



**Research Article** 

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### ABSTRACT

This study was conducted to evaluate the effect of ginger powder on the performance of Salmonella challenged broiler chicks and to compare its efficacy as growth promoter with an antibiotic, avilamycin. In a completely randomized design, 300 chicks were divided into six groups, five replicates with 10 birds per each replicate. The treatments included negative control (basal diet without ginger or antibiotic and challenging), positive control (basal diet without ginger or antibiotic, but with challenging), ginger treated group (5.0 g/kg ginger, without challenging), challenged ginger treated group (5.0 g/kg ginger, with challenging), antibiotic treated group (100 mg/kg avilamycin, without challenging) and challenged antibiotic treated group (100 mg/kg avilamycin, with challenging). The lowest mean of mortality was recorded in the antibiotic treated group and the highest in the positive control. Inclusion of ginger to the diet of challenged chicks decreased numerically the mortality as compared with the positive control. Broilers in the positive control had the lowest overall daily gain. Dietary inclusion of ginger or avilamycin increased (P<0.05) daily weight gain, but the effect of avilamycin was greater than ginger. In the overall period (days 0-42), challenging increased feed conversion ratio and inclusion of avilamycin and ginger to the diet decreased (P<0.05) it, but the effect of avilamycin was greater than ginger. Finally, inclusion of ginger powder in the diet could improve the performance of unchallenged and challenged chicks, but its efficacy is lower than avilamycin.

KEY WORDS antibiotic, broiler chicks, ginger powder, growth, Salmonella challenging.

# INTRODUCTION

The sub-therapeutic doses of antibiotics were used for decades as a growth promoter and to control of some specific intestinal pathogens such as *Salmonella* and Escherichia coli (Jones *et al.* 2003). Although broiler chicks fed diet supplemented with these additives achieved good performance, their side effects became a serious public health problem worldwide (Soomro *et al.* 2010) and led to the ban of these feed additives by the European Union. The ban on feed additives has stimulated extensive research into the phytobiotic alternatives. Ginger is one of the phytobiotics with several compounds such as gingerol, gingerdiol and gingerdione that possess strong antioxidant (Kikuzaki *et al.* 1993; Zhao *et al.* 2011) and antibacterial activities (Meena and Sethi, 1994; Akoachere *et al.* 2002; Ali *et al.* 2007).

Several studies demonstrated that ginger is beneficial to growth in unchallenged chicks (Zhang *et al.* 2009) and aquatic animals (Venkataramalingam *et al.* 2007; Immanuel *et al.* 2009). In the literature, information about the effect of

ginger on health status and performance of challenged broiler chicks and its efficacy in comparison with antibiotic is lacking. Therefore, the objectives of this study were to assess the effect of supplementation of ginger powder on the growth performance of challenged broiler chicks and to compare its capacity as growth promoter with an antibiotic.

## MATERIALS AND METHODS

#### Salmonella culturing

Salmonella enterica serovar Enteritidis (PTCC 1709) was obtained as freeze-dried from the Persian type culture collection (IROST, Tehran, Iran) isolated from the liver of chickens. Freeze-dried inoculum was grown in tryptic soy broth (Acumedia manufacturers Inc., Baltimore, MD, USA) at 37  $^{\circ}$ C for 8 h and passed to fresh tryptic soy broth for 3 another three periods (37  $^{\circ}$ C, each period 8 h). Determination of the number of colony-forming units (CFU) through decimal dilution series was performed in sterile buffered peptone water with pH 7.2. For this, 0.1 mL of diluted medium was inoculated in petri dishes containing *Shigella–Salmonella* agar (SS agar) and cultivated for 24 h at 37  $^{\circ}$ C, then CFU counted.

#### Birds and experimental design

Day-old Ross 308 broiler chicks (n=300, mixed sex) were obtained from commercial hatchery. In a completely randomized design, the chicks were randomly divided into six treatment groups, five replicates, and each 10 chicks placed in separate pens. The treatments included negative control (basal diet without ginger or antibiotic and challenging), positive control (basal diet without ginger or antibiotic, but with challenging), ginger treated group (5.0 g/kg ginger, without challenging), challenged ginger treated group (5.0 g/kg ginger, with challenging), antibiotic treated group (100 mg/kg avilamycin as the sub-therapeutic dose, without challenging) and challenged antibiotic treated group (100 mg/kg avilamycin, with challenging). Environmental temperature in the first week of life was 33 °C and decreased to 20 °C until the end of the experiment. During the first week, 22 h of light as continuous was provided with a reduction to 20 h afterward. Chicks were provided ad libitum access to water and experimental diets based on corn and soybean meal (Table 1). At day 7 of age, chicks in challenged groups received by oral gavage  $1.0 \times 10^5$  CFU / chick of passaged medium. Unchallenged birds received the same amount of sterile buffered peptone water by oral gavage.

#### Moisture content of litter

At d 20 and 40 of age, moisture content of litter was determined in each pen by collecting three samples (300 g) in areas of the pen away from feeders and drinkers. The three samples were mixed together and one homogeneous subsample was weighed (50 g), oven dried at 105  $^{\circ}$ C for 24 h, and reweighed to determine moisture content.

#### **Growth performance**

Growth performances of broilers were evaluated by recording body weight gain, feed intake and feed conversion ratio on d 21 and 42 of age. Feed intakes of birds were recorded on a per pen basis, the uneaten feed was weighed and discarded, and then fresh feed replaced in feeders at the end of each day. Total feed intake and feed intake of each period were recorded and it was divided to its consumption period for computing daily feed intake.

Chicken body weight was measured on 21 and 42 day of age. After correction for the initial body weight, it was divided to the related period for computing daily body gain. Feed conversion ratio (FCR) was calculated as the amount of feed consumed per unit of body weight gain for total and each period. FCR was corrected for body weight of mortality.

| Tabla 1  | Ingradiants | and | abamiaal  | 000 | nosition | of  | basal | diate |
|----------|-------------|-----|-----------|-----|----------|-----|-------|-------|
| I able I | ingreuients | anu | chennical | com | position | OI. | Dasai | ulets |

| Ingredients (%)                     | Starter | Grower |  |  |  |  |  |
|-------------------------------------|---------|--------|--|--|--|--|--|
| Yellow corn                         | 58.13   | 62.21  |  |  |  |  |  |
| Soybean meal                        | 32.81   | 25.84  |  |  |  |  |  |
| Fish meal                           | 3.00    | 0.00   |  |  |  |  |  |
| Soybean oil                         | 2.04    | 2.76   |  |  |  |  |  |
| Corn gluten meal                    | -       | 5.00   |  |  |  |  |  |
| Dicalcium phosphate                 | 1.54    | 1.76   |  |  |  |  |  |
| CaCO3                               | 1.10    | 1.17   |  |  |  |  |  |
| Salt                                | 0.36    | 0.26   |  |  |  |  |  |
| Mineral premix                      | 0.25    | 0.25   |  |  |  |  |  |
| Vitamin premix                      | 0.10    | 0.20   |  |  |  |  |  |
| DL-methionine                       | 0.17    | 0.00   |  |  |  |  |  |
| L-lysine HCl                        | -       | 0.06   |  |  |  |  |  |
| Celite <sup>b</sup>                 | 0.50    | 0.50   |  |  |  |  |  |
| Calculated chemical composition (%) |         |        |  |  |  |  |  |
| ME (kcal/kg) 2920 3060              |         |        |  |  |  |  |  |
| Crude protein                       | 21.00   | 19.50  |  |  |  |  |  |
| Calcium                             | 1.00    | 0.90   |  |  |  |  |  |
| Available phosphorus                | 0.50    | 0.45   |  |  |  |  |  |
| Methionine                          | 0.52    | 0.37   |  |  |  |  |  |
| Methionine + cystine                | 0.87    | 0.75   |  |  |  |  |  |
| Lysine                              | 1.2     | 0.96   |  |  |  |  |  |
| Threonine                           | 0.86    | 0.76   |  |  |  |  |  |

<sup>a</sup>On an as-fed basis

<sup>b</sup> Ginger or antibiotic was added to diets in replace of celite.

#### **Intestinal microflora**

On d 10 of age, samples collected from the ileo-cecum of an eviscerated bird in each pen were put into sterilized vials and conveyed immediately to the laboratory. Then 1.0 g of digesta taken from each sample was added to 9 mL tryptic soy broth and mixed for 1 minute. A tenfold serial dilution was made by transferring 1 mL from each mixture to the same broth volumes. Finally 1 mL was pipetted from the 1 / 1000 dilution test tube of each sample and inoculated on the solid culture medium prepared in petri dishes the previous day. Dispersion was done using a sterile spreader sterilised after each step over a Bunsen flame. *Salmonella* counts were performed using *Shigella-Salmonella* agar medium.

#### Statistical analysis

The Kolmogorov-Smirnov test was used to test the normal distribution of the data before statistical analysis. Statistical analyses were conducted with the general linear model procedure of SAS for windows version 9.1. (SAS Institute Inc., Cary, NC). Whenever significant differences were found, mean values were compared by Tukey test. A probability value of less than 0.05 was considered statistically significant.

## **RESULTS AND DISCUSSION**

#### Effect on Salmonella count

The effect of dietary treatments on *Salmonella* count in the samples collected from the ileo-cecum is shown in Figure 1. The highest *Salmonella* count was observed in the positive control. A significant reduction of *Salmonella* count was observed from antibiotic treated chicks as compared with that in the positive control or ginger treated group (Figure 1). Inclusion of antibiotic to the diet of challenged chicks statistically reduced *Salmonella* count, but inclusion of ginger reduced it numerically, as compared with the positive control. Birds not gavaged with *Salmonella* had any detectable *Salmonella* in the lower intestinal tract.



Figure 1 Salmonella count of chicks at the challenged groups

#### Effect on moisture content of litter

Two days after challenging, diarrhea was observed in the majority of birds reared in the challenged groups; whereas no birds reared in unchallenged groups showing signs of disease. Diarrhea severity as moisture content of the litter in challenged chicks was lower in those fed diets containing avilamycin compared with those fed ginger. In the chicks fed diet containing ginger, diarrhea severity was lower than those were in the positive control. For qualification of diarrhea status, moisture content of litter was determined. Moisture content of litter at days 20 and 40 of age are in

Figure 2. At d 20 of age, the highest moisture content of litter was related to the positive control and the lowest to antibiotic treated group. Moisture content of litter in the pens of challenged chicks fed diet containing antibiotic or ginger decreased (P<0.05) as compared with that of positive control. There were no differences among treatments for moisture content of litter at d 40 of age (Figure 2).



Figure 2 Moisture contents of the litter that were measured at days 20 and 40 of raising period

#### Effect on chick mortality

The lowest mean of mortality was recorded for the antibiotic treated groups and the highest mean for the positive control (Figure 3). The inclusion of ginger to the diets of challenged chicks decreased numerically the mortality of chicks compared with the positive control.



Figure 3 Mortality percentages of chicks during the overall period

#### Effect on growth performance

Average initial body weight of broilers chicks was  $42.3 \pm 0.91$  g and did not differ among treatments. Body weight of chicks on d 21 and 42 of age were affected by treatments. In both periods, the lowest body weight was recorded in the positive control and the highest mean was found in the antibiotic treated group. The inclusion of ginger to the diet of unchallenged chicks increased (P<0.05) the body weight compare with the negative control, but its effect was lower

than that of antibiotic treatment. Challenged broilers fed diet containing antibiotic or ginger had significantly higher body weight than the positive control. There was no difference in the body weight of challenged birds treated with antibiotic or ginger on d 42 of age, but there was on d 21 of age (Table 2). Average feed intake of broilers during the phases of starter, grower and the overall of the experiment is shown in Table 2. There were no significant differences among feed intake of broilers in the starter phase and the overall of the experiment. Significant differences in feed intake (P<0.05) were noted among treatments during the grower phase. Challenging of broilers with *Salmonella* resulted in a decrease of feed intake. As seen in Table 3, broilers in the positive control had significantly lower feed intake compared with the negative control.

Inclusion of antibiotic or ginger increased numerically feed intake in the challenged broilers compare with that in the positive control. Average daily gain and feed conversion ratio of broilers are shown in Table 3. Among treatments, there were significant differences regarding daily gain in the starter, grower and the overall of the experiment. Broilers in the positive control had the lowest daily gain at either phases or the overall period. Inclusion of antibiotic or ginger to the diet of unchallenged or challenged chicks increased significantly average daily gain, but the effect of antibiotic was greater than ginger. At the starter phase, there was no significant difference among feed conversion ratio of broilers (Table 3). At the grower and the overall period, challenging increased feed conversion ratio and inclusion of antibiotic and ginger to the diet decreased it significantly. The overall feed conversion ratio for challenged birds fed diets containing antibiotic or ginger was not differ, but for unchallenged ones was differ significantly.

The present study was designed to assess the effect of supplementation of ginger powder on growth performance of *Salmonella* challenged broiler chicks and to evaluate its capacity as growth promoter. It was hypothesized that *Salmonella* challenging would decrease performance and the inclusion of ginger or antibiotic to the diet of challenged chicks would enhance it alike.

### Mortality and Salmonella recovery

Mortality in challenged groups increased four days after Salmonella infection. Mortality and diarrhea was observed in challenged chicks, which confirmed the challenge infection with Salmonella Enteritidis. The mortality was observed up to 9 days. Chicks were weak and lethargic with distinct green diarrhea before dead. Dietary inclusion of ginger or antibiotic significantly decreased mortality of challenged chicks may be due to their negative effect on Salmonella colonization in the intestine as shown in Figure 1. In the available literature, there was no study regarding the effect of ginger on Salmonella count or viability of chicks. The result of this study was in agreement with the results obtained in pathogen challenged shrimp (Chang et al. 2012) fed ration containing ginger. Ginger compound acts as antimicrobial agent and immune-stimulants by enhancing immune response (Dügenci et al. 2003; Zhou et al. 2006) and it also reduces mortality against pathogenic

Table 2 Body weight and feed intake of broilers fed diets containing ginger or antibiotic with or without challenging

| Dist       | Challenging —      | Body weig         | ght (g/bird)       | Feed intake (g/bird/d) |                     |      |  |
|------------|--------------------|-------------------|--------------------|------------------------|---------------------|------|--|
| Diet       |                    | 21                | 42                 | 0-21                   | 22-42               | 0-42 |  |
| Control    | Without (negative) | 496 <sup>ab</sup> | 1773 <sup>b</sup>  | 28.1                   | 130.1ª              | 80.4 |  |
|            | With (positive)    | 444 <sup>b</sup>  | 1531°              | 27.3                   | 120.6 <sup>b</sup>  | 74.2 |  |
| <i>C</i> : | Without            | 529 <sup>a</sup>  | 1904 <sup>ab</sup> | 28.9                   | 132.6 <sup>a</sup>  | 80.7 |  |
| Ginger     | With               | 505 <sup>ab</sup> | 1789 <sup>ab</sup> | 28.0                   | 124.1 <sup>ab</sup> | 79.6 |  |
| Avilamycin | Without            | 553 <sup>a</sup>  | 1950 <sup>a</sup>  | 29.7                   | 129.6 <sup>a</sup>  | 79.2 |  |
|            | With               | 514 <sup>a</sup>  | 1801 <sup>ab</sup> | 28.5                   | $124.8^{ab}$        | 77.1 |  |
| SEM        | -                  | 35.1              | 88.4               | 1.16                   | 4.77                | 3.39 |  |

SEM: standard error of the mean.

Means in the same column with different superscripts are differ (P<0.05).

| Table 3 <mark>A</mark> v | verage daily | gain and feed | conversion ratio | of broilers f | ed diets | containing ginge | r or antibiotic | with or without | challenging |
|--------------------------|--------------|---------------|------------------|---------------|----------|------------------|-----------------|-----------------|-------------|
|--------------------------|--------------|---------------|------------------|---------------|----------|------------------|-----------------|-----------------|-------------|

| Dist       | Challensing        | Avera              | ige daily gain (g/bird | Feed conversion ratio (g:g) |       |                    |                    |
|------------|--------------------|--------------------|------------------------|-----------------------------|-------|--------------------|--------------------|
| Diet       | Challenging        | 0-21 22-42 0-42    | 0-21                   | 22-42                       | 0-42  |                    |                    |
| Control    | Without (negative) | 21.6 <sup>ab</sup> | 60.8 <sup>a</sup>      | 41.2 <sup>b</sup>           | 1.30  | 2.13 <sup>ab</sup> | 1.95 <sup>ab</sup> |
|            | With (positive)    | 19.1 <sup>b</sup>  | 51.7 <sup>b</sup>      | 35.4°                       | 1.43  | 2.33 <sup>a</sup>  | 2.09 <sup>a</sup>  |
| Ginger     | Without            | 23.2ª              | 65.4 <sup>a</sup>      | 44.3 <sup>ab</sup>          | 1.25  | 2.03 <sup>ab</sup> | $1.82^{ab}$        |
|            | With               | 22.0 <sup>ab</sup> | 61.6 <sup>a</sup>      | 41.6 <sup>ab</sup>          | 1.27  | $2.04^{ab}$        | $1.82^{ab}$        |
| Avilamycin | Without            | 24.3 <sup>a</sup>  | 66.5 <sup>a</sup>      | 45.4 <sup>a</sup>           | 1.22  | 1.94 <sup>b</sup>  | 1.74 <sup>b</sup>  |
|            | With               | 22.4 <sup>a</sup>  | 61.2 <sup>a</sup>      | $41.8^{ab}$                 | 1.27  | $2.06^{ab}$        | $1.85^{ab}$        |
| SEM        | -                  | 1.67               | 4.12                   | 2.10                        | 0.110 | 0.174              | 0.113              |

SEM: standard error of the mean.

Means in the same column with different superscripts are differ (P<0.05).

challenges in shrimp (Chang *et al.* 2012). The antimicrobial mode of action is considered to arise mainly from the potential of the essential oils in ginger (i.e. gingerols), to intrude into the bacterial cell membrane, disintegrate membrane structures and cause ion leakage (Meena and Sethi, 1994; Gugnani and Ezenwanze, 1985). Some studies demonstrated *in vitro* antimicrobial efficacy of ginger compounds against *Escherichia coli* and *Salmonella typhimurium* (Ekwenye *et al.* 2005).

#### **Growth performance**

*Salmonella* challenging showed a significant deterioration in growth performance of chicks, with a body weight gain decrease of 14% and a feed conversion ratio increase of 6.6% at the overall period of experiment.

These results are in accordance with those obtained with *Salmonella* challenged broiler chicks (Hegazy and Adachi, 2000; Chalghoumi *et al.* 2002; Nakamura *et al.* 2009). Many factors such as vaccination, infection and disease can promote the creation of free radicals (Allen, 1997; Kim *et al.* 2007; Stoilova *et al.* 2007), which result in lower growth performance and damage to body tissues. Normally, the body can handle free radicals but if the free radical production becomes excessive or if antioxidants are unavailable, damage can occur extensively.

Dietary supplementation of natural antioxidant such as ginger compounds prevented *in vitro* and *in vivo* production of free radicals (Kim *et al.* 2007) and remedied the clinical symptoms caused by the oxidative stress of infection (Stoilova *et al.* 2007; Chang *et al.* 2012).

In addition to antioxidative activity, the other mode of action of ginger as growth-promoter in challenged chicks arises from beneficially affecting the ecosystem of intestinal microbiota through controlling potential pathogens (Akoachere *et al.* 2002; Ali *et al.* 2007).

Because of a more stabilized intestinal health, animals are less exposed to microbial toxins and other undesired microbial metabolites such as ammonia and biogenic amines.

Intestinal formation of biogenic amines by microbiota is undesirable not only because of toxicity, but also because of the fact that biogenic amines are produced mainly by decarboxylation of limiting essential amino acids.

Consequently, ginger as growth-promoter relieve the birds from immune defense stress during critical situations and increase the intestinal availability of essential nutrients for absorption, thereby helping birds to grow better within the framework of their genetic potential.

Overall, the beneficial effect of ginger treatment on performance parameters of unchallenged broilers are in agreement with a large number of other research studies using ginger powder or extract in broilers (Onimisi *et al.* 2005; Farinu *et al.* 2009; Moorthy *et al.* 2009; Zhang *et al.* 2009), shrimp (Venkataramalingam *et al.* 2007) and fish (Immanuel *et al.* 2009), compared with studies lacking positive effects (El-Deek *et al.* 2002; Al-Homidan, 2005).

However, it is difficult to directly assess different studies using ginger, because the efficacy of ginger application depends on many factors such as administration level, ginger variety, application method and environmental stress factors.

In agreement to our results, the works of Onimisi *et al.* (2005) and Moorthy *et al.* (2009) showed significantly better feed conversion ratio in ginger fed groups of broilers compared to control. El-Deek *et al.* (2002) observed that diet containing 1.0 g/kg of ginger did not affect the growth performance, whereas Farinu *et al.* (2009) reported that supplementation of ginger at the level of 5.0 g/kg improved growth performance of broilers and the improvement rate slightly decreased at the levels of 10 and 15 g/kg. In contrast, Al-Homidan (2005) observed reduced growth rate of starter broilers (1 to 4 wk) when ginger was fed at the rates of 20 and 60 g/kg and similar findings were reported by Ademola *et al.* (2009) who included 20 g/kg ginger in the diet of broilers.

These results suggest that growth performance of broilers may respond to ginger supplementation at the level of 5.0 g/kg and over this level; it had negative effect on body weight gain. Performance parameters of challenged chicks were significantly improved in antibiotic treatments compared with the negative or positive controls. Avilamycin, despite its drawbacks in enhancing antimicrobial resistance, has a documented growth-promoting effect in broiler diets (Aarestrup *et al.* 2000). This was also confirmed in this study, whereby broiler performance in antibiotic treatment was also used as a measure to assess the growth-promoting efficacy of the ginger powder.

### CONCLUSION

The present study clearly showed that *Salmonella* challenge resulted in diarrhea and mortality of chicks, also in a decrease of performance parameters. Inclusion of avilamycin to the diet of challenged chicks compensated the negative effects of *Salmonella* challenge on health status and average daily gain. The effect of ginger on ameliorating the detrimental effect of *Salmonella* challenge was lower than avilamycin; however, it could improve performance parameters in commercial farms.

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