

# Effects of dietary probiotic and synbiotic supplementation on broiler growth performance and intestinal histomorphology

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**Summary:** The effects of *Paenibacillus xylanexedens*, alone or in combination with inulin and lactulose were evaluated on growth performance and intestinal histomorphology of male broilers. A total of 168 one-day-old male broiler chickens were randomly allocated to 4 experimental groups as follows: C, basal diet (control; without supplementation); P,  $1 \times 10^9$  cfu/kg-feed *P. xylanexedens*; IN,  $1 \times 10^9$  cfu/kg-feed *P. xylanexedens* and 1% inulin; LAC,  $1 \times 10^9$  cfu/kg-feed *P. xylanexedens* and 0.5% lactulose. Addition of *P. xylanexedens* and synbiotics significantly increased broiler body weight gain ( $P < 0.001$ ) and feed conversion ratio ( $P = 0.023$ ) from 1 to 21 day of age. Moreover, a significant increase in body weight gain was observed when birds were fed with synbiotic supplemented diets during the overall experimental period ( $P = 0.013$ ). The addition of probiotic and synbiotics increased ( $P = 0.047$ ) villus height in ileum on day 42. Our results indicate that *P. xylanexedens* can be used as a probiotic feed additive in broiler diets and can improve broiler performance and intestinal histomorphology.

Keywords: Broiler, intestinal histomorphology, *Paenibacillus xylanexedens*, performance, probiotic.

## Broyler rasyonlarında probiyotik ve sinbiyotik kullanılmasıın broyler performans ve bağırsak histomorfolojisi üzerine etkisi

**Özet:** *Paenibacillus xylanexedens*'in tek başına veya inulin veya laktuloz ile birlikte kullanılmasının erkek broylerlerin büyüme performansı ve bağırsak histomorfolojisi üzerine olan etkisi incelendi. Toplamda 168 adet günlük erkek civciv dört deneme grubuna ayrıldı: C, bazal rasyon (kontrol); P,  $1 \times 10^9$  cfu/kg-yem *P. xylanexedens*; IN,  $1 \times 10^9$  cfu/kg-yem *P. xylanexedens* ve %1 inulin; LAC,  $1 \times 10^9$  cfu/kg-yem *P. xylanexedens* ve %0.5 laktuloz. *P. xylanexedens* ve sinbiyotik ilavesinin 1-21. günlerde broyler canlı ağırlık artışı ( $P < 0.001$ ) ve yemden yararlanma oranını ( $P = 0.023$ ) önemli düzeyde arttırdığı sonucuna varıldı. Tüm araştırma periyodu dikkate alındığında sinbiyotik ilavesinin canlı ağırlık artışı önemli ölçüde ( $P = 0.013$ ) etkilediği görüldü. Araştırmanın 42. gününde probiyotik ve sinbiyotik ilave edilen gruplarda ileum villus yüksekliğinin daha fazla olduğu tespit edildi ( $P = 0.047$ ). Sonuç olarak, *P. xylanexedens*'in broyler büyüme performansını ve bağırsak histomorfolojisini olumlu yönde etkilediği ve rasyonlarında probiyotik yem katkı maddesi olarak kullanılabilceği görüldü.

Anahtar sözcükler: Bağırsak histomorfolojisi, broyler, *Paenibacillus xylanexedens*, performans, probiyotik.

## Introduction

Recent international legislations and the increasing concern of domestic consumers over the possibility of antibiotic residues in meat and other animal products have put restrictions on the usage of growth-promoting antibiotics and the availability of antibiotics for the treatment of bacterial infections (21). Ban on the prophylactic use of in-feed antibiotics has encouraged the researchers to search for new and safer alternatives (3, 23). In this sense, supplementation of probiotic microorganisms and prebiotic oligosaccharides as an alternative to growth-promoting antibiotics has received increasing interest due to their promising beneficial effects on the broiler performance and intestinal health (28).

Probiotics influence the host organisms' health by maintaining the normal intestinal microbiota, preventing the growth of pathogenic microorganisms, promoting digestion and intake of feed, and inducing the immune system (24, 27). Dietary probiotic supplementation has remarkable effects on broiler performance (16, 28, 29, 30), gastro-intestinal mucosa integrity (6, 35), composition of the gut microflora (16, 28, 37), and intestinal immunity (8). Spore forming probiotic bacteria's, such as *Bacillus* species, has received much more attention than their non-spore-forming counterparts and this particular advantage making them suitable for use as feed supplements in broiler diets (12, 20). *Paenibacillus* is a genus of facultative anaerobic, endospore-forming bacteria, which

previously distinguished from the other *Bacillus* groups by comparative 16S rRNA sequence analysis (4).

Synbiotics are defined as combination of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of newly added strains in the intestine by activating the metabolism of health-promoting bacteria and/or selectively stimulating their growth (19). Synbiotics encourage the growth of the probiotic organism by providing the specific substrate to the probiotic organism for its fermentation (17). Beneficial effects of synbiotics, when compared with probiotics alone, on broiler growth performance, intestinal microflora population, cecal volatile fatty acid concentration and intestinal histomorphological parameters have been reported previously (6, 28).

To the best of our knowledge, dietary probiotics and synbiotics influence broiler growth performance and intestinal morphology by selectively stimulating the growth of health-promoting bacteria. However, until now, there has been no information on the effects of *P. xylanexedens* on broiler intestinal health parameters. Based on the previously reported favorable effects of probiotics and synbiotics, the current study was designed to evaluate the effect of *P. xylanexedens* and prebiotic combinations on broiler growth performance and intestinal histomorphology during different periods of production.

## Materials and Methods

**Birds and management:** A total of 168 one-day-old male broiler chickens (Ross 308), with an average body weight (BW) of  $41.12 \pm 0.55$  g, were obtained from a commercial hatchery (Beypiliç, Bolu, Turkey). Birds were weighed and randomly allocated to 4 experimental groups with 7 replicate pens (90 x 80 cm) containing 6 birds each. Birds were housed in an environmentally controlled room for 42 days. The ambient temperature was thermostatically controlled and gradually decreased from 32–35°C on the first day to 22°C at 3 weeks of age; the temperature was maintained at 22°C thereafter. All experimental procedures were approved by Ankara University, Ethical Committee for Experimental Research on Animals (2013-3-12).

The starter, grower, and finisher diets were based on maize-soybean meal and were offered to the birds from 1-14, 15-35, and 36-42 days of age, respectively (Table 1). All diets were formulated to meet or exceed NRC (32) nutrient recommendations. Each pen was equipped with manual plastic feeders and automatic nipple drinkers. Water and experimental diets (in mash form) were provided *ad libitum*. Dietary treatments were as follows: C, basal diet (control; without supplementation); P,  $1 \times 10^9$  cfu/kg-feed *P. xylanexedens*; IN,  $1 \times 10^9$  cfu/kg-feed *P.*

*xylanexedens* and 1% inulin (Beneo, Orafit IPS, Oreye, Belgium); LAC,  $1 \times 10^9$  cfu/kg-feed *P. xylanexedens* and 0.5% lactulose (Duphalac, Abbott Biologicals B.V. Olst, Netherland). Approximately 7.5 mL/kg feed lactulose syrup equivalent to 0.5% lactulose.

Table 1. Composition of basal diet.

Tablo 1. Bazal rasyonun kompozisyonu.

Item	Starter	Grower	Finisher
	0 to 14 d	15 to 35 d	36 to 42 d
Ingredient, %			
Corn	39.98	47.05	45.00
Soybean meal, (CP 47%)	30.00	24.00	24.65
Soybean (Full fat)	13.00	13.00	8.50
Wheat	10.00	8.00	13.30
Vegetable oil	2.50	3.90	5.00
Dicalcium phosphate	2.25	2.00	1.85
Limestone	0.86	0.80	0.77
DL-Methionine (98%)	0.40	0.31	0.18
L-Lysine-HCl (78%)	0.26	0.19	0.00
L-Threonine	0.15	0.15	0.15
Salt	0.35	0.35	0.35
Mineral premix <sup>1</sup>	0.10	0.10	0.10
Vitamin premix <sup>2</sup>	0.10	0.10	0.10
Anticoccidial	0.05	0.05	0.05
Total	100.00	100.00	100.00
Chemical composition, calculated			
CP, %	23.00	20.60	19.40
ME, kcal/kg	3,012	3,176	3,227
Calcium, %	1.00	0.90	0.85
Available phosphorus, %	0.50	0.45	0.42
Methionine + cysteine, %	1.10	0.95	0.80
Lysine, %	1.44	1.24	1.01
Threonine, %	0.99	0.91	0.86
Analyzed composition			
CP, %	23.12	20.65	19.50
ME, kcal/kg	3,008	3,180	3,223
Ca, %	1.03	1.00	0.90
Total P, %	0.77	0.67	0.63

<sup>1</sup> Provided per kilogram of complete diet: vitamin A, 12,000 IU; vitamin D3, 2,500 IU; vitamin E, 40 IU; vitamin K3, 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 B12, 20 µg.

<sup>2</sup> 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.

<sup>1</sup> Rasyonun her kg'ında: vitamin A, 12,000 IU; vitamin D3, 2,500 IU; vitamin E, 40 IU; vitamin K3, 5 mg; tiamin, 2.5 mg; riboflavin, 6 mg; piridoksin, 5 mg; pantotenik asit, 15 mg; niasin, 25 mg; folik asit 1 mg; biyotin, 50 µg; vitamin B12, 20 µg.

<sup>2</sup> Rasyonun her kg'ında: Cu, 5 mg; I, 1 mg, Co, 200 µg; Se, 150 µg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg.

**Probiotic strain:** *Paenibacillus xylanexedens* strain was isolated from chyme samples of cattles and were collected within sterile tubes. Isolation procedure was adapted from Barbosa et al. (9) The chyme samples were suspended in 1:1 phosphate-buffered saline (PBS) solution and spore forming isolates were then selected by ethanol treatment. For ethanol treatment 50% (v/v) ethanol was added to suspension and incubated at room temperature for one hour. Then 10-fold serial dilutions up to  $10^{-5}$  were made and plated on LB agar. Colonies with distinct morphologies were picked and purified by streaking on LB agar plates. Cultures were incubated at 37°C for colony formation and then isolates stored at -80°C in 30% glycerol.

Further identification of the isolate was carried out by 16S rRNA sequencing method. In 16S rRNA gene sequencing, approximately 1400 bp was analyzed by Bioedit software and compared with BLAST data from National Center for Biotechnology.

After isolation, resistance to simulated gastric fluids (13), tolerance to bile salt (13), sporulation efficiency (9), spore formation (9), adhesion and invasion properties (34) were tested and the results were given in Table 2. Antimicrobial activities (26) of isolate against each representative strain (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) were evaluated and the results were presented in Table 3.

Table 2. Characterization of *P. xylanexedens*.  
Tablo 2. *P. xylanexedens*'in özellikleri.

<i>Paenibacillus xylanexedens</i>	
Sporulation efficiency (%)	81
Survival in simulated gastric fluid (vegetative form)	+
Survival in bile salt (vegetative form)	+
Adhesion (%)	<b>2.6</b>
Invasion (%)	<b>0</b>

Table 3. Antimicrobial activity of isolate *P. xylanexedens*.  
Tablo 3. *P. xylanexedens* izolatlarının antimikrobiyel özellikleri.

Isolate	Pathogenic strains and diameters of inhibition zones (cm)			
	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumoniae</i>
<i>P. xylanexedens</i>	2	2	2	2

**Preparation of *Paenibacillus xylanexedens* as feed additive:** Cultures were grown in 50 mL DSM at 37°C, 250 rpm for 24 h and centrifuged at 3.000 rpm for 5 min. Harvested spores were then inoculated in DSM and

incubated at 37°C and 250 rpm for 48 h. After the incubation period, spores were centrifuged at 6.000 rpm for 30 min; spore pellets were washed with 20 mL sterile distilled water and re-suspended in 5 mL sterile water. Spores of the isolate were then mixed with 10% skim milk for further lyophilization. Lyophilized cultured were then mixed with a composition of  $1 \times 10^9$  viable spores (cfu) of *Paenibacillus xylanexedens* (approximately 0.6% of net weight) as active substance and with calcium carbonate (approximately 99% of net weight product) and corn flour (approximately 0.4% of net weight product) as carriers according to the product specifications of a commercial feed additive.

**Growth performance:** All chicks were weighed individually and feed intake (FI) was recorded at weekly intervals. Body weight gain (BWG), FI and feed conversion ratio (FCR) were subsequently calculated based on the performance values.

**Sampling procedures:** At 21 and 42 days of age one bird from each replicate was selected according to the average body weight (BW) of each treatment group. Birds were slaughtered by exsanguination and the intestinal tract was removed immediately. The tissue samples for histomorphological analysis were taken from the jejunum and ileum. To ensure the uniformity of samples, approximately 2 cm length of the mucosal segments of jejunum and ileum was excised as follows: 8 cm proximal to Meckel's diverticulum (jejunum), and 8 cm proximal to the ileo-cecal junction (ileum), respectively.

**Histomorphologic measurements:** Tissue samples were fixed in 10% neutral buffered formaline for 24 h and washed with tap water subsequently dehydrated in graded ethanol solutions, cleared with xylol and embedded in paraffin, respectively. Intestinal segments were sectioned at the thickness of 5  $\mu$ m with microtome. Cross sections were prepared and stained with Mallory's triple stain modified by Crossman in order to determine the intestinal morphometry (15). Villus height was measured from the top of the villus to crypt mouth and crypt depth was defined as distance between basements of the crypt-to-crypt mouth. Histological sections were examined under light microscope (Leica DM 2500, Leica Microsystems GmbH, Wetzlar, Germany) and photographed with Leica DFC450 (Leica Microsystems, Heerbrug, Germany) digital microscope camera. The images were evaluated using ImageJ software (Image J, US National Institutes of Health, Bethesda, MD, USA).

**Statistical analysis:** Data were analyzed as a completely randomized block design with 4 dietary treatments and 7 replicates using the ANOVA procedure of the SPSS version 14.01 (SPSS Inc., Chicago, IL, USA). Significant differences among treatment groups were tested by Duncan's multiple range tests. Statistical differences were considered significant at  $P < 0.05$ .

Table 4. Effects of dietary *P. xylanexedens* and synbiotic supplementations on broiler performance<sup>1</sup>  
 Tablo 4. Rasyonlara *P. xylanexedens* ve sinbiyotik ilavesinin broiler performansını üzerine etkisi.

Item <sup>3</sup>	Dietary treatment <sup>2</sup>				Statistics	
	C	P	IN	LAC	SEM	P-value
BW, g						
14	325.38	324.90	352.76	334.28	4.38	0.076
21	648.10 <sup>c</sup>	700.03 <sup>b</sup>	748.34 <sup>a</sup>	741.00 <sup>ab</sup>	10.47	0.000
42	2594.71 <sup>b</sup>	2683.43 <sup>ab</sup>	2791.71 <sup>a</sup>	2804.43 <sup>a</sup>	27.65	0.013
BWG, g						
1-14	284.26	283.90	311.48	293.22	4.36	0.078
1-21	606.98 <sup>c</sup>	659.03 <sup>b</sup>	707.06 <sup>a</sup>	699.93 <sup>ab</sup>	10.45	0.000
1-42	2553.59 <sup>b</sup>	2642.43 <sup>ab</sup>	2750.43 <sup>a</sup>	2763.36 <sup>a</sup>	27.63	0.013
FI, g						
1-14	406.86	398.33	432.97	409.72	5.77	0.176
1-21	952.35	942.41	997.65	997.40	11.19	0.161
1-42	4342.81	4325.99	4381.98	4520.80	35.50	0.201
FCR						
1-14	1.43	1.41	1.40	1.40	0.01	0.862
1-21	1.57 <sup>a</sup>	1.43 <sup>b</sup>	1.41 <sup>b</sup>	1.42 <sup>b</sup>	0.02	0.023
1-42	1.70 <sup>a</sup>	1.64 <sup>ab</sup>	1.60 <sup>b</sup>	1.64 <sup>ab</sup>	0.01	0.048
Mortality, %	2.38	0	2.38	2.38	-	0.797

<sup>a-c</sup>Means with different superscripts in the same row are significantly different ( $P < 0.05$ ).

<sup>1</sup>Data represent mean values of 7 replicates per treatment.

<sup>2</sup>C: basal diet (control; without supplementation); P:  $1 \times 10^9$  cfu/kg feed *P. xylanexedens*; IN:  $1 \times 10^9$  cfu/kg feed *P. xylanexedens* and 1% inulin; LAC:  $1 \times 10^9$  cfu/kg feed *P. xylanexedens* and 0.5% lactulose.

<sup>3</sup>BW: body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio.

<sup>a-c</sup>Aynı satırda farklı harf taşıyan ortalama değerler arasındaki farklılıklar istatistiksel olarak önemlidir ( $P < 0.05$ ).

<sup>1</sup>Değerler her bir gruptan seçilen 7 broylerin ortalamasını göstermektedir.

<sup>2</sup>C: bazal rasyon (kontrol; ilave katkı yok); P:  $1 \times 10^9$  cfu/kg yem *P. xylanexedens*; IN:  $1 \times 10^9$  cfu/kg yem *P. xylanexedens* ve %1 inulin; LAC:  $1 \times 10^9$  cfu/kg yem *P. xylanexedens* ve %0.5 laktuloz.

<sup>3</sup>BW: canlı ağırlık; BWG: canlı ağırlık artışı; FI: yem tüketimi; FCR: yemden yararlanma oranı.

Table 5. Effects of dietary *P. xylanexedens* and synbiotic supplementations on histomorphological parameters of the jejunum and ileum<sup>1</sup>  
 Tablo 5. Rasyonlara *P. xylanexedens* ve sinbiyotik ilavesinin jejunum ve ileum histomorfolojisi üzerine etkisi.

Item	Dietary treatment <sup>2</sup>				Statistics	
	C	P	IN	LAC	SEM	P-value
Jejunum						
d 21						
Villus height ( $\mu\text{m}$ )	1061.00	1034.36	1032.79	1081.79	30.42	0.939
Crypt depth ( $\mu\text{m}$ )	184.50	187.43	171.79	195.64	4.41	0.296
VH:CD ratio <sup>3</sup>	5.81	5.54	6.00	5.65	0.17	0.817
d 42						
Villus height ( $\mu\text{m}$ )	1446.40	1540.33	1486.33	1444.30	30.48	0.677
Crypt depth ( $\mu\text{m}$ )	194.60	209.58	215.83	196.20	4.30	0.235
VH:CD ratio	7.47	7.44	6.90	7.37	0.17	0.630
Ileum						
d 21						
Villus height ( $\mu\text{m}$ )	617.71 <sup>ab</sup>	569.50 <sup>b</sup>	523.36 <sup>b</sup>	735.86 <sup>a</sup>	23.00	0.003
Crypt depth ( $\mu\text{m}$ )	174.00 <sup>ab</sup>	163.43 <sup>ab</sup>	148.00 <sup>b</sup>	186.50 <sup>a</sup>	5.18	0.048
VH:CD ratio	3.59	3.51	3.60	4.01	0.12	0.496
d 42						
Villus height ( $\mu\text{m}$ )	1019.60 <sup>b</sup>	1240.36 <sup>a</sup>	1225.60 <sup>a</sup>	1231.33 <sup>a</sup>	32.53	0.047
Crypt depth ( $\mu\text{m}$ )	187.00	190.50	179.70	165.50	4.03	0.100
VH:CD ratio	5.46 <sup>b</sup>	6.58 <sup>ab</sup>	6.86 <sup>a</sup>	7.53 <sup>a</sup>	0.24	0.021

<sup>a,b</sup>Means with different superscripts in the same row are significantly different ( $P < 0.05$ ).

<sup>1</sup>Data represent mean values of 7 replicates per treatment.

<sup>2</sup>C: basal diet (control; without supplementation); P:  $1 \times 10^9$  cfu/kg feed *P. xylanexedens*; IN:  $1 \times 10^9$  cfu/kg feed *P. xylanexedens* and 1% inulin; LAC:  $1 \times 10^9$  cfu/kg feed *P. xylanexedens* and 0.5% lactulose.

<sup>3</sup>Villus height to crypt depth ratio.

<sup>a,b</sup>Aynı satırda farklı harf taşıyan ortalama değerler arasındaki farklılıklar istatistiksel olarak önemlidir ( $P < 0.05$ ).

<sup>1</sup>Değerler her bir gruptan seçilen 7 broylerin ortalamasını göstermektedir.

<sup>2</sup>C: bazal rasyon (kontrol; ilave katkı yok); P:  $1 \times 10^9$  cfu/kg yem *P. xylanexedens*; IN:  $1 \times 10^9$  cfu/kg yem *P. xylanexedens* ve %1 inulin; LAC:  $1 \times 10^9$  cfu/kg yem *P. xylanexedens* ve %0.5 laktuloz.

<sup>3</sup>Villus yüksekliğinin kript derinliğine oranı.

## Results

**Growth performance:** The effect of dietary addition of *P. xylanexedens* and synbiotics on BW, BWG, FI, and FCR are shown in Table 4. Dietary treatments did not affect broiler performance in starter period (1-14 d); however, the addition of *P. xylanexedens* and synbiotics significantly increased ( $P < 0.001$ ) broiler BW, BWG and FCR ( $P = 0.023$ ) from 1 to 21 day of age. Moreover, a significant increase in BW ( $P = 0.013$ ) and BWG ( $P = 0.013$ ) were observed when birds fed with synbiotic-supplemented diets during the overall experimental period. However, FCR value was lower ( $P = 0.048$ ) for the birds fed with *P. xylanexedens* and inulin supplemented diet from 1 to 42 day of age. Feed intake was not significantly different among all dietary treatment groups from 1 to 14, 1 to 21 and 1 to 42 day of age. In addition, no significant mortality rate was observed during the entire experimental period.

**Morphological measurement of the jejunum and the ileum:** Morphological measurements of the jejunum and the ileum are shown in Table 5. Dietary treatments had no significant effect on villus height, crypt depth and villus height: crypt depth (VH:CD) ratio of the jejunum on day 21 and 42. However, ileum villus height ( $P = 0.003$ ) and crypt depth ( $P = 0.048$ ) were significantly increased for the birds fed with *P. xylanexedens* and lactulose supplemented diets on day 21. There were no significant differences among the control and treatment groups regarding the ileum VH:CD ratio on day 21. Moreover, ileum villus height was significantly higher ( $P = 0.047$ ) for the bird fed with probiotic and synbiotic supplemented diets in comparison to control. However, VH:CD ratio was consistently higher ( $P = 0.021$ ) for the birds fed with synbiotic supplemented diets on day 42.

## Discussion and Conclusion

*Bacillus* species are known to produce several extracellular enzymes including  $\alpha$ -amylases and cellulose, which increase nutrient digestibility and absorption. In addition, they stimulate growth and proliferation of beneficial facultative anaerobic bacteria, such as *Lactobacilli*, by creating an anaerobic environment within the gut, which can decrease pathogenic bacteria colonization and improve intestinal integrity (1, 22). Similar to *Bacillus* species, *P. xylanexedens* utilizes several nutrients including D-cellulose, D-fructose, D-galactose,  $\alpha$ -D-glucose, lactose, lactulose, maltose (31). Among the beneficial microorganisms, *Lactobacillus* and *Bacillus* strains receive much more attention than other probiotic strains in the broiler nutrition. However, so far, there are no reported studies evaluating the effect of *P. xylanexedens*, as a probiotic feed additive, on the broiler growth performance. Previous meta-analysis results have showed that the BWG and FCR were improved in birds

that received probiotics in compare to the basal diet without additives (10). Studies with inulin and lactulose also showed pronounced effect on broiler performance by selectively stimulating the beneficial microorganisms (11, 14, 33). The current study indicated that the addition of *P. xylanexedens* alone or in combination with inulin or lactulose significantly increased BWG and FCR of broilers during the first 21 day of age. In agreement with our results, dietary inclusion of *Bacillus* spp. improved the growth performance of broilers (1, 22, 25). However, inulin and lactulose supplemented diets were much more effective on broiler performance than those fed basal diet at the end of the experiment. Contrary to our findings, Mookiah et al. (28) have reported that improvements made by synbiotics were not significantly different from those made by probiotic or prebiotic alone at day 42. According to our results *P. xylanexedens* and prebiotic (especially inulin) combinations showed synergistic effect on broiler overall growth performance. This result is in accordance with the findings of Awad et al. (6) and Ghasemi et al. (18), who found a significant improvement in the broiler performance with the use of synbiotics in broiler diets. It seems that *P. xylanexedens*, as a new promising spore-forming probiotic, may be more efficient on growth performance on broilers when combined with inulin or lactulose. Improved performance of the birds could be related to the beneficial effects of synbiotics on the intestinal microflora and intestinal integrity.

It is widely accepted that the intestinal microbiota and its metabolic activity have significant effects on the broiler health and the performance. In addition there is a growing interest to use of different probiotics to improve intestinal integrity and functionality by selectively altering the intestinal microflora (7). The morphological changes in the small intestine, such as increasing villus height, width, and VH:CD ratio might have beneficial effects on bird's performance. These changes enhance the absorptive surface area, which is important when alternative growth stimulators are applied. However, shorter villus is associated with the presence of toxins (5) and deeper crypts can be regarded as a higher demand for new epithelial intestinal tissue (38). In the present study, ileal villus height was increased by dietary *P. xylanexedens* and synbiotics addition on day 42. However, villus height to crypt depth ratio of ileum was greater for synbiotic supplemented groups. Our results are consistent with Sen et al. (36) who reported increased villus height of ileum in birds fed with *Bacillus subtilis* LS 1-2 supplemented diets. In addition, dietary inclusion of *Bacillus subtilis* increased ileum villus height under thermoneutral and heat stress conditions (2). Moreover, dietary inclusion of several probiotics such as, *Enterococcus faecium* (35), *Lactobacillus salivarius* and *Lactobacillus reuteri* (7), improved broiler intestinal integrity. As reported by the

previous studies (7, 36) greater villus height and VH:CD ratio may be related to the ability of *P. xylanexedens* to create a more favorable intestinal microbial environment. We propose that addition of *P. xylanexedens* with inulin and lactulose much more effective on intestinal structure in male broilers.

In conclusion, *P. xylanexedens*, alone or in combination with inulin and lactulose, exerts beneficial effects on broiler performance and intestinal morphology. Further researches are needed to clarify the effect of between *P. xylanexedens* on intestinal microbiota and bacterial metabolites.

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### References

- Ahmed ST, Islam M, Mun HS, et al. (2014): *Effects of Bacillus amyloliquefaciens as a probiotic strain on growth performance, cecal microflora, and fecal noxious gas emissions of broiler chickens*. *Poult Sci*, **93**, 1963-1971.
- Al-Fataftah AR, Abdelqader A (2014): *Effects of dietary Bacillus subtilis on heat-stressed broilers performance, intestinal morphology and microflora composition*. *Anim Feed Sci Tech*, **198**, 279-285.
- Alzueta C, Rodriguez ML, Ortiz LT, et al. (2010): *Effects of inulin on growth performance, nutrient digestibility and metabolisable energy in broiler chickens*. *Br Poult Sci*, **51**, 393-398.
- Ash C, Priest FG, Collins MD (1993): *Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus Paenibacillus*. *A van Leeuw*, **64**, 253-260.
- Awad WA, Bohm J, Razzazi-Fazeli E, et al. (2006): *Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens*. *Poult Sci*, **85**, 974-979.
- Awad WA, Ghareeb K, Abdel-Raheem S, et al. (2009): *Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens*. *Poult Sci*, **88**, 49-56.
- Awad WA, Ghareeb K, Bohm J (2010): *Effect of addition of a probiotic micro-organism to broiler diet on intestinal mucosal architecture and electrophysiological parameters*. *J Anim Physiol Anim Nutr (Berl)*, **94**, 486-494.
- Bai SP, Wu AM, Ding XM, et al. (2013): *Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens*. *Poult Sci*, **92**, 663-670.
- Barbosa TM, Serra CR, La Ragione RM, et al. (2005): *Screening for bacillus isolates in the broiler gastrointestinal tract*. *Appl Environ Microbiol*, **71**, 968-978.
- Blajman JE, Frizzo LS, Zbrun MV, et al. (2014): *Probiotics and broiler growth performance: A meta-analysis of randomised controlled trials*. *Br Poult Sci*, **55**, 483-494.
- Calik A, Ergun A (2015): *Effect of lactulose supplementation on growth performance, intestinal histomorphology, cecal microbial population, and short-chain fatty acid composition of broiler chickens*. *Poult Sci*, **94**, 2173-2182.
- Cartman ST, La Ragione RM, Woodward MJ (2008): *Bacillus subtilis spores germinate in the chicken gastrointestinal tract*. *Appl Environ Microbiol*, **74**, 5254-5258.
- Chavarri M, Maranon I, Ares R, et al. (2010): *Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions*. *Int J Food Microbiol*, **142**, 185-189.
- Cho JH, Kim IH (2014): *Effects of lactulose supplementation on performance, blood profiles, excreta microbial shedding of Lactobacillus and Escherichia coli, relative organ weight and excreta noxious gas contents in broilers*. *J Anim Physiol Anim Nutr (Berl)*, **98**, 424-430.
- Culling CFA, Allison RT, Bar WT (1985): *Cellular Pathology Technique*, London: Butterworth and Co. Ltd.
- Deniz G, Orman A, Cetinkaya F, et al. (2011): *Effects of probiotic (Bacillus subtilis DSM 17299) supplementation on the caecal microflora and performance in broiler chickens*. *Rev Med Vet*, **11**, 538-545.
- Farnworth ER (2001): *Probiotics and prebiotics*. In: Wildman REC, editor. *Handbook of Nutraceuticals and Functional Foods*. Boca Raton, USA: CRC Press; 2001. pp. 407-422.
- Ghasemi HA, Kasani N, Taherpour K (2014): *Effects of black cumin seed (Nigella sativa L.), a probiotic, a prebiotic and a synbiotic on growth performance, immune response and blood characteristics of male broilers*. *Livest Sci*, **164**, 128-134.
- Gibson GR, Roberfroid MB (1995): *Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics*. *J Nutr*, **125**, 1401-1412.
- Hong HA, Duc le H, Cutting SM (2005): *The use of bacterial spore formers as probiotics*. *FEMS Microbiol Rev*, **29**, 813-835.
- Huff GR, Huff WE, Rath NC, et al. (2006): *Limited treatment with beta-1,3/1,6-glucan improves production values of broiler chickens challenged with Escherichia coli*. *Poult Sci*, **85**, 613-618.
- Jeong JS, Kim IH (2014): *Effect of Bacillus subtilis C-3102 spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers*. *Poult Sci*, **93**, 3097-3103.
- Kim GB, Seo YM, Kim CH, et al. (2011): *Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers*. *Poult Sci*, **90**, 75-82.
- Kim KM, Kim MJ, Kim DH, et al. (2009): *Characterization of Bacillus polyfermenticus KJS-2 as a probiotic*. *J Microbiol Biotechnol*, **19**, 1013-1018.
- Knarreborg A, Brockmann E, Høybye K, et al. (2008): *Bacillus subtilis (DSM17299) modulates the ileal microbial communities and improves growth performance in broilers*. *Int J Probiotics Prebiotics*, **3**, 83-86.
- Lertcanawanichakul M, Sawangnop S (2011): *A comparison of two methods used for measuring the*

- antagonistic activity of *Bacillus* species. *Walailak J Sci Tech*, **5**, 161-171.
27. **Lutful Kabir SM** (2009): *The role of probiotics in the poultry industry*. *Int J Mol Sci*, **10**, 3531-3546.
  28. **Mookiah S, Sieo CC, Ramasamy K, et al.** (2014): *Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens*. *J Sci Food Agric*, **94**, 341-348.
  29. **Mountzouris KC, Tsirtsikos P, Kalamara E, et al.** (2007): *Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities*. *Poult Sci*, **86**, 309-317.
  30. **Mountzouris KC, Tsirtsikos P, Palamidi I, et al.** (2010): *Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition*. *Poult Sci*, **89**, 58-67.
  31. **Nelson DM, Glawe AJ, Labeda DP, et al.** (2009): *Paenibacillus tundrae* sp. nov. and *Paenibacillus xylanexedens* sp. nov., psychrotolerant, xylan-degrading bacteria from Alaskan tundra. *Int J Syst Evol Micr*, **59**, 1708-1714.
  32. **NRC** (1994): *Nutrient Requirements of Poultry*. Washington, DC: Natl. Acad. Press.
  33. **Rebole A, Ortiz LT, Rodriguez ML, et al.** (2010): *Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat- and barley-based diet*. *Poult Sci*, **89**, 276-286.
  34. **Rowan NJ, Deans K, Anderson JG, et al.** (2001): *Putative virulence factor expression by clinical and food isolates of Bacillus spp. after growth in reconstituted infant milk formulae*. *Appl Environ Microbiol*, **67**, 3873-3881.
  35. **Samli HE, Senkoylu N, Koc F, et al.** (2007): *Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota*. *Arch Anim Nutr*, **61**, 42-49.
  36. **Sen S, Ingale SL, Kim YW, et al.** (2012): *Effect of supplementation of Bacillus subtilis LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology*. *Res Vet Sci*, **93**, 264-268.
  37. **Song J, Xiao K, Ke YL, et al.** (2014): *Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress*. *Poult Sci*, **93**, 581-588.
  38. **Yason CV, Summers BA, Schat KA** (1987): *Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: Pathology*. *Am J Vet Res*, **48**, 927-938.

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