & Tyner, 2011). Cereals are the main source of energy for poultry production. Although the prices of corn, wheat, and other cereals have been stabilized by increased production of these foods, costs remain high. Therefore, research has focused on

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developing new alternative feed stuffs with a higher productive efficiency while maintaining good quality (Olmo, Martínez, León, & Leyva, 2012; Roberts et al., 2015).

In this context, wheat is an important crop and about 700 million tons of wheat were gathered each year in the US (Schiermeier, 2015). Wheat gluten, a by-product created during the separation of starch from wheat flour, is composed of 70–85% protein, 5–15% carbohydrates, 3–10% lipids, and 1–2% ash (Apper-Bossard, Feneuil, Wagner, & Respondek, 2013). Wheat gluten proteins include glutenins and gliadins, which are the major storage proteins deposited in the starchy endosperm cells of grain (Wan, Gritsch, Hawkesford, & Shewry, 2014; Wang et al., 2014). Wheat gluten is used as an insoluble protein in a pH-neutral environment to improve baking properties of wheat flour. However, its further utilization in animal feed is limited by its low solubility. Applications of wheat gluten can be expanded by modifying its functional properties; the most important modification methods are enzymatic and chemical methods (Wang et al., 2016). Specifically, the hydrolysis of gluten increases its water solubility and improves its functional properties (Hardt, Boom, & van der Goot, 2015; Zheng et al., 2015).

Thus, several methods are available to produce hydrolysis product of wheat gluten, such as solubilizing with acid or alkali (Jansens et al., 2013); however, this method can increase the degradation of essential amino acids. Another effective method is to hydrolyze the gluten with enzymes (van der Zalm, van der Goot, & Boom, 2009). The enzymes cause a specific peptide bond cleavage and their use is determined by their selectivity, mild reaction conditions, and high conversion rates (Cian, Vioque, & Drago, 2015). Gluten hydrolysates have been used for their emulsifying and foaming properties (Joye & McClements, 2014). These methods could represent a new opportunity for the food industry to improve the quality and protein content of products.

Wheat gluten has also been used commonly in aquaculture feeds as an alternative protein source for fish meal (Apper-Bossard et al., 2013). This product is a highly digestible protein source for several aquaculture species. Using wheat in this context has been shown to increase the apparent digestibility of crude protein, lipid metabolism, and growth performance of both shrimp (*Litopenaeus vannamei*) and Nile tilapia (Lemos, Lawrence, & Siccardi, 2009). Kopec et al. (2013) found that wheat bran contains a high concentration of lysine, glutamine, and proline and its use in broiler diets increases the glutathione peroxidase activity and the essential amino acids in the breast as lysine, threonine, alanine, valine, and isoleucine. However, current knowledge of the field reveals that few higher protein solubility investigations have been developed with wheat gluten and its enzymolysis products in non-ruminant diets. It is hypothesized that poultry growth performance could be enhanced by modulating the immune and antioxidant responses through enzymolysis. This study was conducted to determine the effects of dietary inclusion of enzymolysis products of wheat gluten on growth performance, organ weights, antioxidant indices, and serum biochemical indicators of broiler chickens.

Materials and methods

Animals and experimental design

The protocol for this study was approved by the Committee on the Ethics of Animal Experiments of Hunan Agricultural University and it was conducted at a farm in

Hunan province, China according to the recommendations in the Guide for the Care and Use of Laboratory Animals of Hunan Agricultural University. According to a completely randomized design, a total of 144 one-day-old birds were assigned to six treatments, with six replicates per treatment and four birds per replicate. The treatments administered consisted of a basal control diet (T0); 2% of wheat gluten (T1); and 1%, 2%, 3%, and 4% of enzymolysis products of wheat gluten (T2, T3, T4, and T5 respectively). The feeding period last for 42 days and the diet was formulated according to Chinese chicken feeding standards (NY/T33-2004). The nutrition levels of the diets are shown in Tables 1 and 2, respectively. Each broiler's body weight (BW) was measured on days 21 and 42, with average gains and feed intake subsequently calculated. Health status and mortality during the experiment were recorded within 24 h. On days 21 and 42, one broiler from each replicate was selected at random and slaughtered. The organ index was calculated after collecting and weighing the liver, spleen, bursa of Fabricius, thymus, and pancreas. Serum samples were collected and stored at -80°C until further analyses. Duodenum, jejunum, and ileum tissues were separated and stored at -80°C .

Analysis of serum indicators

The serum level of total protein, uric acid, urea nitrogen, alkaline phosphatase (AKP), IgA, IgM, and IgG were analyzed using commercial apparatus from Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China following the user's manual. The activities of superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) were also analyzed with commercial

Table 1. The composition of ration and nutrition level of 0- to 3-weeks-old broiler chickens.

Ingredients (%)	Control	Wheat gluten 2%	Enzymolysis products of wheat gluten			
			1%	2%	3%	4%
Corn	53.52	55.18	54.06	55.18	55.98	56.39
Soybean meal	33.00	29.44	31.4	29.44	27.53	26.00
Cottonseed meal	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	4.42	4.20	4.40	4.20	4.16	4.23
Wheat gluten	0	2.00	0	0	0	0
Enzymolysis products of wheat gluten	0	0	1.00	2.00	3.00	4.00
Dicalcium phosphate	1.60	1.60	1.60	1.60	1.70	1.70
Stone powder	1.50	1.50	1.50	1.50	1.50	1.50
Multi-vitamins	0.02	0.02	0.02	0.02	0.02	0.02
Minerals	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.18	0.18	0.18	0.18	0.18	0.18
DL-Methionine	0.19	0.18	0.18	0.18	0.18	0.19
L-Lysine	0.11	0.15	0.15	0.15	0.20	0.20
L-Threonine	0.06	0.15	0.11	0.15	0.15	0.19
<i>Calculated nutritional composition</i>						
ME (kcal/kg)	2999	3000	3004	3000	3003	3013
Crude protein (%)	21.4	21.4	21.4	21.4	21.4	21.4
Lysine (%)	1.10	1.10	1.10	1.10	1.10	1.10
Methonine (%)	0.50	0.50	0.50	0.50	0.50	0.50
Threonine (%)	0.90	0.90	0.90	0.90	0.90	0.90
Calcium (%)	0.99	0.98	0.98	0.98	0.99	0.99
Total phosphorus (%)	0.65	0.63	0.64	0.63	0.64	0.63
Available phosphorus (%)	0.44	0.43	0.43	0.43	0.44	0.44

Table 2. The composition of ration and nutrition level of 4- to 6-weeks-old broiler chickens.

Ingredients (%)	Control	Wheat gluten 2%	Enzymolysis products of wheat gluten			
			1%	2%	3%	4%
Corn	57.01	57.27	56.83	57.36	57.92	58.06
Soybean meal	26.80	25.00	26.50	24.80	23.44	22.00
Cottonseed meal	7.20	5.71	6.10	6.03	5.68	5.50
Soybean oil	5.40	6.13	5.84	6.10	6.18	6.55
Gluten	0	2.0	0	0	0	0
Enzymolysis products of wheat gluten	0	0	1.00	2.00	3.00	4.00
Dicalcium phosphate	1.42	1.55	1.55	1.50	1.50	1.59
Stone Powder	1.4	1.42	1.39	1.39	1.39	1.37
Multi-vitamins	0.12	0.17	0.14	0.17	0.2	0.23
Minerals	0.11	0.11	0.11	0.11	0.11	0.10
Choline chloride	0.12	0.12	0.12	0.12	0.12	0.12
Salt	0	0.1	0	0	0.04	0.06
DL-Methionine	0.02	0.02	0.02	0.02	0.02	0.02
L-Lysine	0.20	0.20	0.20	0.20	0.20	0.20
L-Threonine	0.20	0.20	0.20	0.20	0.20	0.20
<i>Calculated nutritional composition</i>						
ME (kcal/kg)	3100	3100	3100	3100	3100	3100
Crude protein (%)	20.00	20.00	20.00	20.00	20.00	20.00
Methionine (%)	1.00	1.00	1.00	1.00	1.00	1.00
Lysine (%)	0.40	0.40	0.40	0.40	0.40	0.40
Threonine (%)	0.72	0.72	0.72	0.72	0.72	0.72
Calcium (%)	0.90	0.90	0.90	0.90	0.90	0.90
Total phosphorus (%)	0.61	0.61	0.62	0.60	0.59	0.60
Available phosphorus (%)	0.41	0.41	0.41	0.41	0.41	0.41

kit from Nanjing Jiancheng Bioengineering Institute following the methods described by Liu, Yang, et al. (2015).

Analysis of anti-oxidative markers in the small intestine

About 1 g tissues of duodenum, jejunum, and ileum were cut and homogenized. Then an ELISA kit from Nanjing Jiancheng Bioengineering Institute was used to determine the level of MDA, CAT, SOD, and GSH-PX activities according to the standard curve. Optical densities of the enzymes were read at 405 nm using a Bio-Rad microplate reader from Bio-Rad Laboratories, USA.

Statistical analysis

All data were presented as the means $\pm$ standard error of the means (SEM). One-way ANOVA was used to analyze the data with Duncan's method, and a *P*-value of less than .05 was used as the criterion of significance.

Results

Growth performance

Data on BW, average daily gain (ADG), and feed efficiency (F/G) during the experimental stage of broiler chicks fed with wheat gluten enzymolysis products are presented in the Table 3. At 21 days old, the BW and ADG increased with the inclusion up to 4% of enzymolysis products of wheat gluten ($P < .05$) with relation

Table 3. Effect of enzymolysis products of wheat gluten on growth performance of broiler chickens.

Items	Control	Wheat gluten 2%	Enzymolysis products of wheat gluten				P-value
			1%	2%	3%	4%	
1–21 days							
BW (g)	684 ± 43 ^{ab}	638 ± 31 ^b	718 ± 27 ^a	711 ± 36 ^a	732 ± 37 ^a	732 ± 61 ^a	.003
ADG (g)	30.5 ± 2.05 ^{ab}	28.4 ± 1.50 ^b	32.2 ± 1.32 ^a	31.8 ± 1.73 ^a	32.8 ± 1.80 ^a	32.8 ± 2.94 ^a	.003
ADFI (g)	44.2 ± 2.74 ^{bc}	41.4 ± 1.67 ^c	45.4 ± 1.89 ^{ab}	45.3 ± 1.49 ^{ab}	45.3 ± 3.32 ^{ab}	47.3 ± 2.22 ^a	.007
F/G	1.44 ± 0.07	1.46 ± 0.03	1.41 ± 0.04	1.42 ± 0.06	1.38 ± 0.05	1.44 ± 0.08	.283
22–42 days							
BW (kg)	2.10 ± 0.06 ^b	1.90 ± 0.17 ^c	2.37 ± 0.09 ^a	2.35 ± 0.06 ^a	2.41 ± 0.15 ^a	2.28 ± 0.22 ^a	<.001
ADG (g)	66.3 ± 3.51 ^b	61.0 ± 6.88 ^b	79.1 ± 4.62 ^a	78.1 ± 2.28 ^a	81.5 ± 6.27 ^a	74.2 ± 7.56 ^a	<.001
ADFI (g)	141 ± 9.76 ^b	131 ± 6.50 ^c	151 ± 6.01 ^a	150 ± 2.06 ^a	154 ± 6.11 ^a	152 ± 6.42 ^a	<.001
F/G	2.13 ± 0.11 ^a	2.17 ± 0.21 ^a	1.92 ± 0.07 ^b	1.93 ± 0.04 ^b	1.90 ± 0.10 ^b	2.07 ± 0.20 ^{ab}	.011
1–42 days							
ADG (g)	48.9 ± 1.88 ^b	44.8 ± 3.92 ^b	55.6 ± 2.31 ^a	55.0 ± 1.64 ^a	56.8 ± 3.37 ^a	53.7 ± 5.18 ^a	<.001
ADFI (g)	92.8 ± 4.66 ^b	86.8 ± 3.39 ^c	98.6 ± 3.34 ^a	98.1 ± 1.65 ^a	99.5 ± 4.09 ^a	100 ± 3.45 ^a	<.001
F/G	1.89 ± 0.07 ^{ab}	1.94 ± 0.14 ^a	1.77 ± 0.06 ^{bc}	1.78 ± 0.03 ^{bc}	1.75 ± 0.08 ^c	1.87 ± 0.15 ^{abc}	.024

Note: Means within the same row with different superscript differ significantly ($P < .05$).

to the inclusion of 2% of wheat gluten (T1), and this inclusion (T5) improved ($P < .05$) the average daily feed intake (ADFI) and indicated significant differences ($P < .05$) compared to the T0 and T1. The F/G did not show any significant change ($P > .05$) by the experimental diets.

From days 22 to 42, the BW, ADG, and ADFI improved ($P < .05$) in the treatments with enzymolysis products. In addition, broilers in the T2, T3, and T4 presented lower F/G ($P < .05$). By the end of the experiment (42 days), the growth performance of broiler chickens had improved with treatments with enzymolysis products of wheat gluten ($P < .05$).

Immune and visceral organ relative weights

In the present experiment, at 21 and 42 days, the immune and visceral organ relative weights of broiler chicks showed no changes ($P > .05$) as an effect of the inclusion of enzymolysis products of wheat gluten (Table 4).

Table 4. Effect of enzymolysis products of wheat gluten on immune and visceral organ relative weights of broiler chickens.

Relative weights (%)	Control	Wheat gluten 2%	Enzymolysis products of wheat				P-value
			1%	2%	3%	4%	
21 days							
Liver	3.05 ± 0.65	2.44 ± 0.21	2.80 ± 0.26	2.60 ± 0.38	2.45 ± 0.29	2.42 ± 0.33	.059
Spleen	0.097 ± 0.015	0.093 ± 0.012	0.100 ± 0.012	0.087 ± 0.019	0.087 ± 0.010	0.093 ± 0.015	.622
Bursa of Fabricius	0.184 ± 0.07	0.187 ± 0.03	0.180 ± 0.03	0.200 ± 0.06	0.212 ± 0.08	0.188 ± 0.06	.945
Pancreas	0.314 ± 0.06	0.253 ± 0.02	0.317 ± 0.02	0.271 ± 0.04	0.284 ± 0.04	0.247 ± 0.06	.071
Thymus	0.213 ± 0.06	0.186 ± 0.02	0.173 ± 0.03	0.147 ± 0.02	0.163 ± 0.02	0.167 ± 0.03	.093
42 days							
Liver	2.20 ± 0.40	2.13 ± 0.35	1.95 ± 0.29	2.28 ± 0.39	2.03 ± 0.19	2.11 ± 0.30	.614
Spleen	0.112 ± 0.02	0.151 ± 0.03	0.123 ± 0.02	0.127 ± 0.02	0.131 ± 0.03	0.117 ± 0.02	.383
Bursa of Fabricius	0.169 ± 0.02	0.163 ± 0.03	0.186 ± 0.10	0.174 ± 0.04	0.158 ± 0.08	0.189 ± 0.04	.974
Pancreas	0.191 ± 0.02	0.160 ± 0.04	0.143 ± 0.05	0.165 ± 0.05	0.141 ± 0.02	0.137 ± 0.04	.494
Thymus	0.170 ± 0.03	0.194 ± 0.05	0.157 ± 0.03	0.156 ± 0.02	0.157 ± 0.05	0.159 ± 0.05	.686

Note: Means within the same row with different superscript differ significantly ($P < .05$).

Serum antioxidant indices

Table 5 shows that MDA did not show significant differences ($P > .05$) among treatments for both ages (21 and 42 days). In addition, the T-SOD at 21 days and T-AOC and GSH-Px at 42 days were not statistically different ($P > .05$). However, the T1 had increased ($P < .05$) T-AOC and GSH-Px concentration at 21 days when compared to T3 and T5. In addition, birds fed with 1% of enzymolysis products of wheat gluten showed a higher concentration ($P < .05$) of T-SOD at 42 days when compared to T0 and T5.

Intestinal antioxidant indices

At 21 days, all intestinal antioxidant indices of broiler chickens showed statistical differences ($P < .05$) as a result of the experimental treatments (Table 6). The T3 treatment increased ($P < .05$) the MDA concentration in relation to the other treatments, and this treatment also increased ($P < .05$) the GSH-Px concentration. However, a significant decrease ($P < .05$) of this enzyme was caused by a higher inclusion of enzymolysis products of wheat gluten. After 42 days, only the MDA concentration indicated statistical differences ($P < .05$), with a lower concentration in the T5.

Serum biochemical indicators

At 21 days, blood urea nitrogen (BUN) and AKP were unaffected ($P > .05$) by the inclusion of enzymolysis products of wheat gluten (Table 7). Similar results were recorded at 42 days for total protein, uric acid, and AKP concentration. However, the total protein concentration increased ($P < .05$) with T4 and T5 and uric acid concentration decreased ($P < .05$) with the T3 and T5. Likewise, the BUN concentration was higher in broilers fed with T3 and T5 with notable differences ($P < .05$) between them and the T1 and T2.

Serum immunoglobulins

At 21 days, the treatments with enzymolysis products of wheat gluten had gradually decreased ($P < .05$) the IgM and IgA concentrations in broiler chickens (Table 8).

Table 5. Effect of enzymolysis products of wheat gluten on serum antioxidant indices and MDA concentration of broiler chickens.

Items	Control	Wheat gluten	Enzymolysis products of wheat gluten				P-value
		2%	1%	2%	3%	4%	
21 days							
MDA (nmol/mg prot)	0.342 ± 0.110	0.320 ± 0.096	0.361 ± 0.069	0.410 ± 0.167	0.368 ± 0.083	0.357 ± 0.121	.853
T-AOC (nmol/mg prot)	5.32 ± 1.11 ^{abc}	6.94 ± 0.66 ^a	5.45 ± 0.72 ^{abc}	4.34 ± 0.69 ^c	6.47 ± 2.29 ^{ab}	5.09 ± 1.42 ^{bc}	.024
GSH-Px (μmol/L)	1509 ± 217 ^a	1379 ± 140 ^a	1344 ± 141 ^a	1368 ± 238 ^a	1269 ± 68 ^{ab}	1085 ± 185 ^b	.020
T-SOD (U/mg prot)	184 ± 16	159 ± 25	147 ± 30	193 ± 18	165 ± 35	190 ± 22	.059
42 days							
MDA (nmol/mg prot)	0.271 ± 0.042	0.289 ± 0.072	0.285 ± 0.096	0.276 ± 0.079	0.267 ± 0.067	0.346 ± 0.116	.596
T-AOC (nmol/mg prot)	5.51 ± 2.34	4.90 ± 2.12	5.34 ± 2.53	5.64 ± 1.01	5.43 ± 1.98	6.84 ± 2.82	.867
GSH-Px (μmol/L)	1349 ± 138	1346 ± 157	1411 ± 158	1377 ± 184	1218 ± 134	1471 ± 203	.195
T-SOD (U/mg prot)	158 ± 25 ^c	181 ± 42 ^{abc}	221 ± 33 ^a	212 ± 24 ^{ab}	212 ± 27 ^{ab}	174 ± 26 ^{bc}	.009

Note: Means within the same row with different superscript differ significantly ($P < .05$).

Table 6. Effect of enzymolysis products of wheat gluten on intestinal antioxidant indices and MDA concentration of broiler chickens.

Items	Control	Wheat gluten 2%	Enzymolysis products of wheat gluten				<i>P</i> -value
			1%	2%	3%	4%	
<i>21 days</i>							
MDA (nmol/ mg prot)	5.06 ± 1.12 ^d	6.39 ± 0.87 ^{cd}	21.2 ± 6.44 ^a	12.8 ± 4.16 ^b	8.68 ± 2.24 ^{bcd}	11.3 ± 4.70 ^{bc}	<.001
GSH-Px (μmol/ L)	12,203 ± 3923 ^{ab}	13,447 ± 2551 ^{ab}	14,453 ± 2574 ^a	10,003 ± 2902 ^{bc}	7557 ± 2160 ^{cd}	5030 ± 1974 ^d	<.001
T-SOD (U/mg prot)	271 ± 50.2 ^a	274 ± 50.5 ^a	281 ± 41.0 ^a	286 ± 29.4 ^a	248 ± 34.2 ^{ab}	213 ± 28.5 ^b	.047
T-AOC (nmol/ mg prot)	15.4 ± 3.26 ^b	18.0 ± 3.02 ^b	19.4 ± 5.27 ^b	28.2 ± 8.40 ^a	17.8 ± 5.49 ^b	16.1 ± 2.34 ^b	.005
<i>42 days</i>							
MDA (nmol/ mg prot)	7.50 ± 3.01 ^{bc}	12.2 ± 3.80 ^{ab}	16.2 ± 5.52 ^a	10.8 ± 3.46 ^{abc}	5.58 ± 1.50 ^c	7.35 ± 2.76 ^{bc}	.005
GSH-Px (μmol/ L)	13,405 ± 6073	10,485 ± 2424	17,701 ± 8236	13,639 ± 1870	9681 ± 1788	10,334 ± 2281	.076
T-SOD (U/mg prot)	272 ± 71	281 ± 25.8	337 ± 96.2	272 ± 43.7	266 ± 60.8	300 ± 63.0	.466
T-AOC (nmol/ mg prot)	18.8 ± 6.44	21.4 ± 6.83	20.6 ± 4.90	14.8 ± 1.36	13.9 ± 3.69	15.6 ± 3.10	.101

Note: Means within the same row with different superscript differ significantly (*P* < .05).

However, the IgG concentration did not show a significant variation (*P* > .05) compared to other treatments. The T1 and T3 indicated a higher (*P* < .05) concentration of IgG when compared to T2 and T5 after 42 days. In addition, a lower concentration IgA was shown in the last treatment (T5). Also, IgM concentration did not change significantly (*P* > .05) during the experimental stage (Table 8).

Discussion

This study showed that dietary inclusion of wheat gluten enzymolysis products from 1% to 4% led to the highest BW, ADG and ADFI after both 21 and 42 days as compared to control treatment, and the results for 2% of wheat gluten suggest that this product may contain chemical compounds that can enhance the growth performance of broiler

Table 7. Effect of enzymolysis products of wheat gluten on serum biochemical indicators of broiler chickens.

Items	Control	Wheat gluten 2%	Enzymolysis products of wheat gluten				P-value
			1%	2%	3%	4%	
21 days							
Total protein (g prot/L)	28.8 ± 6.86 ^b	25.0 ± 2.65 ^b	27.1 ± 5.20 ^b	26.3 ± 5.56 ^b	37.6 ± 3.51 ^a	36.8 ± 5.11 ^a	.001
Uric acid (μmol/L)	492 ± 185 ^a	616 ± 219 ^a	501 ± 83.1 ^a	322 ± 35.5 ^b	437 ± 84.8 ^{ab}	337 ± 174 ^b	.018
BUN (mmol/L)	12.6 ± 3.75	16.2 ± 2.15	13.3 ± 1.95	11.5 ± 2.59	12.8 ± 1.94	12.8 ± 2.95	.128
AKP (U/100 mL)	206 ± 36.9	244 ± 155	218 ± 109	104 ± 30	175 ± 72.4	166 ± 27.9	.139
42 days							
Total protein (g prot/L)	27.6 ± 4.15	28.9 ± 10.1	20.0 ± 9.65	15.7 ± 5.88	24.5 ± 7.90	22.6 ± 6.44	.189
Uric Acid (μmol/L)	380 ± 130	257 ± 106	463 ± 103	255 ± 71.1	342 ± 90.5	282 ± 184	.114
BUN (mmol/L)	12.6 ± 3.66 ^{ab}	11.7 ± 3.32 ^b	10.5 ± 4.91 ^b	17.5 ± 5.16 ^a	14.7 ± 2.46 ^{ab}	29.4 ± 5.20 ^a	<.001
AKP (U/100 mL)	50.1 ± 24.3	55.1 ± 35.5	38.7 ± 20.4	43.9 ± 20.6	32.1 ± 6.38	65.7 ± 20.4	.396

Note: Means within the same row with different superscript differ significantly (*P* < .05).

Table 8. Effect of enzymolysis products of wheat gluten on serum immunological indicators of broiler chickens.

Items	Control	Wheat gluten	Enzymolysis products of wheat gluten				P-value
		2%	1%	2%	3%	4%	
21 days							
IgM (mg/mL)	10.3 ± 2.51 ^a	8.03 ± 2.20 ^{ab}	7.62 ± 1.95 ^b	3.64 ± 1.39 ^c	2.52 ± 0.63 ^c	3.42 ± 2.13 ^c	<.001
IgG (mg/mL)	0.94 ± 0.10	1.02 ± 0.13	1.11 ± 0.10	1.08 ± 0.06	0.97 ± 0.10	0.99 ± 0.11	.114
IgA (mg/mL)	346 ± 19.2 ^a	274 ± 43.3 ^{ab}	230 ± 105 ^b	127 ± 24.2 ^c	84.4 ± 25.6 ^d	65.9 ± 43.1 ^d	<.001
42 days							
IgM (mg/mL)	5.82 ± 2.21	4.64 ± 0.97	5.67 ± 2.81	3.66 ± 1.65	4.69 ± 2.26	3.93 ± 0.80	.369
IgG (mg/mL)	1.17 ± 0.02 ^a	1.16 ± 0.05 ^a	1.05 ± 0.05 ^b	1.13 ± 0.04 ^a	1.11 ± 0.08 ^{ab}	0.95 ± 0.05 ^b	<.001
IgA (mg/mL)	179 ± 31.5 ^a	182 ± 35.7 ^a	178 ± 46.3 ^a	140 ± 53.1 ^{ab}	162 ± 75.8 ^a	90.9 ± 22.7 ^b	.049

Note: Means within the same row with different superscript differ significantly ($P < .05$).

chickens. The enzymolysis of gluten improves its water solubility, lowers the viscoelasticity, and increases the functional properties of the proteins (Hardt et al., 2015; Zheng et al., 2015). Thus, the bioavailability and digestibility of crude protein in broiler diets could be improved as in aquaculture species. In contrast, when 2% wheat gluten was used, the growth performance decreased in comparison to the control treatment (Table 3). However, it is clear that further experiments are needed to justify this hypothesis in birds. The findings of Kopec et al. (2013) indicated that the inclusion of 2.9% wheat gluten did not decrease the BW with respect to control treatment.

Also, a high concentration of proteins (70–85%) (Day, Augustin, Batey, & Wrigley, 2006) in the gluten are glutenins and gliadins, consisting mainly of isoleucine, phenylalanine, glutamic acid + glutamine, cysteine, and proline. In this context, isoleucine is used for incorporation into body proteins which may help in maintaining the BW and health status (Ospina-Rojas et al., 2014). Phenylalanine is also a precursor for the synthesis of tyrosine and acetoacetyl-CoA, which are important for increasing protein synthesis and promoting lipid digestion through stimulating cholecystokinin (CCK) secretion (Cowieson, Lu, Ajuwon, Knap, & Adeola, 2017). On the other hand, the increase in the emulsifying power of gluten hydrolysates can assist the lipid metabolism and thus the growth performance, which is important for the early life of the broiler (Zampiga, Meluzzi, & Sirri, 2016).

At 21 and 42 days, the immune organs' relative weight remained unchanged. An increase in the relative weights of the thymus and bursa Fabricius have been associated with a more active immune system in birds (Ifrah, Perelman, Finger, & Uni, 2017). However, as in this study, other authors have failed to identify a relationship between the lymphoid organs' relative weight and immune response (Kubena, Byrd, Young, & Corrier, 2001). The enzymolysis also had no effect on the relative weight of the spleen. This may be due to the fact that major immunological activities are concentrated in the organs that produce the most antibodies (B and T cells) (Mehaisen et al., 2017). In addition, the relative weights of the pancreas and liver did not show changes due to the experimental diets (Table 3). An excessive increase in the activity of these viscera could depress the growth performance by decreasing the organic function and enzymatics of the birds. On the other hand, protein-rich feeds such as canola meal increase the relative weight of the liver in broiler by the high concentration of glucosinolate (Tossou et al., 2016; Woyengo, Kiarie, & Nyachoti, 2011). It is worth noting that the biological responses depend on dietary components.

In the poultry industry, birds are often exposed to different stressful conditions, which increase the production of free radicals and limit the growth performance (Bai et al., 2017; Liu, Qin, et al., 2015; Toro, Aguilar, Bertot, Hurtado, & Nava, 2015). The antioxidant system includes natural or synthetic antioxidants enzymes (Hu et al., 2016), with T-SOD and GSH-Px being the most important of those antioxidant enzymes. The MDA is also related to lipid peroxidation and avian stress (Delles, Xiong, True, Ao, & Dawson, 2014). At 21 days, a high dietary inclusion of enzymolysis products of wheat gluten decreased antioxidant activity by lowering the values of T-AOC and GSH-Px. T3 was the best treatment for both cases; this feed has a high concentration of glutamic acid, but did not elicit a greater antioxidant response. Wang, Zhao, Yang, and Jiang (2006) demonstrated that this amino acid may help improve the antioxidant status as it increased the glutathione synthesis and. However, at 42 days, T3 provoked a high concentration of T-SOD; this enzyme decreases the massive oxidative stress, which benefits the animal response. A greater concentration of essential and non-essential amino acids and a more soluble and bioavailable protein may help depress the antioxidant activity. Several authors have proposed bird diet formulations based on the ideal protein in order to take advantage of amino acids and reduce losses caused by protein nitrogen (Corzo, Moran, & Hoehler, 2003).

In contrast to serum antioxidant indices, the MDA concentration in the intestine was altered with T3 after 21 and 42 days. Intestinal MDA may increase as a result of the metabolic activity, but it did not affect anti-oxidative action because at 21 days, the dietary inclusion of this new feed increased the GSH-Px and T-SOD concentration in the broilers. Moreover, this enzymolysis product is an important source of cysteine, which plays an essential role in the homeostasis of glutathione synthesis and redox pathways (Liu, Chen, Zhong, Teng, & Yin, 2017; Liu, Yu, et al., 2017). However, the relationship between MDA and antioxidant enzymes in the intestines remains poorly understood.

One relevant observation to be made from this study is that the T4 and T5 treatments increased the serum total protein in 21-day-old broiler chickens. This may be due to an improvement in the functional properties of the native protein, increase in the serum circulation of this bio-molecule, and a high dietary inclusion of this product. The biosynthesis of tissue was increased and the growth performance promoted by the increase in amino acids entering the portal vein (Wu et al., 2014). Dietary inclusion of T3 and T5 also decreased ($P < .05$) the serum concentration of uric acid, although only when measured 21 days after the initiation of treatment. Uric acid is an organic compound of carbon, nitrogen, oxygen, and hydrogen. Expelled with feces, it is the bird's main waste product and is also closely related to BUN (Mowrer, Sedlacek, Kim, Ritz, & Kim, 2016). Studies have shown that excess protein feeding during the late growing/prebreeder stage can increase the level of plasma uric acid (Liu, Niu, et al., 2015). Thus, a decrease in uric acid in poultry could indicate a higher utilization of the true protein and/or a higher expulsion of the product of excretion.

At 42 days, the T1 treatment decreased the BUN concentration. The lower concentration of this serum biochemical indicator could elevate the dietary protein efficiency through the decrease of urea synthesis and higher hydration in the liver (Wu et al., 2014). Apparently, a high bioavailability of protein in treatments with 3%, 4%, and 5% at this stage (growing) provoked a higher concentration of this indicator. According to Yang et al., birds fed on the protein-reduced diets have lower BUN concentration (Li

et al., 2017; Ma, Xu, Wang, Kuang, & Xu, 2016; Yang et al., 2009). On the other hand, not change ($P > .05$) was shown in the ALK enzyme at either 21 or 42 days. At earlier ages, the high concentration is due to a higher bone development when compared to older ages (Silva, Freitas Neto, Laurentiz, Junqueira, & Fagliari, 2007), as was observed in this experiment (Table 7).

The level of serum antibodies is currently an important indicator. An increased immunoglobulin concentration has been associated with a benefit in the immune status, because IgM, IgG, and IgA are the main immunoglobulins protecting against pathogenic microorganisms (Herich, 2017; Molinari et al., 2009; Ren et al., 2016). Interestingly, at 21 and 42 days, our results showed a decrease and variations in serum immunoglobulins concentration with a higher inclusion of enzymolysis products of wheat gluten, although this did not provoke a decrease of growth performance in birds. No relationship has been found between the lymphoid organs relative weight and the immunoglobulins (Table 8). Bartell and Batal (2007) and Chen et al. (2017) identified a correlation between the functionality of the thymus and spleen in terms of IgA and IgG production. This result may have been influenced by a decrease of stress in broiler fed with enzymolysis products of wheat gluten rich in functional amino acids. Inflammatory response is the first line of defense against novel pathogens and its concentration has also been associated with both the stress and pathology of many poultry diseases (Jacques-Hamilton et al., 2017). However, further investigations are required: for its multifactoriality, studies of molecular biology and proteomic technologies are recommended to better understand the mechanisms of immunological regulation (Wang et al., 2006).

These results demonstrated the beneficial effects of dietary inclusion of enzymolysis products of wheat gluten at 2% on growth performance and intestinal antioxidant activity of broiler chickens.

Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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