Effects of prebiotics and probiotics on the performance and bacterial colonization of broiler chickens

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Abstract

A study was undertaken to evaluate the effects of Neoxyval (antimicrobial growth promoter (AGP)), TechnoMos (prebiotic), GalliPro (probiotic) and a mixture of TechnoMos and GalliPro (symbiotic) on growth performance, carcass yield, histomorphology and intestinal bacterial counts in broilers (0 d to 42 d). Two hundred day-old Ross (308) broilers were allocated to five experimental treatments: T1 = control (CONT), T2 = T1 + Neoxyval, T3 = T1 + Gallipro, T4 = T1+ TechnoMos and T5 = T1+ Gallipro+ TechnoMos. The results revealed that birds that received T2, T4 and T3 gained more weight and converted feed more efficiently than those in T1 and T5. Longer ileal villi (492.9 µm) were recorded in birds that received T4 compared with T1 (424.7 µm) and T2 (439.9 µm). Conversely, jejunal villi length and width were not influenced by treatment. T3 eliminated *Clostridium perfringens* from the ileum, but not from the caecum. Generally, birds that received T3 and T5 performed similar to the AGP group, T2. The results from this study indicated that the probiotic (T3) and prebiotic (T4) used in this trial could serve as alternatives to AGP (T2). Enhancement in the performance of broilers could be explained partially by improvement in intestinal morphology and microbial balance associated with modulation of intestinal microflora and inhibition of pathogens.

Keywords: Antimicrobial growth promoters (AGPs), broiler performance, prebiotic, probiotic, symbiotic
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Introduction

The use of antibiotics in poultry feed at sub-therapeutic level as an antimicrobial growth promoters (AGP) has been beneficial to growth performance and has reduced the populations in the gastrointestinal tract of potentially pathogenic organisms such as *Clostridium perfringens, Salmonella* and *Escherichia coli* and thus diseases associated with these pathogenic bacteria (Hume, 2011). AGPs have routinely been included in poultry feeds (Butaye *et al*., 2003). However, many antimicrobials given to animals are of the same class as those used to treat human infections, which lead to an increasing concern about cross-resistance and multiple antibiotic resistance in pathogenic bacteria. This would pose a risk to public health (Tollefson *et al*., 1998) with the concern that many antibiotics that are currently available for treating human diseases would not be effective in future (Casewell *et al*., 2003; Smith *et al*., 2003; Castanon, 2007). A parallel concern is that the growth of resistance among bacteria would outstrip the ability of the pharmaceutical industry to develop new antibacterial agents. Consequently, the European Union (EU) banned antibiotic use in animal production in 2006.

Current trends in poultry production point to a reduction in or total banning of the use of AGPs, and the need to increase the application of non-antibiotic feed additives that offer similar benefits, such as improving the growth of broilers and utilization of feed (Mountzouris *et al*., 2007). Several groups of additives are in use such as probiotics, prebiotics, acidifiers, antioxidants and phytogenes.

Probiotics have been reported to prevent pathogenic bacteria such as *C. perfringens* and *Salmonella* from colonizing the gut through the mechanism of competitive exclusion (Teo & Tan, 2006; Abudabos *et al*., 2013). Another possible alternative to AGPs in poultry diets is the use of prebiotics. 'Prebiotics' refers to oligosaccharides, which are not digested by animal enzymes, but can selectively stimulate certain intestinal bacteria species, which have potentially useful effects on the health of the host. Prebiotics have more advantages than probiotics. While probiotics are intended to bring beneficial microbes to the gut, prebiotics are thought to selectively stimulate the beneficial microbes that already live there (Yang *et al*., 2009). A third

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alternative to AGPs is symbiotics, which is a combination of probiotics and prebiotics promoting substances, which exhibits a combined effect on the health of the digestive tract, digestibility and the performance of broilers (Patterson & Burkholder, 2003). The objective of the current study was to examine the effects of prebiotics, probiotics and symbiotics on growth performance in broilers.

Materials and Methods

Ross 308 broiler chicks were obtained from a commercial hatchery. On arrival, chicks were grouped by weight to reduce variations in mean bodyweight. A total of 200 males and females were distributed among 40 experimental cages. The chicks were vaccinated against Marek's disease, Newcastle Disease and Infectious Bronchitis. Typical iso-energetic and iso-nitrogenous starter (0 - 14 d) and finisher (15 - 42 d) diets, based on maize-soybean meal were formulated in mash form and offered ad libitum (Table 1). At 0 d, birds received one of five dietary treatments: i) T1 = control; ii) T2 = control + 0.05 g Neoxyval/kg; iii) T 3 = control + 0.2 g GalliPro/kg; iv) T 4 = control + 0.75 g TechnoMos/kg and v) T 5 = control + 0.2 g GalliPro/kg + 0.6 g TechnoMos/kg (Table 2).

Table 1 Dietary composition of broiler chick starter and finisher diets

Ingredients	Treatment period (1 - 42) days	
	Starter	Finisher
Yellow maize	62.5	69.9
Soybean meal	31.0	26.7
Palm oil	2.19	2.80
Di-calcium phosphate	2.50	2.0
Ground limestone	0.73	0.59
Choline chloride	0.05	0.04
DL-methionine	0.26	0.20
L- lysine	0.18	0.24
Salt	0.25	0.25
Threonine	0.07	0.25
Vitamin, mineral premix[1]	0.20	0.12
Total	100	100
Calculated analysis		
ME, MJ/kg	12.56	12.98
Crude protein, g/kg	215	210
Non-phytate P, g/kg	5.0	4.0
Calcium, g/kg	10	9.0
Lysine, g/kg	12	11
Methionine, g/kg	5.5	4.7
Sulphur amino acids, g/kg	9.0	8.0
Threonine, g/kg	8.5	8.0

[1] Vitamin-mineral premix contents per kg: 2400000 IU vitamin A; 1000000 IU vitamin D; 16000 IU vitamin E; 800 mg vitamin K; 600 mg vitamin B_1; 1600 mg vitamin B_2; 1000 mg vitamin B_6; 6 mg vitamin B_{12}; 8000 mg niacin; 400 mg folic acid; 3000 mg pantothenic acid; 40 mg biotin; 3000 mg antioxidant; 80 mg cobalt; 2000 mg copper; 400 mg iodine; 1200 mg iron; 18000 mg manganese; 60 mg selenium; 14000 mg zinc.

The experiment was conducted in the environmentally controlled battery room at the Animal Production Department, College of Food and Agriculture Sciences, King Saud University, Saudi Arabia. The chicks were placed in a four-deck cage system and received the experimental diets in electrically heated battery brooders with raised wire floors. Broiler chicks were reared in cages under similar managerial and hygienic conditions with a 24 hour light programme.

Environmental temperature was set at 33 ºC at the beginning of the trial and decreased to 22 ºC until the end of the experiment. Ambient temperature and relative humidity were concurrently and continuously recorded at three-hour intervals using two data loggers (HOBO Pro Series Data Logger, Model H08-032- 08, Onset Colo., USA) inside the chamber. The average temperature and relative humidity for the whole period were 25.0 ± 0.26 ºC (SD) and 26.6 ± 3.30% (SD), respectively. The study was conducted in November and December 2013, under a protocol approved by King Saud University and complies with the current laws of Saudi Arabia.

Table 2 Inclusion rates of antibiotic, probiotic, prebiotic and symbiotic products in treatments for broilers

		Inclusion rate (g/kg)	
Item	**Additive**	**Starter (d 0 to d 14)**	**Finisher (d 15 to d 42)**
T1	Control	0	0
T2	Neoxyval	0.05	0.05
T3	Gallipro	0.2	0.2
T4	TechnoMos	0.75	0.5
T5	Symbiotic	0.2 + 0.6	0.2 + 0.5

Feed consumption and bodyweight gain (BWG) were recorded weekly by pen, and feed conversion ratio (FCR) was computed per week. Mortality was checked daily and weight of dead birds was used to adjust FCR. At the conclusion of the trial, eight birds per treatment were selected. After euthanasia, feathers, heads, necks and shanks were removed, and the remaining carcasses were dissected into breast and leg quarters and weighed. The yield percentage of each part was calculated based on dressed weight. At 42 d, the entire gastrointestinal tracts from eight birds per treatment were removed aseptically, the small intestines were weighed, and the total length was measured, then separated into duodenum, jejunum (proximal to Meckel's diverticulum) and ileum (proximal to ileo-caecal junction) and measurements of length and weight for each part were taken. Two-cm-long samples from the proximal of each portion of the small intestine were collected for histology measurements. Samples were fixed in phosphate-buffered formalin for at least 48 h, after which they were embedded in paraffin. Sections of 5 mm were cut and stained with haematoxylin and eosin. Measurements of height and width were based on at least 10 well-oriented villi per sample, using an IX71 Inverted Olympus Microscope (Eyepiece: WH10X, Objective Lens: 4X) and a PC-based image analysis system (Olympus DP72 Microscope Digital Camera; Olympus NV, Aartselaar, Belgium) with software analysis (cellSens Digital Imaging Software).

Ileal digesta and caecal contents were aseptically emptied into new sterile bags and were kept on ice until time of analysis. Samples were diluted in 0.9% saline, and 0.1 mL of each sample was plated in duplicates, using selective agar media to enumerate target bacterial groups (Table 3). *C. perfringens* was counted on tryptose sulphite cycloserine (TSC) agar (Oxoid CM587) with the addition of SR88 and SR47) (Table 4). Colonies on TSC agar that were suspected to be *C. perfringens* were plated secondarily on blood agar (Garridol *et al*., 2004). *Enterobacteriaceae* was isolated on MacConkey agar (Oxoid CM7) after an incubation time of 24 h in an aerobic atmosphere at 37 ºC (Garridol *et al.,* 2004). Isolates of *Enterobacteriaceae* and *Salmonella* were identified by API 20E (bioMérieux Vitek). The API 20E strips were inoculated, incubated at 37 ºC for 18 - 48 h, and interpreted as recommended by the manufacturer. Results were expressed as $\log_{10}$ colony forming units per gram of sample ($\log_{10}$ CFU/g). Fermentation of carbohydrates was determined using API 50 CHL, a standardized system, consisting of 50 biochemical tests to study carbohydrate metabolism by microorganisms. API 50 CH was used in conjunction with API 50 CHL medium to identify *Lactobacillus* and related genera strips according to the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France). The set-up system was incubated at an appropriate temperature of 35 ºC for 48 h. Bacterial strains were identified based on the fermentation of carbohydrates. Identification tables were prepared as (+/-) according to colour change in the evaluation of the results of API strips reaction. Species designations were identified by evaluating with software identification.

Data were evaluated by ANOVA for a complete randomized block design using the general linear models procedure of SAS software (SAS, 2003). When the ANOVA showed significant differences, Fisher's

protected test was applied. The overall level for statistical significance was set at $P < 0.05$. All values were expressed as means ± standard error of the mean (SEM).

Results

Body weight gain, food intake (FI) and mortality corrected FCR of the birds at different ages are shown in Tables 3 - 5. The performance results were measured weekly and cumulatively for starter (0 - 14 d), finisher (15 - 42 d) and starter and finisher (0 - 42 d).

Table 3 shows the cumulative performance for the starter period (weeks 1 and 2). The results revealed that FI was not affected by any dietary treatments ($P > 0.05$) while BWG and FCR were influenced by dietary treatments ($P < 0.05$ and $P < 0.05$, respectively). Chicks that received T2 and T4 had the highest BWG (319.9 and 308.3 g, respectively) but it was not different from the gain obtained from those that received T3 (299.7 g). Chicks that received T1 and T5 had the lowest BWG during the two-week period (269.7 and 293.7 g, respectively).

The combined FCR for the first two-week period was the best for chicks that received T2, T3 and T4 (1.278, 1.279 and 1.292 g/g, respectively) but it was not different from T5 (1.360 g/g). Chicks on T1 (unsupplemented diet) had the worst FCR (1.451 g/g).

Table 3 Feed intake (FI), bodyweight gain (BWG) and feed conversion ratio (FCR) of broiler chickens given experimental diets from d 0 to d 14

Treatment	Measurements		
	FI (g)	BWG (g)	FCR (g/g)
T1	384.4	269.7^{b}	1.451^{a}
T2	408.4	319.9^{a}	1.278^{b}
T3	383.0	299.7^{ab}	1.279^{b}
T4	397.7	308.3^{a}	1.292^{b}
T5	398.9	293.7^{b}	1.360^{ab}
SEM	± 11.56	± 10.84	± 0.043
P-value	NS	*	*

^{ab} means in the same column with different superscripts differ significantly (* $P < 0.05$); NS: not significant.

Table 4 Feed intake (FI), bodyweight gain (BWG) and feed conversion ratio (FCR) of broiler chickens given experimental diets at different ages (d 15 to d 42)

Treatment	Measurements		
	FI (g)	BWG (g)	FCR (g/g)
T1	3066.9	1660.2^{c}	1.852^{a}
T2	3304.4	1922.1^{a}	1.720^{b}
T3	3200.6	1828^{ab}	1.752^{b}
T4	3241.2	1854.2^{a}	1.749^{b}
T5	3089.8	1694.9^{bc}	1.825^{b}
SEM	± 77.27	± 47.15	± 0.019
P-value	NS	**	**

^{abc} means in the same column with different superscripts differ significantly (** $P < 0.01$); NS: not significant.

The performance results for the cumulative finisher period (15 - 42 d) showed no significant differences ($P > 0.05$) in FI because of treatment (Table 4). BWG was highest for birds that received T2, T4

and T3 (1922.1, 1854.2 ad 1828 g, respectively), intermediate for birds that received T5 (1694.9) and lowest for the T1 group (1660.2 g) (P <0.01). The combined FCR for the finisher period was best for chicks that received T2, T4, T3 and T5 (1.720, 1.749, 1.752 and 1.825 g/g, respectively). Chicks on T1 had the worst FCR for the finisher period (1.852 g/g).

Table 5 shows the performance for the cumulative period (0 - 42 d). Birds that received T2, T4 and T3 gained more weight than the T1 group (2469.3, 2380, 2338.2 and 2105.0 g, respectively) (P <0.01), while those that had received T5 (2194.4 g) were intermediate and not significantly different from T3 and T1. On the other hand, birds that received T2, T4 and T3 converted feed more efficiently (1.638, 1.650 and 1.651 g/g, respectively) compared with those that received T5 (1.718 g/g) and T1 (1.771 g/g) (P <0.001).

The mean percentage of carcass parts in the various treatments is shown in Table 6. Treatment had no effect on dressing percentage, breast muscle yield, leg quarter yield and abdominal fat, (P >0.05). However, relative liver weight was influenced by diet. Birds that received T4 had the highest weight of all treatments (P <0.05).

Table 5 Feed intake (FI), bodyweight gain (BWG) and feed conversion ratio (FCR) of broiler chickens given experimental diets from d 0 to d 42

Treatment	Measurements			
	FI (g)	BWG (g)	FCR (g/g)	Survival rate (%)
T1	3715.1^{b}	2105^{c}	1.771^{a}	98.57
T2	4006.1^{a}	2469.3^{a}	1.623^{c}	99.05
T3	3859.0^{ab}	2338.2^{ab}	1.651^{c}	99.05
T4	3926.3^{ab}	2380.3^{a}	1.650^{c}	98.45
T5	3765.4^{ab}	2194.4^{bc}	1.718^{b}	99.52
SEM	± 88.81	± 59.58	± 0.018	± 0.66
P-value	NS	**	**	NS

^{abc} means in the same column with different superscripts differ significantly (**P <0.01); NS: not significant.

Table 6 Effects of different treatments on carcass parts as percentages of broiler dressed weight at 42 days old

	Treatment					SEM	*P*
	T1	T2	T3	T4	T5		
Dressed yield (%)	73.0	74.6	74.4	74.1	73.2	± 0.60	NS
Breast (%)[1]	35.2	39.2	39.4	39.2	36.6	± 1.41	NS
Leg quarter (%)[1]	43.1	40.9	41.9	41.5	42.9	± 1.05	NS
Abdominal fat (%)	3.27	3.11	2.88	2.72	2.82	± 0.31	NS
Liver (%)	2.94^{abc}	2.63^{c}	2.80^{c}	3.33^{a}	3.10^{ab}	± 0.14	*

Breast and leg quarter were expressed as percentage of the carcass weight.
^{abc} means in the row with different. Superscripts differ significantly (* P <0.05); NS: not significant.

Table 7 shows the measurements of intestinal morphology. Jejunal and ileal length were affected by treatment (P <0.01). The longest small intestine (jejunal and ileum) was recorded from birds that received T2 and T3 (151.7 and 151.3 cm, respectively). The shortest small intestine was measured in birds that received T4 (131.0 cm). However, insignificant differences were found between birds that received T1 (132.5 cm) and T5 (142.8 cm). On the other hand, small intestine weight (g), intestinal weight (g/cm) and intestine relative weight (g/100 g body weight) were not influenced by treatment (P <0.05).

Ileal villi height was influenced by treatment (P <0.001). Longer ileal villi were obtained from birds that received T4 (492.9 µm) compared with T1 (424.7 µm) and T2 (439.9 µm) (P <0.05). Insignificant differences were found in ileal villi length between T3 (454.8 µm), T5 (444.9 µm) and T4 (492.9 µm). Conversely, jejunal villi length was not influenced by treatment (P >0.05). Jejunal and ileal villus width were not affected by dietary treatments (P >0.05).

Table 7 Intestinal morphology and histology of broilers at 42 d old

	Treatment					SEM	*P*
	T1	T2	T3	T4	T5		
Jej+Ile length (cm)	132.5^{bc}	151.7^{a}	151.3^{a}	131.0^{c}	142.8^{bc}	± 3.33	**
Jejunum length (%)	48.7	51.0	50.3	49.9	49.5	± 0.98	NS
Ileum length (%)	51.3	48.9	49.7	50.0	50.5	± 0.98	NS
Jej+Ile weight (g)	33.6	41.2	37.2	34.2	33.9	± 2.25	NS
Jejunum weight (%)	51.7	52.1	51.5	54.7	55.1	± 1.48	NS
Ileum weight (%)	48.3	47.9	48.5	45.3	44.8	± 0.012	NS
Intestine weight (g/ cm)	0.42	0.27	0.25	0.26	0.24	± 0.03	NS
IRW^{A} (g/100g BW)	2.25	2.34	2.34	2.28	2.17	± 0.135	NS
Villus height ^{B} (µm)							
Jejunum	520.9	600.1	625.2	572.6	611.6	± 18.22	NS
Ileum	424.7^{b}	439.9^{b}	454.8^{ab}	492.9^{a}	444.9^{ab}	± 17.88	*
Villus width^{B} (µm)							
Jejunum	66.7	69.1	74.3	72.6	67.6	± 2.75	NS
Ileum	73.7	71.9	71.4	74.2	82.9	± 3.47	NS

^{A} IRW: intestine relative weight.
^{B} Measurements of height and width were based on at least five well-oriented villi per section per broiler for five birds per treatment.
^{abc} means in the row with different superscripts differ significantly (*****P <0.05); ******P <0.01.

Table 8 presents data related to the ileal and caecal bacterial count of broilers. Gram negative *Bacilli* were found to be the same in the ileum and caecum among all groups (P >0.05), which was an indication that the antibiotic, probiotic, prebiotic and symbiotic tested in this trial had no influence on gram negative bacteria. The probiotic (T3) eliminated *C. perfringens* from the ileum but it did not differ significantly from birds that received T5 (0.0 and 1.15 log CFU/g, respectively). Conversely, *C. perfringens* count in the caecum was not influenced by dietary treatment (P <0.05).

Lactic acid bacteria increased significantly (P <0.05) in the ileum for T4 (6.87 CFU/g), which was not different from the T2, T3 and T5 groups (5.87, 6.47 and 6.39 CFU/g, respectively), while T1 had the lowest count (5.52 CFU/g). Lactic acid bacteria increased significantly (P <0.01) in the caecum for T2, T3, T4 and T5 groups (6.99, 7.14, 7.11 and 6.93 CFU/g, respectively) compared with T1 group (4.48 CFU/g).

Discussion

The application of in-feed AGPs in poultry diets threatens consumer health. Resistant microbial populations are the outcome of antibiotic use in poultry. There has been a big push to find alternative treatment methods for common poultry ailments. These include probiotics, prebiotics and symbiotics. The results of the present feeding trial provided evidence that the dietary inclusion of probiotics (T3) and prebiotic (T4) improved the cumulative BWG and FCR of broiler chickens, similarly to the AGP (T2).

The probiotic group performed similarly to the antibiotic group and it was concluded that GalliPro (T3) could replace the antibiotic without any negative effect on bird performance. The results of the current study agree with previous reports (Teo & Tan, 2006; 2007), which showed that *B. subtilis* based-probiotic improved broiler performance, and explained the improvements by different mechanisms. Mountzouris *et al*. (2007)

Table 8 Ileal and caecal bacterial count in broilers at 42 days old (mean, $\log_{10}$ CFU/g)

	Treatments					SEM	*P*
	T1	T2	T3	T4	T5		
Ileum							
Gram neg. *bacilli* ¥	3.95	4.76	4.38	5.20	3.36	± 0.197	NS
C. perfringens	3.34^{a}	2.34^{a}	0.00^{b}	3.01^{a}	1.15^{ab}	± 0.027	*
Lactic acid	5.52^{c}	5.87^{bc}	6.47^{ab}	6.87^{a}	6.39^{ab}	± 0.003	**
Caecum							
Gram neg. *bacilli* ¥	7.42	6.54	7.08	7.04	7.12	± 0.17	NS
C. perfringens	4.48	3.35	4.44	2.81	4.33	± 0.11	NS
Lactic acid	4.48^{b}	4.30^{b}	7.14^{a}	7.11^{a}	6.93^{a}	± 0.003	**

¥ Gram negative *bacilli*, ^{abc} means in the row with different superscripts differ significantly (*****P <0.05; ******P <0.01).

reported that the overall FCR for probiotic treatment was not different from antibiotic treatment. Lund *et al*. (2005) conducted four feeding trials at various locations in EU between 2003 and 2005 and concluded that *B. subtilis* improved bodyweight of broilers from 1% to 7%. Later, Rostagno *et al*. (2006) examined the efficacy of GalliPro® (8×10^5 CFU *B. subtilis*/g feed) and an antibiotic supplemented diet (Avilamycin), and concluded that broilers fed GalliPro® performed similarly to those fed the antibiotic diet. However, other reports disagree with the current findings. For example, Kwakernaak *et al*. (2007) concluded that *B. subtilis* spores supplemented to wheat-SBM diet did not improve cumulative BWG and FCR at d 36.

The prebiotic (T4) improved the performance to a similar level as the AGP. The results revealed a significant improvement in FCR at 42 d for birds that had received the T4. This could be explained by the significant improvement in BWG, which was associated with birds that received T4. These results are in line with the findings of Podmaniczky *et al*. (2006) and Rosen (2007). Both groups reported a positive effect for yeast cell wall products on the performance of broilers. Hooge (2003) came to the same conclusion when he analysed the effects of a prebiotic (mannan-oligosaccharides) from 24 trials. It was reported that FCR improved by 2.27% because of prebiotic supplementation.

Supplementing probiotics (T3) and prebiotics (T4) individually appeared to be superior to symbiotic supplementation (T5) in improving broiler performance. Li *et al*. (2008) showed that combinations of pre- and probiotics (symbiotics) are often more effective than individual additives. Similarly, Awad *et al*. (2009) reported beneficial effects of a symbiotic over a probiotic product on broiler performance. However, this was not the case in the current study. When the symbiotic (T5) was used, the performance was lower compared with the individual probiotic (T3) and prebiotic (T5).

Histological examination revealed a significant difference in villi height among the treatment groups at d 42. Birds that received prebiotics (T4), probiotics (T3) and symbiotics (T5) showed improved ileal villi height to a level that was superior to the control (T1) and antibiotic (T2) supplemented groups. These results indicated that changes occurred in villi morphology between treatments during the trial period, and thus increased the absorptive surface area in the small intestine. Longer villi are usually equated with excellent gut health, high absorptive efficiency and healthier intestinal tracts of chickens (Alfaro *et al.,* 2007). According to Cera *et al.* (1988), maximum absorption and digestion capacity are provided by a large luminal area with villus height and mature enterocytes, and is essential to animal development. Several reports observed greater villi height and superior ileal mucosa development in chickens supplemented with a yeast cell wall product prepared from *Saccharomyces cerevisiae*, particularly in the first week of the chicken's life (Santin *et al*., 2001; Zhang *et al*., 2005). Similarly, Salim *et al*. (2013) reported that ileal villus height and width were improved when birds were fed probiotics compared with antibiotics and control. On the other hand, antibiotic supplementation caused a decrease in ileal villi height. Similar results were reported by Oliveira *et al*. (2008), who found that antibiotic supplementation caused low villi height. They explained this by the suppressing effect of the antibiotic on beneficial bacteria in the gut, such as *Lactobacillus* and *Bifidobacteria*. Longer villi indicate more mature epithelia and enhanced absorptive function due to increased absorptive area of the villus.

Wilson *et al*. (2005) explained that the growth-suppressing effect of intestinal bacteria was due to the production of toxic metabolites that irritate the gut mucosa, thereby inhibiting nutrient absorption. Gram negative *Bacilli* were found to be the same among all groups in the ileum and caecum, which was an

indication that the antibiotic, probiotic, prebiotic and symbiotic tested in this trial had no influence on gram negative bacteria. According to Bedford (2000), most AGPs target gram positive bacteria, and suggest that this group of bacteria is an important cause of impaired performance. The anticlostridial properties of the antibiotic, prebiotic, probiotic and symbiotic were tested in this study. *C. perfringens* is known to be a main causative for necrotic enteritis and could cause major health problems to the birds if antibiotics were removed without being replaced with products that could prevent the proliferation of this bacterium (Ficken & Wages, 1997; Porter, 1998). A subclinical disease associated with necrotic enteritis could damage the intestinal mucosa, which results in a decrease in digestion and absorption and, as a result, poor performance (Kaldhusdal *et al*., 2001). The growth-suppressing effect of intestinal bacteria was explained by the toxin produced by the bacteria, which could irritate the gut mucosa, thus constraining nutrient absorption. Feighner & Dashkevicz (1987) explained the growth depression caused by *C. perfringens* infection was related to the high level of bile salt hydrolase activity in *C. perfringens*. The results show that probiotic supplementation alone (T3) or as part of the symbiotic (T5) controlled the proliferation of *C. perfringens* in the ileum. The probiotic (T3) eliminated *C. perfringens* (0.0 log CFU/g) in the ileum, but not in the caecum. This elimination could be explained by competitive exclusion and stimulation of the immune system.

Several studies used a *Bacillus subtilis*-based probiotic supplement and came to the same conclusions (Jack *et al*., 1995; Teo & Tan, 2007). Strains of *B. subtilis* have been shown to eliminate the colonizing of several pathogenic bacteria in the gut, such as *E. coli*, *Salmonella*, *Clostridium*, *Streptococcus* and *Campylobacter* (La Ragione *et al*., 2001; Teo & Tan, 2006; Knapp *et al*., 2011). The antimicrobial factor produced by *Bacillus subtilis* is typical of gram positive bacteriocins in being broadly active against various strains of *Clostridium* species.

The ileal *C. perfringens* populations of birds were not altered when the prebiotic (T4) was added to their diets. This disagrees with the findings of Spring *et al*. (2000) and Yang *et al*. (2007; 2008), where prebiotics were found to have a positive effect in lowering *C. perfringens* populations. The prebiotic reduction ability of *C. perfringens* was explained by its capability of controlling the pathogens in the small intestine by offering competitive binding sites for undesirable pathogens (Newman, 1994). On the other hand, the improvement in BWG and FCR could be related to the lower microbial population in the gastrointestinal tract in broilers (Thongsong *et al*., 2008).

Prebiotics are not digested in the small intestine. Therefore, bacteria bound to prebiotics are likely to exit the intestine without attaching to the epithelium and this causes a reduction or prevention of colonization of undesirable bacteria in the small intestine (Spring *et al*., 2000).

Lactic acid bacteria, which are regarded as good bacteria in the gut, increased in the probiotic (T3), prebiotic (T4) and symbiotic (T5) treatments compared with the control (T1) and AGP (T2) treatments. This finding agrees with those of others. Teo & Tan (2006) reported that *B. subtilis* produces lactic and acetic acid from glucose. The significant increases in the lactic acid colony counts in the ileum and the caecum in the prebiotic (T4) and probiotic (T3) supplemented groups provide new evidence about changing the intestinal flora through prebiotics and probiotics. Probiotics contain yeast cells, bacterial cultures both that stimulate microorganisms capable of modifying the gastrointestinal environment to a healthy status and improving feed efficiency. Probiotics induce alterations in intestinal flora, enhance the growth of non-pathogenic facultative anaerobic and gram positive bacteria forming lactic acid, and suppress the growth of intestinal pathogens.

Conclusions and Applications

Poor performance was associated with broilers that did not receive any kind of medication (T1). Birds that received the T3 and T4 performed similarly to the AGP group (T2). The order of treatments based on cumulative FCR results were T1 > T5 > T3 ≥ T4 ≥ T2. The combination of the probiotic and the prebiotic (T5) reduced performance compared with each one separately (T3 and T4). The height of intestinal villus is associated with digestion and absorption capacity and could be regulated through dietary means. Ileal villi height reduced in T1 and T2 groups while it was the longest in the T3 and T4 groups. The morphology improvements in the intestinal tract as observed in this study supported the concept that broilers gut health and function can be improved by dietary supplementation such as probiotics and prebiotics. The probiotic eliminated *C. perfringens* from the ileum, while lactic acid increased in the ileum and caecum of broilers because of probiotic and prebiotic supplementation. The probiotic and prebiotic used in this trial could enhance the performance of broilers by improving intestinal morphology and microbial balance associated with modulation of intestinal microflora and pathogen inhibition.

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