Effect of lactulose supplementation on growth performance, intestinal histomorphology, cecal microbial population, and short-chain fatty acid composition of broiler chickens

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ABSTRACT This study investigated the effects of dietary lactulose supplementation on broiler growth performance, intestinal histomorphology, cecal microflora, and cecal short-chain fatty acid (SCFA) concentrations. A total of 245 one-day-old male broiler chickens were randomly assigned to 5 different treatments, with 7 replicates including 7 birds each. The birds received the same basal diet based on corn-soybean meal, and lactulose was included in the diet at 0, 0.2, 0.4, 0.6, or 0.8% at the expense of corn and/or soybean meal. The body weight gain (linear, \( P = 0.027 \)) and feed conversion (linear, \( P = 0.003 \)) from 0 to 21 d showed significant improvement as dietary lactulose was increased from 0.2 to 0.8%. However, dietary lactulose did not affect broiler performance at the end of the experiment (42 d). Furthermore, intestinal measurements and the goblet cell count of broilers fed a lactulose-containing diet differed from those of birds fed a diet that did not contain lactulose. In addition, a significant quadratic response in the Lactobacillus count (\( P \leq 0.001 \)) was observed at 42 d on increasing the level of lactulose. The cecal coliform bacterial population was not affected by the dietary treatments. Supplementation with lactulose significantly increased the concentrations of acetate, propionate, butyrate, and total SCFA measured on d 7 and d 42. In conclusion, inclusion of lactulose in the diet can enhance broiler performance and intestinal morphology by selectively stimulating intestinal microflora and increasing cecal SCFA concentrations.

Key words: broiler, lactulose, intestinal histomorphology, cecal microflora, short-chain fatty acid

INTRODUCTION

Recent international legislation and the increasing concern of domestic consumers over the possibility of antibiotic residues in meat and other animal products have put restrictions on the use of growth-promoting antibiotics, and the availability of antibiotics for the treatment of bacterial infections. In addition, growing numbers of consumers have indicated their willingness to pay for antibiotic-free animal products (Ewing and Tucker, 2009; Huff et al., 2006). In this regard, the beneficial effects of feed additives, such as organic acids, enzymes, probiotics, prebiotics, synbiotics, immunity enhancers, highly available minerals, and phytogenics on gut health and subsequent poultry performance have been reported (Wiseman, 2012).

Prebiotics are defined as non-digestible food or feed ingredients that positively affect the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the intestine (Rehman et al., 2009). Due to the absence of suitable gastrointestinal enzymes, non-ruminants cannot digest prebiotics. However, these products are fermented by beneficial bacteria from genera such as Lactobacillus, Bifidobacterium, and Bacteroides. Thus prebiotics are thought to have the potential to modulate the composition of the microbial community in the gut (Ohimain and Ofongo, 2012). Previous studies have revealed that dietary prebiotics may increase the intestinal population of beneficial bacteria (Kim et al., 2011), alter cecal microbial activity (Rehman et al., 2008b), improve gut integrity (Baurhoo et al., 2009), and improve the digestibility of proteins and fats in a maize-soybean meal-based diet for broiler chickens (Alzueta et al., 2010). Thus, the health of the gastrointestinal tract plays a significant role in achieving optimum productivity and welfare in poultry production (Yegani and Korver, 2008).

Lactulose (\( \beta\)-D-galactopyranosyl-(1→4)-\( \beta\)-D-fructofuranose) is a non-digestible, synthetic disaccharide which is metabolized by gas-producing colonic microorganisms (Bird et al., 1990; Modler, 1994). Lactulose can be found in the prebiotic list defined by the Food and Agriculture Organization of the United

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Nations (Pineiro et al., 2008). The positive effect of lactulose on the intestinal microbiota of humans (Bouhnik et al., 2004), rats (Bovee-Oudenhoven et al., 1997), and pigs (Krueger et al., 2002) have been previously documented. Guerra-Ordaz et al. (2013) reported that the enhanced growth performance of piglets receiving 1% lactulose dietary supplementation might be related to increased feed intake (FI) and improved gut integrity. In addition, marked changes were observed in SCFA concentrations in the cecal digesta of rats (Zdunczyk et al., 2004) and pigs (Kamphues et al., 2007) fed with lactulose. Moreover, lactulose supplementation of the feed of weaned piglets that were orally challenged with Salmonella Typhimurium was found to lead to significant increases in humoral immune responses (Naqid et al., 2015). Only a limited number of studies however have evaluated the use of lactulose in broiler diets. Recently, Cho and Kim (2014) reported that 0.1 or 0.2% dietary lactulose supplementation can improve the growth performance and decrease the Escherichia coli, NH\textsubscript{3}, and H\textsubscript{2}S content of the excreta of 28-day-old broilers.

To the best of our knowledge, dietary prebiotics influence broiler growth performance and intestinal morphology by selectively stimulating the growth of health-promoting bacteria. However, until now, there has been little information on the effects of lactulose on broiler intestinal health parameters. Based on the previously reported favorable effects of prebiotics, the current study was designed to evaluate the effects of a graded concentration of lactulose on broiler growth performance, intestinal histomorphology, cecal bacterial population, and SCFA concentration during different periods of production.

**MATERIALS AND METHODS**

**Birds and Management**

Two hundred and forty-five 1-day-old male broiler chickens (Ross 308), with an average body weight of 41.02 ± 0.12 g, were obtained from a commercial hatchery (Beypiçi, Bolu, Turkey). The birds were randomly allocated to 5 experimental groups, with each group comprising 7 replicate pens (90 × 80 cm) containing 7 birds each. Birds were housed in a controlled environment for 42 d. The ambient temperature was thermostatically controlled and gradually decreased from 32 to 35°C on the first day, to 22°C when the broilers were 3-weeks-old. The temperature was maintained at 22°C thereafter. The relative humidity of the broiler house during the experiment was 50 ± 5%. The broiler house was artificially ventilated and a continuous light regimen was provided during the study. Chicks were vaccinated in the hatchery by a combination spray vaccine against the Newcastle disease virus (La Sota strain) and the infectious bronchitis virus. All experimental procedures were approved by the Animal Ethics Committee of Ankara University (2012-15-96).

**Table 1. Composition of basal diet.**

<table>
<thead>
<tr>
<th>Item</th>
<th>Starter 0 to 14 d</th>
<th>Grower 15 to 35 d</th>
<th>Finisher 36 to 42 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>46.60</td>
<td>49.00</td>
<td>53.60</td>
</tr>
<tr>
<td>Soybean meal (CP, 47%)</td>
<td>30.00</td>
<td>26.60</td>
<td>22.00</td>
</tr>
<tr>
<td>Soybean (Full fat)</td>
<td>15.00</td>
<td>15.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>4.00</td>
<td>5.50</td>
<td>5.50</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.90</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.35</td>
<td>2.20</td>
<td>2.20</td>
</tr>
<tr>
<td>Di-Metithionine (98%)</td>
<td>0.35</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>L-lysine-HCl (78%)</td>
<td>0.15</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.10</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Salt</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Vitamin premix(^2)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Mineral premix(^2)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

1. As-fed basis.
2. Provided per kilogram of complete diet: vitamin A, 12,000 IU; vitamin D\textsubscript{3}, 2,500 IU; vitamin E, 40 IU; vitamin K\textsubscript{3}, 5 mg; thiamin, 2.5 mg; riboflavin, 6 mg; pyridoxine, 5 mg; pantothenic acid, 15 mg; niacin, 25 mg; folic acid, 1 mg; biotin, 50 μg; vitamin B\textsubscript{12}, 20 μg.
3. Provided per kilogram of complete diet: Cu, 5 mg; I, 1 mg, Co, 200 μg; Se, 150 μg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg.

The starter, grower, and finisher diets were based on maize-soybean meal and were offered to the birds from 0 to 14, 15 to 35, and 36 to 42 d of age, respectively (Table 1). All diets were formulated to meet or exceed NRC (1994) and Aviagen (2009) nutrient recommendations. Each pen was equipped with a manual plastic feeder and an automatic nipple drinker. Water and the experimental diet (in mash form) were provided ad libitum throughout the experimental period. The lactulose product (Lactusat, Milei GmbH, Leutkirch, Germany) contained lactulose (42%), lactose (7%), galactose (3%), epigalactose (2%), and fructose (<1%) according to the manufacturer’s data sheet. The detailed ingredient composition of the product was reported by Fleige et al. (2007). Lactusat was added to the diet at a 0.48, 0.95, 1.43 or 1.90% concentration to provide 0.2, 0.4, 0.6, and 0.8% lactulose (dietary treatments: L2, L4, L6, and L8, respectively) at the expense of corn and/or soybean meal. Lactulose levels were selected based on previous prebiotic trials, such as those of isomalto-oligosaccharide (Zhang et al., 2003), fructo-oligosaccharide (FOS) (Xu et al., 2003), and inulin (Alzueta et al., 2010; Rebole et al., 2010), reporting on broiler growth performance and gut health.

All chicks were individually weighed and FI was recorded at weekly intervals. Body weight gain (BWG), FI, and the feed conversion ratio (FCR) were subsequently calculated based on performance values. The European production efficiency factor (EPEF)
was calculated using the following formula: 
\[
\text{livelability}\% \times \frac{\text{live weight (kg)} \times \text{age (d)} / \text{FCR} \times 100}{100}
\]
(Awad et al., 2009).

**Sampling Procedures**

At 7, 21, and 42 d of age, one bird from each replicate was selected according to the average body weight of each treatment group. Birds were euthanized by exsanguination and the intestinal tract was immediately removed. Tissue samples were obtained from the jejunum and ileum for histomorphological analysis. To ensure the uniformity of the samples, approximately 1 cm long mucosal segments of the jejunum and ileum were excised, 8 cm proximal to Meckel’s diverticulum (jejunum) and 8 cm proximal to the ileoceleal junction (ileum). The tissue samples were then flushed with saline solution to remove adherent intestinal contents and fixed in 10% neutral buffered formalin solution for 24 h. Cecal digesta were collected for SCFA and branched-chain fatty acid (BCFA) analyses. Samples were stored at −20°C until further analysis. In addition, at 21 and 42 d of age, 1 g of cecal content from each selected bird was collected in a sterile tube for bacterial enumeration.

**Histomorphological Measurements**

Tissue samples in the formalin solution were dehydrated in graded ethanol solutions, cleared with xylol, and then embedded in paraffin. The intestinal segments were sectioned at a thickness of 5 μm with a microtome. Cross sections were prepared and stained with Mallory’s triple stain, as modified by Crossman, in order to determine the jejunal and ileal morphometry (Culling et al., 1985). Villus height was measured from the top of the villus to the crypt mouth, and crypt depth was defined as the depth of the invagination between adjacent crypt mouths. Villus width was measured at the bottom of the villus. Villus surface area was calculated according to the following geometric formula: 
\[
2\pi \times \left(\frac{\text{villus width}}{2}\right) \times \left(\text{villus height}\right)
\]
(Sakamoto et al., 2000). Goblet cells were analyzed by staining with combined Alcian Blue (AB) and periodic acid Schiff (PAS) reagent. Cells were identified as follows: acid mucin was stained by AB (blue), neutral mucin was stained by PAS (pink), and intermediate mucin, which includes both acid and neutral mucins, was stained by AB and PAS (purple) (Geier et al., 2011). All positive cells along the villus were counted, regardless of the mucin type.

A total of 10 well-oriented villi and crypts were randomly selected for histological measurements. Histological sections were examined under a light microscope (Leica DM 2500, Leica Microsystems GmbH, Wetzlar, Germany) and photographed with a digital microscope camera (Leica DFC450, Leica Microsystems GmbH, Wetzlar, Germany). The images were evaluated using the ImageJ software (US National Institutes of Health, Bethesda, MD).

**Determination of Cecal Bacterial Populations**

One gram of fresh cecal digesta was transferred to 9 mL sterile physiological saline solution and homogenized. Inoculants were serially diluted up to 10⁻⁸. Subsequently, dilutions of 10⁻⁶, 10⁻⁷, and 10⁻⁸ were inoculated (100 μL of each dilution) onto appropriate selective agar media to determine total aerobe, coliform, and *Lactobacillus* counts. Nutrient agar (105450, Merck, Darmstadt, Germany) was used to determine the total number of aerobic bacteria, and MacConkey agar (105465, Merck, Darmstadt, Germany) and MRS agar (110660, Merck, Darmstadt, Germany) were used to enumerate coliform and *Lactobacillus* bacteria, respectively. All dilutions were inoculated onto selective agar in triplicate. Bacterial colonies were counted and averaged. Data have been expressed as log₁₀ colony-forming units/g cecal digesta.

**SCFA and BCFA Analyses**

Frozen cecal digesta (0.5 g at d 7 and 2 g at both d 21 and d 42) were thawed at 4°C and diluted 4-fold with double-distilled water in sterile screw-cap tubes. Cecal digesta were homogenized and centrifuged at 4,000 × g for 15 min at 4°C. One milliliter of supernatant was then transferred to an Eppendorf tube and mixed with 0.2 mL ice-cold 25% metaphosphoric acid solution. The tubes were placed in an ice bath for 30 min and samples were then centrifuged at 11,000 × g for 10 min at 4°C. Supernatants were analyzed using a gas chromatograph (Shimadzu GC-2010, Shimadzu Co., Kyoto, Japan) coupled with a 30 m × 0.53 mm internal diameter column (Teknokroma TRB-FFAP, Teknokroma, Barcelona, Spain) and flame ionization detector to determine SCFA and BCFA concentrations in cecal digesta (Rebole et al., 2010; Zhang et al., 2003). The injector-port and flame ionization detector temperatures were fixed at 230°C and 250°C, respectively. In the temperature program, the initial temperature was held at 120°C for 4 min after injection and then increased at 4°C/min to 160°C, where it was held for 4 min. Helium was used as the carrier gas. The injection volume was set at 1 μL and analyses were performed in duplicate. Total SCFA represents the sum of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate derivatives. BCFA is the total isobutyrate, valerate, and isovalerate content (Zdunczyk et al., 2013).

**Statistical Analysis**

Data were analyzed as a completely randomized block design, with 5 dietary treatments and 7 replicates, using the ANOVA procedure of the SPSS software,
Table 2. Effects of graded dietary supplementation of lactulose on body weight gain, feed intake, and feed conversion ratio of broiler chickens.1

<table>
<thead>
<tr>
<th>Item4</th>
<th>Dietary treatment2</th>
<th>P-value3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>L2</td>
</tr>
<tr>
<td>0 to 14 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWG (g)</td>
<td>371.23</td>
<td>377.02</td>
</tr>
<tr>
<td>FI (g)</td>
<td>477.98</td>
<td>471.96</td>
</tr>
<tr>
<td>FCR</td>
<td>1.29</td>
<td>1.25</td>
</tr>
<tr>
<td>0 to 21 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWG (g)</td>
<td>839.76</td>
<td>831.52</td>
</tr>
<tr>
<td>FI (g)</td>
<td>1,214.17</td>
<td>1,150.87</td>
</tr>
<tr>
<td>FCR</td>
<td>1.45</td>
<td>1.39</td>
</tr>
<tr>
<td>22 to 42 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BWG (g)</td>
<td>2,159.12</td>
<td>2,201.27</td>
</tr>
<tr>
<td>FI (g)</td>
<td>3,491.98</td>
<td>3,511.06</td>
</tr>
<tr>
<td>FCR</td>
<td>1.62</td>
<td>1.60</td>
</tr>
<tr>
<td>0 to 42 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW (g)</td>
<td>2,998.88</td>
<td>3,032.79</td>
</tr>
<tr>
<td>FI (g)</td>
<td>4,706.15</td>
<td>4,661.93</td>
</tr>
<tr>
<td>FCR</td>
<td>1.57</td>
<td>1.54</td>
</tr>
<tr>
<td>EPEF5</td>
<td>4.42</td>
<td>4.66</td>
</tr>
<tr>
<td>Mortality,%</td>
<td>4.08</td>
<td>2.04</td>
</tr>
</tbody>
</table>

1Data represent mean values of 7 replicates per treatment.
2Control = corn–soybean meal basal diet (free of lactulose); L2 = basal diet containing 0.2% lactulose; L4 = basal diet containing 0.4% lactulose; L6 = basal diet containing 0.6% lactulose; L8 = basal diet containing 0.8% lactulose.
3Polynomial contrasts: L = linear, Q = quadratic, and C = cubic effect of supplemental lactulose.
4BWG = body weight gain; BW = body weight; FI = feed intake; FCR = feed conversion ratio.
5EPEF = European Production Efficiency Factor [Liveability (%) × live weight (kg) × age (d)/FCR × 100].

version 14.01 (SPSS Inc., Chicago, IL). The effect of graded levels of dietary lactulose on different variables was analyzed using polynomial contrasts. Mortality rates were compared using a chi-square test. Statistical differences were considered significant at $P \leq 0.05$.

RESULTS

Growth Performance

The effect of graded levels of lactulose on broiler performance is presented in Table 2. Birds supplemented with an increasing level of lactulose showed increase in BWG from d 0 to 14 and d 0 to 21 (linear, $P = 0.002$ and $P = 0.027$, respectively) and improved FCR (linear, $P = 0.002$ and $P = 0.003$, respectively). However, dietary lactulose did not affect the BWG, FI, FCR, or EPEF measured at the end of the experiment (d 42). No significant mortality was observed during the entire experimental period. Furthermore, the dry-matter content of excreta was not altered by the lactulose treatment (data not shown).

Morphological Measurements of the Jejunum and Ileum

Jejunum. Morphological measurements of the jejunum are shown in Table 3. Villus height (linear, $P = 0.021$; quadratic, $P = 0.014$) and villus surface area (linear, $P = 0.011$; quadratic, $P = 0.041$) had increased with increase in the level of dietary lactulose on d 7. There were no significant differences in crypt depth, villus height:crypt depth (VH:CD) ratio, or villus width at d 7 for birds fed different levels of lactulose. Significant quadratic responses in crypt depth ($P = 0.006$) and the VH:CD ratio ($P = 0.003$) were observed with the increasing level of lactulose at d 21. Villus width ($P = 0.011$) and villus surface area ($P = 0.027$) had linearly increased with the increasing level of lactulose on d 21. Villus height at d 21 and d 42 was not affected by dietary lactulose treatment. Significant quadratic responses in crypt depth ($P \leq 0.001$) and the VH:CD ratio ($P \leq 0.001$) were observed with the increasing level of lactulose at d 42. Villus width ($P \leq 0.001$) and villus surface area ($P = 0.003$) had increased linearly with the increasing level of lactulose on d 42.

Ileum. Morphological measurements of the ileum are shown in Table 4. At d 7, the villus surface area had increased with the increasing dietary lactulose level (quadratic, $P = 0.041$). In comparison to the control diet, supplementation with lactulose had no significant effect on villus height, crypt depth, or the VH:CD ratio at d 7. Significant linear responses in villus width ($P = 0.002$) and villus surface area ($P = 0.011$) were observed with the increasing level of lactulose at d 21. There were no significant differences between the control and treatment groups regarding villus height, crypt depth, or the VH:CD ratio at d 21. Specifically, polynomial contrasts analysis revealed that crypt depth was affected both linearly ($P \leq 0.001$) and quadratically ($P \leq 0.001$) at d 42.
by the increased lactulose level. In addition, the VH:CD ratio was linearly affected \((P = 0.003)\) in response to the increased lactulose level at d 42. There were no significant differences between the control and treatment groups regarding villus height, width, or surface area at d 42.

**Goblet Cells**

Goblet cell counts for the jejunum and ileum are shown in Table 5. Polynomial contrasts analysis revealed that goblet cells of the jejunum \((d 21, P = 0.002)\) and ileum \((d 7 \text{ and } d 21, P \leq 0.001 \text{ and } P = 0.006,\)
respectively) exhibited a linear increase in response with the increase in the level of dietary lactulose. However, a significant quadratic response in the ileal goblet cell number \((P \leq 0.001)\) was observed at d 42. In comparison with the control diet, the addition of lactulose had no significant effect on the goblet cell number measured in the jejunum at d 7 or d 42.

**Cecal Microflora**

The microflora composition of the cecum at d 21 and d 42 is shown in Table 6. With the increase in dietary lactulose, the total aerobe count exhibited a cubic response \((P = 0.036)\) and the coliform count exhibited a quadratic response \((P = 0.024)\) at d 21. However, the *Lactobacillus* count was not affected by lactulose treatment at d 21. The total aerobe count in the cecum of 42-day-old broilers showed a linear \((P = 0.002)\) and quadratic \((P = 0.031)\) pattern of increase with increase in the level of lactulose supplementation. Furthermore, a significant quadratic response in the *Lactobacillus* count \((P \leq 0.001)\) was observed with the increase in the level of lactulose at d 42. In addition, the cecal coliform bacterial population was not affected by the dietary treatment at d 42.

**Cecal Concentrations of SCFA and BCFA**

The SCFA and BCFA concentrations in cecal digesta (μmol/g) are shown in Table 7. At d 7, concentrations of acetate \((P = 0.003)\), propionate \((P = 0.050)\), butyrate \((P = 0.002)\), and total SCFA \((P = 0.002)\) linearly increased in birds fed diets supplemented with increasing levels of lactulose. With the increase in dietary lactulose, the cecal isovalerate concentration exhibited both quadratic \((P = 0.010)\) and cubic \((P = 0.012)\) responses at d 21. There were no apparent differences in cecal acetate, propionate, butyrate, total SCFA, or BCFA concentrations on d 21. On d 42, birds fed a diet supplemented with increasing levels of lactulose exhibited an increase (quadratic, \(P = 0.007\)) in cecal acetate concentration. A linear increase \((P \leq 0.001)\) in cecal propionate, valerate, isovalerate, and BCFA concentrations was observed on d 42. In addition, both linear and quadratic increases in cecal butyrate (linear, \(P = 0.020\); quadratic, \(P = 0.027\)) and total SCFA (linear, \(P = 0.002\); quadratic, \(P = 0.027\)) concentrations were observed on d 42.

**DISCUSSION**

Lactulose is a synthetic disaccharide derived from lactose by a chemical reaction and has long been used in clinical settings as a laxative (Tuohy et al., 2002). However, at low doses, lactulose acts as a prebiotic that stimulates intestinal microflora and alters microbial end products (Schumann, 2002; Tuohy et al., 2002). As a new and promising prebiotic, the effect of lactulose on broiler intestinal health has not previously been reported.

In the present study, supplementation with an increasing level of lactulose resulted in a linear improvement in BWG and the FCR from d 0 to 21. Our results are in agreement with those of Cho and Kim (2014), who reported that supplementation with 0.2% lactulose resulted in an improvement in FCR from d 0 to 28. A previous study on piglets reported that those receiving 1% lactulose in the diet showed a better growth performance than those not receiving the prebiotic (Guerra-Ordaz et al., 2013). Furthermore, dietary lactulose addition increased average daily weight gain after an oral enterotoxigenic *E. coli* K88 challenge in piglets (Guerra-Ordaz et al., 2014). The exact mechanism(s) underlying the growth-promoting effects of prebiotics is still unclear but might be attributable to the ability of prebiotics to induce beneficial changes in intestinal microflora and intestinal integrity (Alzueva et al., 2010; Mookiah et al., 2014). Therefore, it is not surprising that, in the present study, the growth performance was improved by lactulose supplementation from d 0 to 21. However, this effect of lactulose on broiler performance was not apparent at the end of the experiment (d 42). Previous studies have revealed that increased prebiotic
levels have a detrimental effect on broiler performance and intestinal microflora. Xu et al. (2003) demonstrated that feeding 8 g/kg FOS resulted in poorer broiler performance than feeding 2 and 4 g/kg FOS-supplemented diets. Biggs et al. (2007) suggested that inclusion of an oligosaccharide at 8 g/kg probably approaches the highest tolerable level for broilers. According to our results, inclusion of lactulose at up to 0.8% in the broiler diet is well tolerated and had no detrimental effect on broiler performance. The observed improvement in broiler performance tended to be associated with broiler intestinal integrity.
As is already established, morphological changes in the small intestine, such as increased villus height, villus width, and VH:CD ratio, can have beneficial effects on the performance of birds. These changes enhance the absorptive surface area, which is important when alternative growth stimulators are applied. However, shorter villus length has been associated with the presence of toxins (Awad et al., 2006), and deeper crypts can be regarded as reflecting a higher demand for new epithelial cells, which influence the differentiation and proliferation of enterocytes.

In addition to its effect on intestinal architecture, dietary lactulose supplementation may have a pronounced effect on goblet cell count in the jejunum and ileum. The mucus layer that is synthesized and secreted by goblet cells protects the brush border area, which is the first line of defense against attack by enteric pathogens, decreasing their adherence to the intestinal mucosa. It also provides protection against bacterial and environmental toxins and against other dietary components that may damage the mucosa. The mucus layer also has a function as a digestion and absorption assisting medium (Baurhoo et al., 2009; Cheled-Shoval et al., 2014; Solis De Los Santos et al., 2007). Our results revealed that dietary supplementation with lactulose resulted in an increase in the goblet cell number in the jejunum and the ileum. In agreement with the current study, the number of goblet cells was previously found to increase in birds fed with diets containing 0.2% (Baurhoo et al., 2007), 0.5% (Baurhoo et al., 2009), and 0.05–0.1% (Solis De Los Santos et al., 2007) mannan-oligosaccharide products. Smirnov et al. (2005) concluded that *Lactobacillus* and *Bifidobacterium* species could increase the synthesis and secretion of mucins in the intestine of chickens. The observed increase in goblet cell numbers due to lactulose supplementation can be attributed to beneficial bacterial growth that influences mucin dynamics (Cheled-Shoval et al., 2014). To the best of our knowledge, the histomorphological results of this study provide new insights regarding the potential prebiotic effects of lactulose in broilers.

It is widely accepted that the intestinal microflora and their metabolic activity have significant effects on broiler health and performance. Previous studies have shown that gastrointestinal enzymatic activity, ileal villus height (Xu et al., 2003), and cecal SCFA levels were increased (Mookiah et al., 2014; Rehman et al., 2008b) and pathogenic bacterial count in the cecum was significantly decreased (Kim et al., 2011) by the stimulatory effect of several prebiotics on intestinal beneficial bacterial growth. However, published data on the response of intestinal bacteria to dietary lactulose are very limited and contradictory. As described in a previous study, dietary lactulose supplementation influenced *Lactobacillus* and *Bifidobacterium* species in piglets (Guerra-Ordaz et al., 2013). In contrast, studies on dietary lactulose supplementation by other researchers suggested that there was no pronounced effect on the population of lactic acid bacteria in the piglet hindgut (Kamphues et al., 2007; Martin-Pelaez et al., 2010). Recently, Cho and Kim (2014) revealed that the numbers of *Lactobacilli* in the excreta increased and of *E. coli* decreased in birds fed diets containing 0.2% lactulose, compared to those for control birds, at d 28 (P < 0.05). Studies conducted with different prebiotics, such as inulin, isomalt-oligosaccharide, and FOS, have suggested that the intestinal *Lactobacillus* count of birds on a supplemented diet was higher than that of birds on the same diet without prebiotics (Li et al., 2008; Mookiah et al., 2014; Rebole et al., 2010). In agreement with these previous studies, dietary lactulose was found to increase the cecal *Lactobacillus* population at d 42 in the current study. Further research using molecular techniques could reveal more details regarding cecal microflora.

In poultry, the cecum harbors a wide variety of microbiota and within the gastrointestinal tract, stands out as the most stable environment for bacterial growth (Mead, 1989; Meimandipour et al., 2010). Bacterial fermentation in the cecum leads to the formation of SCFAs, which are necessary for intestinal functionality and the integrity of the intestine (Meimandipour et al., 2010). In addition, these fermentation by-products contribute to broiler energy metabolism and lower the pH of the intestinal environment, which may limit the growth of bacterial pathogens (van Der Wielen et al., 2000). Meimandipour et al. (2010) suggested that lactate, produced by *Lactobacillus* species in the cecal digesta, promotes the growth of butyrate-producing bacteria, which notably increases the cecal butyrate concentration. Such positive effects of lactulose as a prebiotic on the content of cecal digesta were also reported by other researchers in which cases the broiler chicks were fed with other prebiotic sources such as inulin (Rebole
et al., 2010; Rehman et al., 2008b). Among the SCFAs, butyric acid stands out as a preferred energy source for enterocytes and takes part in cellular differentiation and proliferation within the intestinal mucosa (Rinttilä and Apajalaiti, 2013). Similarly, the results of the current study clearly suggest that the addition of lactulose to the diet improves intestinal histomorphology in correlation with the increased levels of cecal butyrate. The butyrogenic effect of different prebiotics in the broiler cecum has been previously reported (Rehman et al., 2008a). A previous study in pigs revealed that the inclusion of lactulose in the diet increased the SCFA concentration in the large intestine (Kamphues et al., 2007). However, Martin-Pelaez et al. (2010) found that dietary 1% lactulose supplementation did not affect cecal butyric acid concentration in piglets that were orally challenged with Salmonella. In the present study, increased SCFA concentrations were observed in broiler chickens provided with lactulose supplementation, which corresponded to an increased beneficial bacterial population.

Contrary to our expectations, cecal BCFA concentrations linearly increased compared to those for the control group at the end of the experiment (d 42) in the present study. Researchers have reported that the detection of BCFAs in the colonic or cecal content is an indicator of protein fermentation (Cummings et al., 1987; Macfarlane et al., 1992; Zdunczyk et al., 2013). A linear relationship has also been noted between the branched chain ratio1 and the cecal NH3 concentration (Guo et al., 2003). Recently, it has been shown that the dietary inclusion of 1% lactulose did not change the BCFA level in piglets (Guerra-Ordaz et al., 2013). The exact mechanism underlying the increase in cecal BCFA concentration in the present study is unclear, although it might be due to the whey protein (30% CP) in the commercial lactulose product. In this regard, the increase in BCFA concentration might be eliminated by the use of protein-free lactulose products.

In conclusion, supplementation of the diet with lactulose can enhance broiler performance and intestinal morphology by selectively stimulating intestinal microflora and increasing cecal SCFA concentrations.

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