Effect of dietary supplementation with onion (*Allium cepa* L.) on performance and intestinal microflora composition in broiler chickens
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Abbreviated Title: Onion as an antibiotic alternative

Summary
This experiment was conducted to examine the effect of onion (*Allium cepa* L.) on growth performance and microflora composition in broiler chickens. A total of 192 one-d-old mixed sex 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), control+15 mg Virginiamycin/kg, or control+10 or 30 g fresh onions bulb/kg diet. Dietary supplementation of 30 g/kg onion increased final body weight of broilers at 42 d of age compared to the other treatments (*P*<0.05). The feed intake during starter, grower and 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). The *Lactobacilli* spp. population in birds supplemented with onion at the level of 30 g/kg significantly was higher than other groups at 42 d of age (*P*<0.05). The lowest *Escherichia coli* loads were detected in broilers fed diets containing 15 mg Virginiamycin/kg. The *Escherichia coli* loads significantly decreased in broilers fed diets containing 10 or 30 g onion/kg (*P*<0.05). In conclusion, the results of the current study indicated that supplementing broiler diet with 30 g onion/kg could induce favorable influences on performance and ileum microflora composition.

Key words: *Allium cepa* L., Broiler, Performance, Carcass characteristics, Intestinal microflora composition

Objective
Antibiotic growth promoters are used worldwide within the poultry industry to promote growth performance and protect flock health (ENGBERG et al., 2000). Antibiotic growth promoters were supposed to increase growth rate as a result of improved gut health, resulting in better nutrient utilization and decreased feed conversion ratio (VISEK, 1978). However, the use of dietary antibiotics have resulted in controversial problems such as development of antibiotic resistant bacteria (NASIR and GRASHORN, 2006), and drug residue in the final products (BURGAT, 1999) 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 consequence, it has become necessary to develop alternative substances and strategies for animal growth promotion and disease prevention. Phytogenic and herbal products, also known phytobiotic products which are plant derived products, used in animal feeding to improve performance through amelioration of feed properties, promotion of production performance, and improving the quality of animal origin food (LANDY et al., 2011).

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 production in China, India and United States (EBESUNUN et al., 2007). Onion bulbs possess numerous organic sulphur compounds including Trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, S-propylcysteine 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 (MELVIN et al., 2009). Most of the plant parts contain compounds with proven antibacterial, antiviral, antiparasitic, antifungal
properties and has antihypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti-inflammatory and antioxidant activity (LAMPE, 1999).

AJI et al (2011) observed the beneficial influence of onion bulbs on growth performance of broiler chickens. Despite these findings, there has been a dearth of information on the effect of fresh onion bulbs on performance, and intestinal microflora in comparison with an antibiotic growth promoter in broiler chickens. Therefore the present study was designed to compare the efficacy of two levels of fresh onion bulbs a growth promoter agent on growth performance and ileum microflora in broiler chickens when used as supplements in the diet.

Materials and Methods

Animals and dietary treatments

One hundred ninety two, 1-d-old broiler chickens 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. The basal diet formulated according to nutrient requirements of broilers provided by National Research Council (NRC, 1994). 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 × 120 × 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.

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.

Enumeration of bacteria populations in ileum

Intestinal samples were collected and fresh digesta samples from ileum were taken for bacterial analyses within an hour from collection. Digesta samples were serially diluted in 0.85% sterile saline solution for enumeration of Lactobacilli spp and Escherichia coli by conventional microbiological techniques using selective agar media. All microbiological analyses were performed in duplicate and the average values were used for statistical analysis. Lactobacilli spp were anaerobically assayed using MRS agar (Fluka 80961). In cases of doubt confirmation of Lactobacilli spp. was performed by using API 50 CH kit (Biomerieux_ SA, Marcy-l’Etoile/France). E. coli were enumerated through the use of Plate Count MUG Agar (Fluka 80961) and TBX Agar (Fluka 92435). Results were expressed as base-10 logarithm colony-forming units per gram of ileal.

Statistical analysis
The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the general linear model procedures of SAS (SAS Inst. Inc., Cary, NC). The mean differences among different treatments were separated by Duncan’s multiple range tests. A level of \(P<0.05\) was used as the criterion for statistical significance.

**Results**

**Performance**

Impact of dietary treatments on growth performance indices from 1 to 42 day of age is presented in Table 1. Dietary supplementation of 30 g/kg onion increased body weight and feed intake of broilers at different growth periods. Broilers receiving 10 or 30 g onion/kg had lower feed conversion ratio (FCR) compared to broilers receiving antibiotic during starter period \((P<0.05)\), but FCR of broilers in other periods was not affected. As antibiotics, herbs and phytogenic products could control and limit the growth and colonization of a variety of pathogenic and nonpathogenic species of bacteria in chicks' gut. This may lead to a greater efficiency in the utilization of feed, resulting in increased growth and improved feed efficiency (BEDFORD, 2000). 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 (TOGHYANI et al., 2011). Fresh bulbs of *A. cepa* L. consist mainly of water (about 88 %), saccharides (about 6 %) and proteins (about 1.5 %). However, the particular composition depends on a large number of factors, such as growing conditions, time of harvest and length and conditions of storage (WATT and MERRILL, 1963). *A. cepa* L. is a rich source of various compounds and has been thoroughly investigated by phytochemists during the last 100 years. Like other species of the genus *Allium*, e.g. *A. sativum* L. or *A. ursinum* L., *A. cepa* L. is especially characterized by a high content of organosulphur compounds. The most predominant of these genuine sulphur-containing compounds are the amino acids cysteine and methionine, the S-alk(en)yl-substituted cysteine sulphoxides and the \(\gamma\)-glutamyl peptides (STEINEGGER et al., 1999). The improve growth performance of chicks receiving 30 g onion/kg could be due to content of organosulphur compounds of onion. Similar to our results AJI et al (2011) reported an enhancement in BW, FCR and ADFI of broilers offered diets containing fresh onion bulbs in comparison with broilers fed basal diet. Unfortunately, no other reports are available on the effects of onion on bird growth performance.

**Table 1**

Effect of experimental diets on performance indices of broilers at different ages

<table>
<thead>
<tr>
<th>Performance parameters</th>
<th>Dietary treatments</th>
<th>Control</th>
<th>Virginiamycin</th>
<th>10 g/kg Onion</th>
<th>30 g/kg Onion</th>
<th>SEM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily Feed Intake (g per bird/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-21 d</td>
<td></td>
<td>31.4ab</td>
<td>31.4ab</td>
<td>29.4b</td>
<td>32.6a</td>
<td>0.33</td>
</tr>
<tr>
<td>21-42 d</td>
<td></td>
<td>122.5b</td>
<td>124.5b</td>
<td>128.9ab</td>
<td>133.5a</td>
<td>1.19</td>
</tr>
<tr>
<td>0-42 d</td>
<td></td>
<td>77.0b</td>
<td>78.0b</td>
<td>79.2ab</td>
<td>83.0a</td>
<td>0.69</td>
</tr>
<tr>
<td>Feed Conversation Ratio (g/g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-21 d</td>
<td></td>
<td>1.53ab</td>
<td>1.56a</td>
<td>1.51b</td>
<td>1.51b</td>
<td>0.005</td>
</tr>
<tr>
<td>21-42 d</td>
<td></td>
<td>1.87</td>
<td>1.89</td>
<td>1.92</td>
<td>1.89</td>
<td>0.014</td>
</tr>
<tr>
<td>0-42 d</td>
<td></td>
<td>1.79</td>
<td>1.81</td>
<td>1.83</td>
<td>1.81</td>
<td>1.010</td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21d</td>
<td></td>
<td>465.8</td>
<td>463.8</td>
<td>447.1</td>
<td>477.5</td>
<td>4.79</td>
</tr>
<tr>
<td>42d</td>
<td></td>
<td>1838.3b</td>
<td>1845.2b</td>
<td>1859.2b</td>
<td>1955.1a</td>
<td>14.84</td>
</tr>
</tbody>
</table>

1 Values in the same row not sharing a common superscript differ significantly \((P<0.05)\).

1 Standard error of mean.
Intestinal microflora

Data on ileum bacteria populations of broiler chicks at d 42 of age are summarized in Table 2. The Lactobacilli spp. population in birds supplemented with onion at the level of 30 g/kg significantly was higher, and Escherichia coli loads was lower than other groups at 42 d of age ($P<0.05$). The lowest Escherichia coli loads were observed in broilers fed diets containing 15 mg Virginiamycin/kg. Antibiotics may control and limit the growth and colonization of a variety of pathogenic and nonpathogenic species of bacteria in chicks gut (FERKET, 2004). HANNAN et al (2010) reported antimicrobial activity of onion extract against Vibrio Cholerae. In other trials ethanolic extract of onion gave 11 mm zone of inhibition with Minimum Inhibitory Concentration (MIC) 0.8 mg/ml against Pseudomonas aeruginosa and 9 mm zone of inhibition with MIC 0.8 mg/ml (HANNAN et al., 2010). The same results were obtained in another study conducted by Sharma et al in India in 2009. The MIC was determined by disc diffusion method (SHARMA et al., 2009). The onion bulbs contain numerous organic sulphur compounds. Thus, the presence of these compounds may explain the antimicrobial activity of this plant (MELVIN et al., 2009).

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dietary treatments</th>
<th>SEM*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Virginiamycin</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7.35a</td>
<td>5.45d</td>
</tr>
<tr>
<td>Lactobacilli spp.</td>
<td>4.98d</td>
<td>5.53ab</td>
</tr>
</tbody>
</table>

Values in the same row not sharing a common superscript differ significantly ($P<0.05$).
* Standard error of mean.

Conclusion

In conclusion, the results indicate that supplementing broiler diet with 30 g onion/kg could induce favorable influences on performance and ileum microflora composition.

References


