

Effect of Dietary Inclusion of Lemon Balm (*Melissa Officinalis* L.) Extract on Performance, Gut Microflora, Blood Parameters, Immunity and Carcass Traits of Broilers

Monireh Poorghasemi¹, Alireza Seidavi², Mehrdad Mohammadi¹,
João Simões³, Vito Laudadio⁴ and Vincenzo Tufarelli⁴

¹Department of Animal Science, College of Agriculture, University of Guilan, Rasht, Iran

²Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran

³Department of Veterinary Science, University of Trás-os-Montes e Alto Douro, Quinta de prados, Vila Real, Portugal

⁴Department of DETO, Section of Veterinary Science and Animal Production,
University of Bari 'Aldo Moro', Valenzano, Bari, Italy

The aim of the present study was to evaluate the effects of lemon balm (LB; *Melissa officinalis* L.) extract as additive on performance, health status and carcass traits of broilers during a 42-days production cycle. One hundred mixed chicks of Ross 308 strain were assigned for five dietary treatments with four replicates per group and five birds per replicate as follows: control diet, 0.5LB, 1.0LB, 1.5LB and 2.0LB with 0.0, 0.5, 1.0, 1.5 or 2.0 mL of LB extract per liter of drinking water, respectively. In overall, at 42nd day, low feed, energy and protein efficiency ($P < 0.05$) were observed in 1.0LB group than in control diet. However, during the 3rd and 5-6th weeks, feed, energy and protein intakes were improved ($P < 0.05$), without any efficiency enhancement ($P > 0.05$) mainly in group on 1.0LB diet. During the 5th week of rearing, daily weight gain was higher ($P < 0.05$) in groups 0.5LB, 1.0LB and 2.0LB compared to control diet. At the end of feeding period, cecal enterococcus bacteria colony count was higher ($P < 0.05$) and left cecum diameter was lower ($P < 0.05$) in 1.0LB group. Hematological parameters and viscera and carcass traits remained unaffected ($P > 0.05$) by dietary treatments. In conclusion, the supplementation with LB as natural feed additive resulted in a potential positive effect on broilers performance mainly during the grower and finisher periods.

Key words: broiler, growth, lemon balm, microbiota, performance

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Introduction

Lemon balm (*Melissa officinalis* L.; LB) is a perennial herb that grows wild in Mediterranean region and south-central Europe, and it is also successfully cultivated in few countries such as the agricultural research organizations in Taiwan (Lin *et al.*, 2012). In human, LB is widely used as culinary, aromatic and medicinal herb (Kato-Noguchi, 2003), in this last case in both traditional and alternative medicine (Khan *et al.*, 2012; Dhama *et al.*, 2015; Alzawqari

et al., 2016).

The major active compounds present on commercial LB plants are flavonoids such as gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, epicatechin, rutin and quercetin, having antimicrobial and strong antioxidant activities (Mimica-Dukic *et al.*, 2004; Pereira *et al.*, 2014). The use of LB as feed additive in broiler diet, in order to improve meat quality (Marcinčák *et al.*, 2011a, b) or health, and to substitute antibiotics as growth promoter has been recently reported in few studies, even in organic poultry production (Eleroğlu *et al.*, 2013, 2014). A higher relative weight of leg and breast muscles and polyunsaturated fatty acids percentage in meat were observed in broilers supplemented with LB extract by Marcinčáková *et al.* (2011a) confirming its antioxidant activity. Antioxidant effects without performance changes of a clove and LB mixture were also observed by Petrovic *et al.* (2012). Similar observations on performance were also reported by Marcinčák *et al.*

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Correspondence: Dr. Alireza Seidavi, Department of Animal Science, Rasht Branch, Islamic Azad University, Rasht, Iran.

(E-mail: alirezaseidavi@iaurasht.ac.ir), and

Dr. Vincenzo Tufarelli, Department of DETO, Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', Valenzano, Bari, Italy. (E-mail: vincenzo.tufarelli@uniba.it)

(2011b). However, a deep research considering LB use as single feed additive at different dietary inclusion levels was not yet reported. In fact, little research exists evaluating simultaneous both performance and physiologic status of commercial broilers fed LB extract in drinking. Therefore, the objective of this study was to determine the effect of different levels of LB extract in drinking water to broiler chicks performance, immunity, blood constituents, carcass characteristics and gut microflora from hatch to slaughtering age.

Materials and Methods

Animals, Experimental Treatments and Diet

The experiment was performed housing a total of 100 mixed chicks of the Ross 308 strain (Aviagen, Newbridge, Scotland, UK 35805) in a poultry facility at the University of Guilan, Rasht, Iran and at the Faculty of Agriculture, Islamic Azad University, Rasht Branch, Rasht, Iran. The facility was 12×25 meters with four ventilators and 6 windows, and a total 20 land-pens (1.6×0.8 m) were used. Ventilation was turned on to optimize the climate 24 h before the broilers were brought in. A heater was used and temperature program was according to the instructions for Ross 308 broilers (Aviagen, Newbridge, Scotland, UK 35805). Air humidity was kept at 55 to 65% in the early growing period by spraying water on the floor. Lamps were installed at a height of 2.2 meters above the floor. Twenty-three hours lighting was on and daily for one hour between 19:00 and 20:00 the house was left dark till slaughter at day 42. Sanitation principles and health measures for raising chickens were applied.

The experimental design included five dietary treatments with four replicates per treatment. One-day-old chicks were allotted to 20 groups of five birds, such that mean group body weight (BW) was similar for each group. A 12 (female) to 8 (male) ratio for female to male was the same for all treatments. Environmental conditions were also similar for all treatments.

The dietary treatments were as follows: Treatment 1 as control diet with water included no LB extract (0 mL/L); Treatment 2 (0.5LB): water included LB extract at 0.5 mL/L; Treatment 3 (1.0LB): water included LB extract at 1.0 mL/L; Treatment 4 (1.5LB): water included LB extract at 1.5 mL/L and Treatment 5 (2.0LB): water included LB extract at 2.0 mL/L).

All chickens were fed according to the producer's feeding instructions. The composition of diets and their nutrient composition during the starter (1st–14th day of age), grower (15th–28th day of age) and finisher (29th–42nd day of age) periods are given in Table 1.

The LB was acquired by Agricultural-Jihad Organization of Hamadan, Hamadan, Iran and the extract was prepared as described by Bombik *et al.* (2012). A total of 200 g of dried LB per liter of drinking water was daily infused for 10 min, cooled to 40°C and then strained.

Performance Parameters

Daily feed intake (g/day) and BW gain (g/day) were determined weekly. Feed efficiency (total feed intake / total

BW gain), energy intake (kcal/day), energy efficiency (energy intake / BW gain), daily protein intake (g/day), protein efficiency (protein intake/ BW gain) and production index [total BW×livability (%) / (age days×feed conversion ratio ×10)] were calculated.

Immunity Traits

Birds were vaccinated against prevalence diseases. After each vaccination, 1:1000 multivitamin + electrolytes solution was mixed in the drinking water for 24 h. Feed remaining in feeders, after each time was weighed at the end of the week. Vaccination against Avian Influenza disease virus (Avian Influenza- H9N2; Razi Co, Karaj, Iran) in one administration of 10-day old were taken and humoral immune response of chickens to the vaccine at 35-day and 42-day old was sampled and measured based on haemagglutination inhibition method. Meanwhile, vaccination against Infectious Bronchitis disease virus (Infectious Bronchitis Virus -H120; Razi Co, Karaj, Iran) in two administrations of 1-days and 15-days old were taken and humoral immune response of chickens to the vaccine at 25-day old was sampled and measured based on ELISA method (IDEXX Laboratories, B. V., The Netherlands) according to the manufacturer instructions.

Hematology Traits

At 42 days of age, a 5 mL volume of venous blood was collected from the ulnar vein in the wing of one bird taken from each replicate. Care was taken to choose the most representative male birds with respect to body weight compared to the group mean body weight. Before blood collections were made, feed was removed from all the birds for a period of four hours in an attempt to allow stabilization of the various plasma constituents, and all blood sampling was done in the morning to further reduce the variability of the plasma constituents to be measured.

The whole blood sample was transferred from the syringe into a tube coated with 10 mg of the anticoagulant ethylene diamine tetra acetic acid (EDTA). Blood samples were centrifuged at 3000 rpm×20 min to assure separation of the blood cells from the plasma. Plasma was collected and stored at –20°C until plasma constituent analyses (glucose, uric acid, triglycerides, total cholesterol, very low density lipoprotein, high and low density lipoproteins cholesterol, aspartate aminotransferase, alanine aminotransferase, total protein, albumin and globulin) following standard protocols for Roche Cobas Integra 400 Plus analyzer (Roche Diagnostics, GmbH, Mannheim Germany) and commercial kits (Teif Azmoon Pars, Co., Tehran, Iran), according to the manufacturer instructions.

Viscera and Carcass Traits

At the age of 42 days, after 4 hours of fasting for complete evacuation of the gut, the selected birds were euthanized and used for measuring carcass yield and distribution of meat and gastrointestinal tract characteristics. Birds ($n=20$) were fully pecked by dry pecking method. Feet were separated from the carcass in the tibio-tarsal joint. Neck, wingtips, gut and liver were removed and the empty or edible carcass was weighed and intestinal segments dimensions were recorded.

Table 1. Ingredients and nutrient analysis of diets fed to broiler chickens

| Ingredient (%) | Starter period | Grower period | Finisher period |
|-----------------------------------|----------------|---------------|-----------------|
| Corn | 50.53 | 50.96 | 49.34 |
| Soybean Meal | 37.52 | 32.10 | 27.79 |
| Wheat | 5.00 | 10.00 | 15.00 |
| Soybean oil | 2.14 | 2.79 | 3.89 |
| Ca ₆₂₂ P ₁₈ | 1.90 | 1.67 | 1.60 |
| NaCl | 0.34 | 0.33 | 0.32 |
| DL-Methionine | 0.26 | 0.27 | 0.25 |
| Lysine-Hydro-Chloride | 0.29 | 0.21 | 0.19 |
| Mineral premix* | 0.25 | 0.25 | 0.25 |
| Vitamin premix** | 0.25 | 0.25 | 0.25 |
| CaCO ₃ | 1.23 | 1.01 | 0.97 |
| Threonine | 0.09 | 0.06 | 0.05 |
| NaHCO ₃ | 0.10 | 0.10 | 0.10 |
| Nutrient analysis | | | |
| ME (kcal/kg diet) | 2,900 | 3,000 | 3,100 |
| Crude protein (%) | 21.00 | 20.00 | 18.50 |
| Calcium (%) | 1.01 | 0.86 | 0.82 |
| Available P (%) | 0.48 | 0.43 | 0.41 |
| Potassium (%) | 0.38 | 0.38 | 0.38 |
| Chloride (%) | 0.15 | 0.15 | 0.15 |
| Sodium (%) | 0.15 | 0.15 | 0.15 |
| Arginine (%) | 1.39 | 1.21 | 1.10 |
| Lysine (%) | 1.37 | 1.18 | 1.06 |
| Meth + Cys (%) | 1.03 | 0.90 | 0.83 |
| Threonine (%) | 0.90 | 0.79 | 0.72 |
| Tryptophan (%) | 0.23 | 0.19 | 0.17 |

* Calcium Pantothenate: 4 mg/g; Niacin: 15 mg/g; Vitamin B6: 13 mg/g; Cu: 3 mg/g; Zn: 15 mg/g; Mn: 20 mg/g; Fe: 10 mg/g; K: 0.3 mg/g. ** Vitamin A: 5000 IU/g; Vitamin D3: 500 IU/g; Vitamin E: 3 mg/g; Vitamin K3: 1.5 mg/g; Vitamin B2: 1 mg/g.

Breast muscle (including skin and sternum) was dissected from carcass. Legs (including thighs and drumsticks) were dissected by ex-articulation in hip joint and dissecting tissue from iliac bone. Abdominal fat, including that around the rectum, gizzard and proventriculus was collected. The length (cm), width (mm) and wall thickness (mm) of duodenum, ileum jejunum, left and right cecum and colon were recorded. Total weight of all dissected parts and the weights of various segments of the digestive tract were related to the totally eviscerated carcass. Ratios were calculated according to the following formula: [(weight of component(s)/eviscerated carcass weight) × 100].

Microbiota

Agar plates were streaked with cecum and gizzard contents of selected birds and sent to the laboratory. To determine bacterial growth and colony counts, the agar plates streaked on the site were used. Collecting tubes were weighted, wrapped into aluminum sheet and autoclaved for 10 min. The culture mediums were prepared and 24 hours before collecting samples were poured into the petri dish. MRS agar (Man Rogosa Sharpe agar, 1.10660.500) to culture lactic acid producing bacteria, Eosin Metilan Blou

(EMB, 1.01347.0500) to culture *Escherichia coli*, Macconkey agar (105465.0500) to culture coliforms, Azide dextrose broth (101590) to culture enterococcus bacteria, and Nutrient agar (1.05450.0500) was used to culture total aerobic bacteria counts, respectively. Total aerobic bacteria and total acid producing bacteria were also accessed.

All procedures were approved by the Authors' Institution's Ethic Committee, and care was taken to minimize the number of animals used.

Statistical Analysis

Data were analyzed by analysis of variance using a one-way ANOVA procedure based on a completely randomized design. Data were analyzed by SAS[®] 8.0 statistical software (SAS Institute Inc., Cary, NC, USA) and GLM procedure was used. The means (±SEM) were compared by using Duncan's test. Results were considered significantly different at *P* < 0.05.

Results

Performance Traits

No differences (*P* > 0.05) between groups on performance traits were observed during the 1st week of rearing (Table 2).

Table 2. Performance traits of broilers fed different levels of lemon balm extract according to the rearing period

| Item | Daily feed intake (g) | Daily BW gain (g) | Feed efficiency | Daily energy intake (kcal) | Energy efficiency (kcal/g) | Daily protein intake (g) | Protein Efficiency (g/g) |
|-----------------------------------|-----------------------|--------------------|--------------------|----------------------------|----------------------------|--------------------------|--------------------------|
| <i>1st week (1st - 7th day)</i> | | | | | | | |
| Control | 18.9 | 17.6 | 1.14 | 57.5 | 3.32 | 4.0 | 18.9 |
| 0.5 LB | 20.0 | 17.4 | 1.15 | 58.0 | 3.33 | 4.0 | 20.0 |
| 1.0 LB | 19.6 | 15.6 | 1.26 | 57.0 | 3.67 | 3.9 | 19.6 |
| 1.5 LB | 19.8 | 15.9 | 1.25 | 57.4 | 3.62 | 4.0 | 19.8 |
| 2.0 LB | 21.6 | 17.0 | 1.26 | 62.6 | 3.67 | 4.3 | 21.6 |
| (±SEM) | (±0.6) | (±0.5) | (±0.03) | (±1.7) | (±0.07) | (±0.1) | (±0.01) |
| <i>2nd week (8th - 14th day)</i> | | | | | | | |
| Control | 46.7 | 38.1 ^a | 1.23 | 137.8 | 3.64 | 9.6 | 0.25 |
| 0.5 LB | 40.5 | 28.6 ^b | 1.42 | 119.5 | 4.19 | 8.3 | 0.29 |
| 1.0 LB | 39.0 | 29.8 ^b | 1.33 | 115.0 | 3.92 | 8.0 | 0.27 |
| 1.5 LB | 37.8 | 30.2 ^b | 1.25 | 111.6 | 3.70 | 7.8 | 0.25 |
| 2.0 LB | 42.5 | 31.1 ^{ab} | 1.38 | 125.4 | 4.06 | 8.7 | 0.28 |
| (±SEM) | (±1.4) | (±1.2) | (±0.04) | (±4.2) | (±0.11) | (±0.3) | (±0.01) |
| <i>3rd week (15th - 21st day)</i> | | | | | | | |
| Control | 81.1 ^a | 51.6 ^{ab} | 1.46 ^a | 251.3 ^a | 4.55 ^a | 16.2 ^a | 0.29 ^a |
| 0.5 LB | 92.1 ^b | 55.1 ^a | 1.80 ^b | 285.4 ^b | 5.58 ^b | 18.4 ^b | 0.36 ^b |
| 1.0 LB | 91.7 ^b | 53.9 ^{ab} | 1.70 ^{ab} | 284.3 ^b | 5.27 ^{ab} | 18.3 ^b | 0.34 ^{ab} |
| 1.5 LB | 78.2 ^a | 50.5 ^a | 1.55 ^{ab} | 242.3 ^a | 8.41 ^{ab} | 15.6 ^b | 0.31 ^a |
| 2.0 LB | 92.6 ^b | 59.6 ^b | 1.56 ^{ab} | 287.4 ^b | 4.83 ^{ab} | 18.5 ^b | 0.31 ^{ab} |
| (±SEM) | (±1.9) | (±1.1) | (±0.04) | (±5.6) | (±0.12) | (±0.4) | (±0.01) |
| <i>4th week (22nd - 28th day)</i> | | | | | | | |
| Control | 118.6 | 69.5 | 1.71 ^a | 361.6 | 5.23 ^a | 22.8 | 0.33 ^a |
| 0.5 LB | 119.4 | 82.6 | 1.44 ^b | 364.1 | 4.41 ^b | 23.0 | 0.28 ^b |
| 1.0 LB | 122.6 | 68.2 | 1.81 ^a | 373.4 | 5.54 ^a | 23.6 | 0.35 ^a |
| 1.5 LB | 127.3 | 75.0 | 1.70 ^a | 388.1 | 5.19 ^a | 24.5 | 0.32 ^a |
| 2.0 LB | 129.2 | 70.7 | 1.83 ^a | 394.1 | 5.61 ^a | 24.9 | 0.35 ^a |
| (±SEM) | (±2.6) | (±2.2) | (±0.04) | (±8.0) | (±0.13) | (±0.5) | (±0.01) |
| <i>5th week (29th - 35th day)</i> | | | | | | | |
| Control | 168.8 ^a | 87.0 ^a | 1.94 | 523.4 ^a | 6.03 | 31.2 ^a | 0.36 |
| 0.5 LB | 173.9 ^{ab} | 96.0 ^b | 1.81 | 539.0 ^{ab} | 5.61 | 32.2 ^{ab} | 0.33 |
| 1.0 LB | 184.7 ^b | 95.9 ^b | 1.92 | 572.6 ^b | 5.98 | 34.2 ^b | 0.35 |
| 1.5 LB | 172.1 ^{ab} | 92.1 ^{ab} | 1.88 | 533.6 ^{ab} | 5.82 | 31.8 ^{ab} | 0.34 |
| 2.0 LB | 176.9 ^{ab} | 96.0 ^b | 1.84 | 548.5 ^{ab} | 5.71 | 23.7 ^{ab} | 0.34 |
| (±SEM) | (±2.1) | (±1.3) | (±0.02) | (±6.6) | (±0.07) | (±0.4) | (±0.01) |
| <i>6th week (36th - 42nd day)</i> | | | | | | | |
| Control | 188.1 ^a | 99.8 | 1.88 | 583.1 ^a | 5.85 | 34.8 ^a | 0.34 |
| 0.5 LB | 202.1 ^{ab} | 97.0 | 2.08 | 626.6 ^{ab} | 6.46 | 37.4 ^{ab} | 0.38 |
| 1.0 LB | 209.0 ^b | 90.0 | 2.13 | 647.8 ^b | 6.61 | 38.7 ^b | 0.39 |
| 1.5 LB | 190.0 ^a | 96.7 | 2.03 | 589.0 ^a | 6.29 | 35.1 ^a | 0.37 |
| 2.0 LB | 191.4 ^a | 117.4 | 1.71 | 593.3 ^a | 5.30 | 35.4 ^a | 0.31 |
| (±SEM) | (±2.8) | (±4.1) | (±0.06) | (±8.7) | (±0.19) | (±0.5) | (±0.01) |

LB - Lemon balm extract.

Different letters within the same column indicate significant differences among treatment groups ($P < 0.05$)

During the 2nd week and consequently throughout the starter period, the daily weight gain was lower ($P < 0.05$) in groups 0.5LB, 1.0LB and 1.5LB compared to control group. During

the 3rd week, daily feed, energy and protein intakes were higher ($P < 0.05$) in groups 0.5LB and 2.0LB than in control. However, and contrarily to the 4th week, poorly feed, energy

Table 3. Performance mean (\pm SEM) of Ross 308 broilers fed the different levels of lemon balm at the 42-day production cycle

| Item | Daily feed intake (g) | Daily BW gain (g) | Feed efficiency | Daily energy intake (kcal) | Energy efficiency (kcal/g) | Daily protein intake (g) | Protein Efficiency (g/g) | Live BW (g) | Production index |
|--------------|-----------------------|-------------------|--------------------|----------------------------|----------------------------|--------------------------|--------------------------|-------------|------------------|
| Control | 103.9 | 61.2 | 1.69 ^a | 311.6 | 5.09 ^a | 20.6 | 0.35 ^a | 2 553 | 358.5 |
| 0.5 LB | 108.0 | 62.1 | 1.73 ^{ab} | 324.0 | 5.21 ^{ab} | 21.4 | 0.34 ^{ab} | 2 665 | 365.0 |
| 1.0 LB | 111.1 | 60.3 | 1.84 ^b | 333.3 | 5.54 ^b | 20.0 | 0.37 ^b | 2 507 | 324.2 |
| 1.5 LB | 104.2 | 60.6 | 1.73 ^{ab} | 312.6 | 5.21 ^{ab} | 20.7 | 0.34 ^{ab} | 2 568 | 355.8 |
| 2.0 LB | 109.0 | 65.3 | 1.67 ^a | 327.1 | 5.02 ^a | 21.6 | 0.33 ^a | 2 734 | 391.7 |
| (\pm SEM) | (\pm 1.2) | (\pm 0.9) | (\pm 0.02) | (\pm 3.5) | (\pm 0.06) | (\pm 0.22) | (\pm 0.01) | (\pm 44) | (\pm 9.6) |

LB - Lemon balm extract.

Different letters within the same column indicate significant differences among treatment groups ($P < 0.05$).

Table 4. Effect of different levels of lemon balm extract into drinking water on gizzard and cecum microflora (log₁₀ CFU/g) of broilers at 42nd day of age

| Item | Gizzard | | Cecum |
|-----------|-------------------|-------------------------|---------------------|
| | <i>Coliforms</i> | <i>Escherichia coli</i> | <i>Enterococcus</i> |
| Control | 8.53 ^a | 7.89 ^{ab} | 6.62 ^a |
| 0.5 LB | 8.66 ^b | 7.99 ^a | 6.94 ^{ab} |
| 1.0 LB | 8.49 ^a | 7.87 ^{ab} | 7.16 ^b |
| 1.5 LB | 8.69 ^b | 7.87 ^{ab} | 7.09 ^b |
| 2.0 LB | 8.54 ^a | 7.70 ^b | 7.09 ^b |
| \pm SEM | (\pm 0.02) | (\pm 0.03) | (\pm 0.07) |

LB - Lemon balm extract.

Different letters within the same column indicate significant differences among treatment groups ($P < 0.05$).

and protein efficiencies ($P < 0.05$) were observed in group 0.5LB. Considering the whole grower period, low performance efficiency ($P < 0.05$) was observed only in group fed 1.0 LB. During the 5th and 6th weeks, the daily feed, energy and protein intakes were higher ($P < 0.05$) in group 1.0LB than control group, without ($P > 0.05$) feed, energy or protein efficiencies degradation.

In overall, considering the whole production cycle (42 days; Table 3), the group 1.0LB showed poorly feed, energy and protein efficiency ($P < 0.05$) than control group or group 2.0LB, without daily weight gain improvement. No other significant enhancements ($P > 0.05$) were observed in the remained groups.

Gut Microflora

An higher coliforms bacteria colony count ($P < 0.05$) on gizzard was observed in groups 0.5LB and 1.5LB than in control (Table 4). In overall, more cecal enterococcus bacteria colonies ($P < 0.05$) were found when LB additive was supplemented in drinking water.

Hematological, Lymphoid Organs and Humoral Immunity Traits

Total plasma protein and albumin concentration were higher in group 0.5LB (3.45 and 1.47 g/dl, respectively) than

control (2.92 and 1.20 g/dl, respectively; $P < 0.05$). A relative thymus weight improvement ($P < 0.05$) was observed in group 2.0LB when compared with control group (Table 5). However, the relative Bursa of Fabricius weight was lower ($P < 0.05$) in group 1.0LB than control group. The antibody titers against Avian Influenza disease and Infectious Bronchitis disease virus were not affected ($P > 0.05$) by LB supplementation in drinking water.

Gut Gross Morphometry and Carcass Traits

Left cecum diameter was lower in groups 0.5LB (0.27 mm) and 1.0LB (0.26 mm) than in control group (0.35 mm; $P < 0.05$). No other significant differences ($P > 0.05$) among groups on all viscera or carcass traits were observed.

Discussion

In present study, at the end of the production cycle daily feed, energy and proteins intakes, and live body weight or production indexes were similar between groups. These results are in agreement with similar total feed intake, final body weight observed by Marcinčáková *et al.* (2011a), in broilers fed with commercial diets and ground LB at 2% level. However, contrarily to our study, feed conversion ratio remained constant. Marcinčák *et al.* (2011b) also ob-

Table 5. Lymphoid organs weight (g) and relative weight (%) in broilers at 42nd day of age fed different levels of lemon balm extract into drinking water

| Item | Thymus (g) | Thymus (%) | Spleen (g) | Spleen (%) | Bursa Fabricius (g) | Bursa Fabricius (%) |
|---------|-------------------|--------------------|------------|--------------------|---------------------|---------------------|
| Control | 3.7 ^a | 0.20 ^a | 2.9 | 0.16 ^{ab} | 4.9 ^a | 0.26 ^a |
| 0.5 LB | 6.2 ^{ab} | 0.31 ^{ab} | 3.1 | 0.15 ^{ab} | 4.2 ^{ab} | 0.21 ^{ab} |
| 1.0 LB | 5.3 ^{ab} | 0.28 ^{ab} | 2.7 | 0.14 ^{ab} | 2.8 ^b | 0.15 ^b |
| 1.5 LB | 5.6 ^{ab} | 0.30 ^{ab} | 2.3 | 0.12 ^a | 4.1 ^{ab} | 0.22 ^{ab} |
| 2.0 LB | 7.1 ^b | 0.41 ^b | 3.2 | 0.18 ^b | 3.7 ^{ab} | 0.22 ^{ab} |
| (±SEM) | (±0.5) | (±0.03) | (±0.2) | (±0.01) | (±0.3) | (±0.01) |

LB – Lemon balm extract.

Different letters within the same column indicate significant differences among treatment groups ($P < 0.05$).

served unchanged feed consumption, daily weight gain, feed conversion ratio and live body weight, at 42nd day, when 1% clove buds powder (*Syzygium aromaticum* L.) and 0.2% LB were added to feed or water respectively. Similar broiler performance results were observed by Petrov *et al.* (2011) using the same herbal combination. However, Kwiecień and Winiarska-Mieczan (2009) observed a higher live body weight at 42nd day in broilers supplemented with dried 2% LB than in broilers fed with flavomycin (antimicrobial) and dried grass silage (2%), suggesting a positive effect of the herbal on broiler performance.

Although in our study the feed, energy and protein efficiency at 42nd day, were lower in group 1.0LB than control group, greater LB level (2.0LB) seems to attenuate this effect. Additionally, in the first two weeks, during starter period, the daily weight gain was lower in groups 0.5LB, 1.0 LB or 1.5LB than in control group. No other differences between groups were observed. The adverse or neutral effects of LB on performance during this early period can be related with the fact that the gut digestion development is only completed around the 3rd week of broiler life (Noy and Sklan, 1995).

Moreover, on the 3rd week the feed, energy and protein intake were improved in groups 0.5LB, 1.0LB and 2.0LB without efficiency degradation in the last two groups. These improvements, considering the control group as reference, persisted during the 5th and 6th weeks only for group 1.0LB. During 5th week, groups 0.5LB, 1.0LB and 2.0LB showed higher daily weigh gain than control group. In Petrov *et al.* (2011) study, the live body weight remained similar between groups during each one of the six weeks. More studies are necessary in order to evaluate if the LB water or feed supplementation only in finisher period, or even in onset of the grower period, is advantageous for broilers performance.

In overall, our study showed that colony counts of total coliform, but not *E. coli* (some strains are pathogenic), on gizzard (groups 0.5LB and 1.5LB) and enterococcus bacteria on cecum, but not on total lactic acid producing bacteria (groups 1.0LB, 1.5LB and 2.0LB) were improved by LB. Despite some pathogenic species such as *Enterococcus*

faecalis and *Enterococcus faecium* (Tremblay *et al.*, 2011), enterococcus are also lactic acid producing bacteria and can modulate the remaining microflora. In fact, the groups 0.5 LB and 1.0LB negatively affected the left cecum diameter, probably due to a villi underdevelopment, and both findings can be related. The gut microflora enhancement and low cecal development can justify, at least partially (Lu *et al.*, 2003; Lan *et al.*, 2005), the poorly indexes efficiency observed in the present study.

Other than total proteins and albumin plasma levels improvement by group 0.5LB, no differences in hematological parameters were observed in the present study, and in agreement with Petrov *et al.* (2011) results, suggesting that the LB don't affect apparent health status of broilers.

Based on our findings, the group 2.0LB improved the relative weight of thymus, responsible for T lymphocytes production (Le Douarin and Dieterlen-Lièvre, 2013). However, the group 1.0LB had an adverse effect on relative weight of Bursa Fabricius, a critical organ for the B lymphocytes development (Ratcliffe, 2006). Additionally, the tested antibody titers remained unchanged on all LB groups. These results indicate the difficulty to positively modulate cellular and humoral immunity only using LB additive.

The LB at different levels don't influenced the weight of broiler carcass parts. This results are in agreement with Marcinčák *et al.* (2011b) and by Eleroğlu *et al.* (2014) using Hubbard S757 and Hubbard Grey Barred JA slow-growing genotypes until 81st day of organic broiler production and fed with 1% LB in diet. Mašlanko and Pisarski (2009) observed a similar (relative) weight of abdominal fat in chilled carcass between broilers fed with flavomycin as grown promotor and fed with 2% LB powder. However, a higher relative weight of leg and breast muscles were observed by Marcinčáková *et al.* (2011a) with 2% LB powder.

Finally, in order to emphasize the potential impact of LB use on broiler welfare production we also report that the survival rate of 100% during broiler grower period was observed by Skomorucha and Sosnowka-Czajka (2013) when 0.2% LB alcoholic extract was added to drinking water from 21st to 35th day. These researchers also observed that

the feed supplementation with a mixture of goat's rue (*Galega officinalis* L.), common nettle (*Urtica dioica* L.), LB and common sage (*Salvia officinalis* L.), in the last 7 days of production cycle, can alleviate the stress provoked by longer transport distances to slaughter up to 300 km.

Conclusions

The present study suggested that the LB at 1.0 mL/L level added to drinking water during whole production cycle had adverse effect on feed, energy and protein efficiency without weight gain advantages. However, in the finisher period, LB can improve feed, energy and protein intakes without affect health status or carcass gross morphometry. As consequence, dietary 1.0 mL/L LB, only during finisher period can improve broiler performances. However, further researches are need in order to evaluate the potential positive effects of LB, with special emphasis for 0.1% and 0.2% levels, on birds performance, and also considering health status and welfare when supplemented only during the finisher period or even on grower period.

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