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Effects of Xylanase Supplementation and Citric Acid on Performance, Ileal Nutrients Digestibility, and Gene Expression of Intestinal Nutrient Transporters in Broilers Challenged with *Clostridium perfringens*

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This study was conducted to evaluate the effects of xylanase and citric acid (CA) on growth, digesta pH, ileal populations of *Clostridium perfringens* and lactic acid bacteria, ileal nutrient digestibility, digestive enzyme activity, and mRNA expression of intestinal nutrient transporters in starter broilers challenged with *C. perfringens*. The experiment was conducted in a 2×2 factorial arrangement with two levels of CA (0 and 30 g/kg) and 2 levels of xylanase (0 and 200 mg/kg). Each of the four dietary treatments was fed to six replicate pens (15 birds/pen) between 0 and 21 d of age. Dietary CA significantly increased ADFI and ADG; meanwhile, xylanase addition led to a substantial reduction in FCR ($P<0.05$). No differences in digesta pH, *C. perfringens* counts, or quantity of lactic acid bacteria were found between the treatments. Xylanase supplementation increased AME values ($P<0.01$) and ileal digestibility of CP ($P<0.05$) in challenged birds. The inclusion of CA also increased the AME ($P<0.01$), and tended to increased ileal CP digestibility ($P=0.085$). Xylanase supplementation increased α-amylase, trypsin, and sucrose activity in the jejunum ($P<0.01$). Dietary CA significantly increased ($P<0.01$) villi length as well as the villus length to crypt depth ratio in jejunum segments. The jejunal mRNA expression of sodium glucose co-transporter 1 (SGLT1) and H^+-dependent peptide transporter 1 (PepT1) were upregulated by xylanase supplementation ($P<0.01$). The results suggest that dietary CA can promote growth as well as improve intestinal morphology and AME in birds challenged with necrotic enteritis. This study shows that xylanase supplementation improved FCR and AME in birds independent of *C. perfringens* infection; it also elevated the apparent ileal digestibility of CP, digestive enzyme activities, and mRNA expression of nutrient transporters in challenged birds.

Key words: citric acid, necrotic enteritis, nutrient digestibility, nutrient transporter, xylanase

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Introduction

Necrotic enteritis (NE) is an economically important bacterial disease in broilers. The causative agent of NE is *Clostridium perfringens,* which can be found in litter, excreta, soil, and dust, as well as in the intestinal contents of healthy broilers (Williams, 2005). Necrotic enteritis is characterized by severe necrosis of the intestinal mucosa and inflammation of the small intestine, and results in a significant decline in performance as well as increased mortality during acute infection (Van Immerseel *et al*., 2009).

Several dietary components may favor C. perfringens growth, and consequently the incidence of NE, catalyzed by changes in the bacterial community (Liu *et al*., 2002). Research has shown that the use of diets formulated with wheat containing non-starch polysaccharides (NSP) predisposes broilers to NE (Jia *et al*., 2009). While the mechanism by which NSP predisposes birds to NE is unclear, the effects that NSP have on broiler performance, nutrient digestion and absorption, and digesta viscosity have been extensively reported (Choct *et al*., 1999; Annett *et al*., 2002). High digesta viscosity decreases the rate of feed passage and nutrient digestibility, thereby supplying excess substrate for microbial fermentation leading to increased numbers of anaerobic bacteria, particularly *C. perfringens,* in the small intestine (Annett *et al*., 2002). Annett *et al*. (2002) found that wheat-based diets increased proliferation of *Costridia spp.* compared with corn-based diets. Riddell and Kong (1992) found that the presence of arabinoxylans in wheat interfered with nutrients digestion and absorption while also influencing intestinal bacteria. Xylanase may have a positive role on

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broiler performance by increasing the hydrolysis of NSP, reducing intestinal viscosity, increasing the digesta passage rate, and improving nutrient digestion and absorption resulting in reduced availability of substrates for microbial growth in the ileum (Choct *et al*., 1999). Choct *et al*. (1999) found that fermentation products in the small intestine of birds were reduced when diets were supplemented with xylanase. Wang *et al*. (2005) reported that xylanase supplementation increased nutrient retention, chymotrypsin and lipase activities, and glucose absorption via upregulation of sodium glucose cotransporter-1. Thus, the performance of broilers was improved when they were fed wheat-based diets. The positive effects of xylanase may reduce intestinal functional disorders caused by *C. perfringens* infection. Liu *et al*. (2012) noted that the xylanase enzyme reduced the impairment of the intestinal mucosal barrier in birds infected by *C. perfringens*. However, the possible mechanism(s) underlying the influence of xylanase on the nutrient digestibility in subclinical NE of starter broilers fed a wheat-based diet is largely unknown.

Intestinal acidity can also have an effect on the onset of NE. Higher intestinal pH can predispose broilers to NE, as *C. perfringens* growth is inhibited in acidic environments (Kmet *et al*., 1993). Citric acid (CA) is a weak organic acid that has been speculated to cause a decrease in the pH of intestinal contents through an increase in hydrogen ions in the gut of chickens (Nourmohammadi *et al*., 2016). It is possible that the addition of CA may reduce intestinal pH of the broiler gut to an optimal range and enhance xylanase efficacy in broilers (Esmaeilipour *et al*., 2012).

More work focusing on the mode(s) by which dietary feed additives can effectively prevent NE without the addition of antibiotics to diets is required. We hypothesized that the dietary addition of CA and xylanase would ameliorate impairments associated with NE in broiler chickens. Therefore, the aim of this study was to investigate the effects of CA and xylanase supplementation on growth, the digestibility of ileal nutrients, digestive enzyme activity, and mRNA expression of intestinal nutrient transporters in *C. perfringens*-infected starter broilers fed a wheat-based diet.

Materials and Methods

Experimental Design

A total of 360 one-day-old male broiler chicks (Ross 308) were obtained from a commercial hatchery and randomly assigned to 4 treatments, with 6 replicate pens per treatment and 15 birds in each pen. A 2×2 factorial randomized complete design was employed to study the effects of dietary CA (0 and 30 g/kg of feed), xylanase supplementation (0 and 200 mg/kg of diet), and their interactions. Broiler starter diets were formulated based on wheat-soybean meal to meet or exceed NRC (1994) requirements (Table 1). Xylanase (Ronozyme® WX, DSM Nutritional Products Ltd.), which is derived from *Thermomyces Lanuginosus*, was added on the top of the basal diet in powder form and thoroughly mixed. The CA was mixed into a small amount of feed (1 kg) using a premixer. The resulting mixture was then mixed with the rest of the feed using a mechanical blender until a thorough and consistent mixture was obtained. The feeding period lasted 21 d. All experimental diets were free of antibiotics and anti-mycotoxin, and were provided in mash form. The birds had free access to feed and water, and were maintained on a 23-h lighting program. The experiment was approved by the Animal Ethics Committee of the University of Birjand.

Table 1. **Ingredients and compositions of the basal diets**

Item	Value
Ingredients	
Wheat	645.3
Soybean meal (48% CP)	285.2
Soybean oil	28.9
Limestone	9.3
Dicalcium phosphate	19.7
Sodium chloride	3.5
Choline chloride	2.5
L-Lysine	1.7
DL-Methionine	1.4
L-Threonine	0.5
Vitamin-Mineral premix[1]	2.0
Calculated composition	
ME (MJ/kg)	12.14
CP (N×6.25)	21.50
Available P	0.45
Calcium	1.00
Lysine	1.1
Met + Cys	0.93
Thr	0.82

[1] Vitamin and mineral premix supplied the following (per kilogram of diet): vitamin A (from vitamin A acetate), 10,000 IU; vitamin D_3, 9,790 IU; vitamin E (dl-α-tocopheryl acetate), 30 IU; vitamin B_{12}, 20 μg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 μg; thiamin, 4 mg; zinc sulfate, 60 mg; copper sulfate, 100 μg; selenium (sodium selenate), 0.2 mg; iodine, 1 mg, and; manganese oxide, 60 mg.

Necrotic Enteritis Challenge

The NE challenge was a modified version of the method described by Liu *et al*. (2012). A broiler *C. perfringens* type A field strain was isolated from a clinical case of NE at the Iranian Veterinary Culture Center (Mashhad, Iran). The organism was cultured on blood agar base with 5.0% sheep's blood and 100 mg/L neomycin sulphate for 18 h at 37℃, and then aseptically inoculated into cooked meat medium and incubated an aerobically overnight at 37℃. All birds were orally gavaged once daily with active cultures of *C. perfringens* type A (7.0×10^7 CFU/mL, 1.0 mL/bird).

Performance Data

Feed intake and body weight for each replicate were measured on day 21. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated for the 0 to 21 d period. Mortality was

recorded as it occurred, and dead birds were necropsied to analyze for NE.

Metabolism Tracing

From days 18 to 21, titanium dioxide (TiO_2) was added to the diets (3 g/kg) as an indigestible marker to determine the AME as well as the ileal digestibility of crude protein (CP) and dry matter (DM). To maximize feed consumption on day 21, the birds were fasted for 12 h and were then allowed to feed for a one hour period.

Determination of Apparent Ileal Digestibility

On day 21, two birds per replicate (i.e., 12 chicks per treatment) were randomly weighed, euthanized by an intravenous injection of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand), and digesta was collected from the lower half of the ileum following the methods on Ravindran *et al*. (2005). Digesta from birds within a pen were pooled, ground, passed through a 0.5 mm screen, mixed, and freeze-dried for chemical analysis. The dietary samples and digesta were analyzed for DM, titanium (Ti), and CP content.

Chemical Analysis and Calculations

Dry matter content of the diets and ileal digesta were determined using standard procedures (Method 930.15; AOAC, 2005). The crude protein (CP) content (N×6.25) in the diet and digesta samples was determined using the Kjeldahl method. Gross energy content was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardized with benzoic acid. TiO_2 content was determined according to Short *et al*. (1996).

The apparent ileal digestibility was calculated by:

$$AID = 100 \times \frac{(Diet\ component/Ti)_d - (Diet\ component/Ti)_i}{(Diet\ component/Ti)_d}$$

where $(Diet\ component/Ti)_d$ is the ratio of diet component to Ti in the diet and $(Diet\ component/Ti)_i$ is the ratio of diet component to Ti in the ileal digesta.

The AME values of the diets were calculated using the following formula with appropriate corrections made for differences in DM content (Abdollahi *et al*., 2013):

$$AME\ (MJ/kg) = \frac{(Feed\ intake \times GE_{diet}) - (Excreta\ output \times GE_{excreta})}{Feed\ intake}$$

Assay for Digestive and Brush Border Enzyme Activities

To prevent enzyme degradation, the samples were kept on ice throughout the procedure. Jejunal digesta samples were homogenized in 10 mL of ice-cold saline and centrifuged at 2,150×g for 15 min at 4℃. The supernatants were assayed for α-amylase (EC 3.2.1.1) and trypsin (EC 3.4.21.4) activity. α-amylase activity was assayed according to Bernfeld (1955). Trypsin activity was determined according to O'Sullivan *et al*. (1992). The brush border membranes were isolated from the jejuna section of the gut according to Moreno *et al*. (1995). Both sucrose (EC 3.2.1.48) and maltase (EC 3.2.1.20) were assayed according to Dahlqvist (1964).

Jejunum Morphology

Fragments (approximately 3 cm in length) were harvested from the middle of the jejunum (i.e., between the distal portion of the duodenal loop and Meckel's diverticulum). These samples were washed with cold PBS and fixed in 10% neutral buffered formalin at 4℃ for morphometric analysis.

Enumeration of Ilealbacteria and pH Measurements

Pooled digesta (10 g) were homogenized in 90 mL of sterile peptone containing 0.5% cysteine hydrochloride and serially diluted. For enumeration of *C. perfringens*, dilutions were plated on Perfringens agar base (OPSP, Oxoid Inc., Nepean, Ontario, Canada) with SR 76 and SR 7 supplements (Oxoid Inc.) and incubated an aerobically at 38℃ for 48 h (BBL Gas Pak Plus, Becton Dickinson, Sparks, MD). Lactic acid bacteria were counted based on colonies appearing on De Man Rogosa and Sharp (MRS) agar (Oxoid Inc., CM 0361) incubated aerobically at 39℃ for 48 h. After incubation, colonies were carefully counted, converted into the logarithmic equivalent, and expressed as $\log_{10}$ CFU per gram of wet digesta. The ileal digesta samples were also mixed with deionized water (1:10 wt/vol) for pH measurement using a digital pH meter (Model 827, Metrohm, Herisau, Switzerland); measurements took place at room temperature.

Quantitative Real-Time PCR

Total RNA (n=2 per treatment) was extracted from the jejunal mucosa using Trizol (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. The concentration and purity of total RNA was determined using a spectrophotometer (NanoDrop ND 1000, Nano Drop Technologies, Wilmington, DE). One microgram of total RNA was reverse transcribed by a reverse transcription kit (Invitrogen Life Technologies) according to the manufacturer's instructions. Relative quantification PCR was performed using the 7500 Fluorescence Detection System (Applied Biosystems, Foster City, CA) according to optimized PCR protocols given by the SYBR-Green PCR kit (Applied Biosystems). The primers for sodium glucose co-transporter 1 (SGLT1) and H+-dependent peptide transporter 1 were obtained from Guo *et al*. (2014). The PCR conditions were: an initial denaturation step at 95℃ for 10 min, 40 cycles at 95℃ for 30 s, an annealing and extension step at 60℃ for 1 min, and a final extension step of 72℃ for 10 min. Gene expression was quantified using the comparative threshold cycle method, and the data were expressed as relative values.

Statistical Analysis

In this experiment, each pen was considered as an experimental unit and data were analyzed as a completely randomized design with 2×2 factorial treatment arrangements using GLM procedures from SAS (SAS Inst. Inc., Cary, NC, USA). The model included the main effects of CA and xylanase, as well as associated 2-way interactions. The level of significance was set at 5%, and when a significant effect was obtained treatment means were separated using a Tukey-Kramer's test.

Results

The inclusion of 30 g/kg CA significantly increased ADFI and ADG from 0 to 21 d compared to the control group (P<

Table 2. **Effects of citric acid and xylanase supplementation on average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) in broilers challenged with necrotic enteritis from 0 to 21 d of age[1]**

Main effect	ADFI (g/bird/d)	ADG (g/bird/d)	FCR (g/g)
Citric acid (g/kg)			
0	47.1[b]	36.4[b]	1.29
30	48.2[a]	37.7[a]	1.28
Xylanase (mg/kg)			
0	47.6	36.6	1.30[a]
200	47.8	37.3	1.27[b]
SEM	0.64	0.67	0.01
P-value			
Citric acid	0.035	0.042	0.724
Xylanase	0.126	0.085	0.021
Citric acid×xylanase	0.297	0.388	0.855

[a–b] Values in the same column with different superscripts are significantly different ($P<0.05$).
[1] Data are the mean of 6 replicates with 15 chickens per group.

Table 3. **Effects of citric acid and xylanase supplementation on bacterial counts (log_{10}cfu/g of digesta) and pH values of intestinal contents in broilers challenged with necrotic enteritis[1]**

Main effect	*C. perfringens*	Lactic acid bacteria	pH
Citric acid (g/kg)			
0	3.32	7.4	6.29
30	3.10	7.3	6.23
Xylanase (mg/kg)			
0	3.55	7.0	6.28
200	3.17	7.6	6.37
SEM	0.614	0.23	0.15
P-value			
Citric acid	0.217	0.825	0.345
Xylanase	0.522	0.318	0.228
Citric acid×xylanase	0.116	0.255	0.437

[1] Data are the mean of 6 replicates with 2 chickens per group.

0.05; Table 2). Xylanase supplementation decreased FCR ($P<0.05$), but there were no significant differences in the growth of birds supplemented with or without xylanase (Table 2). Birds challenged with *C. perfringens* did not produce clinical signs of NE, and there was no mortality that was directly associated with exposure to *C. perfringens*. Neither CA nor xylanase addition impacted mortality rate (data not shown).

Dietary CA and xylanase supplementation did not affect the number of *C. perfringens* in the ileum (Table 3). In addition, the lactic acid bacterial counts were similar between the treatment groups. Finally, there were no differences in pH values between the treatments ($P>0.05$).

Dietary CA and xylanase supplementation significantly increased AMEvalues ($P<0.01$; Table 4). The supplementation of xylanase significantly increased the apparent ileal digestibility of CP ($P<0.05$). Inclusion of 30 g/kg CA tended to increase the ileal CP digestibility ($P=0.085$).

The activities of α-amylase and trypsin in the jejunum were increased by xylanase supplementation ($P<0.05$; Table 5). Xylanase supplementation significantly increased jejuna sucrose activity compared with the control ($P<0.05$).

Xylanase supplementation did not have a significant effect on jejunal morphology based on the measured criteria (Table 6). In birds fed CA, the villus height and villus height-to-crypt depth ratio were increased compared with those fed the control diet ($P<0.01$).

Xylanase supplementation significantly increased the mRNA expression of SGLT1 and PepT1 in the jejunum ($P<0.01$; Table 7). The jejunal mRNA expression of SGLT1 does not appear to be regulated by the addition of CA ($P=0.087$).

Table 4. **Effects of citric acid and xylanase supplementation on AME and apparent ileal digestibility of DM and CP in broilers challenged with necrotic enteritis at 21 d of age[1]**

Main effect	AME (kcal/kg)	DM (%)	CP (%)
Citric acid (g/kg)			
0	2,886[b]	67.25	75.22
30	2,931[a]	67.40	76.18
Xylanase (mg/kg)			
0	2,828[b]	67.77	75.45[b]
200	2,927[a]	66.61	77.56[a]
SEM	23.5	0.620	1.142
P-value			
Citric acid	0.006	0.102	0.085
Xylanase	0.001	0.311	0.022
Citric acid×xylanase	0.201	0.755	0.310

[a–b] Values in the same column with different superscripts are significantly different ($P<0.05$).
[1] Values are the mean of 6 replicates with 2 chickens per group.

Table 5. **Effects of citric acid and xylanase supplementation on digestive enzyme and brush border enzyme activities in the jejunum of broilers challenged with necrotic enteritis at 21 d of age[1]**

Main effect	Digestive enzymes		Brush border enzymes	
	α-Amylase	Trypsin	Maltose	Sucrase
Citric acid (g/kg)				
0	828.2	229.6	315.2	73.38
30	835.7	223.7	287.5	69.85
Xylanase (mg/kg)				
0	993.3[a]	238.6[a]	399.0	60.92[b]
200	918.8[b]	201.7[b]	305.4	81.77[a]
SEM	35.24	12.02	11.25	3.850
P-value				
Citric acid	0.625	0.254	0.325	0.215
Xylanase	0.020	0.026	0.125	0.034
Citric acid×xylanase	0.436	0.621	0.226	0.905

[a–b] Values in the same column with different superscripts are significantly different ($P<0.05$).
[1] Values are the means of 6 replicates with 2 chickens per group.

Discussion

Supplementation of the wheat-based diet with xylanase improved FCR and AME utilization during the experimental period. These findings agree with the previous study by Esmaeilipour *et al*. (2012), which found that xylanase supplementation improved the FCR and AME values in broilers. The positive effects of xylanase are mainly attributed to the degradation of soluble arabinoxylans, the major component of NSP in the wheat-based diet (Riddell and Kong, 1992), which leads to a higher viscosity of digesta. This consequently has negative effects on the digestion, absorption, and microflora of the gastrointestinal tract (Choct *et al*., 1999). Xylanase supplementation can partially hydrolyze arabinoxylans and release nutrients (Riddell and Kong, 1992). Furthermore, it has been proposed that the use of xylanase can decrease variations in the AME of wheat within animals and significantly increase the overall AME values (Choct *et al*., 1999). We saw increases in ADG and ADFI due to dietary CA that were consistent with previously reports by Boling-Frankenbach *et al*. (2001) and Nourmohammadi *et al*. (2012). Nourmohammadi *et al*. (2012) and Khosravinia *et al*. (2015) attributed improved growth to a decrease dietary energy since CA replaced corn starch in the feed. However, all dietary treatments were isocaloric in our study; therefore, the increases in growth that correspond to CA treatment may be partly associated with the positive effect of CA on improved gut health rather than changes in nutrient availability (Esmaeilipour *et al*., 2012).

We hypothesized that xylanase and CA would facilitate proliferation of lactic acid bacteria and reduce proliferation of *C. perfringens*. However, our results do not fully support

Table 6. **Effects of citric acid and xylanase supplementation on jejunal morphology in broilers challenged with necrotic enteritis at 21 d of age[1]**

Main effect	Villus height (VH)	Crypt depth (CD)	VH:CD
Citric acid (g/kg)			
0	1576[b]	234	6.73[b]
30	1642[a]	231	7.17[a]
Xylanase (mg/kg)			
0	1582	230	6.88
200	1602	231	6.93
SEM	30.2	2.3	0.24
P-value			
Citric acid	0.001	0.092	0.001
Xylanase	0.266	0.109	0.321
Citric acid×xylanase	0.871	0.317	0.418

[a–b] Values in the same column with different superscripts are significantly different ($P<0.05$).
[1] Values are the means of 6 replicates with 2 chickens per group.

Table 7. **Effects of citric acid and xylanase supplementation on relative mRNA expression of SGLT1 and PepT1 in broilers challenged with necrotic enteritis at 21 d of age[1]**

Main effect	SGLT1	PepT1
Citric acid (g/kg)		
0	0.81	0.89
30	0.96	0.93
Xylanase (mg/kg)		
0	0.75[b]	0.85[b]
200	1.38[a]	1.34[a]
SEM	0.185	0.152
P-value		
Citric acid	0.087	0.680
Xylanase	0.009	0.001
Citric acid×xylanase	0.552	0.126

[a–b] Mean in the same column without same superscript differ significantly ($P<0.05$).
[1] Data are means for 6 replicates of 2 chickens per group.
SGLT1=sodium glucose co-transporter 1; PepT1=H+-dependent peptide transporter 1.

this hypothesis; there were no significant differences in *C. Perfringens* counts, digesta pH, or mortality of birds consuming CA or xylanase-supplemented diets compared with the control. Only a few studies have explored the effects of dietary CA and xylanase supplementation on the incidence of NE; furthermore, the results of these studies are contradictory and difficult to compare due to the use of different dietary types, diseases, and enzyme supplements (Riddell and Kong, 1992; Jackson *et al*., 2003; Choct *et al*., 2006). In this study, CA and xylanase supplementation did not impact the growth of *C. perfringens*. However, supplementation did ameliorate any growth inhibition due to disease challenge, as the BW of birds consuming the CA-supplemented diets increased and the FCR of birds consuming the xylanase supplemented diet improved. Discrepancies in the response of birds to xylanase may be due to the differences in diets (i.e., wheat vs. corn) and the content of water-soluble NSP (Choct *et al*., 2006). Foster (1995) suggested that acid-sensitive bacteria can adapt to acid stress in moderately low pH environments that subsequently increases their survival in a high acid conditions; this may explain why CA had no effect on the bacterial populations of the ileal contents.

It has been well documented that feeding wheat-based diets supplemented with xylanase lowers bacterial counts, especially in the small intestine (Jia *et al*., 2009). Therefore, it seems that the xylanase enzyme reduces microbe-host competition for nutrients. Additionally, the enzyme hydrolysis products could act as a prebiotic to facilitate the proliferation of specific beneficial bacteria. Probiotics can indirectly suppress the proliferation of pathogenic bacteria, such as *C. perfringens* (Annett *et al*., 2002). In addition, xylanase had positive effects on morphometric indices and increased the villus height-to-crypt depth ratio in different segments of the small intestine (Liu *et al*., 2012), resulting in a larger surface area available for digestion and absorption of nutrients. All these factors facilitated the digestibility of nutrients and improved the digestibility of protein for challenged broilers.

Endogenous enzymes are essential for nutrient digestion. Few studies have been conducted regarding the effects of xylanase supplementation on the activity of intestinal digestive enzymes in broilers in combination with pathogen infections. Our results indicate that the activity of both α-amylase and tryps in the jejunum decreased as a result of xylanase supplementation. This agrees with the results of Inborr *et al*. (1993) and Fan *et al*. (2009), who both observed a decreased amylase activity in the digesta of weaned piglets fed a wheat-based diet with xylanase. Furthermore, they also observed the partial degradation of arabinoxylan and the

corresponding decrease in digesta viscosity. It has been well documented that trypsin activity depends on the dietary protein source (Moreno *et al*., 1995). In our study the protein source was the same for all four treatments; thus, the elevated secretion of trypsin suggests that the amount of protein available for digestion increased due to xylanase supplementation. Disaccharidase is an extrinsic enzyme that interferes with nutrients digestion and absorption. An increase in disaccharidase activity is associated with an increase in the capacity for disaccharide digestion and absorption in the epithelium (Moreno *et al*., 1995). Liu *et al*. (2012) found that xylanase supplementation improves sucrose digestion in challenged broilers by reducing the number of pathogenic bacteria in the small intestine. In contrast, xylanase decreases the chyme viscosity that may prevent contact between bush border enzymes and their substrates by thickening the unstirred mucosal layer (Wang *et al*., 2005). The elevated sucrose activity in the jejunum of the challenged broilers in our study supports these results.

Villus height was increased with dietary CA supplementation in this study. We believe that this is due to an increase in the rate of nutrient absorption and a decrease in the rate of enterocyte cell migration from the crypt to the villus. The villus height-to-crypt depth ratio is an important indicator of the digestive capacity in the small intestine (Nourmohammadi and Afzali, 2013). Nourmohammadi and Afzali (2013) showed that an increase in this ratio is related to an improvement in nutrient digestion and absorption.

Nutrients transporters in the brush border membrane of the small intestine are important for nutrient absorption. SGLT1 mediates the Na+-dependent uptake of glucose and galactose across the brush border membrane (Debnam *et al*., 1995). In addition, PepT1 is primarily responsible for the absorption from dietary di- and tri-peptides from the small intestinal lumen (Hediger and Rhoads, 1994). There is little available information regarding the effects of dietary CA and xylanase supplementation in NE challenged broilers on the gene expression of nutrient transporters in the small intestine. In our study, xylanase addition increased the jejunal glucose absorption by upregulating SGLT1 mRNA expression in challenged birds; this is consistent with the findings of Wang *et al*. (2005). However, Gal-Garber *et al*. (2000) found that a higher expression of SGLT1 was accompanied by lower SGLT1 affinity and activity in the fasted birds. Therefore, increased glucose absorption in the jejunum is not definitely linked to increased expression of SGLT1 upregulated by *C. perfringens*.

Xylanase supplementation did not significantly alter the growth or feed intake of starter broilers infected with *C. perfringens*. Increases in ileal nutrient digestibility, digestive enzyme activity, and intestinal mRNA expression of nutrient transporters were beneficial for nutrient digestion and absorption in subclinical NE birds. The addition of xylanase to wheat-based diets for commercial poultry could be used as a nutritional strategy for controlling pathogen infection and reducing the economic losses associated with NE. Dietary CA supplementation is an effective strategy for improving intestinal morphology and AME in broiler chickens challenged with *C. perfringens*. This is the first study to assess the changes in the activity of digestive enzymes and gene expression of intestinal nutrient transporters in broilers fed dietary CA and xylanase supplementation under clinical NE conditions; thus, further investigations are required to fully understand the impacts of these supplements.

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