Feed supplementation with some natural products on *Salmonella* infected broilers’ performance and intestinal injury during the starter period

Alaeldein M. Abudabos\(^a\), Mashael R. Aljumaah\(^b\), Abdulaziz Abdullatif\(^a\) and Gamaleldin M. Suliman\(^a\)

\(^a\)Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia; \(^b\)Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia

**ABSTRACT**

Many alternatives are under investigation since 2006 when the European Union banned the use of antimicrobials in animal feed. The objective of this study was to find the effect of different feed additives in comparison with antibiotic on the performance and gut health in broiler infected with *Salmonella* during the starter phase. A total of 240 one day old male Ross 308 chickens were divided into six groups as follow: control treatment (negative control), orally challenged with *Salmonella enterica* subsp. Typhimurium (positive control), T1: oral challenge + 0.1 g/kg antibiotic, T2: oral challenge + 0.15 g/kg Sanguinarine, T3: oral challenge + 1 g/kg oregano powder, T4: oral challenge + calcium montmorillonite. On day second of the experiment, challenge was performed at a dose rate of 1.0 mL of the inoculum (4.5 \(\times\) 10\(^8\) CFU/mL). Data were statistically analysed with the help of Analysis of Variance (ANOVA) for repeated measures using least significant difference (LSD) as post-hoc test. Feed consumption was significantly (\(p < .01\)) improved in both positive and negative control groups and significantly lower in the other treatment groups. Significantly (\(p < .01\)) lower body weight was found in positive control, T3 and T4. Improved feed conversion ratio (FCR) was recorded in T2 and the control groups. Production Efficiency Factor (PEF) was significantly (\(p < .05\)) lower in positive control T2 and negative control. Villus surface area did not differ significantly (\(p > .05\)) between the control and experimental groups. Significantly (\(p < .01\)) higher villus width was found in T1, T4 and negative control. Similarly, villus height was higher (\(p < .05\)) in T2 and T4 compared to the control. It was concluded from the present study that different natural feed additives produced promising results in term of improved growth traits and gut health in *Salmonella* infected broilers. However, Sangrovit Extra produced better results in comparison with other treatments.

**HIGHLIGHTS**

- Body weight, feed efficiency were improved in Sangrovit Extra fed broiler.
- Production efficiency factor was enhanced in Sangrovit Extra fed broiler.
- Villus height was significantly higher in Sangrovit Extra fed broiler.
- Villus width was enhanced in antibiotic treated broiler.

**Introduction**

Salmonellosis is one of the expensive human food born diseases which is transferred from the contaminated environment into the consumers during meat processing and consumption (Abudabos et al. 2017). During the starter phase, broiler may particularly at the risk of Salmonellosis due to the weaker immune system and high growth rate (Wilson et al. 2016; Abudabos et al. 2019). Once entered into the body of the bird, *Salmonella* can easily proliferate in the intestinal tract leading to the stunt growth (Khan and Naz 2013). The disease may cause food poisoning in human due to the contaminated consumption of broiler meat (Abudabos, Alyemni, et al. 2016; Abudabos et al. 2017). Several strategies have been undertaken to reduce the incidence of bacterial infections such as antibiotics, organic acids, and phytobiotics (Abudabos, Alyemni, et al. 2018).

Antimicrobial drugs are frequently used in broiler industry to improve feed consumption and weight gain (Ali et al. 2019; Saleh et al. 2020). The use of these antimicrobial drugs has resulted in better feed consumption and weight gain. However, the emergence of antibiotic resistant pathogens has been a major concern (Ali et al. 2019).

CONTACT

Dr Alaeldein M. Abudabos (aabudabos@ksu.edu.sa) Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 2342342, Saudi Arabia

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efficiency, however, they have also been associated with marked concern of drug residues and antimicrobial resistance in human consumers (Khan et al. 2012a; Nasir et al. 2020). Recently, the European Union suspended the use of antimicrobial drugs in poultry sector, therefore, a dire need has always been advocated to find some suitable alternatives as feed additives to sustain the reasonable growth in broiler (Khan et al. 2012b; Wahab et al. 2019; Ahmad et al. 2020). One such alternative is phytobiotics that could probably compete with these synthetic agents and can maintain optimum growth in broiler industry (Safiullah et al. 2019).

Previous studies have indicated that different feed additives such as Sangrovit Extra, Nor-Spice and Varium produced positive impact on the production performance of broiler exposed to microbial infection (Bravo et al. 2014; Lillehoj et al. 2016; Abudabos, Alyemni, et al. 2016; Abudabos et al. 2017; Abudabos, Hussein, et al. 2018). Different compounds have been used in Salmonella-infected broiler to find non-antibiotic products such as essential oil of thymol, thyme, anise, organic acids, phytopheno, prebiotics and probiotics (Johny et al. 2009; Pickler et al. 2013; Rajani et al. 2016; Wilson et al. 2016; Abudabos, Alyemni, et al. 2016; Abudabos et al. 2017, 2019). These compounds are well known for improving production performance and preventing microbial infection in broiler for variety of mechanisms such as secretion of digestive juices, immune-modulating, antimicrobial and anti-inflammatory effects.

Few studies have evaluated the beneficial effects of Sangrovit Extra, Nor-Spice and Varium on the growth and intestinal histological characteristics in broiler challenged with Salmonella during the early stage of growth. Hypothesis of the current study was to test the comparative effects of different feed additives in Salmonella infected birds. Therefore, the objective of the current study was to find the effect of some natural products in comparison with an antibiotic on the growth and intestinal health of broilers during the induced infection of Salmonella in broiler in the starter phase.

Materials and methods

The local committee of Use and Care of Animals at King Saud University, Riadh, Saudi Arabia approve this study (CFSA/342/19).

Setting and animals

A total of 240 one day old male Ross 308 chickens free of Salmonella were purchased from the hatchery and divided into six groups (five replicates). Each group was consisted of 40 birds and each replicate contained 8 birds. The birds were kept in experimental cages (1.75 m²) with 15 birds per replicate keeping homogeneity in each box. Management guideline was followed as per pedigree manual. Water and feed were provided ad libitum throughout the experiment. The composition of feed is given in Table 1.

Experimental design

A completely randomised design having six treatments and five replicates of 8 birds was followed. All the birds in each group received qualitatively the same feed. The treatments were divided into control treatment (negative control), oral challenge of Salmonella enterica subsp. Typhimurium (positive control), T1: oral challenge + 0.1 g/kg antibiotic (Avilamycin, Maxus, Viena, Austria), T2: oral challenge + 0.15 g/kg Sanguinarine (Sangrovit Extra, Eltville, Germany), T3: oral challenge + 0.25 g/kg Oregano powder (Nor-Spice®, Hvidovre, Denmark), T4: oral challenge + 1.0 g/kg calcium montmorillonite (Varium, Chicago, USA). On the day second of the experiment, challenge was performed at a dose rate of 1.0 mL of the inoculum (4.5 × 10⁸ CFU/mL). Strict measures were taken to prevent the negative control from Salmonella contamination.

Table 1. Ration composition of broiler chickens during the starter phase.

| Ingredient (%) | Starter phase |
|----------------|---------------|
| Yellow corn    | 57.39         |
| Soybean meal (46%) | 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^    | 0.50          |

Analyses

Metabolizable energy, Kcal/kg 3000
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

^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 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.
**Birds performance and parameters**

To determine the performance of the birds in this experiment, chicks were weighed at the beginning of the study and then at the end of each week to obtain average weight gain. The diets were also weighed on daily basis and average daily feed intake was calculated for each batch. Feed consumption also served as the basis for calculation of feed conversion ratio (FCR) of each group of birds. The number of birds died was also recorded for each group as mortality percentage. Production Efficiency Factor (PEF) was calculated as described by Abduabos et al. (2018).

**Tissue preparation for intestinal histomorphology**

At the end of the experimental period, two birds per replicate were slaughtered and the digestive tract was removed to separate the intestinal tissue. About 3 cm section was separated from the ileum, excised and immediately fixed in 10% formalin solution for the next 72 hours followed by dehydration through gradually increased ethanolic content. Finally, the tissue was embedded in paraffin wax and processed to obtain 4 μm thin slices with the help of a microtome. Following staining with Haematoxylin and Eosin, histological features such as villus height, villus width and villus surface area were assessed by viewing the tissue at 40× magnification using light microscope and the images were captured using digital software (Olympus GmbH, Hamburg, Germany).

**Statistical analysis**

Data was statistically analysed with the help of Analysis of Variance (ANOVA) for repeated measures (SAS Institute 2003, Cary, NC, USA) using least significant difference (LSD) as post-hoc test. The results were presented as the mean and pooled standard error of mean (SEM). p value less than .05 was considered statistically significant.

**Results**

**Zootechnical performance**

Growth effects of the treatments in the infected birds are given in Table 2. Feed intake was significantly (p < .01) higher in both negative and positive control and significantly low in the other treatment groups. Significantly (p < .01) improved body weight was found in the negative control and lower (p < .01) in the positive control, T3 and T4. Improved FCR was recorded in T2, positive and negative control in comparison with T4. Production Efficiency Factor improved significantly (p < .05) in T2 and negative control in comparison to the rest of the treatments. No significant (p > .05) difference was observed between the control and treatment groups.

**Intestinal histological features**

The effect of the treatments on the villi health of *Salmonella* treated chickens during the starter phase is given in Table 3. The results showed that villus surface area had no significant (p > .05) effects on the treatments. Villus height was higher (p < .05) in T2 and T4. Villus width was significantly (p < .01) higher in T1, T4 and negative control.

**Discussion**

There is a tremendous pressure on poultry farmers for using alternative to antibiotics to maintain health and performance. The effect of the treatments on the villi health of *Salmonella* treated chickens during the starter phase is given in Table 3. The results showed that villus surface area had no significant (p > .05) effects on the treatments. Villus height was higher (p < .05) in T2 and T4. Villus width was significantly (p < .01) higher in T1, T4 and negative control.

**Table 2.** The effects of treatments on feed intake (FI), body weight (BW), feed conversion ratio (FCR), performance efficiency factor (PEF) and mortality of control and *Salmonella* infected broiler.

| Treatments     | FI (g) | BW (g) | FCR (g: g) | PFE | Mortality (%) |
|----------------|--------|--------|------------|-----|---------------|
| Negative control | 437.0a  | 374.7a  | 1.259c     | 196.6a | 1.9           |
| Positive control | 396.8ab | 308.6c  | 1.285a     | 109.12d | 3.5          |
| T1              | 391.4bc | 331.1bc | 1.390b     | 150.5c  | 2.9          |
| T2              | 401.4bc | 344.3bc | 1.295b     | 174.2bc | 3.4          |
| T3              | 389.8bc | 330.1bc | 1.383b     | 153.3bc | 2.8          |
| T4              | 371.8bc | 309.1bc | 1.407b     | 133.4bc | 3.1          |
| SEM±            | 12.11   | 12.20   | 0.023      | 8.13   | 0.2          |
| P value         | 0.0005  | 0.0001  | 0.0001     | 0.0001 | 0.1          |

T1: infected +0.1 g/kg Maxus; T2: infected +0.15 g/kg Sangrovit Extra; T3: infected +0.25 g/kg Nor-Spice; T4: Infected +1.0 g/kg Varium. Mean values bearing different superscripts in the column differ significantly (p < .01). Each treatment consisted of five replicates.

**Table 3.** The effects of treatments on villi height (L), width (W) and villi total area (TA) of control and *Salmonella* infected broiler.

| Treatments     | Villus height (μm) | Villus width (μm) | Total area (mm²) |
|----------------|---------------------|-------------------|------------------|
| Negative control | 439c               | 76.7abc           | 0.100            |
| Positive control | 425c               | 64.17abc          | 0.085            |
| T1              | 544b               | 73.9a             | 0.124            |
| T2              | 572abc             | 63.5c             | 0.113            |
| T3              | 474c               | 63.2a             | 0.052            |
| T4              | 625a               | 67.8bc            | 0.127            |
| SEM±            | 54.9                | 2.89               | 0.010            |
| P value         | 0.03                | 0.05               | NS               |

NS: Not significant; SEM: Standard error of the mean; T1: infected +0.1 g/kg Maxus; T2: infected +0.15 g/kg Sangrovit Extra; T3: infected +0.25 g/kg Nor-Spice; Infected +1.0 g/kg Varium. Each treatment consisted of five replicates.

abcMeans in the column with different superscripts differ significantly (p < .01)
productivity of birds due to the recent ban on the use of excessive utilisation of antibiotics in poultry feed (Haq et al. 2020). In the current study, we observed that production traits were declined in the infected birds compared to the negative control. Feed intake was significantly reduced in the infected birds. The reason could be less digestion and absorption from the inflamed intestines. In addition, an ameliorative effect was observed when the birds were fed with some feed additives either in the form of synthetic antibiotic or natural products. Stunt growth is a prominent sign during Salmonella infection in broilers and results in heavy economic losses in broiler farms (Wilson et al. 2016). Interestingly, the production performance was much improved with natural products in the current study. Improved weight gain and feed efficiency have been reported in the previous studies in response to different natural products such as Crina Poultry Plus, Fysal Fit, Biostrong, Clostat and Presan during the infections (Abudabos et al. 2017; Abudabos, Alyemni, et al. 2018). Abudabos et al. (2017) reported that Clostat improved the feed intake, weight gain and feed efficiency in broiler infected with Salmonella typhimurium challenge. Abudabos, Alyemni, et al. (2018) concluded that different commercial products such as Crina Poultry Plus, Sangrovit, Fysal Fit 4 and Biostrong enhanced the weight gain in broiler infected with Clostridium perfringens. These positive effects could be due to the presence of different important compounds present in the natural products which improve the health and immune response in birds leading to the higher production performance in broiler (Khan et al. 2012c, 2012d). In addition, different phytocepicics improve the performance of birds by improving the digestion and assimilation of nutrients by increasing the secretion of digestive enzymes and hormones (Saleh et al. 2018; Hafeez et al. 2020). Different studies have reported improved performance in broiler infected with Salmonella when treated with natural products such as Zeolites, Sanguinarine, CRINA Poultry, Sangrovit Extra and Fysal 4 fit (Al-Nass et al. 2011; Pickler et al. 2013; Abudabos, Alyemni, et al. 2016; Abudabos et al. 2017). In the present study, weight gain and feed efficiency were much improved in Sangrovit (T2) compared to the positive control. Sanguinarine is known for a number of biological activities such as gastric motility, production and secretion of gastric juices, fermentation and augmentation of gut histomorphology in broiler (Lee et al. 2014; Bavarsadi et al. 2017). In addition, this product has well known effects as immune-modulating, antimicrobial and anti-inflammatory (Zdunczyk et al. 2010).

In the present study, villus surface area did not improve significantly in the treatment groups. However, villus height and width were significantly higher in Varium (T4) compared to the T2 and T3. These results were also statistically similar to T1. Varium is a heat treated calcium montmorillonite which is composed of microscopic phyllosilicate minerals. This compound has the ability to bind with pathogens and their toxins. Intestinal health is very important for the proper growth of the birds and clinical symptoms such as haemorrhages, ulceration and thick mucosal layer may result in poor absorption of the feed contents leading to the stunt growth. Our results in term of improved villus width in response to phyllosilicate minerals were previously reported by Abudabos, Hussein, et al. (2018) in Salmonella infections. Similar observatories were also recorded by Lillehoj et al. (2016) in broiler during necrotic enteritis. The phytocepicics are well known for improving microstructures, enhancing fermentation process and increasing the intestinal motility in broiler (Saleh 2014; Saleh et al. 2018; Hafeez et al. 2020).

**Conclusions**

It was concluded from the present study that different natural feed additives produced promising results in term of improved growth performance and gut health in broiler infected with Salmonella. However, Sangrovit Extra produced better results in comparison with other treatments.

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**Ethical statement**

This work was approved by the ethical committee on animal welfare and rights King Saud University, Riadh, Saudi Arabia.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).
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ORCID
Gamaleldin M. Suliman http://orcid.org/0000-0001-9865-1589

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