The effect of phytogenics on growth traits, blood biochemical and intestinal histology in broiler chickens exposed to *Clostridium perfringens* challenge

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**ABSTRACT**

A total 560 day-old broiler chicks (Ross 308) was randomly allocated to seven treatments (eight replicates). Control (basal diet), T1 infected with *Clostridium perfringens*, T2 infected + Avilamycin at the rate of 0.2 g/kg T3, infected + essential oil of thyme, T4, infected + Sanguinarine, T5, infected + Anti-Salmonella phytobiotic, T6, infected + essential oils of thyme, anise and others (oregano, carvacol, yucca extract and cinnamaldehyde). Feed conversion ratio (FCR) were significantly (*P*<0.05) high in the T2 during the third week. The dressing percentage decreased significantly (*P*<0.05) and intestinal weight increased (*P*<0.05) in T1. Blood protein, and globulin increased significantly (*P*<0.05) high in the T2 during the fourth weeks, while blood alanine transaminase (ALT) increased significantly (*P*<0.05) in T5. Thiobarbituric acid reactive substances (TBARS) increased significantly (*P*<0.05) in T5 and T6. Similarly, villus height and width increased significantly (*P*<0.05) in T5 and T6. From the results of the present study, it was concluded that different feed additives could be substituted with antibiotics in the feed of broiler exposed to *Clostridium perfringens* challenge.

**Introduction**

To improve feed efficiency and reduce mortality in broiler, antimicrobial growth promoters (AGPs) have been successfully used for the last few decades (Abudabos et al. 2016; Chand et al., 2016; Khan et al. 2016; Alhidary et al. 2017). The use of AGPs has been very useful as controlling strategy; however, acquired resistance and meat residues of these antimicrobial agents is one of the major growing concern (Bajpai et al. 2012). Therefore, in many countries of the world, the use of AGPs has been banned (Tehseen et al. 2016). *Necrotic enteritis* (NE), caused by *Clostridium perfringens*, is one of the most costly diseases in poultry production (Ao et al. 2012). Dietary and management practices such as stocking density, contaminated feed and the damaging factors of the intestinal mucosa are linked with the pathogenesis of the disease (Ao et al. 2012). The ban on the use of antibiotics has led to the spread of NE. Consequently, the poultry researchers are searching for the alternative to antibiotics to improve broiler performance and optimize gut health (Chand et al. 2014; Tanweer et al. 2014).

Phytogenetic feed additives (PFA) have been reported for their positive effects (Amad et al. 2013; Raza et al. 2016; Abudabos et al. 2017). Herbs and spices stimulate feed intake by the secretion of endogenous enzymes, antibacterial effect and antioxidnat potential (Lee et al. 2015; Shahid et al. 2015), resulting in enhanced absorption of nutrients from the gut (Tehseen et al. 2016). Inconsistent results have been reported by the use of different herbs and spices in broiler production (Amad et al. 2011; Lee et al. 2015). Therefore, the objective of the present study was to assess the effect of different phytobiotics in comparison with the standard antimicrobial drug, avilamycin, in broilers exposed to experimentally induced *C. perfringens* challenge during the finisher phase.

**Materials and methods**

This experiment was approved by the Departmental board of Studies on Ethics, Methodology and Welfare, King Saud University, Kingdom of Saudi Arabia.

**Experimental design and management of birds**

A total of 560-day-old broiler chicks (Ross 308) were randomly allocated to seven treatments and eight replicates. Upon arrival, the chicks were confirmed for the absence of *C. perfringes* by the method described by Schocken-iturrino et al. (2013). Briefly, randomly, one bird per replicate from each infected pen was randomly slaughtered. Mucous and stool samples were taken from the intestines and cultured in a test tube containing Tarozzi medium along with brain and heart infusion (Difco). The test tubes were incubated in anaerobiosis at 37°C for 48 h. The materials were cultured, smears were stained and observed under microscope. The presence of typical *Clostridium* characteristic colonies was not seen. The samples were also negative for the typical catalase; indole
Table 1. Dietary composition of broiler chick during the experiment.

| Ingredients                      | (%)       |
|----------------------------------|-----------|
| Yellow corn                      | 57.62     |
| Soybean meal                     | 35.24     |
| Corn oil                         | 2.37      |
| Di-calcium phosphate             | 2.30      |
| Ground limestone                 | 0.83      |
| Choline chloride                 | 0.05      |
| DL-methionine                    | 0.20      |
| L-lysine                         | 0.20      |
| Salt                             | 0.46      |
| Threonine                        | 0.11      |
| Vitamins and minerals premixa    | 0.50      |

Chemical analysis

- ME (kcal/kg) 3000
- Crude protein (%) 22.0
- Non phytate P (%) 0.50
- Calcium (%) 1.05
- Lysine (%) 1.30
- Methionine (%) 0.55
- Sulphur amino acids (%) 0.90
- Threonine (%) 0.95

Vitamin-mineral premix contains the following per kg: vitamin A, 2,400,000 IU; vitamin D, 1,000,000 IU; vitamin E, 16,000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B2, 1600 mg; vitamin B6, 1000 mg; vitamin B12, 6 mg; niacin, 8000 mg; folic acid, 400 mg; pantothenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000 mg; iodine, 400; iron, 1200 mg; manganese, 18,000 mg; selenium, 60 mg, and zinc, 14,000 mg.

Production, nitrate reduction and acid production were also negative in the given samples.

The experiment was conducted under controlled conditions where the temperature and humidity were maintained at 25 ± 0.5°C and 51%, respectively, during the experiment. A starter diet (1–14 days) and finisher (15–30 days) diet were offered in the mash form as recommended for the Ross 308, as shown in Table 1. The chicks were randomly allocated to one of the seven treatments as follows, Control (basal diet with no antibiotic

1979) was determined as follows:

\[ \text{FCR} = \frac{\text{Feed intake}}{\text{Weight gain}} \]

Feed conversion ratio (FCR) was computed for each group by using the following formula: FCR = Feed intake/Weight gain.

Production efficiency factor (PEF) as suggested by Griffin (1979) was determined as follows:

\[ \text{PEF} = \left( \frac{\text{Livability} \times \text{Live weight(kg)}}{\text{Age in days} \times \text{FCR}} \right) \times 100. \]

Carcass measurements
On day 30, eight birds per treatment were selected randomly slaughtered. The carcass was dissected to separate breast, thigh, fat and visceral organs including intestines, spleen, heart, liver, and intestines and weighed to calculate the percentage of the yield.

Biochemical measurements of blood
Blood samples (3 ml) were obtained from the wing vein of the bird per replicate and centrifuged at 3000 rpm for 10 min. Serum was stored at −20°C until analysis. Serum total antioxidant capacity (TAC) and Thiobarbituric acid reactive substances were measured by using ELISA kits (Cayman Chemical Company, MI, USA). Glucose, total protein, albumin, aspartate aminotransferase (AST) and alanine transaminase (ALT) were measured by kits (M di Europa Gmbh Wittekamp 30, D-30163 Hannover, Germany).

Intestinal histology
On day 30, 1 cm section of the lower ileum from two birds per replicate was prepared as described by Abudabos et al. (2016). Measurements of height and width of the villi were based on at least 10 well-oriented villi per section using a microscope (IX71 Inverted Olympus).

Statistical analysis
The data were statistically analysed using analysis of variance in a completely randomized design. All statistical analysis was performed using the Statistical Analysis System (SAS 2003). The overall level of statistical significance was set at \( P < 0.05 \). All values were expressed as statistical means ± standard error of the mean (SEM).

Results

Performance traits
The findings of feed intake, body weight, FCR and PEF during the third and fourth week is given in Tables 2 and 3. FCR was significantly \( (P < 0.05) \) high in the T2 during the third and fourth week. During the fourth week, no significant change was found in performance parameters. The FCR did not change significantly \( (P > 0.05) \) between the control and treated groups during the fourth week, probably, due to the adaptability of the birds during this period.

Challenge inoculum
On day 15, except control group, birds in other groups were challenged by C. perfringens (MicroBiologics, Cloud, MN, USA) at the rate of \( 4 \times 10^8 \) CFU/g as oral gavages (Ao et al. 2012).

Performance measurements
Feed intake on a daily basis was calculated in the post-infection period by subtracting the amount of feed rejected from the feed offered. Total feed intake and body weight were measured at the end of the third and fourth week. Feed conversion ratio (FCR) was computed for each group by using the following formula: FCR = Feed intake/Weight gain.
The result of carcass quality is shown in Table 4. The dressing percentage decreased significantly (P < .05) in the infected group (T1). It is noteworthy that the dressing percentage did not differ between T2 and natural-feed-added groups (T3, T4 and T5). Intestinal weight was significantly (P < .05) higher in the treatment groups compared to the control.

### Table 4. The effect of treatments on carcass quality of broiler chickens at the end of fourth week.

| Treatments | Dressing (%) | Leg (%) | Breast (%) | Fat (%) | Liver (%) | Heart (%) | Spleen (%) | Intestines (%) |
|------------|--------------|---------|------------|---------|-----------|-----------|------------|----------------|
| Control    | 68.9a        | 28.4    | 37.1       | 1.2     | 4.4       | 1.0       | 0.2        | 6.2bc          |
| T1         | 63.7b        | 30.5    | 35.8       | 0.8     | 4.3       | 1.2       | 0.1        | 11.0ab         |
| T2         | 67.5bc       | 28.4    | 36.1       | 1.5     | 4.8       | 1.0       | 0.2        | 9.6a           |
| T3         | 65.6bc       | 27.8    | 37.8       | 1.1     | 4.9       | 1.2       | 0.2        | 9.1a           |
| T4         | 67.5bc       | 28.7    | 38.2       | 1.2     | 4.7       | 1.2       | 0.2        | 9.1a           |
| T5         | 64.8bc       | 27.9    | 39.9       | 0.8     | 4.8       | 1.1       | 0.1        | 8.8abc         |
| T6         | 68.2bc       | 28.4    | 40.8       | 0.8     | 4.3       | 0.9       | 0.2        | 10.6a          |
| SEM        | 0.84         | 0.61    | 0.79       | 0.23    | 0.21      | 0.09      | 0.02       | 0.89           |

Notes: Mean values in a column bearing different superscripts differ significantly (P < .05). T1: Infected; T2: Infected + Maxus; T3: Infected + Crina Poultry plus; T4: Infected + Sangrovit; T5: Infected + Fysal Fit 4; T6: Biostrong 510.

### Blood biochemical parameters

The effect of treatments on the blood biochemical parameters in broiler during the third week are given in Table 5. The result revealed that blood protein increased significantly (P < .05) in birds in T6, while globulin decreased significantly (P < .05) in T1. Blood glucose, protein, triglyceride AST and ALT concentration did not differ significantly (P > .05) between the control and treated birds. Similarly, the results of blood biochemical parameters during the fourth week showed that the total protein concentration increased significantly (P < .05) in T4, T5 and T6. The blood ALT concentration increased significantly (P < .05) in T5 compared to other groups. However, no significant changes (P > .05) were observed in glucose, total protein, globulin and AST concentration between the control and treated groups.

### Serum antioxidant status and ileal histology

The antioxidant status in the form of TAC (at the end of the fourth week) and TBAR during the third and fourth week is shown in Table 6. No significant change (P > .05) was observed in TAC at the end of the experimental period. Villus height and villus width increased significantly (P < .05) in T5 compared to other groups.
width of the ileum increased significantly ($P < .05$) in T6 compared to the control and T1.

**Discussion**

Non-antibiotic feed additives are now used to improve the growth and feed utilization (Dhama et al. 2015). In the present study, we found that that body weight and FCR were similar in control and treated groups. The FCR during the third week increased significantly in T2. In addition, the essential oils and phytobiotics (carvacrol from oregano, cinnamaldehyde from cinnamon and capsaiacin) performed similar to the control group showing the effectiveness since the birds in the treatments were infected. An improved growth performance with phytobiotics without any bacterial infection (Buchanan et al. 2008; Ahmad et al. 2011; Lee et al. 2015) and with a bacterial infection (Abudabos et al. 2016) have been reported previously. The improved performance, gut physiology and blood biochemical profile may be due to the mechanism of action of essential oils supplemented in the current study. The suggested mechanism of action of the essential oils and phytobiotics may be due to the enhanced feed intake, improved nutrient digestion, increased secretion of digestive enzymes and greater absorption in the intestines (Khan et al. 2012a, 2012b; Abudabos et al. 2016, 2017). Few studies have been conducted on the effect of phytobiotics in the infected birds. The improved performance in birds treated with essential oils and phytobiotics has been attributed to the mechanism of action through which they exert their action (Kim et al. 2016.). A considerable number of studies have documented that herbs, spices and various plant extracts have digestion-stimulating and antimicrobial effects (Amad et al. 2011; Khan et al. 2012a, 2012b), cause stimulation of digestive enzymes, improve nutrient utilization and absorption process in the intestines (Buchanan et al. 2008; Khan et al. 2012a). Sanguinarine is well known for its anti-inflammatory, antimicrobial and immune-modulating effects (Zdunczyk et al. 2010). In addition, it influences the gastrointestinal functions such as gut architecture, fermentation process and motility (Jankowski et al. 2009). An improved growth performance and carcass characteristics were noticed in the feed-additives-treated birds compared to the antibiotic-treated birds; this may be due to the multiple beneficial effect of their compounds. In the current study, the higher dressing weight in the photogenic feed additive groups may be due to the better weight gain and feed efficiency in these groups. In addition, the intestinal weight was higher in infected and infected + supplemented groups. The higher intestinal weight in these groups may be due to the inflammation and accumulation of the exudates in the intestines of these infected groups (Abudabos et al. 2016; Alzawqari et al. 2016).

In the present study, blood protein and globulin concentration increased significantly in T6 during the third week with no significant difference in the other metabolites. Amad et al. (2013) also reported significantly increased blood albumin and protein concentration in broilers in response to Biostrong feed additive with no significant change in the blood glucose. Ghazalah and Ali (2008) also reported improved protein, globulin and albumin profile in broiler in response to 0.5% rosemary leaves. The improved protein profile of broiler fed a diet containing phytobiotics may partially be due to the higher body weight, which is linked with the higher protein mass of the body. In addition, the phytobiotics act on the intestinal walls, promoting the absorption of more nutrients on the one side and on the other, digestive enzymes are secreted, which enhance the nutrient digestibility, leading to improved protein profile (Abudabos et al. 2016).

There was no significant effect of treatments on the blood concentration of triglyceride, glucose and AST in broiler. Similar observations were also reported in other studies (Amad et al. 2013). In the present study, serum ALT increased significantly in T5 at the end of the fourth week. Serum AST and ALT are a specific indicator of liver damage and dysfunction. During any pathological manifestation, serum AST and ALT are released from the liver into the bloodstream (Toghyani et al. 2011). The increase concentration of ALT in serum at the end of the fourth week in response to Fysal Fit 4 indicates the negative effect on the liver health. Reduced cholesterol and increased high-density lipoprotein were reported in broiler challenged with *C. perfringens* in response to Biostrong feed additive (Cho et al. 2014). The much discrepancy in the biochemical parameters in broilers in response to different feed additives in the published literature may be due to the differences in the genetic, nutrition, age and experimental designs of the studies.

In the present study, villus height and crypt depth increased significantly in T6 compared to the infected and control groups. It is well documented that microbes can cause a change in the intestinal architecture. Improved villus height and crypt depth ratio was reported by Amad et al. (2013) in broilers fed with Biostrong. An increase in the height of the intestinal villi and the villus crypt may correlate with an increased epithelial turnover. The increased intestinal dimensions in broiler in response to Biostrong indicate a positive effect of the product.

The authors concluded that phytobiotics could be used successfully in comparison of an antibiotic in maintaining the growth and biochemical profile of broiler challenged with *C. perfringens*.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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