

Supplementing dietary *Rosmarinus officinalis* L. powder in quails: the effect on growth performance, carcass traits, plasma constituents, gut microflora, and immunity

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ABSTRACT

This study was conducted to investigate the effects of feed supplementation with rosemary (*Rosmarinus officinalis* L.) powder (RP) on the performance, carcass traits, blood variables, antibody responses, and gut microbiota of quails. 270 one-day-old male Japanese quails were allotted to 6 dietary treatments with 3 replications (15 birds per pen). The diets contained RP at levels of 0 g/kg of feed (CON), 5 g/kg of feed (T05), 10 g/kg of feed (T10), 15 g/kg of feed (T15), 20 g/kg of feed (T20) or 25 g/kg of feed (T25). The trial lasted 42 days. Body weight and feed intake were measured weekly by pen, and the feed conversion ratio (FCR) was calculated. Blood samples were collected for assessing the humoral immune response to Newcastle vaccination at the 29th and 42nd days of age. Analysis of caecal microflora was performed on one quail per replication on the 14th and 42nd days of age. At slaughter, the carcasses and the organs were weighed, and blood constituent analyses were performed. Quails fed with RP had higher weight gain ($P<0.05$) and FCR ($P<0.05$) than CON. T5 and T10 showed higher weight gain than T25 ($P<0.05$). The dietary inclusion of RP did not influence either the weight of organs ($P>0.05$) or the caecal microbiota of the quails ($P>0.05$). RP increased the antibody titre against vaccinations on the 29th day of age ($P<0.05$) and reduced the plasmatic LDL/HDL ratio ($P<0.05$). The dietary supplementation of RP up to 25 g/kg of feed improved the performance and immunity of quails, without affecting their caecal microflora.

Key words: quail; performance; microbiota; immunity; rosemary

Introduction

International production of poultry meat is constantly increasing, with a 31.6% increase in the last decade. On the other hand, in the next ten and twenty years the world population will

increase by 10.3% and 19.5%, respectively (FAO, 2019). Considering that poultry meat is particularly inexpensive, it is easy to understand that its production will increase even more in the

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coming years to meet the increasing world demand for protein of animal origin. At the same time, the increasing amount of poultry raised for food production may favour an increase in antimicrobial consumption. VAN BOECKEL et al. (2015) estimated that antimicrobial consumption in animal rearing will rise worldwide by over 60% from 2010 to 2030. Further, the use of antimicrobials favours antimicrobial resistance, which is widely considered to be a serious health concern even for humans (NHUNG et al., 2017). Essential oils and plant extracts have been proposed as possible alternatives to antimicrobials in poultry production (DALAL et al., 2018; POURNAZARI et al., 2017; SEIDAVI et al., 2018; ZHAI et al., 2018), but the results are often contradictory (PETRIČEVIĆ et al., 2018; REYER et al., 2017).

Rosemary is a shrub belonging to the Lamiaceae family, whose cultivation is increasing worldwide. It is one of the oldest known medicinal plants, known for its beneficial biological activities (RAFIE et al., 2017). This plant is rich in “secondary metabolite compounds” that are useful for protection from biotic and abiotic hazards, and whose amounts are variable, depending on environmental conditions (YESILBAG et al., 2012). In particular, phenolic compounds, such as terpenes, have antioxidant activity, while 1,8-cineol and camphor have antimicrobial activity (RAMIREZ et al., 2004; ROSTAMI et al., 2017; YESILBAG et al., 2012). Very recently, RAFIE et al. (2017) wrote that rosemary is able, not only to inhibit the growth, but also to modify the antibiotic resistance of some bacteria.

ALAGAWANY and EL-HACK (2015) observed that rosemary supplementation in the diet increased the performance of laying hens, along with improvement in immunity. PETRIČEVIĆ et al. (2018) showed that the inclusion of rosemary powder up to 4 g/kg in the diet can improve the performance of broiler chickens, in terms of weight gain and feed conversion, and can modify the microbial population of the cecum. Similar results were also highlighted by GHAZALAH and ALI (2008), using up to 5 g/kg of rosemary in the diet. Conversely, the same authors reported that higher levels of inclusion did not affect the performance

of broiler chickens. Moreover, ROSTAMI et al. (2018) highlighted that the inclusion of rosemary powder at a level of 1% in the diet is not able to affect immunity and blood proteins, but ROSTAMI et al. (2015) stated that, in general, the use of rosemary in broiler chicken nutrition can have beneficial effects on animal performance, immunity and haematological variables. However, not much research is available on the use of rosemary in quail nutrition. CETIN et al. (2017) showed that the *in vivo* performance of quails did not increase with the addition of 25 g/kg of diet of rosemary oil. MAHGOUB et al. (2019) suggested that a level of 2.00 mL/kg of diet of rosemary oil is able to reduce the intestinal pathogenic bacteria of quails. To our knowledge, very little or no information is available on the use of rosemary powder in quail nutrition, and its effects on both the productive performance and health status of these animals.

Therefore, the aim of this study was to investigate the effects of feed supplementation with different levels of rosemary powder on the growth performance, carcass traits, blood variables, antibody responses, and gut microbiota of quail chickens.

Materials and methods

The procedures used were approved by the Ethics Committee of the Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran.

Animals, diets and housing. A total of 270, one-day-old male Japanese quails were allotted to 18 groups/pens (6 treatments, and 3 replicates for each treatment) of 15 birds, that differed in the level of inclusion of rosemary powder (RP) in the diet. Treatments 1 to 6, received 0 g/kg (CON), 5 g/kg (T5), 10 g/kg (T10), 15 g/kg (T15), 20 g/kg (T20), or 25 g/kg (T25) RP in the feed. Treatment 1 was considered as the control group. The experimental diets were formulated according to the NRC (1994) recommendations and are shown in Table 1. No antibiotic was used as a feed additive. RP was analysed for chemical composition according to the AOAC (2000): crude protein (5.08%; method 990.03), crude fibre (19.49%; method 962.09), and it had gross energy of 14.8 MJ/kg (calorimetric

method) and metabolizable energy of 11.3 MJ/kg (calculated according to NRC, 1994). The experimental period lasted 42 days. The animals were reared in pens of 1 × 1 m and subjected to standard rearing conditions, including temperature, humidity, light, stocking density, and health

programmes (SIGOLO et al., 2019). The birds were vaccinated against Newcastle disease virus strain B1 (10th day of age; Razi Co, Karaj, Iran) and Newcastle La Sota (21st day of age; Razi Co, Karaj, Iran) using the spray method.

Table 1. Diets offered to experimental groups of quail chicks according to the different levels of inclusion of rosemary powder: 0 g/kg of feed (CON), 5 g/kg of feed (T5), 10 g/kg of feed (T10), 15 g/kg of feed (T15), 20 g/kg of feed (T20) and 25 g/kg of feed (T25)

| | CON | T5 | T10 | T15 | T20 | T25 |
|--|-------|-------|-------|-------|-------|-------|
| Ingredients (g/kg as-fed) | | | | | | |
| Maize | 517.5 | 512.5 | 507.5 | 502.5 | 497.5 | 492.5 |
| Soybean meal | 340 | 340 | 340 | 340 | 340 | 340 |
| Gluten meal | 87.5 | 87.5 | 87.5 | 87.5 | 87.5 | 87.5 |
| Soybean oil | 20.75 | 20.75 | 20.75 | 20.75 | 20.75 | 20.75 |
| Rosemary powder | 0 | 5 | 10 | 15 | 20 | 25 |
| Enzyme | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| CaCO ₃ | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| Dicalcium phosphate | 10 | 10 | 10 | 10 | 10 | 10 |
| NaCl | 3 | 3 | 3 | 3 | 3 | 3 |
| Mineral mixture ¹ | 3 | 3 | 3 | 3 | 3 | 3 |
| Vitamin mixture ² | 3 | 3 | 3 | 3 | 3 | 3 |
| DL-methionine | 2 | 2 | 2 | 2 | 2 | 2 |
| L-lysine HCL | 1 | 1 | 1 | 1 | 1 | 1 |
| Calculated analysis (g/kg unless stated otherwise) | | | | | | |
| Metabolizable energy (kcal/kg) | 12.1 | 12.1 | 12.1 | 12.1 | 12.1 | 12.1 |
| Crude Protein | 24.0 | 24.0 | 24.0 | 24.0 | 24.0 | 24.0 |
| Extract ether | 4.4 | 4.4 | 4.4 | 4.4 | 4.4 | 4.4 |
| Crude fibre | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Calcium | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| Total phosphorus | 0.74 | 0.74 | 0.74 | 0.74 | 0.74 | 0.74 |
| Available phosphorus | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Potassium | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 |
| Na | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| Chloride | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| Lysine | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 | 1.28 |
| Methionine + Cysteine | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 |

¹Supplied per feed: Cu: 3 mg/g; Zn: 15 mg/g; Mn: 20 mg/g; Fe: 10 mg/g; K: 0.3 mg/g. ²Supplied per feed: Vitamin A: 5000 IU/g; vitamin D₃: 500 IU/g; vitamin E: 3 mg/g; vitamin K₃: 1.5 mg/g; vitamin B2: 1.0 mg/g; calcium pantothenate: 15 mg/g; niacin: 15 mg/g; vitamin B6: 13 mg/g.

Sample collection and measurements. Body weight and feed intake were measured weekly by pen from one to six weeks of age. The feed conversion ratio was calculated by dividing the weekly feed consumption by the weekly body weight gain for each pen.

During the trial, one quail per replication was selected on the 29th day of age and on the last day of the trial (42nd day of age) for blood sampling from the ulnaris vein, in order to assess the humoral immune response to the Newcastle vaccination. Moreover, one quail per replication was selected on the 14th day of age and on the last day of the trial, and euthanized to perform caecal microflora analysis.

At 42 days of age, one bird per replicate was selected and euthanized for carcass and organ weight and for analyses of the blood constituents. The feet were separated from the carcass at the tibio-tarsal joint. The neck, wingtips, gut and liver were removed, and the empty carcasses were weighed. The weights were recorded of the gizzard, pancreas, heart, liver, and of the organs related to immunity (the spleen and Bursa of Fabricius), and the values were expressed as relative weight (RW) with respect to the carcass weight.

For each pen, the selected animals were the most representative with respect to the average body weight of the pen.

Blood analysis. Before blood sampling, feed was removed for a period of four hours in order to allow stabilization of the various plasma constituents, and all blood sampling was done in the morning to further reduce the variability of plasma constituents. Five mL volume of venous blood was collected and transferred from the syringe into a tube coated with 10 mg of an anticoagulant ethylenediaminetetraacetic acid (EDTA). Samples were transferred to the laboratory for analysis within 2 h of collection. The analysis of glucose, cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), uric acid and total protein was performed as reported by SHABANI et al. (2015). Antibody titres against Newcastle disease virus were measured by the hemagglutination inhibition test, according to POURHOSSEIN et al. (2015).

Caecum microflora. The microbial variables examined in this study were *Escherichia coli*, *Lactobacilli* and coliform bacteria. At slaughter, caecal segments were isolated and their contents were streaked on agar plates. The microbial analyses were performed as reported in DIBAJI et al (2014) and SHABANI et al. (2015). The average number of live bacteria was calculated per 1g of original sample. All quantitative data were converted into logarithmic colony forming units (CFU/g).

Statistical analysis. The GLM procedure of SAS 8.0 (SAS, 2000) was used for statistical analyses. The statistical design was $Y_{ij} = \mu + T_j + e_{ij}$, where Y_{ij} is the dependent variable; μ represents the overall mean; T_j is the effect of the treatment; and e_{ij} is the residual error. The least squares means were compared using Duncan's multiple range test. Statistical significance was declared at $P < 0.05$.

Results

The data on the *in vivo* performance of the birds are shown in Table 2. The dietary inclusion of RP did not affect feed intake ($P > 0.05$), but it supported a greater increase in animal weight. In particular, within the experimental groups that received RP, T5 and T10 showed higher weight gain than T25. These results led to a better feed conversion ratio (FCR) in birds fed with RP than those fed the control diet ($P < 0.05$). At slaughter, groups T0.5 and T10 showed higher carcass weights than the other groups ($P < 0.05$).

The dietary inclusion of RP did not influence the weight of organs (Table 3; $P > 0.05$). Considering antibody titres against the Newcastle disease vaccination, a positive effect of RP was observed at 29 ($P < 0.05$), but not at 42 days of age ($P > 0.05$; Table 4). The dietary RP did not affect the weight of the lymphoid organs ($P > 0.05$; Table 4). The plasma biochemical compounds of the experimental groups are reported in Table 5. The highest values for plasma glucose and triglycerides were found in groups T25 and T10, respectively ($P < 0.05$). In comparison with the control, T20 and T25 showed lower values of uric acid ($P < 0.05$), while T5 showed higher value of total protein ($P < 0.05$). The inclusion of RP in the diets of the quails reduced the

LDL/HDL ($P < 0.05$) and did not affect the plasma level of cholesterol ($P > 0.05$). Moreover, RP did not influence the caecal microbiota of the quails ($P > 0.05$; Table 6).

Table 2. Mean \pm SD of feed intake and weight gain in the whole period (1-42 days of age) and carcass weight of quails fed with different levels of dietary inclusion of rosemary powder: 0 g/kg of feed (CON), 5 g/kg of feed (T5), 10 g/kg of feed (T10), 15 g/kg of feed (T15), 20 g/kg of feed (T20) and 25 g/kg of feed (T25)

| Treatment | Feed intake (g) | Weight gain (g) | FCR | Full abdomen carcass weight (g) |
|-----------|-------------------|---------------------------------|------------------------------|---------------------------------|
| CON | 683.0 \pm 12.88 | 165.9 \pm 5.04 ^d | 4.11 \pm 0.07 ^a | 145.1 \pm 6.12 ^b |
| T5 | 697.5 \pm 22.54 | 199.2 \pm 5.46 ^{ab} | 3.50 \pm 0.17 ^b | 191.5 \pm 26.31 ^a |
| T10 | 692.1 \pm 46.86 | 204.8 \pm 6.82 ^a | 3.37 \pm 0.19 ^b | 194.1 \pm 19.16 ^a |
| T15 | 683.2 \pm 37.27 | 189.1 \pm 8.33 ^{bc} | 3.61 \pm 0.10 ^b | 165.9 \pm 18.15 ^{ab} |
| T20 | 695.2 \pm 25.09 | 193.1 \pm 5.58 ^{abc} | 3.58 \pm 0.19 ^b | 155.2 \pm 19.30 ^b |
| T25 | 690.9 \pm 56.14 | 186.1 \pm 4.32 ^c | 3.70 \pm 0.22 ^b | 151.7 \pm 6.04 ^b |

^{a,b,c,d} $P < 0.05$

Table 3. Mean \pm SD of weight and relative weight to carcass weight (RW) of organs of quails fed with different levels of dietary inclusion of rosemary powder: 0 g/kg of feed (CON), 5 g/kg of feed (T5), 10 g/kg of feed (T10), 15 g/kg of feed (T15), 20 g/kg of feed (T20) and 25 g/kg of feed (T25)

| Treat | Gizzard (g) | Gizzard RW (%) | Pancreas (g) | Pancreas RW (%) | Heart (g) | Heart RW (%) | Liver (g) | Liver RW (%) |
|-------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| CON | 3.59 \pm 0.26 | 2.49 \pm 0.29 | 0.45 \pm 0.12 | 0.32 \pm 0.08 | 1.47 \pm 0.13 | 1.02 \pm 0.12 | 3.87 \pm 0.53 | 2.68 \pm 0.48 |
| T5 | 4.09 \pm 0.12 | 2.17 \pm 0.36 | 0.45 \pm 0.10 | 0.24 \pm 0.06 | 1.94 \pm 0.14 | 1.03 \pm 0.21 | 5.02 \pm 1.03 | 2.64 \pm 0.50 |
| T10 | 4.24 \pm 0.77 | 2.20 \pm 0.45 | 0.48 \pm 0.06 | 0.25 \pm 0.02 | 1.60 \pm 0.12 | 0.83 \pm 0.09 | 4.86 \pm 1.84 | 2.46 \pm 0.69 |
| T15 | 3.70 \pm 0.62 | 2.22 \pm 0.14 | 0.42 \pm 0.01 | 0.26 \pm 0.03 | 1.63 \pm 0.09 | 0.99 \pm 0.16 | 4.58 \pm 1.50 | 2.73 \pm 0.63 |
| T20 | 3.55 \pm 1.14 | 2.26 \pm 0.46 | 0.32 \pm 0.10 | 0.20 \pm 0.05 | 1.66 \pm 0.37 | 1.06 \pm 0.12 | 3.61 \pm 1.66 | 2.27 \pm 0.75 |
| T25 | 4.06 \pm 0.49 | 2.69 \pm 0.41 | 0.48 \pm 0.10 | 0.32 \pm 0.06 | 1.48 \pm 0.10 | 1.09 \pm 0.08 | 3.57 \pm 0.53 | 2.35 \pm 0.27 |

Treat - Treatment; ^{a,b,c,d} $P < 0.05$

Table 4. Mean \pm SD of weight and relative weight to carcass weight (RW) of organs related to immunity, and immune response after vaccination for Newcastle disease of quails fed with different levels of dietary inclusion of rosemary powder: 0 g/kg of feed (CON), 5 g/kg of feed (T5), 10 g/kg of feed (T10), 15 g/kg of feed (T15), 20 g/kg of feed (T20) and 25 g/kg of feed (T25)

| Treat | Spleen (g) | Spleen RW (%) | Bursa of Fabricius (g) | Bursa of Fabricius RW (%) | Antibody titre 29 th d of age (lg2) | Antibody titre 42 nd d of age (lg2) |
|-------|-----------------|-----------------|------------------------|---------------------------|--|--|
| CON | 0.11 \pm 0.03 | 0.08 \pm 0.02 | 0.11 \pm 0.01 | 0.08 \pm 0.01 | 0.33 \pm 0.57 ^b | 1.00 \pm 0.00 |
| T5 | 0.09 \pm 0.05 | 0.05 \pm 0.02 | 0.17 \pm 0.05 | 0.09 \pm 0.02 | 1.00 \pm 0.00 ^a | 0.66 \pm 0.57 |
| T10 | 0.09 \pm 0.02 | 0.05 \pm 0.01 | 0.12 \pm 0.03 | 0.06 \pm 0.01 | 1.00 \pm 0.00 ^a | 0.66 \pm 0.57 |
| T15 | 0.09 \pm 0.02 | 0.06 \pm 0.01 | 0.21 \pm 0.10 | 0.13 \pm 0.04 | 1.00 \pm 0.00 ^a | 1.33 \pm 0.57 |
| T20 | 0.08 \pm 0.03 | 0.05 \pm 0.01 | 0.18 \pm 0.08 | 0.12 \pm 0.06 | 0.66 \pm 0.57 ^{ab} | 0.66 \pm 0.57 |
| T25 | 0.10 \pm 0.01 | 0.07 \pm 0.01 | 0.15 \pm 0.02 | 0.10 \pm 0.02 | 1.00 \pm 0.00 ^a | 1.00 \pm 1.00 |

Treat - Treatment; ^{a,b,c,d} $P < 0.05$

Table 5. Mean \pm SD of plasma biochemical compounds of quails fed with different levels of dietary inclusion of rosemary powder: 0 g/kg of feed (CON), 5 g/kg of feed (T5), 10 g/kg of feed (T10), 15 g/kg of feed (T15), 20 g/kg of feed (T20) and 25 g/kg of feed (T25)

| Treat | Glucose (mg/dL) | Cholesterol (mg/dL) | Triglycerides (mg/dL) | LDL/HDL | Uric acid (mg/dL) | Total protein (g/dL) |
|-------|--------------------------------|---------------------|----------------------------------|------------------------------|-------------------------------|-------------------------------|
| CON | 126.2 \pm 38.52 ^b | 296.0 \pm 28.00 | 165.0 \pm 8.92 ^b | 3.19 \pm 0.18 ^a | 7.87 \pm 1.62 ^a | 1.81 \pm 0.36 ^b |
| T5 | 107.1 \pm 6.88 ^b | 268.9 \pm 68.42 | 417.1 \pm 239.95 ^{ab} | 1.42 \pm 0.81 ^b | 5.87 \pm 1.57 ^{ab} | 3.58 \pm 0.34 ^a |
| T10 | 99.6 \pm 5.85 ^b | 236.0 \pm 9.61 | 542.0 \pm 22.95 ^a | 1.51 \pm 0.68 ^b | 7.10 \pm 0.78 ^{ab} | 2.66 \pm 1.20 ^{ab} |
| T15 | 88.7 \pm 3.11 ^b | 192.4 \pm 24.01 | 211.9 \pm 181.95 ^b | 0.73 \pm 0.14 ^b | 6.90 \pm 1.79 ^{ab} | 1.81 \pm 0.67 ^b |
| T20 | 115.5 \pm 32.82 ^b | 206.2 \pm 25.24 | 227.7 \pm 108.91 ^b | 0.96 \pm 0.22 ^b | 4.84 \pm 0.33 ^b | 2.39 \pm 1.10 ^{ab} |
| T25 | 170.2 \pm 25.33 ^a | 232.4 \pm 100.54 | 205.9 \pm 82.38 ^b | 1.23 \pm 1.12 ^b | 5.22 \pm 0.88 ^b | 1.39 \pm 1.16 ^{ab} |

Treat - Treatment; HDL - High Density Lipoproteins; LDL - Low Density Lipoproteins; ^{a,b,c,d} P<0.05

Table 6. Mean \pm SD of caecal microbiota (CFU/g) of quails fed with different levels of dietary inclusion of rosemary powder: 0 g/kg of feed (CON), 5 g/kg of feed (T5), 10 g/kg of feed (T10), 15 g/kg of feed (T15), 20 g/kg of feed (T20) and 25 g/kg of feed (T25)

| Treatment | Lactobacilli (log ₁₀ cfu/g) | <i>Escherichia coli</i> (log ₁₀ cfu/g) | Coliforms (log ₁₀ cfu/g) |
|-----------|--|---|-------------------------------------|
| CON | 6.93 \pm 0.60 | 5.91 \pm 0.55 | 5.60 \pm 0.60 |
| T5 | 6.89 \pm 0.09 | 6.11 \pm 0.21 | 6.62 \pm 0.09 |
| T10 | 7.18 \pm 0.11 | 5.98 \pm 0.33 | 6.69 \pm 0.11 |
| T15 | 7.71 \pm 0.48 | 6.24 \pm 0.42 | 6.76 \pm 0.48 |
| T20 | 7.20 \pm 0.18 | 6.24 \pm 0.49 | 6.84 \pm 0.18 |
| T25 | 6.96 \pm 0.23 | 5.75 \pm 0.46 | 6.42 \pm 0.23 |

^{a,b,c,d} P<0.05

Discussion

In this trial, RP increased the weight gain of quails without affecting the feed intake. These results led to a better FCR in the animals fed with RP. YESILBAG et al. (2012) showed an improvement in live weight gain in quails supplemented with 0.14 g/kg of diet of rosemary oil in comparison with the control. Conversely, ÇIFTÇI et al. (2013) showed that rosemary oil improved the FCR, but not the live weight gain, if added to the diet of quails at a level of 0.125 g/kg of diet and 0.25 g/kg of diet. In general, the effect of rosemary on the *in vivo* performance of birds is complex, and far from understood. Rosemary can modify the diet composition, especially increasing the crude fibre content, and it has been hypothesised that rosemary can have a positive antimicrobial effect, and/or can increase the secretion of enzymes that facilitate the digestion of nutrients in the digestive

tract (PETRIČEVIĆ et al., 2018). Considering that no effect of RP on the caecal microbiota was found, and that the diets had the same level of crude fibre, the results of the present trial seem to confirm the hypothesis that rosemary can facilitate the digestion of dietary nutrients. REYER et al. (2017) showed that phytogetic feed additives, such as rosemary, are able to increase the expression and the mRNA abundance, in particular, of peptide and amino acid transporters, favouring the growth rate and the feed efficiency of animals. Therefore, the results of the present study can probably be explained by an increase in the efficiency of digestion and/or absorption of dietary nutrients, rather than an effect of RP on the gut microbiota. In accordance with the results regarding *in vivo* performance, T5 and T10 showed significantly higher carcass weight compared to the control group, while no

effect of RP on the weight of the organs considered (the gizzard, pancreas, heart and liver), was found. MAHGOUB et al. (2019) showed that dietary rosemary oil supplementation at a level of 1 mL/kg, but not at a level of 2 mL/kg, increased carcass weight. The same authors, while not in agreement with the results of the present study, showed that rosemary oil influenced the RW of the liver, gizzard and heart. However, information is scarce about the effect of rosemary on organs and on carcass traits of quails (SOLTANI et al., 2016).

The RP did not influence the weight of the lymphoid organs, spleen and Bursa of Fabricius. Conversely, RP increased the antibody titre of quails at 29 days of age, but not at 42 days of age. Considering that the vaccination against Newcastle disease took place at 10 and 21 days of age, it is clear that RP temporarily improved the immune status of the quails. ROSTAMI et al. (2018) showed that the dietary supplementation of RP up to 1% did not influence the immunity of broiler chickens. In agreement with the results of the present study, ABD EL-LATIF et al. (2013) failed to detect any effect of rosemary oil on the weight of the spleen and Bursa of Fabricius. KHALIGH et al. (2011) highlighted an increase in the antibody titre against Newcastle disease at 33, but not at 42 days of age, while supplementing the diet of broiler chickens with an herbal blend that contained rosemary. ALAGAWANY and EL-HACK (2015) reported that rosemary may increase the serum immunoglobulins, and wrote that rosemary can activate many immune functions, such as lymphocyte proliferation. From this point of view, the microbiota may influence and regulate both the acquired and the innate immune response (CLAVIJO and VIVES FLÓREZ, 2018). However, in the present trial no effect of RP on the microbiota was found. ABD EL-LATIF et al. (2013) reported that the reasons why rosemary improves bird immunity are still not clear. ROSTAMI et al. (2018) hypothesised that rosemary may have a beneficial effect on the immune response of bird, thanks to the antioxidant activities of phenolic compounds. This hypothesis seems to have been confirmed in the present study, where an decrease was observed in the plasma LDL/HDL ratio, which

is considered an index of higher anti-oxidant status (ALIMOHAMMADI-SARAEI et al., 2018). RP had a limited effect on the level of the other plasma constituents. Total protein, triglycerides and uric acid are related to body condition, fat deposition and protein catabolism (TŮMOVÁ and TEIMOURI, 2010). Our results are in agreement with the findings of ROSTAMI et al. (2015) that failed to detect differences in the plasmatic levels of uric acid and total protein in broiler chickens fed with RP up to a level of 10 g/kg of feed. MAGHOUB et al. (2019) observed a reduction in plasma cholesterol and triacylglycerol levels in the plasma of quails fed with rosemary oil. In general, POLAT et al. (2011) argued that the effect of aromatic plants on the serum biochemical profile of birds is unclear.

In summary, the results of the present study suggest that a dietary supplementation level of RP up to 25 g/kg of feed improves the *in vivo* performance, in terms of live weight gain and feed conversion ratio, and the immunity of quails. These results are probably due to the antioxidant activity of RP rather than its modulatory activity towards the caecal microflora.

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SAŽETAK

Cilj rada bio je istražiti učinak dodatka praha ružmarina (*Rosmarinus officinalis* L.) u prehrani (RP) na rast, svojstva trupa, krvne pokazatelje, stvaranje protutijela i crijevnu mikrofloru prepelica. U ukupno 270 jednodnevnih japanskih prepelica primijenjeno je šest režima prehrane u tri ponavljanja (15 ptica po odjeljku). Hrana je sadržavala prah ružmarina u dozama od 0 g/kg hrane (CON), 5 g/kg hrane (T05), 10 g/kg hrane (T10), 15 g/kg hrane (T15), 20 g/kg hrane (T20) or 25 g/kg hrane (T25). Pokus je trajao 42 dana. Tjelesna masa i unos hrane mjereni su tjedno po odjeljku te je izračunata stopa konverzije hrane (FCR). Uzeti su uzorci krvi kako bi se procijenio humoralni imunostni odgovor na cjepivo protiv njukasljske bolesti 29. i 42. dan. Crijevna mikroflora analizirana je u jedne prepelice po ponavljanju 14. i 42. dan. Pri usmrćivanju izmjerena je masa organa i trupova te su analizirani krvni pokazatelji. Prepelice hranjene prahom ružmarina imale su veći prirast tjelesne mase ($P < 0,05$) i stopu konverzije hrane ($P < 0,05$) od kontrolne skupine (CON). Skupine T5 i T10 pokazale su veći prirast tjelesne mase od skupine T25 ($P < 0,05$). Uključivanje praha ružmarina u prehranu nije utjecalo na masu organa ($P > 0,05$) ni na crijevnu mikrofloru prepelica ($P > 0,05$). Prah ružmarina povećao je titar protutijela na cjepivo 29. dan ($P < 0,05$) i smanjio omjer LDL-a i HDL-a u plazmi ($P < 0,05$). Dodatak praha ružmarina do 25 g/kg po obroku poboljšao je svojstva i imunost prepelica a da nije negativno utjecao na njihovu crijevnu mikrofloru.

Ključne riječi: prepelice; rast; mikroflora; imunost; ružmarin
