Short Communication

Effect of a prebiotic, probiotic and symbiotic on performance of broilers under *Clostridium Perfringens* challenge

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Abstract

The risk of developing antibiotic-resistant pathogenic bacteria is a major concern in poultry production. The aim of this paper was to evaluate a number of commercially available products as alternatives to antimicrobial growth promoters (AGPs) in broiler's feed. Six experimental treatments [T1 = unmediated feed (negative control, NC); T2 = unmediated feed plus bacterial challenge (positive control, PC); T3 = feed plus antibiotic (NEOX); T4 = feed plus probiotic (GALI); T5 = feed plus prebiotic (TECHNO); and T6 = feed plus probiotic and prebiotic (SYM)] were used in this study. A total of 240 one-day-old chicks were utilized in the growth trial for 42 days. The birds in T2 to T6 were challenged with an oral dose of *Clostridium perfringens*. Data demonstrated that poor performance was found in the broilers infected with *C. perfringens* that did not receive any types of medication (negative control). In conclusion, the antibiotic (NEOX), probiotic (GALI) and prebiotic (TECHNO) were able to eradicate the negative impacts of bacterial stress. In addition, the birds receiving GALI and TECHNO had similar performance to those receiving NEOX.

Keywords: antimicrobial growth promoters (AGPs), Eubiotic, probiotic, prebiotic, *clostridium perfringens*, broilers

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Introduction

Supplementation of poultry diet with antibiotics at sub-therapeutic amounts as antimicrobial growth promoters (AGPs) was found to be useful for improving growth and reducing the incidence of pathogens, e.g. *E. coli*, *Clostridium perfringens* (*C. perfringens*) and *Salmonella* (Hume, 2011). Antimicrobial growth promoters (AGPs) have been regularly supplemented in poultry feeds (Butaye et al., 2003). However, lately concern has been raised about the risks surrounding the development of antibiotic-resistant pathogens, which might lead to wide multiplication and distribution of antibiotic-resistant bacteria. Resistant pathogen transfer from animals to humans is probable and could pose a possible health risk (Tollefson et al., 1998). Due to the fact that most of the antimicrobial chemicals given to animals are of the same class as those used to treat human diseases, the European Union banned antibiotic use for growth production in 2006. There are concerns that many antibiotics now used for human treatment will no longer be effective in the future (Smith et al., 2003; Castanon, 2007).

Current developments in poultry nutrition focus on total or partial substitution of non-antibiotic supplements that have similar effects on broiler performance for AGPs (Mountzouris et al., 2007). Many feed additives, such as probiotics, prebiotics and symbiotics, are presently in use in the industry.

Probiotics are among the non-antibiotic supplements that are currently used as alternative growth promoters in the poultry industry (Teo and Tan, 2006; Abudabos et al., 2013). Probiotics have been claimed to prevent *C. perfringens* and *Salmonella* colonization in the gut via a mechanism for the inevitable elimination from a habitat of one of the two different species that have identical needs for resources (Teo and Tan, 2006 and Abudabos et al., 2013). A second potential substitute for AGPs in poultry nutrition is prebiotics, which are oligosaccharides that cannot be digested by animal's enzymes, but can stimulate the growth of certain bacteria in the intestines. These microorganisms have potentially useful impact on the host animal's health. Probiotics are incorporated in order to introduce useful microorganisms into the intestines, but prebiotics are concerned with the selective stimulation of useful microorganisms that are already present in the intestines (Yang et al., 2009). A third potential substitute for AGPs is symbiotics, which are mainly a combination primarily of prebiotics and probiotics with other promoting substances that together exhibit a joint effect on the digestive tract health, digestibility as well as broilers' performance (Patterson and Burkholder, 2003). This study was conducted to determine the effects of the addition of antibiotic, prebiotic, probiotic and symbiotic to the feed of broilers which were challenged with 4 x 10^8 CFU of *C. perfringens*.

Materials and Methods

Growth experiment and treatments: Prebiotic, probiotic, symbiotic and antibiotic samples were obtained from commercial sources. Neoxyval (NEOX) (Sogeval Laboratory, France) was the reference AGP; each gram of NEOX contains 200 mg each of oxytetracycline and neomycin. GalliPro (GALI) is a probiotic (Biochem, Germany) that has *Bacillus subtilis* as its active component. TechnoMos (TECHNO) is a prebiotic (Biochem, Germany) rich in glucans and mannose and is derived from the cell membrane of the yeast, *Saccharomyces cerevisiae*. This trial was conducted under environmentally controlled conditions in a battery room at King Saud University. The procedures were approved by King Saud University Ethic Committee and care was taken to minimize the number of animals used.

Ross 308 broiler chicks were purchased from a local hatchery. Two hundred and forty chicks were allotted into 48 experimental cages with 5 chicks per cage; each treatment was replicated eight times. The chicks were vaccinated against infectious bronchitis, Marek's disease and Newcastle disease, in the hatchery. The chicks were grouped according to their weight and were then placed into 48 experimental four-deck cage systems with electrically heated battery brooders. The temperature inside the room was kept at 24°C. Diet and water were supplied *ad libitum*, and the birds were maintained at a 24 h light schedule. Vitamins were supplemented in the drinking water for the first three days. The growth experiment was conducted from day 1 to day 42 and the chicks were randomly sampled at the end of the experiment.

Before mixing the trial diets, the raw ingredient samples were analyzed to determine their chemical constituents and the values were used to formulate the experimental diets. The chicks were provided with the experimental starter diets from 1 to 14 days of age and finisher diets from 15 to 42 days of age in mashed form. The diets were based on corn-soybean meal and were calculated to meet the recommended doses of the commercial practices and NRC (1994). The control starter and finisher diets contained 3000 and 3100 kcal/kg ME and 21.5% and 21.0% crude protein, respectively (Table 1). The chicks received one of the six dietary treatments for each growing phase as follows:

1) Negative Control (NC)
2) Positive Control + *C. perfringens* challenge (PC)
3) PC + 0.05 g Neoxyval/kg (NEOX)
4) PC + 0.2 g GalliPro/kg (GALI)
5) PC + 0.75 (starter) and 0.6 (finisher) g echnoMos/kg (TECHNO)
6) PC + 0.2 g GalliPro/kg + 0.6 g TechnoMos/kg for starter and 0.2 g GalliPro/kg + 0.5 g TechnoMos/kg for finisher (SYM).

The birds in T2 to T6 were challenged using a 10-fold dose of anticoccidial vaccine (Paracox-8) combined with an oral dose of 1 ml of *C. perfringens* inoculations (4 x 10^8 CFU/ml) to induce necrotic enteritis (NE) challenge at 14 days of age (Abudabos et al., 2013).

Measurement:

Feed consumption, body weight and carcass: Body weight and feed consumption were weekly recorded, and then feed conversion ratio (FCR) values were obtained. Mortality was recorded on a daily basis, and dead birds' weights were used to adjust the FCR values. At day 35, 10 birds from each treatment were

kept without feeding for 12 h and then processed manually to determine the dressing and parts yield. The yield percentage of each part was calculated on a dressed weight basis.

Table 1 Dietary composition of broiler chick starter and finisher diets

Ingredients	Starter	Finisher
Yellow corn	62.45	69.90
Soybean meal	31.0	26.73
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
V-M premix[1]	0.20	0.12
Calculated Analysis		
ME, kcal/kg	3000	3100
Crude protein, %	21.5	21.0
Non phytate P, %	0.50	0.40
Calcium, %	1.0	0.9
Lysine, %	1.20	1.1
Methionine, %	0.55	0.47
SAA[2], %	0.90	0.80
Threonine, %	0.85	0.80

[1]Vitamin supplemented per kg of feed: vitamin A, 2400000 IU; vitamin D, 1000000 IU; vitamin E, 16000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B_2, 1600 mg; vitamin B_6, 1000 mg; vitamin B_{12}, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin, 40 mg; and antioxidant, 3000 mg. Trace minerals supplemented per kg of feed: cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg; and zinc, 14000 mg in accordance with NRC (1994) recommendations.
[2]Sulfur amino acid

Nutrient retention and apparent metabolizable energy: At 25 days of age, additional 36 birds were placed in 18 cages with wired bottoms (two birds per cage), three replicates per treatment, and provided one of the six experimental diets. The diets were supplemented with chromic oxide at a rate of 3 g/kg diet as an analytical marker for the retention trials. The birds were fed the chromium oxide diet for 5 days and on the 5th day (30 days of age) the birds were euthanized and the ileal contents were collected and stored at -20°C until the time of analysis for nutrient retention. To determine the nutrient retention, the feed and ileal digesta were dried at 60°C and were then analyzed. Chromium content was determined using AOAC International (2000) methods. Gross energy of the feed and digesta samples was determined using a Parr adiabatic bomb calorimeter (Parr Instruments Co., Moline, IL). Nitrogen content and ether extract of the feed and ileum samples were determined according to AOAC (1984). AMEn values were calculated using the following equation: AME (kcal/kg of diet) = GE diet - [GE excreta x Marker diet/Marker excreta]. The following equation was used to calculate percent retention according to Scott et al. (1976): % Nutrient retention = 100 - (Diet Cr_2O_3/Fecal Cr_2O_3 x Fecal nutrient/diet nutrient) x 100.

Statistical analysis: Data were subjected to the analysis of variance (ANOVA) for a complete randomized block design (SAS, 2000-2003). When the ANOVA showed significant differences, Tukey's Studentized Range (HSD) test was applied.

The following statistical model was used for the experiment:

$$Y_{ijk} = \mu + T_i + + B_k + e_{ijk}$$

where μ = general mean, T_i = effect of treatment (i= 1, ..., 6), B_k = effect of block (1, ..., 10), and e_{ijk} = random error associated with Y_{ijk} observation.

Results and Discussion

Performance results which included feed intake (FI), body weight gain (BWG) and mortality corrected FCR of the birds at different ages are shown in Table 2. The performance results were measured weekly during the starter (0-14 days), finisher (15-42 days) and cumulative (0-42 days) periods. The results revealed that FI and BWG were not affected by any dietary treatments (P>0.05) during the starter period, but FCR was significantly affected (P<0.05). The birds which received NEOX (T3) or GALI (T4) converted feed more efficiently (1.314±0.043 and 1.318±0.043 g:g, respectively) compared to those which received the control (1.393±0.043 g: g). On the other hand, the birds that received TECHNO (T5) or SYM (T6) converted feed moderately (1.376±0.043 and 1.381±0.043 g:g, respectively).

The performance results for the finisher period showed insignificant differences in the FI or BWG among the treatments (P>0.05). Numerically, the birds in the PC group (T2) gained the least during this period (1264.9±43.1 g), and thus had the highest FCR (1.741±0.021 g:g) (Table 2). The effect of the *C. perfringens* challenge was clear on the birds in the PC group (T2), and the treatments significantly influenced FCR (P<0.05) (Table 2). The birds receiving NEOX (T3) or GALI (T4) converted feed more efficiently (1.635±0.021 and 1.642±0.021 g:g, respectively) compared to those in the PC group (T2) (1.741±0.021 g:g). The birds receiving NEOX (T3) or GALI (T4) were subjected to the bacterial challenge and yet performed numerically better than the unchallenged NC group (T1) (Table 2). However, the birds receiving TECHNO (T5) or SYM (T6) performed moderately and were not significantly different from the birds receiving NEOX (T3) or GALI (T4) (1.687±0.021 and 1.688±0.021 g:g, respectively).

The cumulative performance data for the 0- to 42-day period are presented in Table 2. The trend during 0-42 days (cumulative period) for FI, BWG and FCR was the same as that of the starter and finisher periods. The data indicated that the differences in FI and BWG among the treatments were insignificant (P>0.05). Also, insignificant differences were recorded for FCR among the birds in T3 and T4 as well as in T1 and T5 (1.58±0.018, 1.59±0.018 and 1.62±0.018, 1.63±0.018 g:g, respectively). Conversely, the birds in T2 recorded the highest FCR compared to all the other treatments (1.68±0.018). Even with the bacterial challenge, the birds in T3 or T4 performed similarly to the unchallenged birds (T1).

The mean percentages of carcass parameters are shown in Table 3. No significant differences were reported for breast muscle, dressing percentage, abdominal fat, livers and leg quarter.

Table 2 Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broiler chickens

	Treatment						SEM	P
	T1	T2	T3	T4	T5	T6		
Starter								
FI (g)	385.7	388.7	382.5	366.5	393.7	389.8	±8.90	0.35
BWG (g)	276.8	275.0	291.0	278.1	286.2	282.2	±7.04	0.58
FCR (g:g)	1.393^{a}	1.413^{a}	1.314^{b}	1.318^{b}	1.376^{ab}	1.381^{ab}	±0.043	0.04
Finisher								
FI (g)	2308	2201.6	2219.9	2152.8	2181	2178.5	±54.9	0.44
BWG (g)	1385.4	1264.9	1357.6	1311.4	1293.1	1290.7	±43.1	0.37
FCR (g:g)	1.666^{ab}	1.741^{a}	1.635^{b}	1.642^{b}	1.687^{ab}	1.688^{ab}	±0.021	0.022
Cumulative								
FI (g)	2693.8	2590.3	2602.4	2519.3	2574.7	2568.3	±56.2	0.41
BWG (g)	1662.2	1539.9	1648.7	1589.5	1579.3	1572.9	±42.9	0.33
FCR (g:g)	1.621^{ab}	1.682^{a}	1.578^{b}	1.585^{b}	1.630^{ab}	1.633^{ab}	±0.018	0.003
Liv[1] (%)	98.57	99.05	99.05	98.45	99.52	99.52	±0.66	0.34

T1 = unmediated feed (negative control, NC); T2 = unmediated feed plus bacterial challenge (positive control, PC); T3 = feed plus antibiotic (NEOX); T4 = feed plus probiotic (GALI); T5 = feed plus prebiotic (TECHNO); and T6 = feed plus probiotic and prebiotic (SYM).
[1]Liv: livability. Liv = 100 - mortality (%).
Means within each row followed by the same letters are not significantly different.

Table 3 Effect of different treatments on parts yield as percentages of broiler dressed weight at 35 days of age

	Treatment						SEM	P
	T1	T2	T3	T4	T5	T6		
Dressed yield (%)	73.0	70.9	74.9	72.9	73.7	74.1	±1.5	0.54
Breast (%)[1]	36.9	38.4	38.4	38.8	38.2	37.9	±0.93	0.76
Leg quarter (%)[1]	43.2	42.3	42.2	42.3	42.6	42.0	±0.75	0.91
Abdominal fat (%)	3.28	2.38	2.85	2.88	2.63	2.76	±0.27	0.32
Liver (%)	2.96	3.31	3.62	3.20	3.43	3.47	±0.28	0.63

T1 = unmediated feed (negative control, NC); T2 = unmediated feed plus bacterial challenge (positive control, PC); T3 = feed plus antibiotic (NEOX); T4 = feed plus probiotic (GALI); T5 = feed plus prebiotic (TECHNO); and T6 = feed plus probiotic and prebiotic (SYM).
[1]Results are expressed as percentage of the carcass weight.
Means within each row followed by the same letters are not significantly different.

The findings of this study are in line with previous studies (Teo and Tan, 2006 and Teo and Tan, 2007) which indicated improvement in broiler performance by the supplementation of diet with *B. subtilis* based-probiotic, but these two studies claimed that different mechanisms were involved in the broiler

performance improvements. In another report, Kwakernaak et al. (2007) reported that *B. subtilis* spores supplementation in a wheat-SB based diet did not affect the overall BWG and FCR at 36 days. However, the findings of this study are confirmatory to those reported by Mountzouris et al. (2007), who claimed that the cumulative FCR of the probiotic treatment did not significantly differ from that of the antibiotic treatment. The results of Bai et al. (2013) specified that dietary addition of probiotic to 21-day-old chicks improved body weight and feed intake during the starter period. Also, the results of Kehlet et al. (2014) showed that GalliPro® supplementation to broiler's feed improved live weight.

Lund et al. (2005) carried out four feeding experiments at different sites in the EU between 2003 and 2005 and found that supplementation of diet with *B. subtilis* enhanced broilers' body weight in the range of 1-7%. Later, Rostagno et al. (2006) examined the efficacy of GalliPro® (8×10^5 CFU *B. subtilis*/g feed) and antibiotic (Avilamycin) supplemented diets and concluded that broilers receiving GalliPro® had similar performance to those receiving the antibiotic diet. In the current study, the birds receiving probiotic had identical performance to those birds receiving antibiotic in their diet; therefore, GalliPro (T4) could be used as a substitute for antibiotic without any negative responses on bird's performance. In a recent work published by our group, *B. subtilis* improved the performance similar to the antibiotic in unchallenged birds (Abudabos et al., 2015).

However, prebiotic (TechnoMos) used in the current trial (T5) enhanced the birds' performance to the same degree as the antibiotic. Similar conclusion was reached by Hooge (2003) when he determined the influence of prebiotic (mannan-oligosaccharides) in 24 experiments. He stated that FCR was improved by 2.27% due to the supplementation of diet with prebiotic.

Li et al. (2008) showed that symbiotic supplementation (pre- and probiotic mixture) was usually more effective than individual supplementation of prebiotic or probiotic. Also, Awad et al. (2009) reported positive impact of symbiotic supplementation over supplementation of probiotic product on broiler performance. However, this disagrees with the findings of this study. When symbiotic was supplemented, the performance was lower when compared to the values obtained by the addition of individual probiotic (T4) or prebiotic (T5).

Necrotic enteritis (NE) challenge was induced using *C. perfringens* after the starter period in order to examine the anticlostridial effects of the antibiotic, prebiotic, probiotic and symbiotic used in this study. *C. perfringens* is the main cause of NE, which could cause major health problems in birds if antibiotics are administered without being substituted by another product that can prevent the growth of this bacterium (Ficken and Wages, 1997; Porter, 1998). A subclinical disease that is associated with NE can destroy intestinal mucosa, thus reducing digestion and absorption efficiency, resulting in poor performance (Kaldhusdal et al., 2001). The suppression of growth caused by intestinal bacteria may be due to toxins produced by the bacteria causing irritation of gut mucosa, leading to constraint of nutrient absorption. Feighner et al. (1987) claimed that growth retardation caused by *C. perfringens* infection was caused by the high activity level of bile salt hydrolase in *C. perfringens*.

Many challenged birds (T2) exhibited noticeably distinct pathological symptoms in their intestinal tissue as a result of the bacterial challenge. The challenged birds' responses showed sub-clinical inflammatory reactions throughout various sections of the digestive system, particularly the small intestine, with intestinal lesions and hemorrhages. This was the case for the birds that were challenged and did not receive any supplements (T2), but the challenged birds that were given probiotic, antibiotic, prebiotic or symbiotic did not show any signs of hemorrhages or lesions, i.e. these supplements successfully reversed the negative effects of the bacterial challenge.

Table 4 shows data related to nutrient retention and AME affected by the studied feed additives. No significant differences were observed in the protein or ether extract retention in all groups ($P>0.05$). However, compared with the NC (2,807±50.6 kcal/kg) or PC (2,777±50.6 kcal/kg) birds, the birds fed on GALI (T4) and TECHNO (T5) had higher AMEn values (3,027±50.6 and 2,973±50.6 kcal/kg, respectively). The birds fed on NEOX (T3) or SYM (T6) had moderate AMEn values (2,925±50.6 and 2,925±50.6 kcal/kg, respectively) that did not significantly differ from those of the birds fed on GALI (T4) or TECHNO (T5). The AMEn values increased in the GALI and TECHNO groups, which indicates that more digestion and absorption took place in the small intestine as a result of the decrease in pathogenic bacteria in the small intestine in these two groups. Similarly, Waititu et al. (2014) reported that *Bacillus*-strain probiotics increased AMEn of the diet by possibly increasing dry matter and fat retention.

Table 4 Apparent nutrient retention of broiler chickens at 30 days

	Treatment							
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**SEM**	**P**
					(%)			
Protein	70.5	68.8	70.1	70.5	70.8	69.7	±0.98	0.73
Ether Ex.	79.9	77.4	78.9	79.8	80.5	79.5	±0.77	0.16
AME	2807^{ab}	2777^{b}	2925^{ab}	3027^{a}	2973^{ab}	2925^{ab}	±50.6	0.03

T1 = unmediated feed (negative control, NC); T2 = unmediated feed plus bacterial challenge (positive control, PC); T3 = feed plus antibiotic (NEOX); T4 = feed plus probiotic (GALI); T5 = feed plus prebiotic (TECHNO); and T6 = feed plus probiotic and prebiotic (SYM).

Means within each row followed by the same letters are not significantly different.

Conclusion

The impact of the treatments on performance were clear during all growing periods for the feed conversion ratio (FCR). Poor performance was associated with the broilers infected with *C. perfringens* that did not receive any types of medication (T2). The birds that received the probiotic, prebiotic and their mixture had similar performance to those that received the antibiotic. GALI or TECHNO can be used as a substitute for antibiotics in broiler's feeding.

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บทคัดย่อ

ผลของ พรีไบโอติก โปรไบโอติก และ ซิมไบโอติก ต่อสมรรถนะของไก่เนื้อที่ได้รับเชื้อ *Clostridium perfringens*

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ความเสี่ยงของเชื้อแบคทีเรียก่อโรคที่ดื้อต่อยาปฏิชีวนะมีความสำคัญในการผลิตสัตว์ปีก วัตถุประสงค์ของการศึกษานี้คือ การประเมินผลิตภัณฑ์ที่มีจำหน่ายในท้องตลาด เป็นทางเลือกมาใช้เป็นสารต้านจุลชีพที่เร่งการเจริญเติบโต (antimicrobial growth promoter (AGPs) ในอาหารไก่เนื้อ ประกอบด้วย 6 การทดลอง คือ [T1 = อาหารที่ไม่ได้ใส่สาร (กลุ่มควบคุมเชิงลบ, NC); T2 = อาหารที่ไม่ได้ใส่สารและรับเชื้อแบคทีเรีย (กลุ่มควบคุมบวก, PC) T3 = อาหารร่วมกับยาปฏิชีวนะ (NEOX); T4 = อาหารร่วมกับโปรไบโอติก (GALI); T5 = อาหารร่วมกับพรีไบโอติก (TECHNO); และ T6 = อาหารร่วมกับโปรไบโอติกและพรีไบโอติก (SYM)] โดยการทดลองในลูกไก่อายุ 1 วันจำนวน 240 ตัวเป็นเวลา 42 วัน โดยไก่ในกลุ่มที่ T2 ถึง T6 ได้รับเชื้อแบคทีเรีย Clostridium perfringens โดยการกิน ผลการศึกษาแสดงให้เห็นว่าในไก่เนื้อที่ติดเชื้อ C. perfringens ที่ไม่ได้รับยาทุกประเภท (การควบคุมเชิงลบ) มีประสิทธิภาพการเติบโตต่ำ ส่วนกลุ่มได้รับยาปฏิชีวนะ (NEOX), โปรไบโอติก (GALI) และ พรีไบโอติก (TECHNO) สามารถขจัดผลกระทบเชิงลบของความเครียดจากแบคทีเรียได้ นอกจากนี้ในไก่ที่ได้รับโปรไบโอติก (GALI) และ พรีไบโอติก (TECHNO) มีประสิทธิภาพการเติบโตใกล้เคียงกับไก่ที่รับยาปฏิชีวนะ NEOX

คำสำคัญ: สารต้านจุลชีพที่เร่งการเจริญเติบโต โปรไบโอติก พรีไบโอติก Clostridium perfringens ไก่เนื้อ

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