The effect of some natural alternative to antibiotics on growth and changes in intestinal histology in broiler exposed to *Salmonella* challenge

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ABSTRACT This study was conducted to find the effect of different feed additives on the production performance and intestinal histology in Salmonella challenged birds. A total of 600 day-old-broiler chicks (Ross 308) were assignment to 10 treatments. Each treatment was further divided into 10 replicates. The chicks were randomly divided into one of the following 10 treatments as follow: Negative control; positive control infected with *Salmonella enterica subsp. typhimurium*; T1, infected + avilamycin at the rate of 0.2 g/kg; T2, infected + probiotic having viable spores (2×10^7 CFU/g) of *Bacillus subtilis* (ATCC PTA-6737); T3, infected + Sanguinarine consisting of benzo phenanthridine alkaloids from Macleaya cordata; T4, *B. subtilis* (ATCC PTA-6737) + Sanguinarine; T5, infected + *B. subtilis* 500 g/T of feed (1.2×10^6 cfu/g); T6, prebiotic, *Saccharomyces boulardii* (1×10^8cfu/g); T7, infected + oregano at the rate of 1 g/kg; T8, infected + thermally processed clay calcium montmorillonite. The results showed that feed intake was significantly ($P < 0.01$) high in negative control and T2 compared to the positive control. Body weight gain was significantly ($P < 0.01$) higher in negative control and significantly ($P < 0.05$) low in T8. Feed conversion ratio was significantly ($P < 0.05$) high in negative control and significanlty ($P < 0.05$) high in T6. Similarly, PEF was also significantly ($P < 0.05$) high in negative control and significantly ($P < 0.05$) low in positive control and T8. Villus width was significantly ($P < 0.05$) high in negative control followed by T8. Dietary supplementation of different feed additives may be useful in broiler chicks challenged with Salmonella infection.

Key words: broiler, feed additives, intestinal histology, production, Salmonlla

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INTRODUCTION

Salmonella is an expensive human born disease, which can easily transfer into the chicken from the environment (Abudabos et al., 2016, 2017). During the starter phase, chicks are more vulnerable to the infection of Salmonella probably due to the low immunity (Wilson et al., 2016). Chicks may be predominantly exposed to the infection of Salmonella from different sources such as hatchery and poultry house. After entry into the body, Salmonella make colonies in the intestinal tract of the chicken during the starter phase and gradually declines during the later life (Wilson et al., 2016). Salmonellosis is easily transferred to the human through contaminated meat leading to the sever condition of food poisoning (Bajpai et al., 2012). Several practices are in vogue to control Salmonella such as prophylatic measures such as antibiotics and vaccination (Bajpai et al., 2012; Abudabos et al., 2017).

Antimicrobial growth promoters (**AGPs**) are the most frequently used chemical agents, which enhance feed conversion ratio and reduce chicken mortality (Khan et al., 2012a). The use of AGPs has been associated with acquired resistance and meat residues that jeopardize human health (Ayisi et al., 2017). Consequently, in many advanced countries, the unlimited use of these AGPs has been discouraged, therefore, the poultry producers are looking for alternative to antibiotics such as phytogentics (Khan et al., 2012b,c; Abudabos et al., 2016; Alzawqari et al., 2016). These natural products mostly originate from plant sources are potent source of improved growth performance and health in broilers (Khan et al., 2012d; Chand et al., 2016; Rahman et al., 2017; Abudabos et al., 2018). It has been suggested that plants derived extract, polyphenol and oils enhance the absorption of nutrients, secrete the digestive enzymes, improve the immune response and antioxidant status in broiler (Khan et al., 2012a; Tehseen et al., 2016).

The objective of the present study is to compare the effect of a standard AGP with plant derived compounds on growth traits and intestinal histology of broiler during the starter phase.

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Table 1. Dietary composition of broiler chickens starter diets.

Ingredient (%)	Starter phase
Yellow corn	57.39
Soybean meal	27.00
Palm oil	2.20
Corn gluten meal	8.80
Wheat bran	0.00
Dicalcium phosphate	2.30
Ground limestone	0.70
Choline chloride	0.05
DL-methionine	0.105
L-lysine	0.39
Salt	0.40
Threonine	0.17
V-M premix[1]	0.50
Analyses	
ME, kcal/kg	3,000
Crude protein, %	23.0
Non phytate P, %	0.48
Calcium, %	0.96
D. Lysine, %	1.28
D. Methionine, %	0.60
Sulfur amino acids, %	0.95
Threonine, %	0.86

[1]Vitamin-mineral premix contains in 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 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; 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.

MATERIALS AND METHODS

The study was approved by the departmental committee on ethics and welfare of animals approved by the Department of Animal Production, King Saud University, Saudi Arabia.

Husbandry of Birds and Experimental Design

A total of 600 day-old-broiler chicks (Ross, 308) were assigned to 10 treatments and further into 10 replicates. Upon arrival, the day old chicks were tested for the absence of Salmonella infection (Abudabos et al., 2017). The experiment was carried out with optimum temperature as recommended by Ross guide. A standard ration was prepared according to the recommendation of National Research Council (1994). The composition and analyses of starter ration is given in Table 1. The chicks were randomly divided into one of the following 10 treatments as follow: Negative control; positive control infected with *Salmonella enterica subsp. typhimurium*; T1, infected + avilamycin at the rate of 0.2 g/kg (Maxus, Viena, Austria); T2, infected + probiotic having viable spores (2×10^7 CFU/g) of *Bacillus subtilis* (ATCC PTA-6737) (Clostat, Kemin Industries, Inc., Des Moines, IA, USA); T3, infected + Sanguinarine (Sangrovit ®, Phytobiotics, Eltville, Germany), consisting of benzo phenanthridine alkaloids from Macleaya cordata (Willd.); T4, *B. subtilis* (ATCC PTA-6737) + Sanguinarine; T5, infected + *B. subtilis* 500 g/T of feed (1.2×10^6 cfu/g) (Gallipro Tect, Zusatzstoffe GmbH, Germany); T6, prebiotic, *Saccharomyces boulardii* (1×10^8 cfu/g); T7, infected + oregano at the rate of 1 g/kg (NOR-FEED ApS, Hvidovre, Denmark); T8, infected + thermally processed clay calcium montmorillonite (CaMM, Calibrin-Z®, Amlan International, Chicago, IL 60,611).

Challenge Inoculum

On day 2, all birds except control group (T1) were orally inoculated with 3×10^9 CFU/mL of *Salmonella enterica* subsp. *Typhimurium*.

Performance and Carcass Measurements

Measurement of feed intake (**FI**), body weight gain (**BWG**), and feed conversion rate (**FCR**) were taken at 5 d interval. Production Efficiency Factor (**PEF**) was calculated weekly by using the formula: PEF = (Livability × live weigh (kg)/Age in days × FCR × 100.

Hitomorphological Measurements

The tissue samples for histology were taken from the small intestine of 5 birds per treatment and processed for histological examination as described by Abudabos et al. (2016). Briefly, a 2-cm-long sample from the proximal ileum were collected, fixed in phosphate-buffered formalin for at least 48 h and embedded in paraffin. Five mm sections were cut and stained by using haematoxylin and eosin. For the measurement of height and width, 10 well-oriented villi per sample were chosen and observed under an inverted Olympus Microscope and a PC-based image analysis system (Olympus DP72 microscope digital camera; Olympus NV, Aartselaar, Belgium) with software analysis (cellSens digital imaging software for research application).

Statistical Analysis

All statistical analysis was performed for analysis of variance using the Statistical Analysis System (SAS, 2003). Means for measurements showing significant differences in the analysis of variance were tested using the PDIFF option. The overall level for statistical significance was set at $P < 0.05$.

RESULTS

Production Performance

The result of FI, BWG, FCR, BW, and PFE are given in Table 2. The results showed that FI was significantly ($P < 0.01$) high in negative control and T2 compared to the positive control. Body weight gain was significantly ($P < 0.01$) higher in negative control and significantly

Table 2. The effects of treatments on feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), body weight (BW), and performance efficiency factor (PEF) for the cumulative starter period (0 to 15 d of age).

Treatment	FI (g)	BWG (g)	FCR (g: g)	PFE
Negative control	437.0^{a}	299.9^{a}	1.46^{e}	196.6^{a}
Positive control	356.8^{e}	$262.9^{d,e}$	1.35^{a}	134.6^{e}
T1	$391.4^{d,e}$	$261.9^{d,e}$	$1.49^{b,c}$	$150.5^{c,d,e}$
T2	$420.8^{a,b}$	$291.5^{a,b}$	$1.44^{b,c}$	$171.3^{b,c}$
T3	$401.4^{c,d,e}$	$281.9^{b,c}$	$1.42^{e,d}$	$174.2^{a,b}$
T4	$407.5^{b,c,d}$	$278.4^{b,c,d}$	$1.46^{b,c,d}$	$162.5^{b,c,d}$
T5	$416.3^{a,b,c,d}$	285.0^{b}	$1.44^{c,d}$	$165.8^{b,c,d}$
T6	$429.23^{a,b}$	$290.43^{a,b}$	1.53^{a}	$167.65^{b,c}$
T7	$389.8^{c,d,e}$	$283.2^{c,d,e}$	$1.37^{b,c}$	$153.3^{b,c,d,e}$
T8	371.8^{e}	265.1^{e}	1.407^{b}	133.4^{e}
SEM±	11.27	9.31	0.022	8.13
P-value	0.0001	0.0001	0.0001	0.0001

Mean values bearing different superscripts in the column differ significantly ($P < 0.01$).

T1: Maxus; T2: CloSTAT; T3: Sangrovit; T4: CloSTAT + Sangrovit; T5: Gallipro Tect; T6, *Saccharomyces boulardii*, T7: Oregano; T8; Varium.

Table 3. The effects of treatments on villi height (L), width (W), and villi total area (TA) of broiler chickens at 15 d.

Treatment	Villus height (μm)	Villus width (μm)	Total area (mm^2)
Negative control	439.1	76.7^{a}	0.100
Positive control	425.3	$64.17^{b,c}$	0.085
T1	544.1	73.9^{a}	0.124
T2	614.8	57.6^{d}	0.110
T3	572.3	$63.5^{b,c}$	0.113
T4	536.1	$60.9^{b,c,d}$	0.104
T5	562.2	$61.1^{b,c,d}$	0.108
T6	474.4	$63.2^{b,c}$	0.152
T7	461.6	$62.4^{b,c}$	0.101
T8	625	$67.8^{a,b}$	0.127
SEM±	54.9	2.84	0.010
P-value	NS	0.0001	NS

NS: Not significant, SEM: Standard error of the mean; ^{abcd}Means in the column with different superscripts differ significantly.

T1: Maxus; T2: CloSTAT; T3: Sangrovit; T4: CloSTAT + Sangrovit; T5: Gallipro Tect; T6, *Saccharomyces boulardii*, T7: Oregano; T8; Varium.

($P < 0.05$) low in T8. Feed conversion ratio was significantly ($P < 0.05$) high in negative control and significantly ($P < 0.05$) high in T6. Similarly, PEF was also significantly ($P < 0.05$) high in negative control and significantly ($P < 0.05$) low in positive control and T8.

Intestinal Histology

The effect of villus height, width, and total surface area of broiler chicken is given in Table 3. There was no significant difference in villus height and total surface area between the control and treatment groups. Villus width was significantly ($P < 0.05$) high in negative control followed by T8.

Intestine of control chicken showed normal intestinal villi with normal enterocytes (Figure 1A). Intestine of chicken with Salmonella challenge during starter period showed severe sloughing and desquamation of lining epithelium with metaplasia into goblet cells (Figure 1B). Extensive metaplasia of columnar epithelium into goblet cells was detected (Figure 1C). Moderate hemorrhage was seen among the intestinal glands (Figure 1D). Intestine of chicken with Salmonella challenge during starter period then supplemented with Maxus showed extensive hyperplasia of enterocytes with severe metaplasia into goblet cells with mild desquamation of superficial epithelium (Figure 1E). Intestine of chicken with Salmonella challenge during starter period then supplemented with Clostat showed mostly normal tissue architecture with normal villi and enterocytes (Figure 1F). Intestine of chicken with Salmonella challenge during starter period then supplemented with Sangrovit showed shortening of intestinal villi with slight thickening due to moderate hyperplasia and metaplasia of columnar cells into goblet cells (Figure 1G). Intestine of chicken with Salmonella challenge during starter period then supplemented with Clostat and Sangrovit showed thickening of intestinal villi with hyperplasia and metaplasia of columnar cells into goblet cells together with desquamation of superficial lining epithelium (Figure 1H). Intestine of chicken with Salmonella challenge during starter period then supplemented with Gallipro showed severe thickening and shortening of intestinal villi with extensive hyperplasia and metaplasia of columnar cells into goblet cells together with desquamation and degeneration of superficial lining epithelium (Figure 1I). Intestine of chicken with Salmonella challenge during starter period then supplemented with *Saccharomyces boulardii* showed severe thickening of intestinal villi with extensive hyperplasia and metaplasia of columnar cells into goblet cells (Figure 1J). Intestine of chicken with Salmonella challenge during starter period then supplemented with Nor-spice showed hyperplasia of the lymphoid aggregations (Figure 1K). Intestine of chicken with Salmonella challenge during starter period then supplemented with Varium showed extensive degeneration and sloughing of intestinal villi with hyperplasia and metaplasia of columnar cells into goblet cells (Figure 1L).

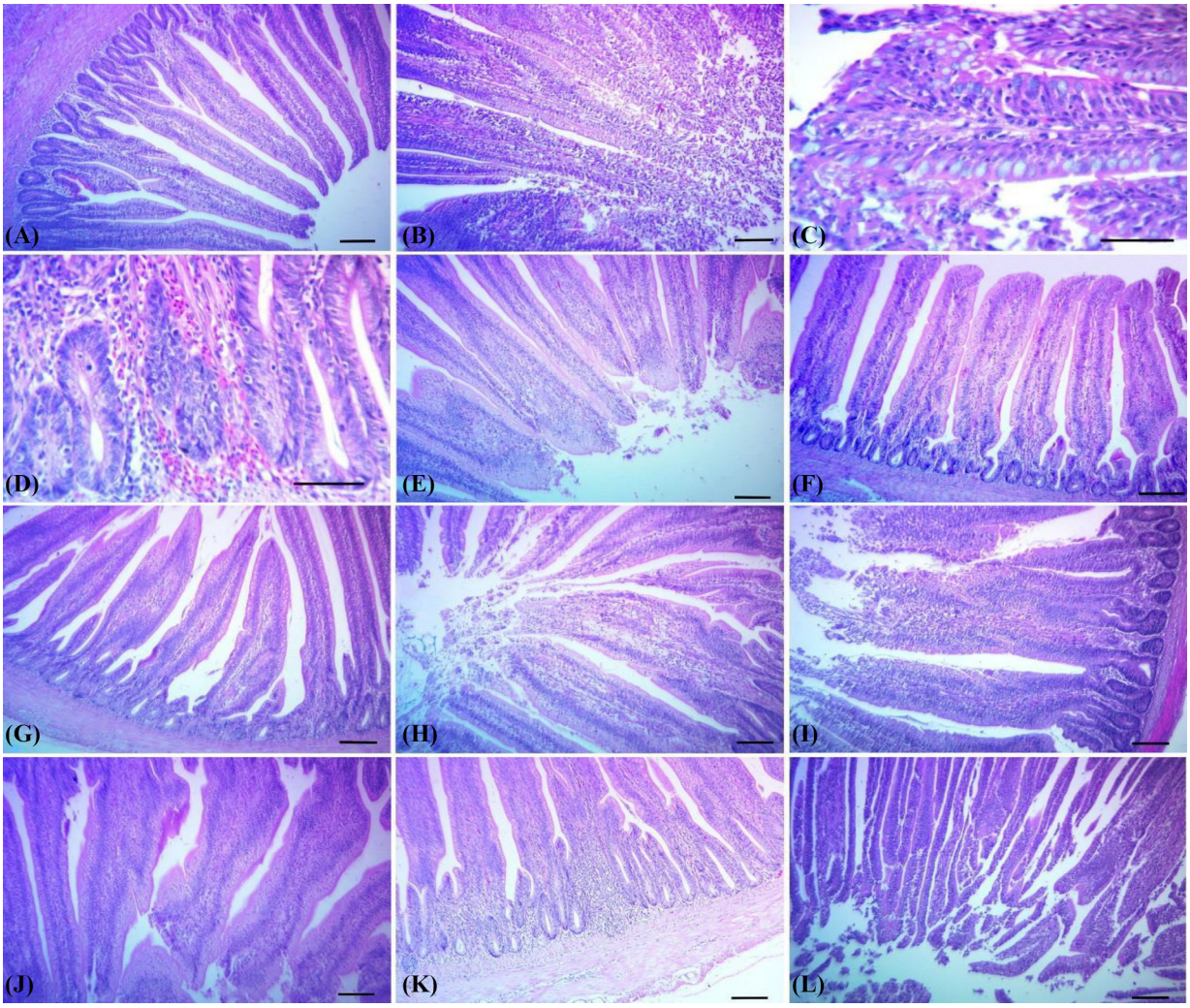

Figure 1. A, Intestine of negative control group showed normal intestinal villi with normal enterocytes. B, C, and D, Intestine of birds with Salmonella challenge during starter period. B, Showed severe sloughing and desquamation of lining epithelium with metaplasia into goblet cells. C, Showed extensive metaplasia of columnar epithelium into goblet cells. D, Showed moderate hemorrhage was seen among the intestinal glands. E, F, G, H, I, J, K, and L Intestine of chicken with Salmonella challenge during starter period co-supplemented with: E, Maxus showed extensive hyperplasia of enterocytes with severe metaplasia into goblet cells with mild desquamation of superficial epithelium. F, Clostat showed mostly normal tissue architecture with normal villi and enterocytes. G, Sangrovit showed shortening of intestinal villi with slight thickening due to moderate hyperplasia and metaplasia of columnar cells into goblet cells. H, Clostat and Sangrovit showed thickening of intestinal villi with hyperplasia and metaplasia of columnar cells into goblet cells together with desquamation of superficial lining epithelium. I, Gallipro showed severe thickening and shortening of intestinal villi with extensive hyperplasia and metaplasia of columnar cells into goblet cells together with desquamation and degeneration of superficial lining epithelium. J, *Saccharomyces boulardii* treated group showed severe thickening of intestinal villi with extensive hyperplasia and metaplasia of columnar cells into goblet cells. K, Nor-spice showed hyperplasia of the lymphoid aggregations. L, Varium showed extensive degeneration and sloughing of intestinal villi with hyperplasia and metaplasia of columnar cells into goblet cells. (A, B, E, F, G, H, I, J, K, and L Scale bar = 100 μm & C, and D Scale bar = 50 μm).

DISCUSSION

Due to the recent ban on the use of antibiotics in poultry feed, there is an increasing trend in the use of non-antibiotic feed additives to improve growth and health of the birds (Alhidary et al., 2016; Abudabos et al., 2016). In the present study, production parameters were declined in the infected birds, however, these parameters were much improved when they were also treated with different natural feed additives. In the current experiment, in the infected groups of birds given the basal unsupplemented diet had significantly reduce body weight compared to the unsupplemented infected birds. Decreased growth is a prominent symptom of Salmonellosis and the cause of major economic losses in poultry production (Abudabos et al., 2017).

In addition, the production performance in the birds fed with natural additives was almost parallel to the birds that were fed with the synthetic antibiotic. An improved performance and feed efficiency in broiler fed with the different feed additives has been reported by previous studies (Abudabos et al., 2016, 2017). The improved production performance in birds fed with herbal oil is due to the presence of different important alkaloids, which produce positive effects on the health of the broiler. A considerable studies have documented the positive effect of different phytogenic feed additives in broiler (Khan et al., 2012a; Alzawqari et al., 2016).

Sanguinarine is a well known herbal product with excellent biological properties (Abudabos et al., 2016). In addition, it influence gastric motility, fermentation process, and gut histomorphology (Jankowski et al., 2009). The effect of other feed additives such as probiotic and prebiotic are well known for the their positive effects on the broiler performance. It has been well documented that probiotic and prebiotic improve body weight gain and feed efficiency, reduce food born bacteria and stimulate immunity (Khan and Naz, 2013).

In the present study, villus width was influenced by the supplementation of different feed additives. The highest villus width was found in negative control group and T1. In addition, the villus width was deteriorated in the infected birds. In some of the treatment groups such as T8 (Calcium Montmorillonite), the villus width was much restored compared to the infected group of birds. Calcium Montmorillonite is comprised of soft and microscopic phyllosilicate minerals. This substance usually binds to the bacteria and the toxins. Intestinal lesions with thick mucus layer, ulceration, hemorrhages result in the death of the birds. In the current study, the infected unsupplemented birds showed the least villus width. It is also clear from the results that villus width was much improved among the supplemented groups especially with supplementation of Varium. Similar observations were reported by Lillehoj et al. (2016) in chicks infected with *Clostridium perfringens* and co-supplemented with Varium. Dietary prebiotic, probiotic, and phytochemicals have been shown to improve gut health in chickens (Abudabos et al., 2017, 2018; Rahman et al., 2017). In a similar study, Lillehoj et al. (2016) reported that dietary supplementation of Calcium Montmorillonite in broiler improved not only feed intake, weight gain, and feed efficiency but also reduced the intestinal lesion of necrotic enteritis in the *C. perfringens* challenged birds. In the same way, Ching and Cravens (2016) reported that Varium reduced the lesion score and mortality in necrotic enteritis infected birds compared to the control.

In conclusion, dietary supplementation of different feed additives improved the growth performance and gut health by mitigating the negative effect of the disease.

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