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Effect of probiotic *Lactobacillus reuteri* XC1 coexpressing endoglucanase and phytase on intestinal pH and morphology, carcass characteristics, meat quality, and serum biochemical indexes of broiler chickens

Lei Wang[1,2], Yuzhe Feng[1], Xiaowei Zhang[1], Guofang Wu[1,2]*

[1] Qinghai University, Academy of Animal Science and Veterinary Medicine, Xining, China.
[2] Qinghai University, State Key Laboratory of Plateau Ecology and Agriculture, Xining, China.

***Corresponding author:**
letitbe521@163.com

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ABSTRACT - This study investigated the effect of transformed *Lactobacillys reuteri* on intestinal pH and morphology, carcass characteristics, meat quality, and serum biochemical indexes of broiler chickens. A total of 480 broilers were assigned to six treatment groups and fed a phosphorus-adequate diet, a phosphorus-deficient diet, or a phosphorus-deficient diet containing different *L. reuteri* recombinants. The results showed that transformed *L. reuteri* decreased the pH in the duodenum and jejunum of chickens at day 21, decreased drip loss and cooking loss of muscles, and improved muscle tenderness of chickens at days 21 and 42, but did not affect carcass characteristics and only slightly decreased abdominal fat. Transformed *L. reuteri* also significantly increased calcium, phosphorus, and glucose levels, decreased the uric acid level of serum at day 21, and significantly increased the glucose level and decreased the triglycerides of serum on day 42. *L. reuteri* pLEM4159-cel/phy increased the villi height in the duodenum of chickens at days 21 and 42. The transformed *L. reuteri* decreased the crypt depth in the duodenum and jejunum of chickens at day 21 and also decreased the crypt depth in the ileum and increased the villi height in the duodenum at day 42. *L. reuteri* pLEM4158 (phy) and *L. reuteri* pLEM4159-cel/phy improved the villi height in the ileum at day 42. Taken together, transformed *L. reuteri* can improve blood calcium, phosphorus, and glucose metabolism and intestinal development in broilers, but does not affect carcass characteristics.

Keywords: bird nutrition, meat quality, microbial additive, phosphorus, pH, villus

Introduction

Glucan and phytic acid (phytate) are two major anti-nutritional factors present in monogastric animal diets that can cause digestion difficulties due to their ability to form insoluble complexes with proteins and minerals in the gastrointestinal tract (GIT). Consequently, their presence in feedstocks can lead to losses in endogenous nutrients that ultimately lead to significant increases in animal feed costs (Humer et al., 2015; Attia et al., 2016; Bueno et al., 2018).

Compared with other cereals, endosperm and aleurone wheat layers contain high levels of high-molecular-weight hydrosoluble arabinoxylan, which can adhere strongly to cell walls to readily form thick granular suspensions. The endosperm cell wall of barley contains abundant glucan that is comprised of multiple β-(1-3) and β-(1-4) glucosidic bonds (Fernandes et al., 2016), which can produce gel-like materials in the GIT of poultry that can impair intestinal movement. This type of

gelation can result in significant decrease in the diffusion and action of digestive enzymes, limit systemic absorption of nutrients through the intestinal villi, and stimulate proliferation of spoilage organisms (Attia et al., 2003; Maisonnier et al., 2001).

Phytic acid is a powerful chelating agent that can directly (or indirectly) bind to many nutrients (e.g., minerals, proteins/amino acids, carbohydrates, and lipids), forming phytate complexes (or liposomes) that prevent efficient nutrient absorption (Attia et al., 2003; Attia et al., 2014). The inhibitory action of phytic acid on Zn^{2+} in the GIT is particularly problematic and can lead to zinc deficiency, dwarfism, and hypogonadism. Acidity is also known to play an important role in determining the stability, solubility, and chelating properties of phytic acid in the gut (Lopez et al., 2002; Gupta et al., 2015).

There are significant concerns about the effect of residual antibiotics, hormones, and other synthetic drugs in meat, egg, and milk products used for human consumption (Attia et al., 2016; Pan et al., 2011; Cabello, 2004; Cabello, 2006). This has resulted in much attention being directed towards identifying alternatives to antibiotic use in livestock, with probiotics having been proposed as a substitute for poultry breeding applications (Stephens et al., 2010; Zhou et al., 2010; Suo et al., 2012). Multifunctional transgenic probiotics have been proposed as a feasible method to overcome problems caused by anti-nutritional factors and antibiotics in animal husbandry. For example, a multifunctional transgenic *Lactobacillus reuteri* XC1 strain that is capable of degrading β-glucan and absorbing phytic acid has been evaluated as a probiotic in broilers, with its effect on growth performance, tibia parameters, and the cecum microflora population being investigated. The presence of transformed *L. reuteri* XC1 was found to improve the feed conversion ratio of broilers from 21 to 42 days; however, no effect on the body weight gain of new-born chicks was observed (Wang et al., 2014).

This study investigates the positive effects of adding transformed *L. reuteri* XC1 probiotics to the diet of broiler chickens, and reports the beneficial changes in their intestinal pH and morphology, meat quality, and serum biochemical indexes.

Material and Methods

Lactobacillus reuteri strain XC1 was isolated from the GIT of healthy broilers. *L. reuteri* pLEM4156 containing an empty plasmid, *L. reuteri* pLEM4157 (cel) expressing an endoglucanase gene, *L. reuteri* pLEM4158 (phy) expressing a phytase gene, and *L. reuteri* pLEM4159-cel/phy coexpressing endoglucanase and phytase genes were constructed using previously described methods (Wang et al., 2014). Different recombinant *L. reuteri* strains were prepared according to the method described by Mappley et al. (2013).

In total, 480 similarly sized 1-day-old male Arbor Acres (AA) broilers were weighed and randomly assigned to six treatment groups comprising four replicates of 20 birds each. The six groups were offered six dietary treatments of barley-wheat-based diets, which were formulated as isonitrogenous and isoenergetic according to NRC (1994) requirements (Table 1). Control groups were fed a phosphorus-adequate diet (as a positive control) or a phosphorus-deficient diet (as negative control) and were administered distilled water from day 1. Four experimental treatment groups were fed a phosphorus-deficient diet and were administered distilled water containing recombinant *L. reuteri* pLEM4156, *L. reuteri* pLEM4157 (cel), *L. reuteri* pLEM4158 (phy), or *L. reuteri* pLEM4159-cel/phy (2.5×10^8 cfu/mL) from day 1. The mixture was provided in the water container, and water was freely accessible to all birds and changed frequently to maintain freshness. Feed and water were supplied for *ad libitum* intake. Vaccination and light regimens were performed according to routine procedures. Chicks were raised using typical commercial conditions over the 0-42 days of the experiment. All experimental methods and protocols were approved by the local Research Ethics Committee (case no. 2014ZX08008002). The study was conducted in Yangling district (34°28' N; 108°07' E), Xianyang, China.

Two birds were randomly selected at 21 and 42 days old from each replicate and were anesthetized to a surgical plane by an intramuscular injection of xylazine chlorhydrate to minimize suffering, and

Table 1 - Composition of the experimental diet

Item	Days 1-21		Days 22-42	
	Positive control	Negative control	Positive control	Negative control
Composition (g/kg)				
Barley	390.60	382.20	410.00	400.00
Wheat	300.00	300.00	310.00	287.80
Soybean meal	180.00	200.00	125.40	170.50
Fish meal	65.40	52.00	68.70	40.00
Soybean oil	33.90	35.40	52.90	57.60
Limestone	10.90	14.60	16.00	18.50
Dicalcium phosphate	5.50	1.70	2.00	1.00
Salt	1.60	1.90	2.80	2.10
Choline-Cl (50%)	1.00	1.00	1.00	1.00
Methionine	1.00	-	0.30	0.50
Lysine	-	0.10	1.00	1.00
Premix[1]	10.00	10.00	10.00	10.00
Nutrient level				
Metabolizable energy (kcal/kg)	2900	2900	3041	3041
Crude protein (%)	21.00	21.00	19.00	19.00
Calcium (%)	0.90	0.90	1.00	0.96
Total phosphorus (%)	0.65	0.56	0.58	0.50
Available phosphorus (%)	0.45	0.35	0.40	0.30

[1] The premix provided per kilogram of diets: manganese, 120 mg; iron, 100 mg; iodine, 0.7 mg; zinc, 100 mg; selenium, 0.3 mg; copper, 8 mg; vitamin A, 8,000 IU; vitamin E, 20 IU; vitamin D_3, 1,000 IU; flavin, 8.0 mg; menadione, 0.5 mg; thiamine, 2.0 mg; niacin, 35 mg; pyridoxine, 3.5 mg; biotin, 0.18 mg; vitamin B_{12}, 0.01 mg; pantothenic acid, 10.0 mg; antioxidant, 0.4 g; folic acid, 0.55 mg in the phase of 1-21 d, and manganese, 60 mg; iron, 60 mg; iodine, 0.6 mg; zinc, 80 mg; selenium, 0.3 mg; copper, 8 mg; and vitamin A, 6,000 IU; vitamin E, 30 IU; vitamin D_3, 500 IU; flavin, 5.0 mg; menadione, 0.5 mg; thiamine, 2.0 mg; niacin, 30 mg; pyridoxine, 3.0 mg; biotin, 0.15 mg; vitamin B_{12}, 0.01 mg; pantothenic acid, 10.0 mg; antioxidant, 0.5 g; folic acid, 0.55 mg in the phase of 22-42 d.

their duodenums, jejunums, and ileums were then surgically removed. The pH of the contents of each intestinal segment at three different points was determined using a handheld digital display pH meter. A 2-5 cm segment from the middle of each intestinal sample was dissected, its enteric cavity was washed with physiological saline, the sample was then fixed with 4% paraformaldehyde for 12 h, gradient dehydrated by 20% and 30% sucrose for 1 day, then dried on absorbent paper towel. Then, the intestinal sample was trimmed to the appropriate size, placed on an object stage, and embedded in 100% optimal cutting temperature compound. Frozen embedded samples were serially sectioned at 10-μm thickness along the cross-section (three cross-sections from each sample), and then fixed on slides, and stained with hematoxylin and eosin. Photomicrographs of the intestinal slides were obtained using an Eclipse E200 microscope (Nikon, Tokyo, Japan) equipped with a Motic 2300 capture system (Sony Super Steady Shot, 9.1 megapixels). A total of 10 intact, well-oriented crypt-villus units from each cross-section were selected and analyzed using Image ProPlus software (Version 4.5.0.29, 2001). Measurement of the villus height and crypt depths was performed according to the method of Villanueva et al. (2016).

Another two birds of 42 days old from each replicate were randomly chosen and after 10-h of overnight fasting, were anesthetized as previously described. Their carcasses were defeathered and weighed, with subsequent treatment of their semi-eviscerated and fully eviscerated carcasses using the method described by Mosca et al. (2016). Their left breasts (and associated tightly bound abdominal fat) were weighed to calculate the dressing percentages, semi-eviscerated yields, fully eviscerated yields, breast percentages, tight percentages, and abdominal fat percentages. Simultaneously, their right breasts were used to determine meat quality (pH, drainage percentage, drip loss, cooking loss, and shear force) using the assay methods described by Choo et al. (2014).

Blood samples (5 mL) were collected from the brachial veins of two birds from each replicate after 21 and 42 days, which were then centrifuged at 3000 rpm for 5 min to obtain serum samples that were stored at −20 °C. Serum concentrations of urea nitrogen, calcium, phosphorus, glucose, total cholesterol, and uric acid were determined using a clinical chemistry analyzer (Gilford Impact 400E, Gilford Systems, Oberlin, OH, USA).

Each replicate was considered as the experimental unit. The data were analyzed by one-way ANOVA and Duncan's multiple range test (Dawson and Trapp, 2001), using the General Linear Model of the SPSS22.0 software (SPSS Inc., Chicago, IL, USA). The significance level was taken as $P<0.05$. The statistical model was as follows:

$$Y_{ij} = \mu + G_i + \varepsilon_{ij},$$

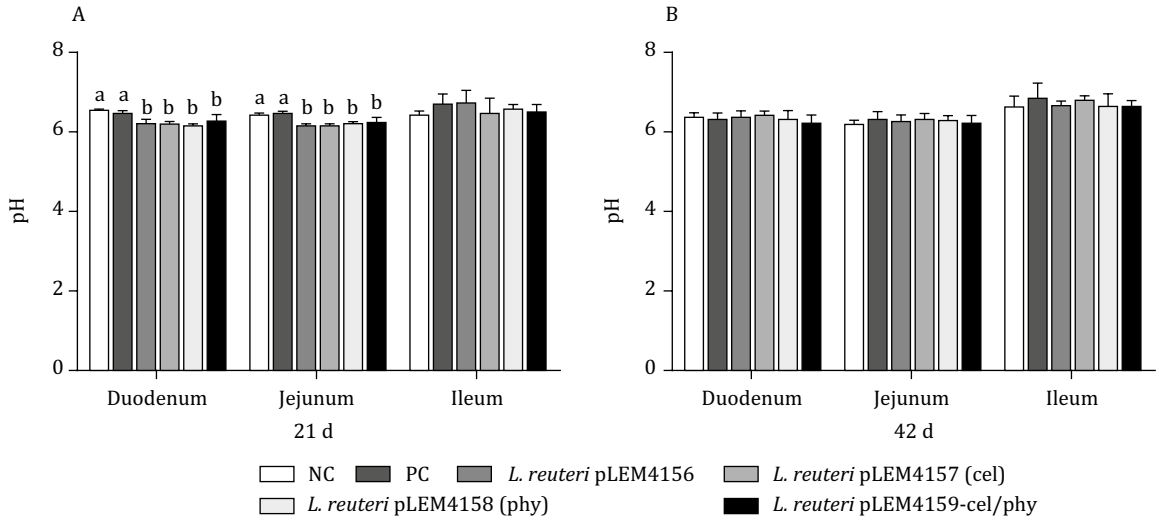
in which Y_{ij} = observed value of the dependent variables, μ = overall mean, G_i = effect of presence or absence of different transformed *L. reuteri*, and ε_{ij} = random error associated with each observation.

Results

Broilers were fed a barley-wheat-based diet containing transformed *L. reuteri* strains, and changes in the pH of their intestinal tracts were monitored over time (Figure 1). At day 21, the pH values of duodenums and jejunums of chicks that received *L. reuteri* pLEM4156, *L. reuteri* pLEM4157 (cel), *L. reuteri* pLEM4158 (phy), or *L. reuteri* pLEM4159-cel/phy were significantly lower than those of the positive and negative control groups ($P<0.05$). However, there were no significant differences in the pH values or the duodenums, jejunums, and ileums of birds fed the different treatments at day 42 ($P>0.05$).

No significant differences in carcass composition were found in chicks fed different treatments at day 42 (Table 2), although they all exhibited slightly decreased abdominal fat levels ($P>0.05$) relative to the control groups. Amongst them, chickens treated with *L. reuteri* pLEM4159-cel/phy had the lowest abdominal fat content. Compared with the control group, all treatment groups exhibited no significant differences in pH and drainage rates ($P>0.05$). However, there were significant decreases in drip losses, cooking losses, and shear force, as well as an improvement in overall intramuscular quality ($P<0.05$).

The use of *L. reuteri* pLEM4159-cel/phy resulted in a significant increase in the duodenum villus height of chicks at day 21 ($P<0.05$) (Figure 2). Supplementation with *L. reuteri* pLEM4158 (phy), *L. reuteri* pLEM4159-cel/phy, or *L. reuteri* pLEM4157 (cel) resulted in a significant decrease in duodenum crypt



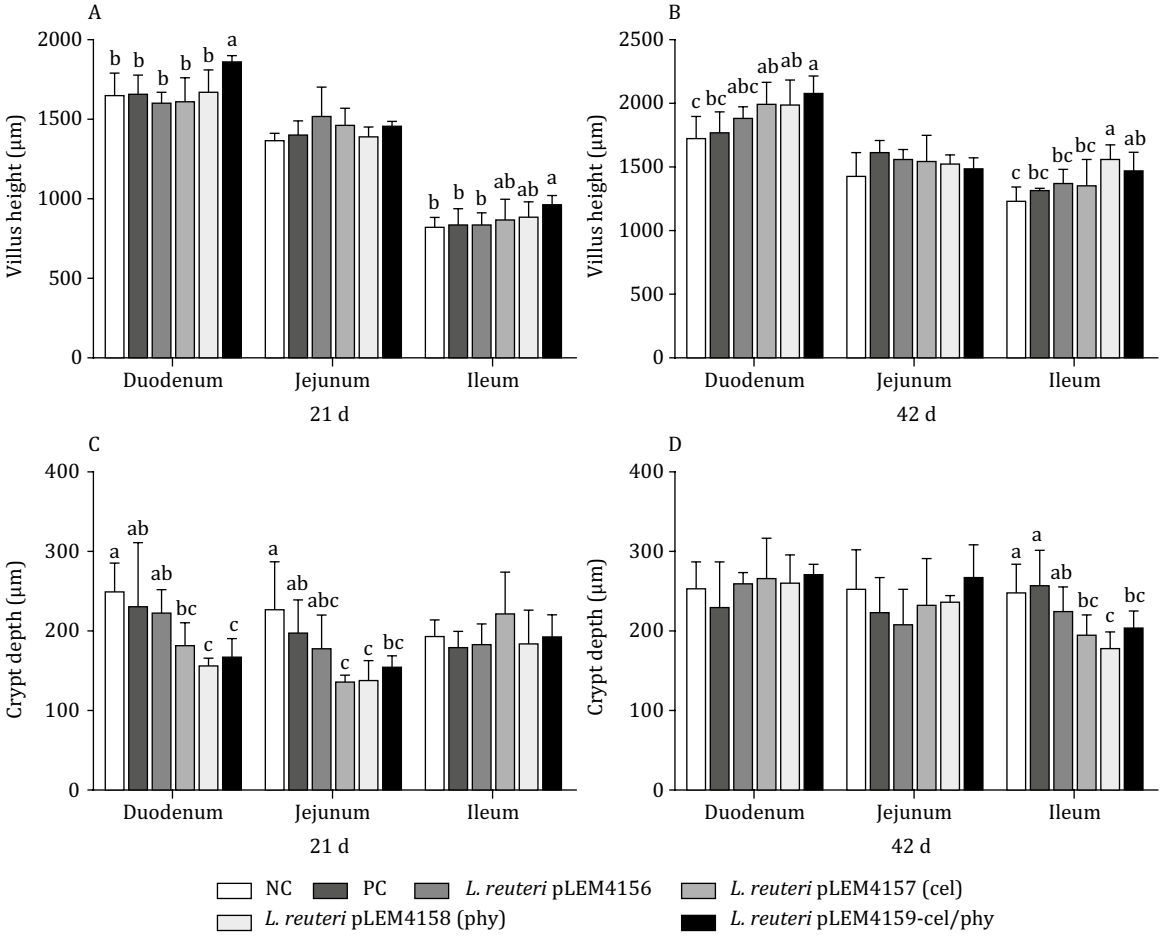
A: pH in the small intestine of broilers at day 21; B: pH in the small intestine of broilers at day 42.
NC - negative control; PC - positive control.
Data are expressed as mean±SD.
a,b - Means on the error bar with different letters are significantly different ($P<0.05$).

Figure 1 - Effects of transformed *L. reuteri* strain supplementation on intestinal tract pH of broilers.

Table 2 - Effects of transformed *L. reuteri* strain supplementation on carcass characteristics and meat quality of broilers

Carcass characteristic	Control group		*L. reuteri* group				SEM[1]	P-value
	NC	PC	pLEM4156	pLEM4157 (cel)	pLEM4158 (phy)	pLEM4159-cel/phy		
Live weight (kg)	2.04	2.20	1.99	2.13	2.22	2.14	0.038	0.552
Carcass weight (kg)	1.88	2.07	1.84	1.98	2.06	1.98	0.036	0.360
Dressing percentage (%)	92.11	93.78	92.20	93.13	92.63	92.80	0.240	0.373
Semi-eviscerated yield (%)	85.93	87.12	86.46	86.17	86.79	87.01	0.269	0.802
Fully eviscerated yield (%)	69.23	72.54	69.58	71.12	71.67	71.91	0.440	0.171
Breast (%)	12.27	11.27	11.53	12.20	11.39	11.86	0.195	0.602
Thighs (%)	13.06	12.79	13.29	13.29	14.24	13.74	0.253	0.675
Abdominal fat (%)	6.77	6.76	6.11	6.05	6.43	5.81	0.301	0.940
Meat quality								
pH 45 min	5.87	6.11	6.05	6.08	6.26	6.05	0.058	0.655
pH 24 h	5.32	5.29	5.38	5.53	5.52	5.37	0.039	0.392
Drainage (%)	32.72	32.83	32.23	31.32	30.54	31.47	0.529	0.857
Drip loss (%)	4.63	4.69	3.68	3.75	3.31	3.13	0.197	0.094
Cooking loss (%)	25.77a	25.74a	21.65b	22.64b	20.39b	20.45b	0.629	0.008
Shear force (N)	49.49a	42.92a	33.34b	34.38b	30.10b	29.60b	2.196	0.028

NC - negative control; PC - positive control.
a,b,c - Means within the same row with different letters are significantly different (P<0.05).
[1] SEM - pooled standard error of the mean (n = 8 birds for each replicate).



A: villus height in the small intestine of broilers at day 21; B: villus height in the small intestine of broilers at day 42; C: crypt depth in the small intestine of broilers at day 21; D: crypt depth in the small intestine of broilers at day 42.
NC - negative control; PC - positive control.
Data are expressed as the mean±SD.
a,b,c - Means on the error bar with different letters are significantly different (P<0.05).

Figure 2 - Effects of transformed *L. reuteri* strain supplementation on intestinal villus height and crypt depth of broilers.

depth at day 21 (P<0.05). The duodenum crypt depth of chicks treated with *L. reuteri* pLEM4158 (phy) or *L. reuteri* pLEM4159-cel/phy at day 21 were significantly lower than of those treated with *L. reuteri* pLEM4156 and of the positive control group (P<0.05).

No notable differences in jejunum villus height were observed for the chicks fed different treatments at day 21. However, supplementation with *L. reuteri* pLEM4158 (phy), *L. reuteri* pLEM4159-cel/phy, or *L. reuteri* pLEM4157 (cel) significantly decreased crypt depth in the jejunum at day 21 (P<0.05). Supplementation with *L. reuteri* pLEM4159-cel/phy significantly increased the ileum villus height of chicks at day 21 (P<0.05); however, there was no significant effect on the ileum crypt depth at day 21 for all other treatment groups (P<0.05). Compared with the negative control group, supplementation with *L. reuteri* pLEM4158 (phy), *L. reuteri* pLEM4159-cel/phy, or *L. reuteri* pLEM4157 (cel) resulted in a significant increase in duodenum villus height at day 42 (P<0.05). However, no significant effects on the ileal crypt depth, jejunal villus height, and crypt depth of chicks fed different treatments at day 42 were observed (P>0.05). Compared with the positive and negative control groups, supplementation with *L. reuteri* pLEM4158 (phy) or *L. reuteri* pLEM4159-cel/phy significantly increased the villus height of the ileum of chicks at day 42 (P<0.05). Analogously, supplementation with *L. reuteri* pLEM4158 (phy), *L. reuteri* pLEM4159-cel/phy, or *L. reuteri* pLEM4157 (cel) significantly decreased ileum crypt depth at day 42 (P<0.05).

No significant differences in urea nitrogen, total cholesterol, or triglyceride levels were observed in the serum of chicks fed different treatments at day 21 (P>0.05) (Table 3). The calcium content of serum from chicks treated with *L. reuteri* pLEM4159-cel/phy, *L. reuteri* pLEM4156, *L. reuteri* pLEM4157 (cel), or *L. reuteri* pLEM4158 (phy) were all significantly higher after 21 days. The phosphorus content of serum of chicks treated with *L. reuteri* pLEM4158 (phy), *L. reuteri* pLEM4159-cel/phy, *L. reuteri* pLEM4156, or *L. reuteri* pLEM4157 (cel) was also greater (P<0.05). The glucose content of serum in chicks treated with *L. reuteri* pLEM4157 (cel) or *L. reuteri* pLEM4159-cel/phy at day 21 was significantly higher than that of chicks treated with *L. reuteri* pLEM4156 or chicks in the negative and positive control groups (P<0.05). There were no significant differences in urea nitrogen, calcium,

Table 3 - Effects of transformed *L. reuteri* strain supplementation on serum biochemical indexes (SBI; mmol/L)

SBI	Control group		*L. reuteri* group				SEM[1]	P-value
	NC	PC	pLEM4156	pLEM4157 (cel)	pLEM4158 (phy)	pLEM4159-cel/phy		
Day 21								
BUN	0.37	0.29	0.33	0.30	0.28	0.35	0.017	0.734
Ca	1.61c	1.69bc	2.05ab	2.01ab	2.09ab	2.32a	0.071	0.014
P	1.34c	1.57bc	1.75b	1.85b	2.33a	2.22a	0.093	0.001
GLU	9.27b	10.38b	10.06b	13.14a	10.74ab	14.00a	0.420	0.000
TC	3.25	3.33	3.60	3.91	3.44	3.48	0.193	0.957
TG	0.47	0.43	0.45	0.36	0.43	0.35	0.022	0.574
UA	0.34a	0.32ab	0.25abc	0.21c	0.25bc	0.19c	0.015	0.013
Day 42								
BUN	0.33	0.29	0.28	0.36	0.45	0.27	0.025	0.312
Ca	2.57	2.53	2.53	2.55	2.43	2.57	0.052	0.973
P	2.33	2.57	2.20	2.46	2.40	2.31	0.055	0.505
GLU	5.71c	6.81bc	8.56ab	10.33a	8.89ab	10.06a	0.472	0.004
TC	3.44	4.07	3.31	2.99	3.33	3.41	0.122	0.282
TG	0.56a	0.50a	0.45ab	0.51ab	0.47ab	0.40b	0.016	0.045
UA	0.28	0.31	0.26	0.24	0.24	0.27	0.061	0.862

NC - negative control; PC - positive control; BUN - blood urea nitrogen; GLU - glucose; TC - total cholesterol; TG - triglyceride; UA - uric acid.
a,b,c - Means within the same row with different letters are significantly different (P<0.05).
[1] SEM - pooled standard error of the mean (n = 8 birds for each replicate).

phosphorus, total cholesterol, and uric acid in the serum of chicks fed different treatments at day 42 (P>0.05). However, the glucose content of the serum of chicks treated with *L. reuteri* pLEM4157 (cel) or *L. reuteri* pLEM4159-cel/phy was greater than of chicks in the negative and positive control groups (P<0.05). The glucose content of the serum of chicks treated with *L. reuteri* pLEM4156 or *L. reuteri* pLEM4158 (phy) was higher than of chicks in the negative control group (P<0.05). Supplementation with *L. reuteri* pLEM4159-cel/phy was found to decrease the triglyceride content of serum in chicks at day 42 (P<0.05).

Discussion

The small intestine is the main site of nutrient absorption, with intestinal morphology, structure, and function determining the rate of nutrient absorption (Xu et al., 2003). Villus height and width, crypt depth, villus height:crypt depth ratio (VH:CD), and mucosal/muscle layer thickness in the small intestine are all important factors affecting the rate of nutrient absorption. Villus height and width determine the contact area with nutrients, with thinner, shorter villi normally resulting in reduced absorption capacity (Santin et al., 2001; Montagne et al., 2003).

Zhang et al. (2005) previously reported that administration of *Saccharomyces cerevisiae* to the diets of broilers can increase their VH:CD ratio. Lei et al. (2015) reported that birds fed *Bacillus amyloliquefaciens* showed significantly increased villus height, crypt depth, and VH:CD ratio of the small intestine on day 21. Al-Sultan et al. (2016) found that probiotics increased villus length and decreased crypt depth in the small intestine of broilers. Mathlouthi et al. (2002) reported that the addition of xylanase improved the morphology of the ileal villi, leading to increased VH:CD ratio. Wu et al. (2004) reported that supplementation with microbial phytase increased villus height in the duodenum and reduced the number of goblet cells present in the jejunum, with xylanase supplementation causing an increase in goblet cell numbers in the duodenum and decreased crypt depths in the jejunum. Administration of both enzymes increased villus height in the ileum and crypt depths in the jejunum and ileum.

Jaroni et al. (1999) reported that feeding chickens a wheat diet resulted in the formation of short villi in the jejunum that were thick and atrophic. Conversely, Iji et al. (2001) reported that high wheat diets had no effect on the small intestine morphology of broilers. In the present study, the addition of *L. reuteri* pLEM4159-cel/phy was found to increase villus height in the duodenum of chickens at days 21 and 42, whereas the administration of *L. reuteri* pLEM4158 (phy), *L. reuteri* pLEM4159-cel/phy, or *L. reuteri* pLEM4157 (cel) resulted in decreased crypt depths in their duodenums and jejunums at day 21. The use of these probiotics also led to decreased ileum crypt depths and increased duodenal villus heights at day 42. *L. reuteri* pLEM4158 (phy) or *L. reuteri* pLEM4159-cel/phy supplementation led to improved villus height in the ileum of chickens at day 42.

The administration of exogenous enzymes is known to result in changes in bacterial activity in the GIT that can lead to changes in the morphology and composition of microflora (Attia et al., 2014). These exogenous enzymes produce monosaccharides and oligosaccharides in the intestine that are known to promote the growth of beneficial bacteria that can improve animal growth (Galdeano et al., 2007).

In the current study, supplementation of a barley-wheat-based diet with transformed *L. reuteri* strains was found to significantly increase calcium, phosphorus, and glucose levels and decrease uric acid levels in serum at day 21, whilst further increasing glucose levels and decreasing the triglyceride content at day 42.

It has been suggested that transformed *L. reuteri* can secrete phytase, endoglucanase, and organic acids to influence the electrolyte (Ca, P), glucose, and lipid composition of the blood of the host. Phytase can directly hydrolyze the phosphate groups of phytate, whilst cellulolytic enzymes hydrolyze oligosaccharides to decrease chymous viscosity (Attia et al., 2014). The action of these digestive enzymes can combine to facilitate increased host digestive enzyme activity. The presence of organic

acids in the diet results in a lower pH environment in the gut, which can result in decreased stability and increased solubility of chelating phytate. However, Aureli et al. (2011) reported that supplementation of phosphorus-deficient diets with microbial phytase had no significant effect on serum calcium and glucose, although a significant increase in the blood phosphorus concentration and a decrease in blood uric acid levels was observed. Kies et al. (2005) found that supplementation with phytase resulted in a significant increase in blood glucose and phosphorus levels, with no effect on blood calcium levels in piglets.

However, Mondal et al. (2007) showed that phytase supplementation of a low-phosphorus diet could increase blood phosphorus and calcium concentration levels in broilers, with no changes observed in their blood glucose and cholesterol levels. Kashani et al. (2014) reported that supplementation of low-protein diets with phytase resulted in increased total cholesterol and triglycerides levels in the serum of laying hens. Liu et al. (2009) reported that supplementation of a low-phosphorus diet with phytase increased total blood cholesterol levels of broilers. Balamurugan and Chandrasekaran (2010) found that combined administration of non-starch polysaccharides hydrolyzing enzyme and phytase improved the blood glucose levels of broilers. Józefiak et al. (2010) reported that supplementation with multi-carbohydrase and phytase had no significant effect on the total cholesterol and glucose levels in the blood of broilers. Cowieson et al. (2013) observed that addition of microbial phytase and myo-inositol to wheat/corn-based diets resulted in increased blood glucose levels and decreased total blood cholesterol and triglyceride levels in broilers. Mansoub (2010) and Pourakbari et al. (2016) found that probiotic supplementation led to lower triglyceride and cholesterol concentrations in the serum of broilers. Kalavathy et al. (2003) also showed that a diet supplemented with *Lactobacillus* culture could significantly decrease the levels of total cholesterol and triglycerides in the blood of broilers.

In our study, no differences in carcass characteristics were observed in broilers in all of the treatment groups, which was in agreement with previous studies on the effects of yeast (Attia et al., 2014) and probiotics (Pourakbari et al., 2016) supplementation. The overall quality of broilers produced was directly related to their growth performance, especially their live weight. All treatment groups displayed a tendency towards slightly decreased abdominal fat content, consistent with lower triglyceride and cholesterol blood levels leading to reduced abdominal fat deposition (Anjum et al. 2005; Mehr et al., 2007).

Meat quality is related to pre- and post-slaughter practices, age, strain and sex of the bird, and the environment and nutrition that the bird is exposed to during rearing (Attia et al., 2014). Shear force and drip loss tests are important indicators used to estimate the texture, tenderness, water-holding capacity, and juiciness of chicken meat (Rasmussen and Andersson, 1996). The addition of *L. reuteri* to the diet resulted in improved intramuscular quality that led to significant decrease in drip and weight losses during cooking. Zhou et al. (2010) previously reported that supplementation with *Bacillus coagulans* ZjU0616 improved resistance to shear force in chicken breast. Mehr et al. (2007) observed that a high level of probiotic supplementation resulted in higher body and carcass weights and breast percentages in broilers. However, Zhang et al. (2005) reported that *S. cerevisiae* had no beneficial effects on shear force resistance in male broiler breast muscle.

Conclusions

This study showed that supplementation of broiler diets with transformed *L. reuteri* results in improved blood calcium and phosphorus levels, accompanied by better overall glucose metabolism and intestinal development. However, it revealed that short-term feeding with transformed *L. reuteri* does not improve carcass morphology or meat quality in broilers.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Data curation: Y. Feng. Methodology: X. Zhang. Writing-original draft: L. Wang. Writing-review & editing: G. Wu.

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