# Effect of onion (*Allium cepa* L.) as an antibiotic growth promoter substitution on performance, immune responses and serum biochemical parameters in broiler chicks

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# ABSTRACT

This experiment was conducted to evaluate the effect of onion (Allium cepa L.) as an antibiotic growth promoter substitute on growth performance, immune responses and serum biochemistry in broilers. A total of 192 one-d-old as hatched broiler chicks (Ross 308) were weighed and randomly allocated to four treatment groups, each with 4 replicate pens of 12 chicks. The dietary treatments consisted of the basal diet (control), antibiotic (15 mg Virginiamycin/kg), and control +10 or 30 g fresh onion bulb/kg diet. Body weights of broilers were determined at d 1, 21 and 42, feed intake was determined at the same periods, and feed conversion ratio was calculated accordingly. At 14th and 21st days blood samples were taken for measuring antibody titers against NDV and at 42nd day for biochemical analysis. At d 42, two birds per replicate were slaughtered for determination of lymphoid organ weights. Dietary supplementation of 30 g/kg onion increased final body weight of broilers at 42nd d of age compared to the other treatments (P < 0.05). Birds fed 30 g onion/kg in the diet had the highest feed intake than other treatments at different growth periods (P < 0.05). Dietary treatments failed to induce any significant effect on antibody titers against NDV, although the weight of lymphoid organs was significantly (P < 0.05) higher for birds fed diets supplemented with 30 g/kg Onion. Broilers receiving 30 g/kg onion had a significantly higher HDL and lower triglyceride concentrations compared to control groups (P < 0.05). Feeding 30 g/kg onion resulted in a marked reduction in the concentration of the glucose compared to control groups (P < 0.05). The results suggested that dietary inclusion of 30 g/kg onion can be applied as alternatives to in-feed antibiotics for broiler diets.

**Keywords:** *Allium cepa*; Onion; Broiler Chickens; Immune Response; Serum Biochemical Parameters

## **1. INTRODUCTION**

Subtherapeutic feeding of antibiotics has historically been a practice in some sectors of the commercial broiler industry to promote growth performance and protect flock health [1,2]. However, the use of dietary antibiotics has resulted in controversial problems such as development of antibiotic resistant bacteria and drug residue in the final products [3] which can be harmful to consumers. Thus, the use of antibiotics as a growth promoter is no longer acceptable and it is forbidden in European Union countries. As a result new alternatives are being introduced to livestock producers, among which phytogenic and herbal products have been given considerable attention as possible in-feed antibiotics substitutions. In recent years, the use of phytogenic compounds has gained momentum for their potential role as natural alternatives to antibiotic growth promoters in animal nutriation [4,5].

The genus *Allium* includes about 550 species. A few of these are important as food plants and as drugs in folk medicine, notably onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). Onion is a bulbous plant widely cultivated in almost every country of the world with leading

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production in China, India and United States [6]. Onion bulbs possess numerous organic sulphur compounds including Trans-S-(1-propenyl) cysteine sulfoxide, S-methylcysteine sulfoxide, Spropylcycteine sulfoxides and cycloallicin, flavinoids, phenolic acids, sterols including cholesterol, stigma sterol, b-sitosterol, saponins, sugars and a trace of volatile oil compounds mainly of sulphur compounds [7]. Most of the plant parts contain compounds with proven antibacterial, antiviral, antiparasitic, antifungal properties and have antihypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti inflammatory and antioxidant activity [8].

Aji *et al.* [9] observed the beneficial influence of onion bulbs on growth performance of broiler chickens. Sebastian *et al.* [10] reported that administration of onion extract in rabbits significantly reduced serum, liver and aorta triglycerides and serum and liver proteins. The aim of this work was to evaluate the effects of the inclusion of two levels of fresh onion bulbs on broiler responses regarding growth, immune responses and serum biochemistry.

## 2. MATERIALS AND METHODS

## 2.1. Animals and Dietary Treatments

One hundred ninety two, 1-d-old broiler chickens (mean initial weight:  $35.5 \pm 1$  g) of mixed sex (Ross-308) were weighed and randomly assigned to each of the 4 treatment groups, each with 4 replicate pens of 12 chicks. The dietary treatments included the basal diet (control), control + 15 mg Virginiamycin/kg, or control + 10 or 30 g fresh onions (Allium cepa) bulb/kg diet. Table 1 lists the basal diet formulated according to the nutrient requirements of broilers provided by National Research Council [11]. The birds were fed a starter diet from 0 to 21 d and grower diet from 22 to 42 d. All the dietary treatments were added to the basal diets at the expense of sand. Chicks were raised on floor pens  $(120 \times 120 \times 80)$ cm) for 6 wk and had free access to feed and water throughout the entire experimental period. The lighting program consisted of a period of 23 h light and 1 h of darkness. The ambient temperature in experimental house was maintained at 32°C during the first week and gradually decreased by 3°C in the second and third weeks, and finally fixed at 22°C thereafter.

## 2.2. Performance

Body weights of broilers were determined at d 1, 21, and 42 of age. Feed intake and weight gain were recorded in different periods and feed conversion ratio (FCR) was calculated. Mortality was recorded as it occurred and was used to adjust the total number of birds to determine the total feed intake per bird and FCR.

| arter and grower diets.           |         |        |   |
|-----------------------------------|---------|--------|---|
| Item                              | Starter | Grower | - |
| Ingredient, g/kg                  |         |        |   |
| Corn                              | 505.1   | 524.6  |   |
| Soybean meal                      | 385     | 350    |   |
| Soybean oil                       | 35.8    | 59     |   |
| Mono calcium phosphate            | 14.2    | 10     |   |
| CaCO <sub>3</sub>                 | 17.3    | 16.7   |   |
| NaCl                              | 3.1     | 2.1    |   |
| NaHco <sub>3</sub>                | 2       | 1.6    |   |
| Trace mineral premix <sup>1</sup> | 2.5     | 2.5    |   |
| Vitamin premix <sup>2</sup>       | 2.5     | 2.5    |   |
| DL-Methionine                     | 2.5     | 1      |   |
| L-Lysine                          | -       | -      |   |
| Sand                              | 30      | 30     |   |
| Calculated composition            |         |        |   |
| Metabolizable energy (kcal/kg)    | 2,900   | 3,100  |   |
| Crude protein (g/kg)              | 215     | 200    |   |
| Calcium (g/kg)                    | 10      | 9      |   |
| Available phosphorus (g/kg)       | 4.5     | 3.5    |   |
| Methionine + cysteine (g/kg)      | 9       | 7.2    |   |
| Lysine (ø/kø)                     | 11.8    | 10.9   |   |

Table 1. The ingredient and calculated composition of basal

<sup>1</sup>Provided the following per kg of diet: Mg, 56 mg; Fe, 20 mg; Cu, 10 mg; Zn, 50 mg; Co, 125 mg; I, 0.8 mg. <sup>2</sup>Provided the following per kg of diet: vitamin A, 10,000 IU; vitamin D<sub>3</sub>, 2000 IU; vitamin E, 5 IU; vitamin K, 2 mg; riboflavin, 4.20 mg; vitamin B<sub>12</sub>, 0.01 mg; pantothenic acid, 5 mg; nicotinic acid, 20 mg; folic acid, 0.5 mg; choline, 3 mg.

FCR = Feed intake/weight gain

#### 2.3. Immune Parameters

The commercially available oil-adjuvant injectable emulsion against Newcastle Disease virus (NDV) and Avian Influenza virus (AIV) were used (H9N2 subtype) for vaccinating broiler chicks, and they were injected subcutaneously with 0.2 mL per chick at 9 d of age. At 14 and 21 days of age two birds per replicate were randomly chosen and blood samples were collected from the brachial vein and centrifuged at 2000 ×g for 15 min to obtain serum (SIGMA 4 - 15 Lab Centrifuge, Germany). Antibody titers against NDV were measured using Hemagglutination Inhibition Test according to the method of Thayer and Beard [12].

At 42 d of age, three birds per replicate were randomly

chosen, based on the average weight of the group and slaughtered through cutting carotid arteries and partial slicing of the neck by a manual neck cutter; bursa and spleen were collected, weighed and calculated as a percentage of live body weight.

#### 2.4. Serum Biochemistry

After 12 h of fasting, blood samples were collected in non-heparinised tubes at day 42 of age from 8 birds in each treatment by puncturing the brachial vein and the blood was centrifuged at 2000  $\times$ g for 15 min to obtain serum (SIGMA 4 - 15 Lab Centrifuge, Germany). Individual serum samples were analyzed for glucose, total cholesterol, high-density lipoprotein (HDL) and lowdensity lipoprotein (LDL) cholesterol and triglyceride (Pars-Azmoon Co., Tehran).

#### 2.5. Statistical Analysis

The obtained data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute (2008). Means were compared using Duncan multiple test. Statements of statistical significance are based on P < 0.05.

## 3. RESULTS

#### 3.1. Performance Parameters

The impact of dietary treatments on growth performance indices from 1 to 42 day of age is presented in **Table 2**. At 21 d of age BW of chicks did not differ (P > 0.05) between the dietary treatments, although BW of broiler supplemented with 30 g onion/kg was higher than other groups. At the end of the trial (d 42), birds supplemented with the 30 g onion/kg had a greater BW compared with other groups (P < 0.05). The average daily feed intake (from d 1 to 21) was increased (P < 0.05) for bird supplemented with 30 g onion/ kg of diet. The average daily feed intake (ADFI) during grower and the entire experimental period was higher for broilers supplemented with 30 g onion/kg compared with control birds and birds supplemented with antibiotic (P < 0.05). Broilers receiving 10 or 30 g onion/ kg had a lower feed conversion ratio (FCR) compared to broilers receiving antibiotic during the starter period (P < 0.05), but FCR of broilers in other periods was not affected. No differences because of treatment effects were observed on mortality.

#### 3.2. Immunity

The effects of treatments on immune related parameters are shown in **Tables 3** and **4**. The additives used in the current study failed to induce any significant impact on antibody titers against NDV at 14 and 21 days of age (P > 0.05). The weight of lymphoid organs was signed (P < 0.05) higher for birds fed diets supplemented with 30 g/kg Onion.

## 3.3. Serum Biochemistry

**Table 5** summarizes the impact of treatments on serum constituents at day 42 of age. Treatments did not induce any significant effect on the serum concentration of total cholesterol and LDL-cholesterol. Broilers receiving 30

Table 2. Effect of experimental diets on performance indices of broilers at different ages.

| Performance parameters             | Dietary treatments  |                     |                     |                    |                  |
|------------------------------------|---------------------|---------------------|---------------------|--------------------|------------------|
|                                    | Control             | Virginiamycin       | 10 g/kg Onion       | 30 g/kg Onion      | SEM <sup>1</sup> |
| Daily Feed Intake (g per bird/day) |                     |                     |                     |                    |                  |
| 0 - 21 d                           | 31.4 <sup>ab</sup>  | 31.4 <sup>ab</sup>  | 29.4 <sup>b</sup>   | 32.6 <sup>a</sup>  | 0.33             |
| 21 - 42 d                          | 122.5 <sup>b</sup>  | 124.5 <sup>b</sup>  | 128.9 <sup>ab</sup> | 133.5 <sup>a</sup> | 3.29             |
| 0 - 42 d                           | 77.0 <sup>b</sup>   | 78.0 <sup>b</sup>   | 79.2 <sup>ab</sup>  | 83.0 <sup>a</sup>  | 1.96             |
| Feed Conversation Ratio (g/g)      |                     |                     |                     |                    |                  |
| 0 - 21 d                           | 1.53 <sup>ab</sup>  | 1.56 <sup>a</sup>   | 1.51 <sup>b</sup>   | 1.51 <sup>b</sup>  | 0.005            |
| 21 - 42 d                          | 1.87                | 1.89                | 1.92                | 1.89               | 0.014            |
| 0 - 42 d                           | 1.79                | 1.81                | 1.83                | 1.81               | 1.010            |
| Body Weight (g)                    |                     |                     |                     |                    |                  |
| 21d                                | 465.8               | 463.8               | 447.1               | 477.5              | 16.79            |
| 42 d                               | 1838.3 <sup>b</sup> | 1845.2 <sup>b</sup> | 1859.2 <sup>b</sup> | 1955.1ª            | 14.84            |

Values in the same row not sharing a common superscript differ significantly (P < 0.05). <sup>1</sup>Standard error of mean.

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| Lymphoid organs Co  |                    |                     | Dietary treatments  |               |                  |
|---------------------|--------------------|---------------------|---------------------|---------------|------------------|
|                     | Control            | Antibiotic          | 10 g/kg Onion       | 30 g/kg Onion | SEM <sup>1</sup> |
| Bursa <sup>a</sup>  | 0.137 <sup>d</sup> | 0.165 <sup>b</sup>  | 0.149 <sup>cd</sup> | $0.190^{a}$   | 0.010            |
| Spleen <sup>a</sup> | 0.111 <sup>b</sup> | 0.139 <sup>ab</sup> | 0.138 <sup>ab</sup> | $0.148^{a}$   | 0.005            |

Table 3. Effect of experimental diets on lymphoid organs at 42nd day.

Values in the same row not sharing a common superscript differ significantly (P < 0.05). <sup>a</sup>Percentage of live body weight. <sup>1</sup>Standard error of mean.

Table 4. Effect of experimental diets on antibody titers against Newcastle Disease Virus at 14th and 21st days.

| Antibody titers (log) | _       |            | Dietary treatments |               |         |
|-----------------------|---------|------------|--------------------|---------------|---------|
|                       | Control | Antibiotic | 10 g/kg Onion      | 30 g/kg Onion | $SEM^1$ |
| 14 days               | 0.619   | 0.650      | 0.643              | 0.650         | 0.016   |
| 21 days               | 0.739   | 0.775      | 0.775              | 0.775         | 0.019   |

<sup>1</sup>Standard error of mean.

Table 5. Effect of experimental diets on serum biochemical parameters of broilers at day 42.

| Serum biochemistry —           |                     |                      | Dietary treatments   |                    |         |
|--------------------------------|---------------------|----------------------|----------------------|--------------------|---------|
|                                | Control             | Antibiotic           | 10 g/kg Onion        | 30 g/kg Onion      | $SEM^1$ |
| Triglyceride <sup>a</sup>      | 120.67 <sup>a</sup> | 111.25 <sup>ab</sup> | 104.25 <sup>ab</sup> | 95.75 <sup>b</sup> | 7.59    |
| Total cholesterol <sup>a</sup> | 146.29              | 153.14               | 130.60               | 134.00             | 11.83   |
| LDL-cholesterol <sup>a</sup>   | 36.75               | 32.75                | 30.50                | 41.75              | 6.77    |
| HDL-cholesterol <sup>a</sup>   | 77.75 <sup>b</sup>  | 94.25 <sup>a</sup>   | 86.25 <sup>ab</sup>  | 88.75ª             | 2.89    |
| Glucose <sup>a</sup>           | 93.75 <sup>a</sup>  | 81.75 <sup>ab</sup>  | 75.00 <sup>ab</sup>  | 59.00 <sup>b</sup> | 7.37    |

Values in the same row not sharing a common superscript differ significantly (P < 0.05). <sup>1</sup>Standard error of mean. <sup>a</sup>(mg/100 mL).

g/kg onion had a significantly higher HDL and lower triglyceride concentrations compared to control groups (P < 0.05). Feeding 30 g/kg onion resulted in a marked reduction in the concentration of the glucose compared to control groups (P < 0.05).

## 4. DISCUSSION

#### 4.1. Performance

Dietary supplementation of 30 g/kg onion increased body weight and feed intake of broilers at different growth periods. Also, feeding 30 g/kg onion resulted in a marked reduction in the concentration of the glucose. Onion containing sulfur organic compounds including *S-Methylcysteine sulfoxide* (SMCS) and *S-allylcysteine* suiloxide (SACS) is related to decreasing of blood lipid, liver protein and glucose. Hypoglycemia stimulates a nerve center for intake whereas hyperglycemia stimulates the center for satiety. Shurlock and Forbes [13] observed reductions in feed intake after they infused glucose into the hepatic portal vein of fasted chickens at physiological rates, whereas no effect was observed when glucose was infused into the jugular vein. Onion stimulated growth by increasing the inflow of glucose into tissues, thyroid like activity. The results of the present experiment are consistent with Al-homidan [14]. Similar to our results Aji *et al.* [9] reported an enhancement in BW, FCR and ADFI of broilers offered diets containing fresh onion bulbs in comparison with broilers fed basal diet. In this trial the positive impact of the onion on the feed utilization was observed at starter period, but the improved FCR obtained in broilers supplemented with 30 g onion/kg was not reflected at grower period probably due to the facts that older birds' nutrient requirements decrease with age and also they have a better developed digestive tracts and organs [4].

#### 4.2. Immunity

With respect to a higher weight of lymphoid organs recorded in chicks fed onion diets, it is concluded that the active components of onion which have antibacterial, antiviral, antifungal, anti inflammatory and antioxidant activities [8] induce positive effects on these organs. These results are in agreement with those obtained by

Ibrahiem et al. [15] who reported that bursa weights were magnified by feeding onion to broiler muscovy ducks. Onions have a mode of action which is similar to antibiotics. Yamamoto and Glick [16] reported that the synthesis of immunoglobulins was higher in chicken with larger Bursa. Dafwang et al. [17] reported that the effects of onion and garlic on immunoglobulins were similar to antibiotics. But in the present trial that antibody titers measured against NDV, neither positive nor negative effect was affected. Since antimicrobial agents started to be used as growth promoters, researchers [18,19] working with broilers and swine respectively understood that the presence of an important health challenge in the field was essential to reveal the significant effects of these products. This was while the current trial was conducted in optimum conditions and no external challenges or stresses were impelled to the broilers.

#### 4.3. Serum Biochemistry

Broilers receiving 30 g/kg onion had a significantly higher HDL and lower triglyceride concentrations compared to control groups. Suresh and Srinivasan [20] found that 3% onion powder reduced blood lipids, lipid peroxides and cholesterol. Al-homidan [14] and Sebastian *et al.* [10] also observed the reduced serum cholesterol in their experiments by using of onion. In contrast to the foreign experiments, Sklan *et al.* [21] did not observe any effect of onion on hepatic cholesterol. The effects of onion have been ascribed to its sulfur containing principles which oxidize thiol compounds either present free or combined with a protein and NADPH which are necessary for lipid synthesis [10].

In conclusion, the results suggested that the dietary inclusion of 30 g/kg onion can be applied as an alternative to in-feed antibiotics for broiler diets.

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#### Supplement

FCR = Feed conversion ratio; NDV = Newcastle Disease virus; AIV = Avian Influenza virus; HDL = High-density lipoprotein; LDL = Low-density lipoprotein; BW = Body Weight; ADFI = Average daily feed intake; NADPH = Nicotinamide adenine dinucleotide phosphate; SEM = Standard error of mean.