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Hydrolyzed wheat gluten for piglets

Effects of enzymatically hydrolyzed wheat gluten on growth performance, antioxidant status and immune function in weaned pigs¹

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ABSTRACT: The aim of this study was to determine the effects of enzymatically hydrolyzed wheat gluten (HWG) on growth performance, serum antioxidant indices, immune functions and small intestinal mucosal morphology in weaned pigs. A total of 96 weaned piglets were randomly allocated to 3 diets with 4 pens/diet, and 8 piglets/pen (4 barrows and 4 gilts). The diets were: basal diet (CON), basal diet supplemented with 2% wheat gluten (WG), and basal diet supplemented with 2% HWG. The experimental period was 28 days. Pigs fed HWG diet had greater ($P < 0.05$) average daily gain, average daily feed intake and feed efficiency, and lower ($P < 0.05$) diarrhea rates than those fed CON diet or WG diet. On d 28 after weaning, serum total antioxidative capacity, glutathione peroxidase and superoxide dismutase activities of pigs fed the HWG diet were greater ($P < 0.05$) than those fed CON diet or 2% WG diet. Pigs fed HWG diet had larger ($P < 0.05$) spleen and abdominal lymph nodes than pigs fed CON diet or WG diet. In conclusion, dietary WG enhanced growth performance and health of weaned pigs when it was enzymatically hydrolyzed, implying peptides form of glutamine is more beneficial to the nutrient absorption.

Key words: enzymatically hydrolyzed wheat gluten, growth performance, immune organ index, small intestine morphology, weaned pigs

INTRODUCTION

Wheat gluten, also called gluten meal, or simply gluten, is an important byproduct of wheat processing with a high crude protein level. In recent years, it has attracted worldwide attention for its film-forming, viscoelastic and extension properties, as well as its renewability and biodegradability (Matsudomi et al. 1995). More interestingly, gluten is a rich natural source of glutamine (Gln), which is the most abundant free amino acid in the plasma of mammals and in the milk of sows (Wu and Knabe 1994). After weaning, the endogenous Gln synthesis becomes inadequate for piglets as their diets change from mother's milk to solid feed. Results from previous studies showed that adding appropriate amounts of exogenous Gln in diets for weaned pigs can improve the structure and function of small intestine, reduce diarrhea rate, and improve growth performance of piglets (Wu et al. 1996; Liu et al. 2002; Kitt et al. 2002). Moreover, Gln is conditionally essential amino acid in early weaned piglets (Wu et al. 1996; Liu et al. 2002; Kitt et al. 2002). Therefore, it is critical to supplement diets for weaned pigs with Gln to ensure adequate dietary supply of Gln (Wu et al. 2011).

Glutamine dipeptides, such as alanyl-glutamine (Ala-Gln) and glycyl-glutamine (Gly-Gln), which are not completely hydrolyzed in the jejunum of weaned piglets can be absorbed by intestinal epithelial cell. The absorption rate of Gln in peptide-bound form is faster than in free form (Wang 2010). Both Gln and Ala-Gln can prevent oxidant- and endotoxin-induced deaths of neonatal enterocytes, and prevent possible intestinal oxidative injury and inflammatory disease in neonates (Haynes et al. 2009).

The protein of gluten can be hydrolyzed to produce short glutamine peptides, which are more easily absorbed through the mucous membrane of small intestine. Thus, it is possible that peptide-bound glutamine from enzymatically hydrolyzed wheat gluten (HWG) can be rapidly used by enterocytes of weaned piglets.

Therefore, the aim of this study was to investigate the effects of HWG on growth performance, antioxidant activities and immune function in weaned piglets.

MATERIALS AND METHODS

This study was approved by the China State Administration of Grain Animal Care and Use Committee.

Preparation of HWG

The materials and all reagents used in this study were food-grade or reagent-grade. Wheat gluten was supplied by Kangdi Flour Industry Co., Ltd (Henan, China). Wheat gluten was dissolved in tap water at 10% (w/v) solution, and the enzymatical hydrolysis was performed at temperature of $55 \pm 1^\circ\text{C}$ and pH of $8.0 \pm 1^\circ\text{C}$ using Alcalase 2.4 L (EC 3.4.21.62, from *Bacillus licheniformis*, 2.4 AU/g; Novozymes, Denmark) for 120 min in a fermentor that was stirred continuously (250 rpm). The final enzyme-to-substrate ratio for this reaction was 1:200 (w/w). After the reaction period, the mixture was heated to 85°C for 10 min to inactivate the enzyme. Then the mixture was centrifuged and the supernatant was spray-dried and stored at 4°C .

Efficiency of Enzymatic Hydrolysis of WG (Wheat Gluten)

The efficiency of short peptides production from WG enzymolysis was evaluated by trichloroaceticacid-nitrogen soluble index (TCA-NSI) as previously reported (Jang

and Lee, 2005) with slight modification. Briefly, 3 g of HWG sample ($n = 5$) were mixed with 15 mL of 20% TCA and then centrifuged at $7,000 \times g$ for 10 min at 4 °C. Nitrogen content in the supernatant was measured using Automatic Kieldahl Apparatus (Kjeltec 8400, Foss, Denmark). TCA-NSI was calculated as:

$$\text{TCA-NSI} = N_1/N_0 \times 100\%$$

Where N_1 is the nitrogen content of hydrolyzed proteins solubilized in TCA, and N_0 is the total nitrogen content in HWG samples.

Determination of glutamine in small peptides

Peptide-bound glutamine concentration was determined as described previously (Kuhn et al. 1996) with some modifications. Briefly, HWG samples ($n = 5$) were diluted to a final concentration of 2.5 g/L, and an aliquot of the diluted HWG was mixed with an equal amount of a freshly prepared solution of bis-1,1-(trifluoroacetoxy)-iodobenzene (BTI, Sigma, Germany) in acetonitrile (10 mg/mL) and a half amount of pyridine solution (50 mmol/L). After 4 h of reaction (50 °C), the sample, as BTI-treated specimen (BS), was then evaporated and hydrolyzed by 6 mol/L of HCl for 24 h (110 °C). Another one aliquot of HWG protein solution sample, as control specimen (CS), was only evaporated and hydrolyzed by 6 mol/L of HCl for 24 h (110 °C). Both BS and CS were washed three times by water and evaporated. Then the sample residues were dissolved in sodium citrate buffer solution (0.1 mol/L), filtrated, and concentration of glutamate in them was determined by ion-exchange chromatography using an automatic amino acid analyzer (L-8900 Hitachi automatic amino acid analyzer, Tokyo, Japan) as previously

described (Wang et al. 2010).

Animals, Management and Study Design

The experiment was conducted at the Resource Pig Breeding Farm (Beijing, China). A total 96 crossbred (Duroc × Landrace × Yorkshire) pigs (48 barrows and 48 gilts) with initial body weight of 8.29 ± 0.63 kg and weaned at 28 d of age were used in the study. The pigs were housed in 12 pens in a mechanically ventilated room with a 12 h:12 h light:dark cycle. The room temperature was initially set at 30 °C for one week and lowered by 1.5 °C/week until it reached 26 °C. The pens (2.5 × 2.5 m) with plastic-coated, expanded metal floors are equipped with a feeder and a nipple waterer. The pigs were randomly allocated to 3 diets with 4 pens/diet, and 8 piglets/pen (4 barrows and 4 gilts). The diets were: basal diet (CON), basal diet supplemented with 2% WG (WG was purchased from Kangdi Flour Industry Co., Ltd, Henan, China), and basal diet supplemented with 2% HWG. All pigs had unlimited access to feed and water during the 28-d experimental period.

The diets were formulated to meet or exceed the National Research Council requirements for weaned pigs (NRC 2012). The ingredient and nutrient composition of the experimental diets are presented in Table 1.

Data Collection

On d 0 and 28, individual body weight (BW) and feed intake were recorded to calculate average daily gain (ADG), average daily feed intake (ADFI) and the feed conversion ratio ($FCR = ADFI/ADG$). Diarrhea rate of piglets was calculated as follows:

The diarrhea rate (%) = total number of diarrhea pigs / (number of experimental pigs × number of experimental days) × 100%.

Blood Sampling and Analysis

On d 28, blood samples were taken from one barrow selected from each group by venipuncture of anterior vena cava after food deprivation for 8 h. Blood samples were collected in anticoagulant vacutainers (Greiner in Bio-One GmbH, Austria). The blood samples were centrifuged at $2,000 \times g$ for 10 min to obtain serum, and serum was stored at -20°C for analysis of serum superoxide dismutase (SOD) level, total antioxidant capacity (T-AOC) and glutathione peroxidase (GSH-Px). Serum SOD, T-AOC and GSH-Px activities levels were analyzed by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively.

Relative Organ and Tissue Weights

After collecting blood samples, one barrow from each pen were slaughtered by a lethal injection of sodium pentobarbital. The spleen, kidney and abdominal lymph node were removed and weighed to calculate immune organ index [organ weight (g) / BW (kg)].

Histological Analysis of Small Intestine

The duodenum (10 cm away from the pyloric junction), jejunum (55 cm distal to the pyloric junction) and ileum (15 cm proximal to the ileocaecal junction) were quickly removed and flushed with ice-cold saline, fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned into 4 μm -thick sections and stained with hematoxylin-eosin for histomorphological examination. The villus height and crypt

depth were determined using a light microscope (Olympus, Southend-on-Sea, UK) fitted with a Canon EOS60D camera (Canon Inc., Tokyo, Japan) using NIS-Elements BR software (Nikon, Tokyo, Japan).

Statistical Analysis

All data were analyzed by one-way ANOVA using the General Linear Model procedure of SPSS Statistics 17.0 software. Each pen was considered as the experimental unit for all the parameters measured. Results were expressed as means $\pm$ SD. Duncan's multiple comparison method was performed to identify differences among groups. $P \leq 0.05$ were considered significant.

RESULTS

The efficiency of short peptides production from WG enzymolysis was $81.06 \pm 0.21\%$. Glutamine concentration in small peptides of HWG was $17.47 \pm 0.58\%$.

Pigs fed the HWG diet had greater ($P < 0.05$) BW, ADG and ADFI, and better ($P < 0.05$) feed to gain ratio than pigs fed CON diet or WG diet on d 28 (Table 2). The diarrhea rate of pigs fed the HWG diet was lower ($P < 0.05$) than that of pigs fed CON diet or WG diet during the first week after weaning and the whole trial period (Table 3).

Dietary treatment had no significant effect on serum T-AOC and SOD activity at 7 d after weaning. However, GSH-Px activity was greater ($P < 0.05$) in pigs fed HWG diet than in pigs fed CON diet or WG diet. The serum T-AOC, SOD and GSH-Px activities of pigs fed the HWG diet were greater ($P < 0.05$) than those of pigs fed CON diet or WG diet at 28 d after weaning (Table 4).

The spleen index and abdominal lymph node index of pigs fed the HWG diet were greater ($P < 0.05$) than those of pigs fed the other diets (Table 5). But dietary treatment had no effect on kidney index.

The villus height and villus height/Crypt depth ratio of duodenum and ileum were greater ($P < 0.05$) in pigs fed HWG diet than those fed CON diet or WG diet (Table 6).

DISCUSSION

Glutamine is a conditionally essential amino acid for pigs and humans (Wu et al. 2010). It serves as a major fuel for rapidly differentiated cells, such as intestinal epithelial cells and activated lymphocytes (Wu et al. 1996). Wheat gluten is a rich natural source of Gln (Wu and Knabe 1994). But the solubility of WG in water is very poor (Day et al. 2006), so it is inappropriate to add glutamine in intact protein form. Besides, free Gln is less soluble, and can be converted to toxic pyroglutamic acid and ammonia under heat, pressure, sterilization and some storage conditions (Acree and Lee 1975; Airaud et al. 1987; Schneider et al. 2003). However, the negative effects of Gln can be avoided if short Gln peptides and non-free Gln are fed (Fürst et al. 1997). Thus, our study focused on the Gln content in the small peptide, which can be called “effective glutamine”. In addition, trichloroacetic acid (TCA) can only dissolve small peptides rather than proteins and long-chain peptides (Chavan et al. 2001). Thus we used TCA-NSI to determine the efficiency of enzymatic hydrolysis of WG, i.e. the small peptide production in HWG (Zhang et al. 2012).

After weaning, pigs suffer from multiple stresses, including nutritional,

psychological and environmental stresses. Besides, both their digestive and immune functions are not well developed, resulting in growth inhibition and poor feed efficiency (Dong and Pluske 2007; Moeser et al. 2007). However, the Gln peptide may effectively alleviate piglet early weaning stress syndrome and promote the growth. Xi et al. (2005) reported that dietary supplementation of 0.125–0.5% Gln dipeptide improved feed conversion ratio and increased serum growth hormone concentrations in weaned piglets. In addition, long-term consumption of diet supplemented with Gly-Gln dipeptide can enhance growth performance and decrease the weight loss in stressed piglets (Jiang et al. 2009). Similarly, the current results showed that dietary supplementation with 2% HWG and not 2% WG increased ADG and ADFI, and decreased feed to gain ratio, indicating that supplemental HWG reduced the swine industry losses that are caused by weaning stress. However, the optimal HWG supplementation amount should be determined in future studies.

Under normal circumstances, the production and elimination of free radicals in body are maintained at a balanced state. Excessive production of free radicals in body can lead to cell membrane damage, disrupt the body's normal metabolism, and result in disease (Valko et al. 2007). In vivo, free radicals can be neutralized by either enzyme systems or non-enzyme systems. Among the antioxidant enzyme systems, SOD and GSH-Px are the most important. Serum SOD can convert free radicals to hydrogen peroxide, and GSH-Px can continue to decompose hydrogen peroxide into water (Lobo et al. 2010). Piglet weaning stress can disrupt oxidative balance and cause oxidative injury. Oxidative injury is accompanied with increased

malondialdehyde, suppressed SOD and GSH-Px activities (Yin et al. 2014). Mechanisms by which weaning process increases diarrhea occurrence in piglets are unclear; however, stress theory has been widely suggested. Stress can destabilized the redox homeostasis, resulting in disruption of the mucosal structure and function, which in turn can lead to pathogen invasion and diarrhea (Spirt 2004).

In the present study, HWG increased serum GSH-Px activity of piglets at 7 d post-weaning, but did not increase serum SOD activity. Besides, HWG increased serum GSH-Px and SOD activities in piglets at 28 d post-weaning. These results were consistent with those of a previous study in which HWG increased the serum GSH-Px, SOD, IgG, IL-2 and IGF-1 levels in mice (Fang and Wang 2009). In addition, wheat peptides may increase SOD and GSH-Px activities in rats (Yin et al. 2015).

The spleen and lymph nodes are peripheral immune organs where T cells and B cells are harbored prior to immune responses (Witmer and Steinman 1984). The relative weight of immune organs can reflect the overall immune function. Weaning stress results in early inflammatory response and reduced immune organ weights (Mejía-Medina et al. 2012). In the current study, the indices of spleen and abdominal lymph nodes of pigs fed HWG diet were greater than those of pigs fed CON diet or WG diet, which was consistent with results from previous studies, implying that gluten hydrolysates increases the weight of immune organs (Fang and Wang 2009). Feed supplemented with HWG can promote the growth of immune organs in weaned piglets, and the reason may be attributed to that Gln is a major energy supplier for intestinal epithelial cells and activated lymphocytes (Wu et al. 1996), besides, HWG

can enhance intestinal digestive enzyme activity and increase nutrient digestibility (Houdijk et al. 1994; Fang and Wang 2009).

Weaning stress in piglets increases their sensitivity to potential pathogenic microorganisms, often causing post-weaning diarrhea. According to the report of Pluske et al. (1996), weaning stress can cause serious harm to the function of intestinal tract of piglet. However, dietary Gln supplementation can alleviate the intestinal dysfunction and atrophy caused by weaning stress (Wu et al. 1994). Wu et al. (1996) reported that 1.0% of glutamine supplementation can prevent jejunal atrophy. The current results showed that diarrhea rate of piglets fed 2% HWG was significantly lower than that piglets fed the other two diets on post-weaning, which may be attributed to the fact that high content of glutamine in the diet can increase villus height and V/C ratio in the duodenum and ileum, and decreased crypt depth in the duodenum of weaned pigs (Wang et al. 2010). It was suggested that dietary Gln could stimulate intestinal mucosal protein synthesis and prevent apoptosis and necrosis of villus cells (Labow and Souba 2000). The microarray analysis revealed that early weaning could up-regulate the gene expression related to oxidative stress and immune responses (Wang et al. 2008). However, dietary supplementation of Gln can up-regulate the gene expression related to nutrient absorption, cell growth and anti-oxidative stress (Wang et al. 2008). Thus, our results showed that dietary supplementation of WHG could strengthen the function and structural integrity of intestinal of weaned piglets. Wheat gluten supplementation did not reduce the diarrhea rate of weaned piglets, probably because intestinal epithelial cell prefers to

absorb Gln in a peptide form. Therefore, dietary supplementation of WG had no significant effect on diarrhea rate of piglets.

In conclusion, dietary supplementation with 2% HWG could significantly improve the growth performance of weaned piglets, reduce their diarrhea rate and feed conversion ratio. In addition, HWG could significantly increase GSH-Px, SOD, and T-AOC activities of serum, and the relative weight of immune organs in weaned piglets. Supplementation of HWG in diets had more positive effects on growth performance and intestinal function of weaning piglets than WG, which indicated that peptides form of glutamine is more beneficial to the nutrient absorption.

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Table 1 Diets composition and nutrient levels

Item	Diets		
	composition (g		
	kg ⁻¹) ^a		
	CON	WG	HWG
Ingredient			
Wheat protein hydrolysis	0	0	20
Gluten	0	20	0
Corn	556	574	570
Soybean meal	100	70	70
Extruded soybean	140	130	130
Fish meal	40	40	40
Whey powder	40	40	40
Sucrose	10	10	12
Spray-dried porcine plasma	40	40	40
Soybean oil	30	32	34
Dicalcium phosphate	12	12	12
Limestone	5	5	5
Salt	2.5	2.5	2.5
L-Lysine-HCl	5	5	5
DL-Methionine	1.5	1.5	1.5
L-Threonine	1.5	1.5	1.5

L-Tryptophan	1	1	1
Zinc oxide	3	3	3
Vitamin and mineral premix ^b	10	10	10
Antibiotic ^c	2.5	2.5	2.5
Total	1000	1000	1000
Analyzed composition ^d (g kg ⁻¹)			
Digestible energy ^e (MJ kg ⁻¹)	14.90	14.95	15.00
Crude protein	206	207	208
Calcium	8.6	8.5	8.7
Phosphorus	7.9	8.0	8.0
Lysine	15.8	15.6	15.7
Methionine	4.6	4.7	4.8
Threonine	9.1	8.9	9.0
Tryptophan	3.2	3.1	3.2

^aCON: the control group; WG: wheat gluten; HWG: enzymatically hydrolyzed wheat gluten.

^bVitamin and mineral premix provided the following per kg of feed: vitamin A, 9,000 IU; vitamin D₃, 2,400 IU; vitamin E, 20 IU; vitamin K₃, 3 mg; vitamin B₁, 1.4 mg; vitamin B₂, 4 mg; vitamin B₆, 3 mg; vitamin B₁₂, 12 µg; nicotinic acid, 30 mg; pantothenic acid, 14 mg; folic acid, 0.8 mg; biotin, 44 µg; choline chloride, 0.5 g; Fe, 76 mg; Cu, 240 mg; Zn, 76 mg; Mn, 20 mg; I, 0.48 mg; Se, 0.4 mg.

^cAntibiotic mixture contained: 15% chlortetracycline, 0.12 g kg⁻¹; 10% colistin

sulfate, 0.10 g kg⁻¹; 50% kitasamycin 0.03 g kg⁻¹.

^dAnalyzed value was as-fed basis.

^eDigestible energy was calculated data.

For Review Only

Table 2 The effects of wheat gluten and enzymatically hydrolyzed wheat gluten on the growth performance of weaned pigs^a

Item	Control	WG	HWG
BW ^b (kg/pig)			
Day			
0	7.92 ± 0.26	7.98 ± 0.28	8.02 ± 0.16
28	16.64 ± 0.26	16.89 ± 0.71	19.44 ± 0.73
ADG ^c (g)	311.4 ± 6.9 ^b	318.0 ± 34.3 ^b	407.9 ± 22.2 ^a
ADFI ^d (g)	572.9 ± 38.4 ^b	556.9 ± 19.2 ^b	633.5 ± 20.6 ^a
FCR ^e	1.84 ± 0.09 ^a	1.75 ± 0.04 ^a	1.55 ± 0.02 ^b

Note: Means within a row not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level.

^aValues are means ± SD; n = 4 for BW, ADG, ADFI and FCR. CON: the control group; WG: wheat gluten; HWG: enzymatically hydrolyzed wheat gluten.

^bBW= body weight.

^cADG= average daily gain.

^dADFI= average daily feed intake.

^eFCR= feed:gain ratio.

Table 3 The effects of wheat gluten and enzymatically hydrolyzed wheat gluten on the diarrhea rate in weaned pigs ^a

Item	CON	WG	HWG
Diarrhea rate (%)			
Day			
0–7	10.71 ± 0.86 <i>a</i>	9.82 ± 0.78 <i>a</i>	6.14 ± 1.03 <i>b</i>
7–28	1.94 ± 0.37	1.49 ± 0.91	0.90 ± 0.27
0–28	4.13 ± 0.97 <i>a</i>	3.57 ± 0.89 <i>a</i>	2.46 ± 0.53 <i>b</i>

Note: Means within a row not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level.

^aValues are means ± SD, n = 4. CON: the control group; WG: wheat gluten; HWG: enzymatically hydrolyzed wheat gluten.

Table 4 The effects of wheat gluten and enzymatically hydrolyzed wheat gluten on serum antioxidant activities in weaned piglets^a

Item	CON	WG	HWG
T-AOC ^b (U mL ⁻¹)			
Day			
7	8.20 ± 1.25	8.69 ± 2.38	8.32 ± 3.28
28	10.76 ± 0.76 <i>b</i>	9.93 ± 1.31 <i>b</i>	12.91 ± 0.50 <i>a</i>
SOD ^c (U mL ⁻¹)			
Day			
7	93.44 ± 7.54	90.71 ± 4.66	96.764 ± 5.65
28	100.53 ± 10.36 <i>b</i>	99.60 ± 8.07 <i>b</i>	114.38 ± 9.70 <i>a</i>
GSH-Px ^d (U mL ⁻¹)			
Day			
7	67.98 ± 1.45 <i>b</i>	62.92 ± 3.50 <i>b</i>	74.78 ± 0.99 <i>a</i>
28	83.13 ± 3.07 <i>b</i>	79.6 8 ± 6.64 <i>b</i>	124.90 ± 4.28 <i>a</i>

Note: Means within a row not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level.

^aValues are means ± SD, n = 4. CON: the control group; WG: wheat gluten; HWG: enzymatically hydrolyzed wheat gluten.

^bT-AOC = total antioxidative capacity.

^cSOD= superoxide dismutase.

^dGSH-Px= glutathione peroxidase.

Table 5 The effects of wheat gluten and enzymatically hydrolyzed wheat gluten on relative organ and tissue weights in weaned pigs ^a

Item	CON	WG	HWG
Relative organ weight (g kg ⁻¹)			
Organ			
Spleen	2.32 ± 0.18 _c	2.67 ± 0.19 _b	3.18 ± 0.13 _a
Kidney	5.85 ± 0.41	5.51 ± 0.19	5.92 ± 0.19
Lymph	0.28 ± 0.06 _a	0.25 ± 0.09 _a	0.32 ± 0.13 _b

Note: Means within a row not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level.

^aValues are means ± SD, n = 4. CON: the control group; WG: wheat gluten; HWG: enzymatically hydrolyzed wheat gluten.

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Table 6 The effects of wheat gluten and enzymatically hydrolyzed wheat gluten on the intestinal morphology of weaned piglets^a

Item	CON	WG	HWG
Duodenum			
Villus height (μm)	337.03 ± 21.50 _c	402.49 ± 17.12 _b	488.80 ± 13.64 _a
Crypt depth (μm)	312.29 ± 15.74 _a	165.38 ± 12.68 _c	249.61 ± 11.28 _b
V/C	1.11 ± 0.08 _c	2.58 ± 0.43 _a	1.91 ± 0.15 _b
Jejunum			
Villus height (μm)	419.10 ± 11.89 _a	337.44 ± 11.42 _b	371.48 ± 16.69 _b
Crypt depth (μm)	240.62 ± 6.98	215.58 ± 14.21	253.05 ± 14.40
V/C	1.72 ± 0.05 _a	1.49 ± 0.16 _b	1.54 ± 0.17 _b
Ileum			
Villus height (μm)	311.32 ± 12.47 _b	355.89 ± 16.73 _a	350.27 ± 7.65 _a
Crypt depth (μm)	186.40 ± 8.23	173.12 ± 8.14	185.28 ± 9.56
V/C	1.61 ± 0.07 _b	1.96 ± 0.16 _a	1.98 ± 0.21 _a

Note: Means within a row not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level.

^aValues are means ± SD, n = 4. CON: the control group; WG: wheat gluten; HWG: enzymatically hydrolyzed wheat gluten.