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

Jun Fang\textsuperscript{a,b}\textsuperscript{*}, Yordan Martínez\textsuperscript{c}\textsuperscript{*}, Changjian Deng\textsuperscript{a,b}, Dan Zhu\textsuperscript{a,b}, Hanhui Peng\textsuperscript{a,b}, Hongmei Jiang\textsuperscript{a,b} and Aike Li\textsuperscript{d}

\textsuperscript{a}College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, People’s Republic of China; \textsuperscript{b}College of Animal Science and Technology, Hunan Agricultural University, Changsha, People’s Republic of China; \textsuperscript{c}Study Center of Animal Production, Faculty of Veterinary Medicine, University of Granma, Bayamo, Granma, Cuba; \textsuperscript{d}Cereals & Oils Nutrition Research Group, Academy of Science & Technology of State Administration of Grain, Beijing, People’s Republic of China

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

ARTICLE HISTORY

Received 9 April 2017
Accepted 15 May 2017

KEYWORDS

Wheat gluten; enzymolysis; growth performance; immunity; broiler

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
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 Biotechnology 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 | 0.18           |
| 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           |

**Calculated nutritional composition**

| 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 |
| Methionine (%) | 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 |
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 ± 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 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 |

**Calculated nutritional composition**

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

| Items         | Control | Wheat gluten 2% | 1%  | 2%  | 3%  | 4%  | P-value |
|---------------|---------|-----------------|-----|-----|-----|-----|---------|
| 1–21 days     |         |                 |     |     |     |     |         |
| BW (g)        | 684 ± 43ab | 638 ± 31b      | 718 ± 27a | 711 ± 36a | 732 ± 37a | 732 ± 61a | .003    |
| ADG (g)       | 30.5 ± 2.05ab | 28.4 ± 1.50b  | 32.2 ± 1.32a | 31.8 ± 1.73a | 32.8 ± 1.80a | 32.8 ± 2.94a | .003    |
| ADFI (g)      | 44.2 ± 2.74abc | 41.4 ± 1.67a | 45.4 ± 1.89ab | 45.3 ± 1.49ab | 45.3 ± 3.32ab | 47.3 ± 2.22a | .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 ± 0.06b | 1.90 ± 0.17c  | 2.37 ± 0.09a | 2.35 ± 0.06a | 2.41 ± 0.15a | 2.28 ± 0.22a | <.001   |
| ADG (g)       | 66.3 ± 3.51b | 61.0 ± 6.88b  | 79.1 ± 4.62a | 78.1 ± 2.28b | 81.5 ± 6.27a | 74.2 ± 7.56b | <.001   |
| ADFI (g)      | 141 ± 9.76b | 131 ± 6.50a   | 151 ± 6.01a | 150 ± 2.06a | 154 ± 6.11a | 152 ± 6.42a | <.001   |
| F/G           | 2.13 ± 0.11ab | 2.17 ± 0.21a  | 1.92 ± 0.07b | 1.93 ± 0.04b | 1.90 ± 0.10b | 2.07 ± 0.20ab | .11     |

1–42 days:

| ADG (g)       | 48.9 ± 1.88b | 44.8 ± 3.92b  | 55.6 ± 2.31a | 55.0 ± 1.64a | 56.8 ± 3.37a | 53.7 ± 5.18b | <.001   |
| F/G           | 1.89 ± 0.07ab | 1.94 ± 0.14a  | 1.77 ± 0.06bc | 1.78 ± 0.03bc | 1.75 ± 0.08bc | 1.87 ± 0.15abc | .024    |

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

### 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% | 1%  | 2%  | 3%  | 4%  | P-value |
|----------------------|---------|-----------------|-----|-----|-----|-----|---------|
| 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 | .622    |
| 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 | .495    |
| 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 | .871    |
| 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 | .939    |
| 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 | .974    |
| 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 | .945    |
| 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 | .871    |
| 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 | .949    |
| 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 |
|------------------|---------|--------------|-------------------------------------|
|                  | 2%      | 1%           | 2%                                  | 3%                                  | 4%                                  | P-value |
|                  |         |              |                                     |                                     |                                     | .853    |
| 21 days          |         |              |                                     |                                     |                                     | .024    |
| 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 ± 1.11ab | 6.94 ± 0.66a | 5.45 ± 0.72abc | 4.34 ± 0.69c | 6.47 ± 2.29ab | 5.09 ± 1.42bc | .024    |
| GSH-Px (μmol/L)   | 1509 ± 217a | 1379 ± 140a  | 1344 ± 141a  | 1368 ± 238a  | 1269 ± 68ab  | 1085 ± 185b  | .020    |
| T-SOD (U/mg prot) | 184 ± 16 | 159 ± 25     | 147 ± 30     | 193 ± 18     | 165 ± 35     | 190 ± 22     | .059    |
| 42 days          |         |              |                                     |                                     |                                     | .596    |
| 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 ± 25a | 181 ± 42ab   | 221 ± 33a    | 212 ± 24ab   | 212 ± 27ab   | 174 ± 26bc  | .009    |

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 chickens.

### 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    | $P$-value |
|------------------------------|--------------------------|--------------------------------------|-----------------------------------------|-----------|
|                              |                          |                                      | 1%                                      | 2%        | 3%           | 4%           |             |
| **21 days**                  |                          |                                      |                                         |           |              |              |             |
| 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$^{b}$ | 7557 ± 2160$^{cd}$ | 7557 ± 2160$^{cd}$ | <.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 ± 5.22$^{ab}$                     | 28.2 ± 8.40$^a$ | 17.8 ± 5.49$^{b}$ | 16.1 ± 2.34$^b$ | .005       |
| **42 days**                  |                          |                                      |                                         |           |              |              |             |
| 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$^{bc}$ | 7.35 ± 2.76$^{bc}$ | .005       |
| GSH-Px (μmol/L)              | 13,405 ± 6073$^{bc}$     | 10,485 ± 2424$^{ab}$                 | 17,701 ± 8236$^{a}$                    | 13,639 ± 1870$^{bc}$ | 9681 ± 1788$^{b}$ | 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 ($P < .05$).

### 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 ($P < .05$).
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.

**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 |
|----------------|---------------------|--------------|-------------------------------------|---------|
|                | 21 days             | 2%           | 1% 2% 3% 4%                         |         |
| IgM (mg/mL)    | 10.3 ± 2.51ab       | 8.02 ± 2.20ab| 7.62 ± 1.95bc 3.64 ± 1.39c 2.52 ± 0.63c 3.42 ± 2.13c | <.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 ± 0.31 | .114    |
| IgA (mg/mL)    | 346 ± 19.2a         | 274 ± 43.3ab | 230 ± 105bc 127 ± 24.2c 84.4 ± 25.6d 65.9 ± 43.1d | <.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 ± 0.02a        | 1.16 ± 0.05a | 1.05 ± 0.05bc 1.13 ± 0.04a 1.11 ± 0.08bc 0.95 ± 0.05bc | <.001   |
| IgA (mg/mL)    | 179 ± 31.5a         | 182 ± 35.7a  | 178 ± 46.3a 140 ± 53.1ab 162 ± 75.8b 90.9 ± 22.7b | .049    |

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

**Funding**

This work was supported by National Natural Science Foundation of China [grant number 31672457], Ministry of Agriculture of the People’s Republic of China [grant numbers 2015-Z64, 2016-X47], and Hunan Provincial Science and Technology Department [grant numbers 2016NK2101, 2016WK2008, 2016TP2005].

**Notes on contributors**

**Jun Fang** is a professor at Hunan Agricultural University in China, and mainly studies on the research of animal nutrition and microbiology.

**Yordan Martínez** is an assistant professor at University of Granma in Cuba, and mainly studies on the research of animal nutrition.

**Changjian Deng** is a master student at Hunan Agricultural University in China, and mainly studies on the research of animal nutrition.

**Dan Zhu** is a master student at Hunan Agricultural University in China, and mainly studies on the research of animal nutrition and microbiology.
**Hanhui Peng** is a PhD student at Hunan Agricultural University in China, and mainly studies on the research of animal nutrition.

**Hongmei Jiang** is an associate professor at Hunan Agricultural University in China, and mainly studies on the research of animal nutrition and microbiology.

**Aike Li** is a professor at Cereals & Oils Nutrition Research Group, Academy of Science & Technology of State Administration of Grain in China, and mainly studies on the research of processing of the grains.

**References**

Apper-Bossard, E., Feneuil, A., Wagner, A., & Respondek, F. (2013). Use of vital wheat gluten in aquaculture feeds. *Aquatic Biosystems*, 9(1), 21.

Bai, K. W., Huang, Q., Zhang, J. F., He, J. T., Zhang, L. L., & Wang, T. (2017). Supplemental effects of probiotic *Bacillus subtilis* fmbJ on growth performance, antioxidant capacity, and meat quality of broiler chickens. *Poultry Science*, 96(1), 74–82.

Bartell, S. M., & Batal, A. B. (2007). The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract, and humoral immune response of broilers. *Poultry Science*, 86(9), 1940–1947.

Chen, Y. P., Cheng, Y. F., Li, X. H., Yang, W. L., Wen, C., Zhuang, S., & Zhou, Y. M. (2017). Effects of threonine supplementation on the growth performance, immunity, oxidative status, intestinal integrity, and barrier function of broilers at the early age. *Poultry Science*, 96(2), 405–413.

Cian, R. E., Vioque, J., & Drago, S. R. (2015). Structure-mechanism relationship of antioxidant and ACE I inhibitory peptides from wheat gluten hydrolysate fractionated by pH. *Food Research International*, 69, 216–223.

Corzo, A., Moran, E., Jr, & Hoeher, D. (2003). Arginine need of heavy broiler males: Applying the ideal protein concept. *Poultry Science*, 82(12), 402–407.

Cowieson, A. J., Lu, H., Ajiuwon, K. M., Knapp, I., & Adeola, O. (2017). Interactive effects of dietary protein source and exogenous protease on growth performance, immune competence and jejunal health of broiler chickens. *Animal Production Science*, 57(2), 252–261.

Day, L., Augustin, M. A., Batey, I. L., & Wrigley, C. W. (2006). Wheat-gluten uses and industry needs. *Trends in Food Science & Technology*, 17(2), 82–90.

Delles, R. M., Xiong, Y. L. L., True, A. D., Ao, T. Y., & Dawson, K. A. (2014). Dietary antioxidant supplementation enhances lipid and protein oxidative stability of chicken broiler meat through promotion of antioxidant enzyme activity. *Poultry Science*, 93(6), 1561–1570.

Gorter, H. D., Drabik, D., Just, D. R., Kliauga, E. M., Swinnen, J., & Weersink, A. (2013). The impact of OECD biofuels policies on developing countries. *Agricultural Economics*, 44(4–5), 477–486.

Hardt, N. A., Boom, R. M., & van der Goot, A. J. (2015). Starch facilitates enzymatic wheat gluten hydrolysis. *LWT – Food Science and Technology*, 61(2), 557–563.

Herich, R. (2017). Is the role of IgA in local immunity completely known? *Food and Agricultural Immunology*, 28(2), 223–237.

Hu, Y. N., Wang, Y. W., Li, A. K., Wang, Z. S., Zhang, X. L., Yun, T. T., … Yin, Y. H. (2016). Effects of fermented rapeseed meal on antioxidant functions, serum biochemical parameters and intestinal morphology in broilers. *Food and Agricultural Immunology*, 27(2), 182–193.

Ifrah, M. E., Perelman, B., Finger, A., & Uni, Z. (2017). The role of the bursa of Fabricius in the immune response to vaccinal antigens and the development of immune tolerance in chicks (*Gallus domesticus*) vaccinated at a very young age. *Poultry Science*, 96(1), 51–57.

Jacques-Hamilton, R., Hall, M. L., Buttemer, W. A., Matson, K. D., da Silva, A. G., Mulder, R. A., & Peters, A. (2017). Personality and innate immune defenses in a wild bird: Evidence for the pace-of-life hypothesis. *Hormones and Behavior*, 88, 31–40.

Jansens, K. J. A., Lagrain, B., Brijs, K., Goderis, B., Smet, M., & Delcour, J. A. (2013). Impact of acid and alkaline pretreatments on the molecular network of wheat gluten and on the mechanical properties of compression-molded glassy wheat gluten bioplastics. *Journal of Agricultural and Food Chemistry*, 61(39), 9393–9400.
Joye, I. J., & McClements, D. J. (2014). Emulsifying and emulsion-stabilizing properties of gluten hydrolysates. *Journal of Agricultural and Food Chemistry, 62*(12), 2623–2630.

Kopec, W., Jamroz, D., Wiliczkiewicz, A., Biazik, E., Hikawczuk, T., Skiba, T., ... Orda, J. (2013). Antioxidation status and histidine dipeptides content in broiler blood and muscles depending on protein sources in feed. *Journal of Animal Physiology and Animal Nutrition, 97*(3), 586–598.

Kubena, L. F., Byrd, J. A., Young, C. R., & Corrier, D. E. (2001). Effects of tannic acid on cecal volatile fatty acids and susceptibility to salmonella typhimurium colonization in broiler chicks. *Poultry Science, 80*(9), 1293–1298.

Lemos, D., Lawrence, A. L., & Siccardi, A. J. (2009). Prediction of apparent protein digestibility of ingredients and diets by in vitro pH-stat degree of protein hydrolysis with species-specific enzymes for juvenile Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture, 295*(1–2), 89–98.

Liu, G., Chen, S., Zhong, J., Teng, K., & Yin, Y. (2017). Crosstalk between tryptophan metabolism and cardiovascular disease, mechanisms, and therapeutic implications. *Oxidative Medicine and Cellular Longevity, 2017*, 1–5. 1602074.

Liu, S. K., Niu, Z. Y., Min, Y. N., Wang, Z. P., Zhang, J., He, Z. F., ... Liu, F. Z. (2015). Effects of dietary crude protein on the growth performance, carcass characteristics and serum biochemical indexes of luang black-boned chickens from seven to twelve weeks of Age. *Brazilian Journal of Poultry Science, 17*(1), 103–108.

Liu, L., Qin, D. K., Wang, X. F., Feng, Y., Yang, X. J., & Yao., J. H. (2015). Effect of immune stress on growth performance and energy metabolism in broiler chickens. *Food and Agricultural Immunology, 26*(2), 194–203.

Liu, G., Yang, G., Guan, G., Zhang, Y., Ren, W., Yin, J., ... Yin, Y. (2015). Effect of dietary selenium yeast supplementation on porcine circovirus type 2 (PCV2) infections in mice. *PLoS One, 10*(2), e0115833.

Liu, G., Yu, L., Martinez, Y., Ren, W., Ni, H., Abdullah Al-Dhabi, N., ... Yin, Y. (2017). Dietary saccharomyces cerevisiae cell wall extract supplementation alleviates oxidative stress and modulates serum amino acids profiles in weaned piglets. *Oxidative Medicine and Cellular Longevity, 2017*, 1–7. 3967439.

Li, S., Xu, L., Sun, M., Wu, X., Liu, L., Kuang, H., & Xu, C. (2017). Hybrid nanoparticle pyramids for intracellular dual MicroRNAs biosensing and bioimaging. *Advanced Materials, 29*, 1606086.

Ma, W., Xu, L. G., Wang, L. B., Kuang, H., & Xu, C. L. (2016). Orientational nanoparticle assemblies and biosensors. *Biosensors & Bioelectronics, 79*, 220–236.

Mehaisen, G. M. K., Eshak, M. G., Elkaiaty, A. M., Atta, A. R. M. M., Mashaly, M. M., & Abass, A. O. (2017). Comprehensive growth performance, immune function, plasma biochemistry, gene expressions and cell death morphology responses to a daily corticosterone injection course in broiler chickens. *Plos One, 12*(2), e0172684.

Molinari, R., Manzi, L., Ricci, S., D’Aquino, M., Tomassi, G., Papeschi, C., & Merendino, N. (2009). Diets rich in whole wheat improve redox status and enhance immune responses in rats. *Food and Agricultural Immunology, 20*(2), 95–104.

Mowrer, J. E., Sedlacek, P., Kim, J., Ritz, C., & Kim, W. K. (2016). Supplementation of nitrocompounds in broiler diets: Effects on bird performance, ammonia volatilization and nitrogen retention in broiler manure. *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes, 51*(2), 126–131.

Olmo, C., Martinez, Y., León, E., & Leyva, L. (2012). Effect of mulberry foliage (*Morus alba*) meal on growth performance and edible portions in hybrid chickens. *International Journal of Animal & Veterinary Advances, 4*(4), 263–268.

Ospina-Rojas, I. C., Murakami, A. E., Duarte, C. R. A., Eyng, C., Oliveira, C. A. L., & Janeiro, V. (2014). Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases. *British Poultry Science, 55*(6), 766–773.

Ren, W., Yin, J., Chen, S., Duan, J., Liu, G., Li, T., ... Yin, Y. (2016). Proteome analysis for the global proteins in the jejunum tissues of enterotoxigenic *Escherichia coli* – infected piglets. *Scientific Reports, 6*, 465, 25640.
Roberts, T., Wilson, J., Guthrie, A., Cookson, K., Vancraeynest, D., Schaeffer, J., … Clark, S. (2015). New issues and science in broiler chicken intestinal health: Emerging technology and alternative interventions. *Journal of Applied Poultry Research, 24*(2), 257–266.

Schiermeier, Q. (2015). Quest for climate-proof farms. *Nature, 523*(7561), 396–397.

Silva, P. R. I., Freitas Neto, O. C., Laurentiz, A. C., Junqueira, O. M., & Fagliari, J. J. (2007). Blood serum components and serum protein test of hybro-PG broilers of different ages. *Revista Brasileira De Ciência Avícola, 9*(4), 229–232.

Taheripour, F., Hertel, T. W., & Tyner, W. E. (2011). Implications of biofuels mandates for the global livestock industry: A computable general equilibrium analysis. *Agricultural Economics, 42*(3), 325–342.

Toro, D. M., Aguilar, Y. M., Bertot, R. R., Hurtado, C. B., & Nava, O. R. (2015). Effect of dietary supplementation with *Morinda citrifolia* on productivity and egg quality of laying hens. *Ciencia Y Agricultura, 12*(2), 7.

Tossou, M. C., Liu, H., Bai, M., Chen, S., Cai, Y., Duraiapandiyam, V., … Yin, Y. (2016). Effect of high dietary tryptophan on intestinal morphology and tight junction protein of weaned Pig. *BioMed Research International, 2016*, 1–6, 2912418.

van der Zalm, E. E. J., van der Goot, A. J., & Boom, R. M. (2009). Influence of process conditions on the separation behaviour of starch-gluten systems. *Journal of Food Engineering, 95*(4), 572–578.

Wang, P., Chen, H. Y., Mohanad, B., Xu, L., Ning, Y. W., Xu, J., … Xu, X. M. (2014). Effect of frozen storage on physico-chemistry of wheat gluten proteins: Studies on gluten-, glutenin- and gliadin-rich fractions. *Food Hydrocolloids, 39*, 187–194.

Wang, K. Q., Luo, S. Z., Cai, J., Sun, Q. Q., Zhao, Y. Y., Zhong, X. Y., … Zheng, Z. (2016). Effects of partial hydrolysis and subsequent cross-linking on wheat gluten physicochemical properties and structure. *Food Chemistry, 197*, 168–174.

Wan, Y., Gritsch, C. S., Hawkesford, M. J., & Shewry, P. R. (2014). Effects of nitrogen nutrition on the synthesis and deposition of the ω-gliadins of wheat. *Annals of Botany, 113*(4), 607–615.

Wang, J. S., Zhao, M. M., Yang, X. Q., & Jiang, Y. M. (2006). Improvement on functional properties of wheat gluten by enzymatic hydrolysis and ultrafiltration. *Journal of Cereal Science, 44*(1), 93–100.

Woyengo, T. A., Kiarie, E., & Nyachoti, C. M. (2011). Growth performance, organ weights, and blood parameters of broilers fed diets containing expeller-extracted canola meal. *Poultry Science, 90*(11), 2520–2527.

Wu, G. Y., Bazer, F. W., Dai, Z. L., Li, D. F., Wang, J. J., & Wu, Z. L. (2014). Amino acid nutrition in animals: Protein synthesis and beyond. *Annual Review of Animal Biosciences, 2*(2), 387–417.

Yang, Y. X., Guo, J., Yoon, S. Y., Jin, Z., Choi, J. Y., Piao, X. S., … Chae, B. J. (2009). Early energy and protein reduction: Effects on growth, blood profiles and expression of genes related to protein and fat metabolism in broilers. *British Poultry Science, 50*(2), 218–227.

Zampiga, M., Meluzzi, A., & Sirri, F. (2016). Effect of dietary supplementation of lysophospholipids on productive performance, nutrient digestibility and carcass quality traits of broiler chickens. *Italian Journal of Animal Science, 15*(3), 521–528.

Zheng, X. Q., Wang, J. T., Liu, X. L., Sun, Y., Zheng, Y. J., Wang, X. J., & Liu, Y. (2015). Effect of hydrolysis time on the physicochemical and functional properties of corn glutelin by Protamex hydrolysis. *Food Chemistry, 172*, 407–415.