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Effects of lactulose on growth, carcass characteristics, faecal microbiota, and blood constituents in broilers

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ABSTRACT: This study was conducted to determine the effect of supplementing diets with lactulose on growth performance, carcass characteristics, faecal microbiota, and blood constituents. A total of 324 one-day-old Ross 308 mixed-sex broiler chicks with an average initial body weight of 38 g were used in a 35-day growth assay. There were 18 birds/pen and six pens/treatment with food and water available *ad libitum*. Treatments consisted of a corn-soybean-meal-based diet with 0, 0.25 and 0.5% of lactulose. The results indicated that body weight gain (BWG) was improved (linear effect, $P < 0.05$) by increasing the concentration of lactulose in the diet from zero to 0.5% while the feed conversion ratio (FCR) decreased (linear effect, $P < 0.05$) for Days 8 to 21, 21 to 35, and overall (Day 0 to 35). Chickens fed the diet supplemented with 0.5% lactulose showed a higher relative weight of breast meat compared to other groups. Inclusion of lactulose decreased the count of *Salmonella* and *E. coli* in excreta of chickens fed diets containing 0.25 or 0.5% lactulose ($P < 0.05$), but the count of *Lactobacillus* was not affected. Drip loss percentage was decreased ($P < 0.05$) on Day 1 by addition of 0.5% lactulose, but there was no effect on meat colour. Blood characteristics were not influenced. Thus, it was concluded that inclusion of lactulose improves growth performance and alters excreta microbial populations with no adverse effect on broilers.

Keywords: broiler; carcass characteristics; excreta microbiota; growth performance; lactulose

Concern about antibiotics as growth promoters has led to a desire to identify alternatives. Prebiotics, probiotics, and synbiotics are being increasingly adopted as promoters of growth and gut health in poultry and swine (Kleesen et al. 2001; Patterson and Burkholder 2003; Higgins et al. 2008; Markovic et al. 2009; Zhang et al. 2013; Zhang and Kim 2013; Zhao et al. 2013). Huang et al. (2004) reported that supplementing diets with a probiotic (*Lactobacillus acidophilus* and *Lactobacillus casei*) may enhance the development and function of immune cells in calves.

Prebiotics are feed additives which may stimulate the growth and activity of beneficial microorganisms such as *Bifidobacteria* and *Lactobacillus* in the gut (Cummings and MacFarlane 2002). Steiner (2006) reported that prebiotics may improve performance and nutrient digestibility by creating suitable conditions for beneficial microorganisms. Kermanshahi and Rostami (2006) reported that using dried whey as a substrate for acidophilic flora (e.g. *Lactobacilli*) as prebiotics or fermentable sugars improved the useful microbial population of the gastrointestinal tract. The results of other studies have demonstrated that supplementing diets with probiotics can improve the broiler growth performance and may enhance the activity of digestive enzymes such as proteases, lipases, and amylases resulting in better nutrient utilisation and consequently, improved growth performance (Fuller 2001).

Several carbohydrates including NSPs, resistant starch and non-digestible oligosaccharides that may be fermented by intestinal microorganisms can be classified as prebiotics (Bauer et al. 2006). Lactulose (4-*O*-β-D-galactopyranosyl-D-fructose) is a synthetic disaccharide that is classified as a non-digestible carbohydrate (Bird et al. 1990). However, it can be metabolised in the colon by saccharolytic microbiota. A benefit of using non-

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digestible carbohydrates is stimulation of growth of *Lactobacilli* in the gut and eventual fermentation by *Lactobacilli, Bifidobacteria,* and other bacteria species in the hind-gut (Mitsuoka et al. 1987; Fleige et al. 2007). Experiments with pigs indicate that lactulose can indeed not be digested and absorbed in the small intestine and that it passes to the large intestine where microorganisms use it to produce acetic and lactic acid. Consequently, lactulose can stimulate the growth of *Lactobacillus* and *Bifidobacterium* and reduce the activity of proteolytic bacteria (Gibson 2004; Marinho et al. 2007). The ingestion of lactulose has been reported to exert beneficial effects by increasing probiotic bacteria and putrefactive bacteria and significantly reducing potential pathogens and consequently reducing the activity of pro-carcinogenic enzymes (e.g. azoreductase, 7-alpha-dehydroxylase) in humans, mice, rats, sows and pigs (Ballongue et al. 1997; Bianchi et al. 1997; Krueger et al. 2002). It was hypothesised that through its prebiotic effects, dietary lactulose will improve the performance of broiler chickens. Thus, the objective of the current study was to determine the effect of supplementing broiler diets with lactulose on growth performance, carcass characteristics, excreta microbiota, and blood constituents.

MATERIAL AND METHODS

Animals, diets, and facilities. Use and management of the broiler chickens used in this experiment were approved by the Animal Care and Use Committee of Dankook University. In this study 324 one-day-old Ross 308 broiler chicks (mixed gender) with an average initial BW of 37.8 g were used in a 35 days growth assay. Chicks were sorted into pens with 18 birds/pen and six pens/treatment. Treatments consisted of a corn-soybean-meal-based diet with 0, 0.25 or 0.5% of lactulose. Diets (Table 1) were fed in three phases with ME 3015, 3114, and 3180 Kcal/kg, Met + Cys 1.06, 0.95, and 0.93%, and P 0.53, 0.50, and 0.50% for phase 1 (Day 0 to 7), phase 2 (Day 7 to 21), and phase 3 (Day 21 to 35), respectively. All other nutrients met or exceeded nutrient concentrations recommended by the National Research Council (NRC 1994). Birds were housed in battery cages (1.75 × 1.55 m/pen) in an environmentally controlled room (temperature started at 32 °C and was reduced by 2 °C every week down to 24 °C, and 65% relative humidity) and were allowed free access to feed and water during the experiment. Relative breast meat, abdominal fat and organ weights were described as a percentage of live body weight.

Table 1. Composition of diets in percent, (as-fed basis)

Item (%)	Phase 1 Day 0–7	Phase 2 Day 7–21	Phase 3 Day 21–35
Corn	45.49	36.74	40.85
Wheat	10.00	20.00	20.00
Soybean meal (CP 48%)	34.25	33.62	25.48
Corn gluten meal (CP 60%)	2.00	–	–
Rapeseed meal	–	–	3.50
Tallow	1.90	5.54	6.01
Soybean oil	1.50	–	–
Limestone	1.06	1.12	1.17
Dicalcium phosphate	2.23	1.90	1.84
Salt	0.35	0.32	0.29
DL-Methionine	0.46	0.39	0.41
L-Lysine-HCl	0.42	0.15	0.20
Threonine	0.17	0.09	0.12
Vitamin mix[1]	0.03	0.03	0.03
Vitamin E (10%)	0.04	–	–
Mineral mix[2]	0.10	0.10	0.10
Total	100	100	100
Calculated nutritional content			
ME (kcal/kg)	3015	3114	3180
Analysed nutritional content (%)			
CP	22.12	20.43	18.55
Lysine	1.45	1.22	1.10
Met + Cys	1.06	0.95	0.93
Ca	1.05	1.00	1.00
Available P	0.53	0.50	0.50
Crude Fat	5.55	7.27	7.96
Crude fibre	3.24	3.29	3.25

[1]Provided per kg of diet: 15 000 IU of vitamin A, 3750 IU of vitamin D_3, 37.5 mg of vitamin E, 2.55 mg of vitamin K_3, 3 mg of B_1, 7.5 mg of B_2, 4.5 mg of B_6, 24 μg of B_{12}, 51 mg of niacin, 1.5 mg of folic acid, 126 mg of biotin, and 13.5 mg of pantothenic acid

[2]Provided per kg of complete diet: 37.5 mg of Zn, 137.5 mg of Mn, 37.5 mg of Fe, 0.83 mg of I, 0.23 mg of Se, and 1408 mg of choline

Sampling and measurements. On Day 0, 7, 21, and 35, chickens and feeders were weighed to allow calculations of body weight gain (BWG), feed

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intake (FI) and feed conversion ratio (FCR). Upon completion of the growth assay, 36 chickens (two/pen) were selected randomly and blood samples were collected from a wing vein into K_3EDTA vacuum tubes (Becton Dickinson Vacutainer Systems, Fraklin Lake, NJ). The samples were centrifuged at 3000 × *g* for 15 min to recover plasma. Whole blood cell counts [white blood cells (WBC), red blood cells (RBC), and lymphocytes] were determined using an automatic blood analyser (ADVIA 120, Bayer, NY).

The chickens used for blood samples were weighed and slaughtered so that the breast meat, abdominal fat, gizzard, liver, spleen, bursa of Fabricius, and heart could be removed by trained personnel. All samples were patted dry to remove excess moisture and were weighed. Hunter *L** (lightness), *a** (redness), and *b** (yellowness) of breast meat were measured using a Minolta CR410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). Drip loss percentage was determined on Day 1, 3, 5, and 7 by using the procedure described by Honikel (1998). Before slaughter six birds from each treatment group (one bird per pen) were chosen randomly and by massaging their abdominal area, excreta samples were collected from the cloacae into microtubes. One gram of the excreta sample from each pen was diluted with 9 ml of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenised. Counts of viable bacteria in excreta samples were determined by plating 10-fold serial dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and *Lactobacilli* medium agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate *E. coli* and *Lactobacillus*, respectively. The *Lactobacilli* medium agar plates were then incubated for 48 h at 39 °C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37 °C. The *E. coli* and *Lactobacillus* colonies were counted immediately after removal from the incubator. For *Salmonella*, the serially diluted peptone broth tubes were incubated overnight at 37 °C, after which 1 ml was transferred to 9 ml of tetratinate broth (Neogen Corporation, Lansing, MI) which was then incubated for 48 h at 42 °C. From these tubes, 1 ml was used to inoculate 9 ml of Rappaport-Vassiliadis *Salmonella* Enrichment broth (Neogen Corporation, Lansing, MI) which was then incubated for 48 h at 42 °C. The Rappaport was used to inoculate XLT4 plates for *Salmonella* isolation, and *Salmonella* were identified using LIS (VIDAS Listeria) and TSI (Triple Sugar Iron) agar tubes (Difco Laboratories, Detroit, MI).

Table 2. Effect of dietary lactulose supplementation on growth performance in broiler chickens

Item	Lactulose (%)			SE	*P*-value	
	0	0.25	0.5		linear	quadratic
Day 1 to 7						
BWG (g)	101	102	105	3	0.392	0.795
FI (g)	131	133	135	3	0.350	0.936
F : G	1.30	1.30	1.29	0.03	0.968	0.869
Day 7 to 21						
BWG (g)	586	617	638	14	0.018	0.777
FI (g)	894	899	911	15	0.443	0.848
F : G	1.53	1.46	1.43	0.03	0.016	0.515
Day 21 to 35						
BWG (g)	1,028	1,046	1,100	18	0.012	0.439
FI (g)	1,764	1,788	1,800	17	0.164	0.552
F : G	1.72	1.69	1.64	0.03	0.046	0.622
Overall						
BWG (g)	1,715	1,765	1,843	25	0.002	0.664
FI (g)	2,789	2,820	2,846	25	0.134	0.589
F : G	1.63	1.58	1.55	0.02	0.003	0.903

SE = standard error, BWG = gain in BW per bird, FI = feed intake per bird

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Table 3. Effect of dietary lactulose supplementation on relative weight of breast meat, abdominal fat, and organs in broilers

Item[1] (% of live body weight)	Lactulose (%)			SE	*P*-value	
	0	0.25	0.5		linear	quadratic
Breast muscle	14.52	17	17.28	0.49	0.001	0.095
Abdominal fat	1.49	1.18	1.21	0.11	0.079	0.217
Gizzard	1.19	1.17	1.17	0.06	0.853	0.881
Heart	0.48	0.49	0.48	0.03	0.967	0.869
Liver	2.09	2.04	2.04	0.17	0.818	0.900
Spleen	0.09	0.07	0.09	0.01	0.922	0.115
Bursa of Fabricius	0.12	0.13	0.13	0.02	0.868	0.924

SE = standard error

Statistical analysis. Data were analysed by ANOVA using the GLM procedure of SAS (SAS Institute 1996), with the pen being defined as the experimental unit. The linear and quadratic effect of lactulose among treatments was analysed by using a polynomial regression to describe the shape of the response to increasing concentrations of lactulose in the diet.

RESULTS

Growth performance

The results of growth performance presented in Table 2 indicate that from Day 1 to Day 7, gain in body weight (BWG), feed intake (FI), and feed conversion ratio (FCR) were not affected by treatments. From Day 7 to Day 21, BWG increased (P = 0.018) and FCR decreased (P = 0.016) linearly in response to increasing concentrations of lactulose. From Day 21 to Day 35 there was a linear increase (P = 0.012) in BWG and decrease (P = 0.046) in FCR, and overall BWG increased (P = 0.002) and FCR decreased (P = 0.003) linearly in response to increasing concentrations of lactulose in the diets. FI was not affected from Day 0 to 7, Day 7 to 21, Day 21 to 35, or for the overall experimental period.

Carcass characteristics

There was a linear (P = 0.001) increase in breast muscle weight and a trend for a linear (P = 0.079) decrease in abdominal fat by in response to increasing dietary levels of lactulose (Table 3). Relative weights of organs were not affected by treatments. Supplementation of diets with 0.5% lactulose linearly decreased (P = 0.002) drip loss percentage on Day 1 and tended (linear, P < 0.10) to reduce

Table 4. Effect of dietary lactulose supplementation on colour and drip loss of breast meat

Item	Lactulose (%)			SE	*P*-value	
	0	0.25	0.5		linear	quadratic
Breast meat colour						
Lightness (L^*)	57.14	55.40	55.54	1.22	0.370	0.544
Redness (a^*)	14.84	15.45	15.58	0.53	0.341	0.721
Yellowness (b^*)	11.28	10.40	11.81	1.01	0.716	0.368
Drip loss (%)						
Day 1	3.98	2.94	2.35	0.30	0.002	0.559
Day 3	6.32	5.59	5.21	0.40	0.070	0.717
Day 5	9.35	8.81	8.10	0.47	0.080	0.873
Day 7	11.08	11.08	10.41	0.51	0.375	0.604

SE = standard error

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Table 5. Effect of dietary lactulose supplementation on excreta microbiota in broiler chickens

Item (log_{10}cfu/g)	Lactulose (%)			SE	*P*-value	
	0	0.25	0.5		linear	quadratic
Lactobacillus	7.65	7.77	7.83	0.11	0.263	0.829
E. coli	6.57	6.39	6.40	0.05	0.027	0.143
Salmonella	2.73	2.50	2.47	0.04	0.001	0.106

SE = standard error

drip loss on Day 3. These results demonstrate that addition of lactulose to the diets did not have any significant impact on breast meat colour (Table 4).

Excreta microbiota

Data presented in Table 5 indicate that there was a linear decrease in the count of excreta *E. coli* (P = 0.027) and *Salmonella* (P = 0.001). The count of *Lactobacillus* was not affected by experimental diets.

Blood constituents

Counts of blood cells and haptoglobin concentrations in blood were not affected by treatments but RBC counts increased (linear effect, P = 0.164) with increasing lactulose concentrations in the diet.

DISCUSSION

Several researchers have reported that supplementing diets with prebiotics may improve growth performance and possibly decrease mortality in chickens, pigs, and calves (Fairchild et al. 2001; Hooge et al. 2004; Fleige et al. 2007; Cho and Kim 2014). Our results are in agreement with these previous studies and indicate that supplementing diets with 0.5% lactulose improves the growth performance of broiler chickens.

Previous research demonstrated that prebiotics can modulate the gut environment by increasing the number of beneficial microorganisms and inhibiting the proliferation of pathogens in the intestine (Patterson and Burkholder 2003; Higgins et al. 2008). Prebiotics in the diet reduced the population of *Salmonella* in the intestine of chickens (Bailey et al. 1991; Pascual et al. 1999; Stern at al. 2001) and prebiotics supported competitive exclusion and immune modulation (Jin et al. 1997; Simon et al. 2001). Our observations that feeding broiler chickens diets supplemented with lactulose increased excreta *Lactobacilli* whereas the counts of *E.coli* and *Salmonella* were reduced support the argument that lactulose acts as a prebiotic in chickens and this may partly explain the improved growth performance observed for the birds fed treatment diets.

Heckert et al. (2002) suggested measuring the weight of immune organs as a method for estimating the immune status of chickens. The bursa of Fabricius and spleen are the main lymphoid organs in broilers but these authors concluded that inclusion of prebiotics did not increase the relative weight of these organs. In the current study the lymphocyte counts were not influenced by dietary treatment, which is consistent with the lack of an effect on the relative weights of the spleen and bursa of Fabricius. These findings are consistent with the results reported by Mohebbifar et al. (2013).

In conclusion, supplementing diets with lactulose improved the growth performance and altered the

Table 6. Effect of dietary lactulose supplementation on blood constituents in broilers

Item	Lactulose (%)			SE	*P*-value	
	0	0.25	0.5		linear	quadratic
WBC (10^3/µl)	451.7	410.5	536.3	82.5	0.479	0.421
RBC (10^6/µl)	2.29	2.73	2.82	0.3	0.164	0.572
Lymphocyte (%)	77.4	57.3	68.5	13.8	0.656	0.371
Haptoglobin (mg/dl)	16	17	18	0.9	0.094	0.565

SE = standard error, VBC = white blood cells, RBC = red blood cells

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excreta microbiota by increasing *Lactobacilli* and decreasing *E. coli* and *Salmonella* counts. However, breast meat colour, relative weights of organs and blood constituents were not affected.

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