Effects of virginiamycin and galbanum (*Ferula gummosa boiss*) on performance, carcass traits, immune system and blood parameters of broiler chickens

This unedited manuscript has been accepted by RCCP for future publication and is provisionally published on our website. The manuscript will undergo copyediting, typesetting, and galley review before final publication. Please note that this advance version may differ from the final version.
Effects of virginiamycin and galbanum (*Ferula gummosa boiss*) on performance, carcass traits, immune system and blood parameters of broiler chickens

Efectos de la virginiamicina y el gálbano (*Ferula gummosa boiss*) sobre la producción, características de la canal, sistema inmune y parámetros sanguíneos en pollos de engorde

Efeitos na adição na dieta do virginiamicina e do gálbano (*Ferula gummosa boiss*) no desempenho, características da carcaça, sistema imunológico e fatores sanguíneos de frangos de corte

Amin Sarchahi\(^1\); Khosro Ghazvinian\(^1\*\); Khatereh Kafshdoozan\(^1\); Reza Jamshidi\(^1\).

Department of Animal Science, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.

To cite this article:

Abstract

Background: Some medicinal plants can stimulate growth in poultry. Objective: To compare the effects of dietary addition of virginiamycin antibiotic and galbanum plant (*Ferula gummosa*) on the performance, carcass characteristics, immune system, and blood factors of broiler chickens.

Received: March 10, 2021; Accepted: October 8, 2021

*Corresponding author: Semnan - Opposite Sokan Park, Campus No. 1, Faculty of Veterinary Medicine, Semnan University, Postal Code: 19111-35131. E-mail: khghazvinian@semnan.ac.ir

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

eISSN: 2256-2958
Methods: A total of 250 one-day-old male Ross 308 broiler chickens were randomly assigned to five treatments with five replicates (10 birds per replicate). The treatments were: control (basal diet), virginiamycin (basal diet plus 0.1 g virginiamycin/kg of diet) or one of three levels of galbanum powder (2.5, 5 and 10 g galbanum/kg of diet). Results: Body weight gain, feed intake and feed conversion ratio improved in the groups containing galbanum compared to the other groups (p<0.05). Differences in the percentage of liver, heart and abdominal fat decreased in the treatments containing 5 and 10 g galbanum compared to the control (p<0.05). Spleen weight and antibody titers against Newcastle disease and SRBC were significantly increased in the treatment containing 10 g of galbanum compared to the other treatments (p<0.05). The addition of galbanum powder resulted in a significant decrease in serum cholesterol, triacylglycerol, and LDL (p<0.05). Additionally, galbanum increased serum total protein, albumin, globulin, and HDL (p<0.05). Conclusion: Galbanum improves performance and the immune system of broiler chickens, and has similar growth promoting effects as virginiamycin.

Keywords: Antibiotic; broiler chicken; carcass; galbanum; growth performance; immune response; medicinal plants; virginiamycin.

Resumen

Antecedentes: algunas plantas medicinales pueden estimular el crecimiento de las aves. Objetivo: comparar los efectos de añadir virginiamicina y gálbano (Ferula gummosa) a la dieta sobre la producción, características de la canal, sistema inmune y parámetros sanguíneos de pollos de engorde. Métodos: un total de 250 pollos machos Ross 308 de engorde con un día de vida se asignaron aleatoriamente a 5 tratamientos (5 réplicas y 10 aves por réplica). Los tratamientos fueron: control (dieta basal), virginiamicina (0,1 g virginiamicina/kg dieta) y tres niveles de polvo de gálbano (2,5, 5 y 10 g galvano/kg dieta). Resultados: la ganancia de peso, el consumo de alimento y el índice de conversión mejoraron en los tratamientos que contenían gálbano comparados con los otros grupos (p<0,05). Las diferencias en el porcentaje de grasa hepática, cardíaca y abdominal fueron significativamente reducidas en los tratamientos que contenían 5 y 10 g/kg de gálbano comparados con el control (p<0,05). El peso del bazo y la producción de anticuerpos contra las enfermedades de Newcastle y SRBC aumentaron significativamente en el tratamiento con 10 g/kg de gálbano en comparación con los otros tratamientos (p<0,05). La adición
de gálbano resultó en una disminución significativa de los niveles de colesterol, triglicéridos y LDL (p<0,05), mientras que los niveles séricos de proteína total, albúmina, globulina y HDL aumentaron significativamente (p<0,05). **Conclusion:** los resultados mostraron que el gálbano mejora la producción y refuerza el sistema inmune en pollos de engorde, y promueve el crecimiento de forma similar a la virginiamicina.

**Palabras clave:** antibiótico; canal; gálbano; hierbas medicinales; pollos; rendimiento de crecimiento; respuesta inmune; virginiamicina.

**Resumo**

**Antecedentes:** algumas plantas medicinais podem estimular o crescimento dos pássaros. **Objective:** comparar os efeitos da adição na dieta do antibiótico virginiamicina e do gálbano (*Ferula gummosa*) no desempenho, características de carcaça, sistema imunológico e fatores sanguíneos de frangos de corte. **Métodos:** um total de 250 frangos de corte, machos Ross 308, de um dia de idade foram aleatoriamente divididos em 5 tratamentos e 5 repetições (10 aves por repetição). Os tratamentos foram: grupo controle (dieta base), grupo com adição de antibiótico virginiamicina (0,1 g virginiamicina/kg de dieta) e três outros grupos com diferentes níveis gálbano em pó (2,5, 5 e 10 g gálvano/kg de dieta). **Resultados:** o ganho de peso corporal, o consumo de ração e a taxa de conversão melhoraram nos grupos contendo 5 e 10 gramas de gálbano em comparação com os outros grupos (p<0,05). As diferenças no percentual de gordura hepática, cardíaca e abdominal diminuíram significativamente nos tratamentos contendo 5 e 10 g de gálbano em comparação ao controle (p<0,05). O peso do baço e os títulos de anticorpos gerados contra a doença de Newcastle e SRBC foram significativamente aumentados no tratamento contendo 10 gramas de gálbano em comparação com os outros tratamentos (p<0,05). A adição de gálbano em pó resultou em diminuição significativa do colesterol sérico, triacilglicerol e LDL (p<0,05) e, por outro lado, aumento significativo da proteína sérica total, albumina, globulina e HDL (p<0,05). **Conclusões:** os resultados mostraram que a erva medicinal gálbano melhora o desempenho e estimula o sistema imunológico em frangos de corte e pode ser considerada um factor de crescimento em frangos de corte em comparação com o antibiótico virginiamicina.

**Palavras-chave:** antibiótico; carcaça; desempenho de crescimento; frango de corte; gálbano; planta medicinal; resposta imune; virginiamicina.
Introduction

Nowadays, broiler breeding is one of the largest sources of animal protein in the world, and the industry has been developed in many areas such as nutrition, genetics, and management, and it has reached its maximum production (Chen et al., 2008). At the moment, the industry's attention is focused on the health of the community and environment. Therefore, it is advisable to use additives in poultry feeding in order to achieve high yields and to ensure the health of poultry such that the desired characteristics are held while harmful environmental and health effects are avoided (Ahmadi et al., 2019).

Antibiotics are among the supplements used in poultry nutrition to prevent the growth of intestinal pathogens and improve performance. The effects of the excessive use of antibiotics have created concerns among consumers, mostly related to increasing bacterial resistance and its adverse effects (Abedi et al., 2008). Given the prohibition of using antibiotics in Europe, more attention is being paid to finding suitable alternatives for antibiotics in the poultry industry (Poorghasemi et al., 2018).

Medicinal plants had been neglected in modern feeding of livestock and poultry due to the use of antimicrobial growth promoters, but because of the prohibition of using most antimicrobial growth promoters, medicinal herbs have been attracting a special position in livestock feeding strategies (Poorghasemi et al., 2014). Medicinal plants, due to the effective compounds in their tissues including phenols, polyphenols, terpenoids, volatile oils, alkaloids, antimicrobial compounds can stimulate digestion process, decrease lipid and cholesterol levels, stimulate the immune system, increase antibody generation and finally promote the growth of the body (Soltan et al., 2008).

Due to the importance of economic efficiency in broiler breeding, and considering the ban on the use of antibiotics, many studies have been recently conducted on the alternatives to growth-promoting antibiotics (Greathead, 2003).

Research has shown that herbs and essential oils are used to lower cholesterol and increase their function (Adaszyńska-Skwirzyńska and Szczerbińska, 2019).

*Ferula gummosa boiss*, known as galbanum, is from the family Apiaceae or Umbelliferae. The family has 275 genera and 2,850 species (Klaverand Van Der Meer, 1993). The most important product of galbanum is oleo-gum resin that is obtained from all the parts of the plant, especially
the root. The material contains 5 to 30 percent essential oil, 50 to 70 percent resin, 20 to 40 percent
gummy material, and 1 to 10 percent moisture and minerals (Cross et al., 2007). This plant has a
high antibacterial effect against gram-negative bacteria, which can be considered a stimulant for
the gastrointestinal tract (Dorman and Deans, 2000).
The research carried out by Movahhedkhah et al., 2019 have shown the effect of different levels
of summer savory (Satureja hortensis L.) extract in the diet on broilers performance, immune
response, hematology, and microbiota. Based on findings, dietary supplementation with summer
savory extract, as natural feed additive, sustained growth traits and improved the feed efficiency
and health status of broilers.
Therefore, the present study compared the effects of dietary addition of virginiamycin antibiotic
and galbanum plant (Ferula gummosa boiss) on the performance, carcass characteristics, immune
system, and blood factors of broiler chickens.

Materials and Methods

Ethical considerations
The experimental protocol was ratified by the Animal Ethic Committee of the Semnan University,
Semnan, Iran, and the experiment was performed with respect to the International Guidelines for
research involving animals (Directive 2019/63/EU).

Animals and experimental design
A total of 250 one day old male broiler chickens (Ross 308) were used in a completely randomized
design within 25 experimental units. The chickens were assigned to 5 treatments with 5 replicates
so that 10 chickens with the same weights were used in each experimental unit. The experimental
treatments included control (basal diet), virginiamycin antibiotic (basal diet plus 0.1 g
virginiamycin/kg of diet), and three levels of galbanum powder (2.5, 5 and 10 g/kg of diet). In
order to prepare the galbanum powder, the tops of the plant’s branches were collected and were
used after drying and milling. The experimental diets were starter (1-10 days), grower (11-24 days)
and finisher (25-42 days), in which antibiotics and galbanum powder were added to the basal diet.
All the diets were based on corn-soybean and were arranged based on the advice of Ross Company
to meet the nutrient requirements of the chickens (Ghazvinian et al., 2018). The User-Friendly Feed Formulation, Done Again (UFFDA) software was used to formulate the diets. The composition of experimental diets is shown in Table 1.

Performance

Brooding conditions were the same for all the chickens throughout the period and free access to feed was provided (Poorghasemi et al., 2018). Feeds and chickens were weighed at the end of each period as well as for the whole period (42 days), and then the values of feed intake, weight gain, and feed conversion ratio were determined (Poorghasemi et al., 2018).

Carcass characteristics

At the end of the experimental period (42 days), one chicken from each replicate was randomly selected (close to the mean weight) and its digestive system was removed after slaughtering and the percentage of different components such as carcass, breast, thighs, gizzard, liver, heart, and abdominal fat were calculated based on live weight.

Table 1. Ingredients and nutrient composition of the experimental diets.

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Starter (1-10 days old)</th>
<th>Grower (11-24 days old)</th>
<th>Finisher (25-42 days old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>49.03</td>
<td>51.01</td>
<td>57.29</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>38.28</td>
<td>35.68</td>
<td>29.97</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Canola oil</td>
<td>5.00</td>
<td>6.35</td>
<td>6.00</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>1.42</td>
<td>1.15</td>
<td>1.15</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.73</td>
<td>1.50</td>
<td>1.34</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.38</td>
<td>0.29</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Lysine-HCl</td>
<td>0.33</td>
<td>0.17</td>
<td>0.15</td>
</tr>
<tr>
<td>Salt</td>
<td>0.33</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Mineral premix¹</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin premix²</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Metabolizable energy (kcal/kg)</td>
<td>2,980</td>
<td>3,100</td>
<td>3,150</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>21.63</td>
<td>20.66</td>
<td>18.70</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>1.03</td>
<td>0.88</td>
<td>0.83</td>
</tr>
<tr>
<td>Available Phosphorus (%)</td>
<td>0.49</td>
<td>0.44</td>
<td>0.40</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.41</td>
<td>1.23</td>
<td>1.07</td>
</tr>
<tr>
<td>Methionine (%)</td>
<td>0.71</td>
<td>0.61</td>
<td>0.55</td>
</tr>
<tr>
<td>Methionine + Cysteine (%)</td>
<td>1.05</td>
<td>0.93</td>
<td>0.84</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Threonine (%)</td>
<td>0.86</td>
<td>0.83</td>
<td>0.75</td>
</tr>
</tbody>
</table>

1. Mineral mixture per kg of diet: MnSO4: 100 mg; I: 1 mg; CuSO4: 100 mg and Fe: 50 mg.
2. Vitamin premix per kg of diet: vitamin A: 11,000 IU; vitamin D₃: 23,000 IU; vitamin E: 121 IU; vitamin K₃: 2 mg; vitamin B₆: 4 mg; vitamin B₁₂: 0.020 mg; Thiamine: 4 mg; Riboflavin: 4 mg; Biotin: 0.03 mg; Nicotinic acid: 30,000 mg; Folic acid: 1 mg; Choline chloride: 840 mg and Ethoxyquin: 0.125.

Lymphoid organ weight

The percentage of the spleen to body weight (relative weight) as one of the immune organs was measured at 42 days of age.

Response to Newcastle disease virus vaccine

Newcastle disease vaccine was given to the chickens at 19 days of age, and at the end of the course at 42 days of age, two birds were randomly selected from each replicate and 1 mL of blood was taken from a vein of its wing. Blood serum was separated by placing the specimens in a 3,000 rpm centrifuge machine for 15 minutes. The serum was specified in terms of Newcastle anti-virus antibodies by the Hemagglutination Inhibition (HI) assay (Dorman and Deans, 2000).

Response to sheep red blood cell (SRBC)

To investigate the effect of experimental treatments on specific immune responses, at 28-day of age, two chickens in each experimental unit were injected 0.2 ml of washed 5% suspension sheep red blood cell in sterile phosphate buffer through the breast muscle. Blood samples of the chickens were collected from the wing vein 14 days after injection (42 days of age) and blood serum was isolated by placing the samples in a centrifuge at a speed of 3,000 rpm for 15 minutes, and it was investigated by hemmaglutination assay to assess the concentration of anti-SRBC antibody (Poorghasemi et al., 2015).

Blood factors

At 42 days of age, two chickens were randomly selected for each replicate and blood samples were taken from their wing veins. Then blood serum was used to determine the concentration of total protein, albumin, globulin, cholesterol, triglyceride, LDL, and HDL. The concentrations of those
factors were measured using biochemistry kits and calorimetric enzyme assay (Vase-Khavari et al., 2019).

Statistical analysis

Data were analyzed using the general linear model (GLM) of the SAS software, version 9.4 (SAS Institute, Cary, NC, USA; 2019) and the comparisons of the means were performed using Duncan’s multi-domain test at 5% probability level. The statistical model of the design was $Y_{ij} = \mu + A_i + e_{ij}$, which in this formula, $Y_{ij}$ is the measured attribute value, $\mu$ is the mean of the trait in the target population, $A_i$ is the effect of treatment and $e_{ij}$ is the effect of the test error.

Results

Tables 2, 3 and 4 show the effect of experimental groups on the performance of broilers (body weight gain, feed intake, and feed conversion ratio). In the starter period, there was no significant difference in the weight gain between the treatments ($p<0.05$). During the grower period, weight gains in all the treatments were significantly different compared to the control ($p<0.05$). The treatment containing 10 g of galbanum had the highest weight gain. In the finisher period and during the whole period, the weight gain of the treatments containing 5 and 10 grams of galbanum increased significantly compared to the control ($p<0.05$).

In the starter and finisher periods, feed intake was similar compared to the control ($p>0.05$). In the grower period, the differences in feed intake of all the treatments were significant compared to the control ($p<0.05$). Feed intake of broilers fed galbanum-containing diets was higher than the control and antibiotic-containing treatment. In the whole period, the differences in the feed intake of the treatments containing 5 or 10 g of galbanum were significantly increased compared to the control ($p<0.05$). In contrast, feed intake of the treatments containing 2.5 mg galbanum or antibiotic were significantly lower than that of the control ($p<0.05$). In the starter period, the feed conversion ratios for all of the treatments were not significantly different than the control ($p<0.05$). In the grower period, the conversion ratio of the treatments containing galbanum showed a significant difference compared to the control ($p<0.05$). In the finisher period, the conversion ratio of all the treatments significantly decreased compared to the control ($p<0.05$). In the whole period,
the conversion ratio of the treatments containing 2.5 or 5 g of galbanum and also the treatment containing antibiotic showed a significant decrease compared to the control (p<0.05).

Carcass composition

The results regarding the effects of galbanum and antibiotic on carcass components are shown in Table 5. No significant difference was found for the percentage of carcass, breast, and thigh in any of the treatments compared to the control (p>0.05). The percentage of gizzard in all the treatments did not significantly differ compared to the control (p>0.05). In contrast, the weight of gizzard in galbanum-containing treatments showed a significant increase compared to antibiotic-containing treatment (p<0.05). The difference in the percentage of the liver and ventricular fat in the treatments containing 5 and 10 g of galbanum powder showed a significant decrease compared to the control and antibiotic-containing treatment (p<0.05). Heart weight in all treatments containing galbanum decreased significantly compared to the control and antibiotic-containing treatments (p<0.05)
Table 2. Effects of virginiamycin and galbanum on body weight gain (g) of broiler chickens.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Starter (1-10 days old)</th>
<th>Grower (11-24 days old)</th>
<th>Finisher (25-42 days old)</th>
<th>Whole (1-42 days old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet (control)</td>
<td>216.56±1.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>589.44±0.88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1186.26±1.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2001.0±1.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Virginiamycin (0.1 g/kg of diet)</td>
<td>215.38±1.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>595.47±1.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1186.98±0.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2001.9±0.91&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galbanum (2.5 g/kg of diet)</td>
<td>215.52±0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>598.5±1.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1189.19±1.23&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2002.3±1.18&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galbanum (5 g/kg of diet)</td>
<td>216.58±0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>608.58±1.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1191.45±1.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2011.4±0.96&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galbanum (10 g/kg of diet)</td>
<td>216.58±0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>610.73±1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1196.73±1.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2016.7±1.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

SEM: standard error of the mean.

Means within the same column with at least one common letter, are not significantly different (p<0.05).

Table 3. Effects of virginiamycin and galbanum on feed intake (g/hen/period) of broiler chickens.
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Starter (1-10 days old)</th>
<th>Grower (11-24 days old)</th>
<th>Finisher (25-42 days old)</th>
<th>Whole (1-42 days old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet (control)</td>
<td>366.0±1.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1,173.0±1.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2,444.0±0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4,007.4±1.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Virginiamycin (0.1 g/kg of diet)</td>
<td>364.0±1.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,185.0±1.18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2,444.0±1.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,981.6±1.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galbanum (2.5 g/kg of diet)</td>
<td>366.0±1.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1,197.0±1.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2,444.0±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,993.0±2.12&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galbanum (5 g/kg of diet)</td>
<td>364.60±1.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,205.0±1.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2,444.0±1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4,015.0±1.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galbanum (10 g/kg of diet)</td>
<td>368.2±0.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,252.0±1.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,444.03±1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4,064.2±1.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>1.06</td>
<td>1.42</td>
<td>1.12</td>
<td>1.51</td>
</tr>
</tbody>
</table>

Means within the same column with at least one common letter, are not significantly different (p<0.05).

SEM: standard error of the mean.
Table 4. Effects of virginiamycin and galbanum on feed conversion ratio (g feed: g gain) of broiler chickens.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Starter (1-10 days old)</th>
<th>Grower (11-24 days old)</th>
<th>Finisher (25-42 days old)</th>
<th>Whole (1-42 days old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet (control)</td>
<td>1.69±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.99±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.06±1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00±1.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Virginiamycin (0.1 g/kg of diet)</td>
<td>1.69±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.99±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.05±1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.98±0.08&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galbanum (2.5 g/kg of diet)</td>
<td>1.70±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.00±1.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.05±1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.99±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galbanum (5 g/kg of diet)</td>
<td>1.69±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.098±0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.05±1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.99±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galbanum (10 g/kg of diet)</td>
<td>1.70±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.05±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.04±1.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.01±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.07</td>
<td>0.50</td>
<td>1.11</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Means within the same column with at least one common letter, are not significantly different (p<0.05).

SEM: standard error of the mean.
Table 5. Effects of virginiamycin and galbanum on carcass characteristics of broiler chickens.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carcass (%)</th>
<th>Breast (%)</th>
<th>Thigh (%)</th>
<th>Gizzard (%)</th>
<th>Liver (%)</th>
<th>Heart (%)</th>
<th>Abdominal Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet (control)</td>
<td>73.72±0.72a</td>
<td>26.92±0.58a</td>
<td>19.84±0.54a</td>
<td>1.36±0.015bc</td>
<td>2.19±0.025ab</td>
<td>0.51±0.003a</td>
<td>1.38±0.018ab</td>
</tr>
<tr>
<td>Virginiamycin (0.1 g/kg of diet)</td>
<td>72.85±0.84a</td>
<td>25.87±0.49a</td>
<td>19.00±0.71a</td>
<td>1.33±0.009c</td>
<td>2.23±0.013a</td>
<td>0.51±0.0005a</td>
<td>1.43±0.18a</td>
</tr>
<tr>
<td>Galbanum (2.5 g/kg of diet)</td>
<td>73.38±0.96a</td>
<td>26.31±0.71a</td>
<td>19.14±0.50a</td>
<td>1.37±0.008b</td>
<td>2.16±0.012b</td>
<td>0.49±0.001b</td>
<td>1.38±0.013b</td>
</tr>
<tr>
<td>Galbanum (5 g/kg of diet)</td>
<td>73.50±0.77a</td>
<td>26.53±0.27a</td>
<td>19.23±0.37a</td>
<td>1.38±0.01b</td>
<td>1.98±0.022d</td>
<td>0.49±0.0003b</td>
<td>1.28±0.017c</td>
</tr>
<tr>
<td>Galbanum (10 g/kg of diet)</td>
<td>74.63±0.40a</td>
<td>27.47±0.70a</td>
<td>20.73±0.61a</td>
<td>1.38±0.009b</td>
<td>2.07±0.007c</td>
<td>0.49±0.0008b</td>
<td>1.31±0.012c</td>
</tr>
</tbody>
</table>

SEM: standard error of the mean.

Means within the same column with at least one common letter, are not significantly different (p<0.05).
Immune system

The effects of experimental treatments on aspects of the immune system are presented in Table 6. The weight of spleen in the treatments containing 5 or 10 g of galbanum powder was significantly increased compared to the control and antibiotic-containing treatments (p<0.05). The immune response against the Newcastle disease virus and SRBC for the treatment containing 10 g of galbanum at 42 days of age showed a significant increase compared to the control and the other treatments (p<0.05).

Table 6. Effects of virginiamycin and galbanum on the immune system of broiler chickens.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Spleen (%)</th>
<th>Immune response after Newcastle vaccination</th>
<th>Immune response after SRBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet (control)</td>
<td>0.117±0.0006cd</td>
<td>3.00±0.29b</td>
<td>3.00±0.31b</td>
</tr>
<tr>
<td>Virginiamycin (0.1)</td>
<td>0.116±0.0006d</td>
<td>3.00±0.39b</td>
<td>3.20±0.37b</td>
</tr>
<tr>
<td>Galbanum (2.5 g/kg)</td>
<td>0.119±0.0008c</td>
<td>3.70±0.33b</td>
<td>3.40±0.24b</td>
</tr>
<tr>
<td>Galbanum (5 g/kg of)</td>
<td>0.121±0.0008b</td>
<td>4.00±0.25b</td>
<td>3.80±0.37b</td>
</tr>
<tr>
<td>Galbanum (10 g/kg)</td>
<td>0.124±0.0005a</td>
<td>5.00±0.33a</td>
<td>5.00±0.31a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.0006</td>
<td>0.31</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Means within the same column with at least one common letter, are not significantly different (p<0.05).

SEM: standard error of the mean.

Blood factors

The effects of different treatments on some blood biochemical parameters of broilers at the end of the period (42 days) are presented in Table 7. In this study, there were no significant changes in the level of blood biochemical parameters for the treatment containing antibiotics compared to the control (p>0.05). By adding 10 g of galbanum powder in poultry diet, levels of serum protein variables (total protein, albumin, and globulin) showed a significant increase compared to the control and the other treatments (p<0.05). Also, the serum lipids values (cholesterol, triglyceride, and LDL) in each of the treatments containing galbanum powder showed a significant decrease compared to the control and the antibiotic-containing treatments (p<0.05). Serum HDL concentration of the broilers treated with galbanum showed a significant increase compared to the control and the antibiotic treatment (p<0.05).
Table 7. Effects of virginiamycin and galbanum on blood factors of broiler chickens.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>Total protein (g/dL)</th>
<th>Albumin (g/dL)</th>
<th>Globulin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet (control)</td>
<td>184.29±1.05\textsuperscript{a}</td>
<td>111.32±1.70\textsuperscript{a}</td>
<td>73.56±0.94\textsuperscript{a}</td>
<td>24.77±0.77\textsuperscript{d}</td>
<td>3.29±0.06\textsuperscript{b}</td>
<td>2.20±0.04\textsuperscript{bc}</td>
<td>1.09±0.04\textsuperscript{bc}</td>
</tr>
<tr>
<td>Virginiamycin (0.1 g/kg of diet)</td>
<td>184.39±1.06\textsuperscript{a}</td>
<td>111.32±1.73\textsuperscript{a}</td>
<td>60.20±0.63\textsuperscript{b}</td>
<td>24.53±0.55\textsuperscript{d}</td>
<td>3.25±0.07\textsuperscript{b}</td>
<td>2.13±0.02\textsuperscript{bc}</td>
<td>1.06±0.50\textsuperscript{c}</td>
</tr>
<tr>
<td>Galbanum (2.5 g/kg of diet)</td>
<td>161.98±2.05\textsuperscript{b}</td>
<td>93.87±1.70\textsuperscript{b}</td>
<td>58.18±0.91\textsuperscript{b}</td>
<td>30.03±0.55\textsuperscript{c}</td>
<td>3.25±0.03\textsuperscript{b}</td>
<td>2.03±0.09\textsuperscript{c}</td>
<td>1.12±0.02\textsuperscript{b}</td>
</tr>
<tr>
<td>Galbanum (5 g/kg of diet)</td>
<td>136.23±1.31\textsuperscript{c}</td>
<td>86.94±1.07\textsuperscript{c}</td>
<td>40.81±1.10\textsuperscript{c}</td>
<td>36.00±0.60\textsuperscript{b}</td>
<td>3.27±0.04\textsuperscript{b}</td>
<td>2.19±0.06\textsuperscript{b}</td>
<td>1.12±0.03\textsuperscript{b}</td>
</tr>
<tr>
<td>Galbanum (10 g/kg of diet)</td>
<td>115.79±2.14\textsuperscript{d}</td>
<td>76.56±1.84\textsuperscript{d}</td>
<td>42.38±1.59\textsuperscript{c}</td>
<td>40.83±0.62\textsuperscript{a}</td>
<td>3.72±0.08\textsuperscript{a}</td>
<td>2.43±0.05\textsuperscript{a}</td>
<td>1.29±0.04\textsuperscript{a}</td>
</tr>
<tr>
<td>SEM</td>
<td>1.58</td>
<td>1.42</td>
<td>1.03</td>
<td>0.61</td>
<td>0.05</td>
<td>0.07</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Means within the same column with at least one common letter, are not significantly different (p<0.05).

SEM: standard error of the mean.
Discussion

According to our results, adding galbanum powder to the diet of broiler chickens increased body weight gain and feed intake in the grower, finisher, and total period. This increase was higher than that of antibiotic-containing treatments. Also, feed conversion ratio was improved due to the addition of this plant to the diet of broilers, and this improvement was higher compared with the antibiotic-containing treatment. These results are inconsistent with the report by Hernandez et al. (2004) who observed that medicinal plants and their extracts did not affect feed intake. They stated that the addition of mint leaves to the diet did not have a significant effect on poultry performance. Cross et al. (2007) reported that feeding medicinal plants at concentrations of 1 and 0.1% increased body weight and feed intake, and improved feed conversion ratio compared to the control, which was probably due to the antibacterial and antifungal effects of the compounds existing in the plants used in the experimental groups. The positive effects on body weight using essential oils in the diet have also been observed in other studies (Abudabos et al., 2017). The use of herbal medicines in the diet increased the body weight of the broiler chickens by 3% at 42 days of age (Tiihonen et al., 2010). The effect of herbal ingredients may be due to the use of the nutrients that improve poultry growth. Also, the increase of appetite, stimulation of digestive secretion and antibacterial effects are among the mechanisms that can be considered in justifying the reason for performance improvement (Tiihonen et al., 2010). Adding a mixture of effective oils from several wild medicinal plants to the diet of broiler chickens resulted in the improvement of the conversion ratio compared to the control and the antibiotic-containing treatment (Alçiçek et al., 2003). It has been suggested that herbal ingredients can improve feed conversion ratio through improved nutrient utilization due to increased digestive enzymes; improvement of ileal digestibility, reported by some researchers, confirms the claim (Isabel and Santos, 2009). According to the results, the feed conversion ratio for the whole period in the antibiotic-containing treatment was lower than the control and the other treatments due to reduced feed intake. It has been determined that the effect of antibiotics on improving the performance of broilers is due to the lower need of feed intake per unit of weight gain, which will result in an improvement in the feed conversion ratio (Alhidary et al., 2016).

The results showed that the difference in the percentage of carcass, breast, thigh, and gizzard in galbanum-containing treatments wasn’t significant compared to the control, though numerically it
was significantly higher than antibiotic-containing treatment. The compounds in medicinal herbs have a microbicidal effect and, by decreasing the microbial population in the digestive system of the broilers, prevent amino acid degradation and provide better absorption of them and improve carcass traits (Nobakht et al., 2015). The presence of high levels of microbial mass in the digestive system in various ways, such as secretion of urease, results in the degradation of protein and amino acids through their deamination, and in this way significant amounts of amino acids and proteins are not decomposed and not absorbed (Lee et al., 2003).

However, in a situation where the harmful microbial population is reduced, the conditions for the growth and proliferation of beneficial microbes are provided and the health of the chickens is improved, and this issue can lead to optimization of digestion and absorption and improve carcass traits (Lee et al., 2003). No significant difference in the percentage of carcass, breast, and thigh in the present study may be due to the reason that by providing the sanitary principles of breeding conditions, the activity of harmful microbes has not been up to a limit to allow the addition of galbanum plant and antibiotics to considerably affect carcass components in comparison with the control.

Based on the results of this study, while the relative weight of the liver increased by increasing the levels of galbanum powder in the diet on a specified procedure, liver percentage in the treatments containing 5 and 10 g of galbanum powder decreased significantly compared to the control which is consistent with the results of Nasiri et al. (2011). The diets containing 0.5% of the herbal medicine in the broiler diet did not only increase the size of the liver than the control but also reduced its size relative to the control (Galiband Al-kassie, 2010). Liver is the target for the effects of toxins; the toxins cause liver toxicity and affect its weight (Galiband Al-kassie, 2010). Since galbanum has toxic effects due to its alpha-thujene compound, it can increase liver size with hypertrophy of smooth endoplasmic in hepatocyte (Sayyah et al., 2001). According to the researchers, while the use of high doses of herbal drugs in the long run can have deleterious effects on the liver, the use of low doses in the diet while having low toxicity, improves the function of the bird's organs due to its antioxidant properties. This finding can be a reason for a significant decrease in liver percentage compared to the control in this study (Ocak et al., 2008).

As shown in the results, heart weight decreased significantly in galbanum-containing treatments compared to the other treatments, which is consistent with the results of Cross et al. (2007). The
thymol and phenolic compounds in medicinal plants have antimicrobial and antifungal activity and are considered as an antioxidant and growth stimulant (Cross et al., 2007).

Regarding the theory that lipid peroxidation may cause heart tissue damage and the development of blood pressure syndrome, the role of medicinal plants with antioxidant properties has been investigated in many studies such that of Ciftci et al. (2010), who found that adding 5 g of medicinal herbs with antioxidant property for each kilogram of diet reduces the thick outer wall of the arteries, which subsequently decreases the resistance to blood flow, thus reducing the size of the heart. Also, (Ruiz-Feria, 2009) in their studies stated that the use of 5 ml of lemon balm essential oil in the diet of broilers significantly reduced heart weight. Increasing the antioxidant capacity of the cell, especially in the tissues involved with ascites, such as myocardium cells, can by creating an antioxidant protective effect, stop the peroxidation of the capillary membrane lipid tissue and prevent the hypertrophy of the heart ventricles. It is possible that galbanum’s antioxidant effects could prevent cellular peroxidation in the heart tissue and inhibit increasing its size (Geng et al., 2004b).

It has been reported that the addition of medicinal plants to the diet of broilers resulted in a significant decrease of abdominal cavity fat compared to the control. The researchers stated that this decline was due to the increase in the secretion of pancreatic enzymes (Jamroz et al., 2005). In another report, the effect of herbal material on the reduction of abdominal cavity fat has been attributed to inhibiting the activity of liver enzymes involved in lipogenesis or lipoprotein lipase present in fat storage tissue by active ingredients in plant compounds (Gavanji et al., 2014). Therefore, the decrease in the abdominal cavity fat through the use of galbanum powder can be attributed to those materials.

In the case of using plant additives in the poultry diet, there would be a significant increase in the relative weight of the spleen compared to the control (Moorthy et al., 2009), which is consistent with this study. Medicinal plants are rich in flavonoids and terpenes, which indirectly enhance and stimulate the immune system with their antibacterial effects. When stimulating the immune system, the size of the large spleen and the protein synthesis increases, which leads to the refining and activation of the immune system in response to the disease (Nasiroleslami and Torki, 2010). Regarding the highest titer of antibodies against Newcastle Virus and SRBC in galbanum-containing treatments, it can generally be concluded that in this study, the plant was effective in boosting the immune system as compared to the antibiotic, which is consistent with Feizi and
Nazari (2011) and Ghalamkari et al. (2011). The rate of the immune system’s response will vary based on genetic variation (Webster et al., 2006). Some studies indicate that in addition to genetic factors, non-genetic factors such as supplements in the diet, that affect the growth, can change or modify the expression of the genes responsible for immune sensitivity by altering antibody production level and immune maturation. One of the growth supplements that have been recently used in various ways in the poultry diet is herbal medicines that, by eliminating harmful bacteria in the intestines, help the digestive system to better absorb the nutrients present in the diet and cause further growth of cells, which consequently increases the production of immunization compounds (Okoro et al., 2016). Various compounds in the herbs of the medicinal plants are able to stimulate lymphoid organs due to their nutritional and antioxidant effects. These plants, having specific ingredients, contribute to the amplification and proliferation of embryonic fibroblast cells in the chickens that play a role in the development of the immune system and bone marrow tissue (Mahanta et al., 2017). Also, regarding the effects of medicinal plants on the immunity level of broilers, the use of medicinal plants increases the percentage of lymphocytes and heterophile, which indicates the beneficial effects of this plant on the immunity level of broilers (Al-Jaff, 2011; Nacimento et al., 2020). The damage caused by free radicals is an important mechanism of cell damage (Kumar et al., 2003).

A higher level of total protein, albumin, and globulin under the influence of 10 g of galbanum powder can be attributed to the increased absorption of nutrients such as protein and its presence in blood serum. As it has been reported, galbanum powder in the diet reduces the harmful gastrointestinal microbial population and therefore the degradation rate of protein and amino acids for digestive materials decreases; more of them are absorbed and as the result, improving digestion and absorption of nutrients and protein increases the concentration of total protein, albumin, and globulin in the serum (Lee et al., 2003; Ghazvinian et al., 2018).

Significant decrease in total cholesterol, triglyceride and LDL levels, as well as a significant increase in HDL in the treatments containing galbanum compared to the control and antibiotic-containing treatment demonstrated the effectiveness of the plant in modifying blood lipids. Abdel-Hafez et al. (2017) stated that the decrease in abdominal fat percentage is due to the increase in the population of beneficial bacteria such as Bacillus subtilis as a result of adding prebiotics to the diet. These bacteria further reduce the activity of acetyl coenzyme A carboxylase by excreting bile
acids, thereby reducing the synthesis of fatty acids and their availability for esterification reactions and the production of triglycerides for storage in adipose tissue.

The role of medicinal plants in reducing cholesterol and triglyceride levels has been reported in some research, which is consistent with the results of this study (Hosseini et al., 2013). The supplementation of the medicinal plant with the poultry diet can be effective in lowering cholesterol levels due to the activity of lactic acid bacteria by producing bile-degrading enzymes and their deconjugation, as well as by decreasing the pH of the intestinal tract (Jahromi et al., 2016). The solubility of unconjugated bile acids decreases at low pH, resulting in less intestinal absorption and more secretion in the stool (Jahromi et al., 2016). As a result, the liver converts more cholesterol to bile to rebuild the liver cycle of bile acids, and hence the concentration of cholesterol in tissues and blood decreases (Ali et al., 2007). Herbal ingredients inhibit the activity of 3-hydroxyl 3-methylglutaryl-coenzyme A (HMG-COA) liver reductase (Souri et al., 2015). This is a key enzyme in the regulation of cholesterol synthesis. As it has been reported, there is a link between the activity of HMG-COA reductase, serum cholesterol and lipoproteins in broilers (Iqbal et al., 2018). 5% HMG-COA reductase inhibitory activity can reduce serum cholesterol, triglyceride and LDL of poultry by 1% and also increase HDL (Safa and Al-Beitawi, 2009). In a study by Jeong and Kim (2015) they tested the hypothesis that a medicinal herb used in the diet could restrain the biosynthesis of isopentenyl pyrophosphate, a precursor to cholesterol synthesis. In an experiment, Ajuwon (2016) reported that the diets containing medicinal plants in poultry increased the microbial population of lactobacilli in the intestine and these lactobacilli can metabolize and absorb cholesterol in the small intestine and also reduce its absorption through the bloodstream.

Rostami et al. (2020) investigated the effects of Thymus vulgaris extract in comparison with some common feed additives on performance, blood biochemical parameters and antibody response in broiler chickens under heat stress conditions. The results in this research suggest that thyme extract at the level of 500 mg/kg of diet can be recommended as an alternative to the common feed additives to improve growth performance and health benefits of the heat-stressed broilers. The results of this experiment showed that the application of dried and ground powder of galbanum at the levels 5 and 10 g/kg of diet compared to the diet of using 2.5 g of galbanum and the diet containing virginiamycin antibiotic was increasingly effective in improving the performance and carcass characteristics. Using these recommended levels of galbanum in comparison with
antibiotics led to an increase in the immune response and immune defenses of birds, and also it was revealed that those levels have no harmful effects on blood parameters.

According to the results of this experiment, galbanum can be used in replacement of virginiamycin as a growth stimulant for broiler chickens.

**Declarations**

**Acknowledgments**

The authors wish to acknowledge support from Department of Animal Science, Semnan University, Semnan, Iran.

**Funding**

This study was conducted with personal funding by the authors.

**Conflicts of interest**

The authors declare they have no conflicts of interest with regard to the work presented in this report.

**Author contributions**

All authors contributed extensively to all aspects of this work, including the conception and design of the study, implementation, data analysis and manuscript writing.

**References**


Al-Kassie GA. The role of peppermint (*Mentha piperita*) on performance in broiler diets. ABJNA 2010; 1(5): 1009-1013. DOI: https://doi.org/10.5251/abjna.2010.1.5.1009.1013


Jeong JS, Kim IH. Effect of fermented medicinal plants (Gynura procumbens, Rehmannia glutinosa, Scutellaria baicalensis) as alternative performance enhancers in broilers. J Poult Sci2015; 0140061. DOI: https://doi.org/10.2141/jpsa.0140061

Klaver FA, Van der Meer R. The assumed assimilation of cholesterol by lactobacilli and Bifidobacterium bifidum is due to their bile salt-deconjugating activity. Appl Environ Microbiol 1993; 59(4): 1120-1124. DOI: https://doi.org/0099-2240/93/041120-05$02.00/0


Nacimento RA, Moro ME, Ferrari VB, Sanfelice LV, Pelissari PH, Sartore YG, Araújo LF. Oleoresins from chili pepper and turmeric could substitute for salinomycin in broilers. RCCP 2020; 33(3): 195-201. DOI: https://doi.org/10.17533/udea.rccp.v33n3a03


Okoro VMO, Nwokeocha ACC, Ijezie CO, Mbajorgu CA, Mbajorgu EF. Effect of varying dietary supplemental inclusion levels of onion and garlic on semen quality characteristics of Hubbard white breeder broiler cocks aged 35–41 weeks old. Indian J Anim Res 2016; 50(6): 922-929. DOI: [https://doi.org/10.18805/ijar.9378](https://doi.org/10.18805/ijar.9378)


