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EFFECT OF INCLUSION OF LEMON BALM (*MELISSA OFFICINALIS*) EXTRACT INTO DRINKING WATER ON ILEUM MICROFLORA OF BROILERS

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ABSTRACT

An experiment was conducted in order to investigate the effect of different orally levels of lemon balm (*Melissa officinalis*) extract to broiler chicks on ileum microflora. The lemon balm (*Melissa officinalis*) extract as much as 0, 0.5, 1.0, 1.5, and 2.0 mL/L added into drinking water in treatments 1-5 respectively. To determinate 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 to culture lactic acid producing bacteria, Eosin Metilan Blou to culture *Escherichia coli*, Macconkey agar to culture coliforms, Azide dextrose broth to culture enterococcus bacteria, and Nutrient agar was used to culture total aerobic bacteria counts, respectively. Data were analyzed by analysis of variance based on a completely randomized design. Statistical differences between studied treatments for these bacteria were not significant (P>0.05). From obtained results, it is showed that the highest level of aerobic bacteria belonged to control treatment (0 mL/L extract). Meanwhile, the highest level of lactic acid producing bacteria belonged to treatment 4 (1.5 mL/L extract). Also the highest level of enterococcus bacteria belonged to control treatment (0 mL/L extract). Meanwhile, the highest level of *escherichia coli* belonged to treatment 2 (0.5 mL/L extract), and also the highest level of coliforms bacteria belonged to treatment 2 (0.5 mL/L extract).

Keywords: Lemon Balm, Chick, Aerobic Bacteria, Lactic Acid Producing Bacteria, Enterococcus, *escherichia coli*, Coliforms

INTRODUCTION

Broiler have huge role in human nutrition. Broiler performance is affected by its gut microbiota. Lemon balm (*Melissa officinalis*) is a useful plant **[1]**. There are positive reports about effects of lemon balm (*Melissa officinalis*) on microbiota **[2]**. Meanwhile there are reports on neuroprotective and neurological properties of Melissa officinalis. Also there are side effects such as anti-diabetic effects for lemon balm (*Melissa officinalis*) essential oil on glucose-and lipid-regulating enzymes in type 2 diabetic mice **[3]**.

Little research exists evaluating ileum microbiota of commercial broilers fed water differing in lemon balm (*Melissa officinalis*) extract. The objective of this study was to determine the effect of different orally levels of lemon balm (*Melissa officinalis*) extract to broiler chicks on ileum population of aerobic bacteria, lactic acid producing bacteria total, enterococcus, escherichia coli, and coliforms during a 6-wk period from hatch to 6 wk of age.

MATERIALS AND METHODS

The facility was 12 by 25 meters with four ventilators and 6 window. A total 20 land pens (1.6 m× 0.8 m) were used. Prior to the experiment the facility was carefully cleaned including all drinkers and feeders. Subsequently the facility was disinfected (Multifenelicratio 1:125 and 1:250). All drinkers and feeders were immersed in the 20 % solution of benzalkonium chloride (germokiller). The facility was left to dry for two days. Thereafter nonflammable parts were flamed up, including the floor and metal walls of the pens. Walls were subsequently sprayed with water and lime. After drying, all joints, windows and ventilation was gasified with Formalex solution. During 48 hours doors and windows remained closed. All equipment used during the rearing period including buckets, sandals, cardboard rolls, temperature gauges, and all drinkers and feeders were placed before gasification. Ventilation was turned on to optimize the climate 24 hours 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; infoworldwide@aviagen.com). Air humidity was kept at 55 to 65 % in the early growing period by spraying with water on the floor. Forty and three watt 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. Drinker were washed and cleaned daily. After each vaccination, 1:1000 multivitamin + electrolytes solution was mixed in the drinking water for 24 hours. Feed remaining in feeders, after each time was weighed at the end of the week cleaned thorough with a brush. Birds were vaccinated against prevalence diseases.

The experimental design was 5 treatments with 4 replicates for each treatment. A total of 100 one-day-old mixed chicks of the Ross 308 strain (Aviagen, Newbridge, Scotland, UK 35805) were allotted to 20 groups of 5 birds, such that mean group body weights were similar for each group. Environmental conditions were similar for all treatments. The treatments were as follows:

Treatment 1: water included lemon balm (*Melissa officinalis*) extract (0 mL/L)

Treatment 2: water included lemon balm (*Melissa officinalis*) extract (0.5 mL/L)

Treatment 3: water included lemon balm (*Melissa officinalis*) extract (1.0 mL/L)

Treatment 4: water included lemon balm (*Melissa officinalis*) extract (1.5 mL/L)

Treatment 5: water included lemon balm (*Melissa officinalis*) extract (2.0 mL/L)

All chickens were fed according to the producer`s feeding instructions. The composition of diets and their nutrient composition in the starter (1st-14th days of age), grower (15th-28th days of age) and finisher (29th-42nd days of age) periods are given in **Tables 1** and **2**.

Agar plates were streaked with ileum content and sent to the laboratory. To determinate 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, and Azide dextrose broth (101590) to culture enterococcus bacteria was used. Nutrient agar (1.05450.0500) was used to culture total aerobic bacteria counts, respectively.

Data were analyzed by analysis of variance using a one-way ANOVA procedure based on a completely randomized design (CRD). Data were analyzed by SAS® 8.0 **[4]** statistical software and GLM procedure was used. The means were compared by using Duncan. The

results were considered significantly different when P<0.05. The data showed in **Table 3** are the mean ± standard error values of the mean.

RESULTS AND DISCUSSION

Obtained results are summarized in **Table 3**. From obtained results, it is showed that amount of aerobic bacteria in five studied treatments were between 7.97-8.10 (log 10 CFU/gr). Among studied treatments, the highest level of aerobic bacteria belonged to control treatment (0 mL/L extract), and treatment 3 (1.0 mL/L extract) remained at lower level than other treatments. Other treatments were between these two treatments. Meanwhile statistical differences between studied treatments for this bacteria were not significant (P>0.05). Amount of lactic acid producing bacteria in five studied treatments were between 8.91-8.96 (log 10 CFU/gr). Among studied treatments, the highest level of lactic acid producing bacteria belonged to treatment 4 (1.5 mL/L extract), and treatments 2 and 3 (0.5 and 1.0 mL/L extract) remained at lower level than other treatments. Other treatments were between these treatments. Meanwhile statistical differences between studied treatments for this bacteria were not significant (P>0.05). Amount of enterococcus bacteria in five studied treatments were between 6.75-7.15 (log 10 CFU/gr). Among studied treatments, the highest level of enterococcus bacteria belonged to control treatment (0 mL/L extract), and treatment 4 (1.5 mL/L extract) remained at lower level than other treatments. Other treatments were between these two treatments. Meanwhile statistical differences between studied treatments for this bacteria were not significant (P>0.05). Amount of *escherichia coli* in five studied treatments were between 8.72-8.95 (log 10 CFU/gr). Among studied treatments, the highest level of *escherichia coli* belonged to treatment 2 (0.5 mL/L extract), and treatment 3 (1.0 mL/L extract) remained at lower level than other treatments. Other treatments were between these two treatments. Meanwhile statistical differences between studied treatments for this bacteria were not significant (P>0.05). Amount of coliforms bacteria in five studied treatments were between 8.59-8.95 (log 10 CFU/gr). Among studied treatments, the highest level of coliforms bacteria belonged to treatment 2 (0.5 mL/L extract), and treatment 3 (1.0 mL/L extract) remained at lower level than other treatments. Other treatments were between these two treatments. Meanwhile statistical differences between studied treatments for this bacteria were not significant (P>0.05).

Gut microflora is one of inhibiting agents of broiler production. Antibiotics usage is

resulted to microbial resistance in broiler and also its reminders in products is transmit to human. On the other hand, antibiotics will decrease useful microflora in broiler gut and then decrease broiler productivity **[5]**. Therefore farmers must use alternative agents instead of antibiotics. Medicine plants are novel alternatives for inclusion in broiler feeder and drinker as powder and extract. Medicine plants have anti-microbial and growth promoter properties. Their usage in broiler feeding can improve liver and gastrointestinal functions and so will produce more enzymes. There results supported by other findings that reported positive effects for lemon balm **[6, 7]**. Based on obtained results in this experiment, lemon balm can use for more studies and is a potential alternative for antibiotics.

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REFERENCES

[1] Moradkhani H, Sargsyan E, Bibak H, Naseri B, Sadat-Hosseini M, Fayazi-Barjin A, and Meftahizade H, *Melissa officinalis* L., a valuable medicine plant: A review. J. Medicinal Plants Res., 4(25), 2010, 2753-2759.

[2] Mimica-Dukic, N., Bozin, B., Sokovic, M., & Simin, N, Antimicrobial and antioxidant activities of Melissa officinalis L.(Lamiaceae) essential oil. J. Agri. Food Chem., 52(9), 2004, 2485-2489.

[3] Chung MJ, Cho SY, Bhuiyan MJH, Kim KH, and Lee SJ, Anti-diabetic effects of lemon balm (Melissa officinalis) essential oil on glucose-and lipid-regulating enzymes in type 2 diabetic mice. British J. Nutr., 104(02), 2010, 180-188.

[4] SAS. Versión 8.0 Edition. Cary (NC): SAS institute Inc, 2009.

[5] Doho-mulin M, and Fairbrother JM, Avian pathogenic *Echerichia coli*. Vet. Res., 30(2-3), 1999, 299-316.

[6] Herodez Z, Skerget M, and Kneze Z, Solvent extraction study of antioxidant from balm (*Melissa officinalis* L.). Leaves. Food Chem., 80(2), 2003, 1275-1282.

[7] López V, Martín S, Gómez-Serranillos MP, Carretero ME, Jäger AK, and Calvo MI, Neuroprotective and neurological properties of Melissa officinalis. Neurochemical Res., 34(11), 2009, 1955-1961.

Table 1: Feed Ingredients of Used Diets During the Starter (1st-14th Days of Age), Grower (15th-28th Days of Age), and Finisher (29th-42nd Days of Age) Periods

Ingredient (%)	Starter period (1st-14th days of age)	Finisher period (15th-28th days of age)	Finisher period (29th-42nd days of age)
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%22P%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
CaCO3	1.23	1.01	0.97
Threonine	0.09	0.06	0.05
Sodium bicarbonate (NaHCO3)	0.10	0.10	0.10
Total	100	100	100

NOTE: *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; **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

Table 2: Nutrient Analysis of Used Diets During the Starter (1st-14th days of age), Grower (15th-28th Days of age), and Finisher Periods (29th-42nd days of age)

Nutrient analysis	Starter period (1st-14th days of age)	Finisher period (15th-28th days of age)	Finisher period (29th-42nd days of age)
Metabolizable energy (kcal/kg)	2900	3000	3100
Crude protein (%)	21.00	20.00	18.50
Calcium (%)	1.01	0.86	0.82
Available Phosphorus (%)	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	0.10
Lysine (%)	1.37	1.18	1.06
Methionine + Cysteine (%)	1.03	0.90	0.83
Threonine (%)	0.90	0.79	0.72
Tryptophan (%)	0.23	0.19	0.17

Table 3: Microflora Mean (±SEM) of Ileum at 42nd Days of Age in Ross 308 Broilers Fed the Different Levels of Lemon Balm (*Melissa officinalis*) Extract into Drinking Water From 1st-6th Weeks of Age (log10 CFU/gr)[*]

Bacteria / Treatment	Aerobic bacteria total	Lactic acid producing bacteria total	Enterococcus bacteria	*Escherichia coli*	Coliforms bacteria
Control	8.10[a]	8.94[a]	7.15[a]	8.87[a]	8.90[a]
0.5 mL/L lemon balm extract	8.15[a]	8.91[a]	7.13[a]	8.95[a]	8.95[a]
1.0 mL/L lemon balm extract	7.97[a]	8.91[a]	6.90[a]	8.72[a]	8.59[a]
1.5 mL/L lemon balm extract	8.09[a]	8.96[a]	6.75[a]	8.84[a]	8.91[a]
2.0 mL/L lemon balm extract	8.00[a]	8.94[a]	6.89[a]	8.78[a]	8.76[a]
P	0.70	0.98	0.34	0.79	0.19
SEM (Standard Error of Mean)	0.04	0.28	0.07	0.06	0.05

NOTE: [*] Means (± Standard Error) within each Column of Dietary Treatments with no Common Superscript Differ Significantly at P<0.05