Effect of organic acid blend and *Bacillus subtilis* alone or in combination on growth traits, blood biochemical and antioxidant status in broilers exposed to *Salmonella typhimurium* challenge during the starter phase

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**ABSTRACT**

The present study was designed to find the effect of an antibiotic, an organic acid and a probiotic on performance traits, blood biochemical parameters and antioxidant status during the starter phase exposed to *Salmonella typhimurium* challenge. A total of 300 day-old broiler chicks were randomly allocated to control (basal diet), T1: infected with *Salmonella enterica* subsp. Typhimurium; T2: infected + avilamycin; T3: infected + organic acid; T4: infected + *Bacillus subtilis*; T5: infected + organic acid + probiotic. The results showed that body weight, feed conversion ratio and production efficiency factor did not differ (P > .05) between the control and treated groups. Blood albumin and aspartate aminotransferase increased significantly (P < .05) in birds in T5 during the first week. Similarly, total protein and triglyceride concentration increased significantly (P < .05) in T4 and T5. The total antioxidant capacity in the second week decreased significantly in T4 compared to the control. Thiobarbituric acid reactive substances during the first and second weeks did not differ significantly (P > .05) between the control and treated groups. We concluded that the effect of organic acid blend and *B. subtilis* was similar to that of the antibiotic in broilers during the starter phase exposed to *S. typhimurium* challenge.

1. Introduction

Salmonellosis is one of the most frequent zoonotic disease caused by the bacterium *Salmonella* having more than 2500 serotypes (Calenge et al. 2010). Some of the *Salmonella* stereotypes can attack domestic animals, including poultry, and can cause severe clinical signs ranging from gastroenteritis to death. This bacterium can also be easily transferred to humans through the consumption of contaminated eggs and meat and causes food poisoning (Calenge et al. 2010; Bajpai et al. 2012). The Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) stressed on the need of effective interventions to reduce the incidence of Salmonellosis in broilers (FAO & WHO 2009). In poultry, a number of efforts have been undertaken to control the disease such as prophylactic measures, vaccination, the use of antibiotics and others (Calenge et al. 2010; Bajpai et al. 2012; Abudabos et al. 2015); however, fruitful results have not been achieved.

Antimicrobial growth promoters (AGPs) have been used in animal production for the last few decades to improve the growth and reduce mortality. The use of AGPs in controlling *Salmonella* infection is successful to a large extent; however, acquired resistance of these antimicrobial agents is a major concern (Bajpai et al. 2012; Dhama et al., 2015) and has been phased out by the European Union (Kabploy et al. 2016). Despite the ban, many countries in the world, including the USA, recommends the use of avilamycin in poultry at sub-therapeutic doses (Kabploy et al. 2016). Some studies have shown that the use of AGPs failed to reverse the number of pathogenic bacteria and failed to improve the performance of animals (La-ongkhum et al. 2011; Kabploy et al. 2016).

As a result of these circumstances, the poultry researchers are focusing on the alternative to antibiotics to optimize gut health and improve animal performance (Wu et al. 2011; Chand et al., 2016; Khan et al., 2016; Raza et al., 2016). One such alternative possessing growth-promoting characteristics is organic acid, which can maintain gut health and improve animal performance by balancing normal gut flora (Sultan et al. 2015). Organic acids and their derivatives are considered safe and increase the profitability by providing healthy meat to the consumers (Hayat et al. 2014). In addition, many researchers have documented that the use of *Bacillus subtilis* as a probiotic plays an essential role in animal performance and health by adjusting the intestinal ecological imbalance (Abudabos et al. 2013; Khan & Naz 2013). *Bacillus* species have been considered safe in animal production and can be easily administered to animals as an oral dose for weight gain and feed efficiency (Wu et al. 2011; Nguyen et al. 2015).

The objective of the present study was to assess the effectiveness of the *Bacillus* bacteria and an organic acid blend in comparison with the standard antimicrobial drug, avilamycin.
in broilers exposed to experimentally induced *Salmonella typhimurium* challenge during the starter phase.

2. 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.

2.1. Experimental design and management of birds

A total of 300 day-old broiler chicks (Ross 308) were weighed and randomly allocated to six treatments. Each group was further divided into five replicates having eight birds per replicate. On arrival, all chicks were confirmed for the absence of *Salmonella* spp. The experiment was conducted in an environmentally controlled experimental station where the temperature was maintained at 35 ± 0.5°C during the first week and 25 ± 0.3°C in the following week. A typical starter diet (1–14 days) consisting of isocaloric and isonitrogenous contents was offered in mash form as recommended by the National Research Council (NRC 1994). The ingredients and composition of feed are presented in Table 1. Upon arrival, the chicks were randomly distributed to one of the six treatments as follows: Control (basal diet); T1: infected with *Salmonella enteric* subsp. *Typhimurium*; T2: infected + avilamycin at the rate of 0.2 g/kg (Maxus, Vienna, Austria); T3: infected + organic acid blend containing phenolic compounds stimulating the release of butyrate, medium chain fatty acids and organic acids (Presan, Trouw Nutrition, Ireland); T4: infected + consisting of a probiotic containing phenolic compounds stimulating the release of butyrate, medium chain fatty acids and organic acids (Presan, Trouw Nutrition, Ireland); T5: infected + consisting of a probiotic containing phenolic compounds stimulating the release of butyrate, medium chain fatty acids and organic acids (Presan, Trouw Nutrition, Ireland), and T6: infected + consisting of a probiotic containing phenolic compounds stimulating the release of butyrate, medium chain fatty acids and organic acids (Presan, Trouw Nutrition, Ireland) + dicalcium phosphate at the rate of 0.2 g/kg (Maxus, Vienna, Austria). The challenge inoculum was diluted and adjusted to 3 × 10⁷ CFU/ml. Count of viable bacteria was confirmed before and after inoculation.

Table 1. Dietary composition of broiler chicks during starter.

| Ingredients                          | Starter |
|--------------------------------------|---------|
| Yellow corn                          | 57.62   |
| Soybean meal                         | 35.24   |
| Corn oil                             | 2.37    |
| Dicalcium phosphate                  | 2.30    |
| Ground limestone                     | 0.83    |
| Choline chloride                     | 0.05    |
| dl-Methionine                        | 0.20    |
| I-Lysine                             | 0.20    |
| Salt                                 | 0.46    |
| Threonine                            | 0.11    |
| Vitamins and minerals premix         | 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 mg; iron, 1200 mg; manganese, 18,000 mg; selenium, 60 mg; zinc, 14,000 mg.*

2.2. Challenge inoculum

In this experiment, the chicks were challenged with *S. enteric* subsp. *typhimurium* (MicroBiologics, St. Cloud, MN, USA) as described by Abudabos and Al-Mufarrej (2014). The strain was known for efficient colonization of the gut of broilers. The viability of the bacteria was confirmed before and after the inoculation. Briefly, stored at −80°C, the bacteria were retrieved and plated twice on tryptone soy agar for 24 h at 37°C. A single colony of the bacteria was transferred into sterile pre-warmed tryptone soy broth and incubated at 37°C for 18 h. The challenge inoculum was diluted and adjusted to 3 × 10⁷ CFU/ml. Count of viable bacteria was confirmed before and after inoculation.

2.3. Performance measurements

Feed intake (FI) on a daily basis was calculated in the post-infection period by subtracting the amount of feed rejected from the feed offered. Total FI was computed for each group at the end of the first and second weeks. FI and weight gain for each group were subjected to adjustment in case of mortality of birds. Feed conversion ratio (FCR) was computed for each group by using the following formula: FCR = FI/weight gain.

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

PEF = (liveability × live weight (kg)/(age in days × FCR)) × 100.

2.4. Biochemical measurements of blood

At the end of the first and second weeks, two blood samples (3 ml) were obtained from the wing vein of the bird perlicate and centrifuged at 3000 rpm for 10 min. Serum was harvested and then transferred into clean plastic tubes and stored at −20°C until analysis. Serum total antioxidant capacity (TAC) and thiobarbituric acid reactive substances (TBARS) were measured by using ELISA kits (Cayman Chemical Company, MI, USA). Total protein, albumin, glucose, alanine transaminase (ALT) and aspartate aminotransferase (AST) were measured by using ELISA kits (Cayman Chemical Company, MI, USA). All the analyses were carried out in duplicate.

All statistical analyses was performed using the Statistical Analysis System (SAS 2003). The overall level of statistical significance was set at *P* < .05. All values were expressed as statistical means ± standard error of the mean (SEM).

3. Results

3.1. Performance traits

The findings of FI, body weight (BW), FCR and PEF during the first and second weeks are presented in Tables 2 and 3. The results showed that the performance traits did not change significantly (*P* > .05) during the experiment. In infected birds (T1), the FI and corresponding weight gain, FCR and PEF decreased numerically. It is further important to note that the performance traits were not significantly different in antibiotic-treated (T2) and other AGPs.
### 3.2. Blood biochemical parameters

The effect of treatments on the blood biochemical parameters in broiler during the first week are presented in Table 4. The result revealed that blood albumin and AST increased significantly \( (P < .05) \) in birds in T5. Blood glucose, protein, globulin, triglyceride and ALT concentration did not differ significantly \( (P > .05) \) between the control and treated birds. Similarly, the results of blood biochemical parameters in Table 5 observed during the second week showed that total protein and triglyceride concentration increased significantly \( (P < .05) \) in T4 and T5, respectively. However, no significant changes \( (P > .05) \) were observed in glucose, total protein, globulin, AST and ALT concentrations between the control and treated groups.

### 3.3. Serum antioxidant status

The antioxidant status in the form of TAC (at the end of the second week) and TBAR during the first and second weeks is shown in Figure 1. The result showed that TAC decreased significantly in T4 compared to the control. However, no significant change \( (P > .05) \) was observed in TBAR during the first and second weeks.

### 4. Discussion

The current trend in poultry is to increase the application of non-antibiotic feed additives to improve the growth and feed utilization after banning the use of antibiotics in the feed of animals (Abduabos et al. 2015). The use of antibiotics as a feed additive under the sub-therapeutic level has been found to be useful in poultry production; however, a ban has been imposed on their use due to the possible antibiotic resistance and the presence of their residues in meat and milk products. Due to a big push to search for alternatives, several feed...
additives such as acidifiers, antioxidants, phylogenics, probiotics and prebiotics have been used successfully in poultry production.

In the present study, we found that the addition of an organic acid blend and a probiotic was as effective as the addition of an antibiotic in terms of growth performance and feed utilization, suggesting their possible role as potential alternatives. An improved growth performance and feed utilization fed with probiotics in comparison with antibiotics in broilers challenged with different pathogenic bacteria such as *Salmonella*, *Clostridium perfringens* and *Escherichia coli* have been reported recently (Abudabos et al., 2013, 2015; Al-Owaimer et al. 2014). However, the comparison of an organic acid blend with antibiotic alone or in combination with a probiotic has probably been reported for the first time in this study. The improved performance in birds treated with probiotic bacteria has been attributed to the mechanism of action through which the probiotic bacteria exert their action such as lowering the pH of the gut, suppression of pathogenic bacteria through the production of organic acids, prevention of the colonization of the bacteria through competitive exclusion, production of antibacterial mucin, stimulating the immune system, production of antibacterial enzymes (β-glucosidase and β-glucuronidase), competition for nutrients in the gut and others (Khan & Naz 2013). An improved performance in broilers by the addition of organic acid without any bacterial challenge has also been previously reported (Sultan et al. 2015). It has been suggested that the addition of organic acid significantly reduced the population of Salmonella by the production of acidic environment in the gut that favours the lowering of pH and arrests the growth and proliferation of harmful bacteria but favours the growth of beneficial bacteria (Samanata et al. 2010; Sultan et al. 2015). In addition, acidic environment favours the production and secretion of pepsin, gastrin and cholecystokin, which play a significant role in the nutrient utilization and subsequent growth performance and feed efficiency (Hayat et al. 2014).

In the present study, blood albumin and AST concentration increased significantly in T5 (organic acid + probiotic) during the first week, with no significant difference in the other metabolites. No significant effect on blood metabolites, particularly glucose, in birds fed with organic acid has been previously reported (Mahdavi & Torki 2009; Adil et al. 2010). In the previous studies, an increase in serum protein and albumin has been reported in probiotic-treated birds (Arslan & Saatci 2004; Yesilbag & Colpan 2006; Li et al. 2011; Rajput et al. 2013). We suggest that the increased protein, especially albumin, may be due to the fact that an acidic environment in the gut stimulates peptide-2 (Tappenden & McBurney 1998), which may result in better absorption of protein in the gut. A significant increase in AST during the first week and triglyceride in the second week in T5 was observed in the present study. A reduction in the concentration of triglyceride was documented in response to the supplementation of *Saccharomyces boulardii* and *B. subtilis* in broilers (Rajput et al., 2013). No significant difference in triglyceride was observed in broilers in response to *B. subtilis* and organic acid in birds in the previously documented reports (Adil et al., 2010; Li et al., 2011). The discrepancy may be due to the genetic, dose and duration of the agents and experimental conditions.

The TAC reflects accurately the antioxidant status of the organism; therefore, different antioxidant components in serum of the birds were not measured since it is neither possible nor practical. The TAC precisely measures the antioxidant status of any organism (Erel 2004). The body usually keeps a balance between the production of free radicals and antioxidants; however, under stress conditions, the balance shifts towards free radicals and oxidative stress is developed, which may harm cellular machinery, enzymes and DNA and protein (Abudabos & Al-Mufarrej 2014). In the present study, no significant effect was observed by the treatments on TBARS concentration during the two weeks, however, at the end of the study, TAC decreased significantly in the infected group and infected groups treated with organic acid and probiotic. It is noteworthy that the birds recovered from the infection stress when the organic acid and probiotics were combined (T5), suggesting the synergistic effect of both the entities. Further, an improved TAC concentration was also observed in the birds treated with the antibiotic. Recently the anti-oxidative effect of avilamycin has been linked to its scavenging effect on hydroxyl radicals (Kabploy et al. 2016). Improved TAC was also reported in another study in broilers simultaneously challenged with *S. enterica* subsp. *enterica* *typhimurium* and an organic acid (Abudabos & Al-Mufarrej 2014) and/or in response to *S. boulardii* and *B. subtilis* B10 (Rajput et al. 2013). The probiotic bacteria resist oxidation process, scavenge free radical and enhance the antioxidant potential of the organism (Capcarova et al. 2010).

5. Conclusion
The authors concluded that probiotic and organic acid could be used successfully in comparison to an antibiotic in maintaining the growth and biochemical profile of broilers challenged with *S. typhimurium*.

Disclosure statement
No potential conflict of interest was reported by the authors.

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