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Medicinal Effects of Lemon Balm (*Melissa officinalis* L.) Supplementation on Growth Performance, Immunity, and Intestinal Health in Broiler Chickens

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ABSTRACT

Lemon balm (*Melissa officinalis* L.) is a medicinal plant rich in bioactive compounds such as essential oils, phenolic acids, flavonoids, and triterpenes, which are known for their antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties. This study aimed to evaluate the effects of dietary Lemon balm (LB) supplementation on growth performance, humoral immunity, and intestinal health in broiler chickens. Four hundred male Ross 308 broiler chicks were randomly assigned to five dietary treatments: a basal diet (control), basal diet plus 250 ppm lincomycin (antibiotic), and basal diet supplemented with 1%, 2%, or 3% LB powder. The experimental period lasted 42 days. Results showed that 1% LB supplementation significantly improved economic efficiency by reducing feed cost per kg weight gain and increasing return on investment. LB supplementation, particularly at 1% and 3%, enhanced antibody titers against Newcastle disease and avian influenza, respectively, indicating improved humoral immunity. All levels of LB reduced serum aspartate aminotransferase (AST), uric acid, and creatinine, suggesting better hepatic and renal health. Both antibiotic and LB groups exhibited decreased ileal *E. coli* counts, while no adverse effects on carcass yield or beneficial intestinal Lactobacillus populations were observed. However, 3% LB reduced villus height, indicating potential negative effects at higher doses. In conclusion, *M. officinalis* demonstrates promising potential as a phytogenic feed additive, offering health and economic benefits for broiler production. Its use as a natural alternative to antibiotics could contribute to safer and more sustainable poultry farming.

Keywords: Blood metabolites, Broilers, Immunity, Lemon balm, Performance

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INTRODUCTION

Lemon balm (LB) is an aromatic medicinal plant belonging to the mint family. Its use can alleviate the detrimental effects of stress and infectious diseases, promote digestion, and increase appetite [1]. LB contains various bioactive substances, including essential oils, phenolic acids, flavonoids, triterpenes, tannins, coumarins, and polysaccharides [2]. The antioxidant, anti-inflammatory, and neuroprotective effects of these compounds are well documented [3, 4]. Antioxidants in LB help prevent immune system suppression by counteracting oxidative stress, inhibiting lipid peroxidation, and maintaining cell membrane integrity [5,6]. LB metabolites can chelate metal ions [7] and enhance endogenous antioxidant enzyme activity [8]. Ursolic acid and oleanolic acids, the main triterpenes in LB, exhibit anti-inflammatory and antimicrobial effects [9]. LB is particularly effective against gram-positive bacteria and yeasts and also demonstrates prebiotic and immunomodulatory properties [10-14]. The prebiotic effects of LB support a healthy gut microbiota, a key target in poultry nutrition. Previous studies have shown that LB can improve growth performance, carcass yield, and economic efficiency in poultry [15]. Its essential oils also have antiviral activity against main poultry pathogens significantly [16-19]. LB supplementation has been shown to increase antibody titers and support immune organ development [20, 21]. Given the growing interest in phytogenic feed additives, especially in organic systems [22], this study aimed to evaluate the effects of dietary LB on growth, humoral immunity, and intestinal health in broilers from 1 to 42 days of age.

MATERIALS AND METHODS

All birds´ procedures and ethics considerations were done considering the Guide of the Care and Use of Agricultural Animals in Research and Teaching. This project was reviewed and approved by Iranian ministry of agriculture with experimental authorization NO. ASRI-2016-52039.

Broiler Management

Four hundred broiler chicks (male, Ross 308) were weighed on arrival moment (41.38± 0.39), then allocated to 20 litter pens (1m× 2 m^2) in a completely randomized design with five treatments, 4 replicates, and 20 chicks per pen. Lemon balm *(Melissa Officinalis* L.*)* seed was purchased from a Lemon balm field in the west of Iran, air dried, and ground, and its chemical composition was studied considering the methods of AOAC [23]. AOAC Official Methods 925.09, 978.04, 991.36, and 978.10 were used for dry matter, crude protein, crude fat, and crude fiber, respectively. It had 93.78 dry matter, 18.26% crude protein, 8% crude fat, 20% crude fiber, and 5% crude ash. The studied treatments were included: BD (control); BD plus 250 ppm of lincomycin (positive control); BD containing 1% of LB powder; basal diet containing 2% of LB powder; BD containing 3% of LB powder. All experimental diets (starter, grower, and finisher diets) were isocaloric and iso-nitrogenous (Tables 1, 2, 3). The temperature of the house was kept at 32±1 °C during the first seven days of the experiment and then reduced by 3 °C weekly. The relative humidity of the house was about 55-65% during the study.

Growth Performance

During the experiment, the growth traits: body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded at the end of each rearing phase. The body weight of the birds was measured after six hours of fasting. The FCR was calculated by dividing the FI of each experimental unit by the body weight gain of that unit adjusting for mortalities. The FC/WG and ROI were calculated according to Zaghari et al. [24].

Humoral Immunity

To evaluate the birds' humoral immunity state, antibody titers against ND and Avian Influenza (AI) were studied. On day 40, blood samples from the wing vein of three broilers in each replicate were placed in blood tubes and centrifuged to separate the serum. The humoral immune response to ND and AI was evaluated by performing hemagglutination inhibition (HI) antibody titration on the serum samples [25]. Briefly, serums of the birds were serially diluted, then a determined amount of ND or AI virus antigen was added to each dilution, and incubated to allow antibody-virus interactions. Washed red blood cells of chicken at 0.5% concentration were added to each sample. The plate was incubated at room temperature for 30 minutes. The wells in which hemagglutination was inhibited showed a button of red blood cells at the bottom, as a sign of antibodies presence.

Table 1 Diet formulation and composition during starter period (1-10 d).

Item	B	BA	LB1	LB2	LB3
Ingredient (%)					
Corn	56.74	56.74	55.42	54.1	52.78
Soybean meal	35.14	35.14	34.94	34.75	34.55
gluten	3.0	3.0	3.0	3.0	3.0
Corn oil	1.09	1.09	1.62	2.15	2.67
LB	0	0	1	2	3
Dicalcium Phosphate	1.84	1.84	1.83	1.82	1.81
Calcium carbonate	0.92	0.92	0.91	0.89	0.88
Salt	0.29	0.29	0.29	0.29	0.30
Sodium bicarbonate	0.1	0.1	0.1	0.1	0.1
Vitamin–mineral premix	0.25	0.25	0.25	0.25	0.25
Mineral premix	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.21	0.21	0.21	0.21	0.21
L-lysine HCl	0.16	0.16	0.16	0.17	0.17
L-Threonine	0.01	0.01	0.02	0.02	0.03
Calculated Nutrient Content					
AME (Kcal/Kg)	2900	2900	2900	2900	2900
Crude protein%	22.00	22.00	22.00	22.00	22.00
Available phosphorus%	0.46	0.46	0.46	0.46	0.46
Calcium%	0.93	0.93	0.93	0.93	0.93
Na%	0.16	0.16	0.16	0.16	0.16
Lys (dig)%	1.24	1.24	1.24	1.24	1.24
Met (dig)%	0.55	0.55	0.55	0.55	0.55
Met + Cys (dig)%	0.92	0.92	0.92	0.92	0.92
Thr (dig)%	0.83	0.83	0.83	0.83	0.83

B: BD, BA: BD + 250 ppm dietary antibiotic (Licodan contained 8.8 g Lincomycin HCL/ kg). LB1: diet contained 1 percent LB seed, LB2: diet contained 2 percent LB seed, LB3: diet contained 3 percent LB seed.

Table 2 Diet formulation and composition during the grower period (11-24 d).

Item	B	BA	LB1	LB2	LB3
Ingredient (%)					
Corn	59.83	59.83	58.48	57.20	55.87
Soybean meal	33.8	33.8	33.60	33.39	33.2
Corn oil	2.54	2.54	3.08	3.59	4.12
LB	0.0	0.0	1.0	2.0	3.0
Dicalcium Phosphate	1.64	1.64	1.63	1.62	1.61
Calcium carbonate	0.81	0.81	0.80	0.79	0.78
Salt	0.29	0.29	0.31	0.29	0.3
Sodium bicarbonate	0.1	0.1	0.1	0.1	0.1
Vitamin–mineral premix	0.25	0.25	0.25	0.25	0.25
Mineral premix	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.25	0.25	0.25	0.25	0.25
L-lysine HCl	0.23	0.23	0.23	0.24	0.24
L-Threonine	0.01	0.01	0.02	0.03	0.03
Calculated Nutrient Content					
AME (Kcal/Kg)	3000	3000	3000	3000	3000
Crude protein%	20.02	20.02	20.02	20.02	20.02
Available phosphorus%	0.42	0.42	0.42	0.42	0.42
Calcium%	0.84	0.84	0.84	0.84	0.84
Na%	0.16	0.16	0.16	0.16	0.16
Lys (dig)%	1.24	1.24	1.24	1.24	1.24
Met (dig)%	0.55	0.55	0.55	0.55	0.55
Met + Cys (dig)%	0.84	0.84	0.84	0.84	0.84
Thr (dig)%	0.76	0.76	0.76	0.76	0.76

B: BD, BA: BD + 250 ppm dietary antibiotic (Licodan contained 8.8 g Lincomycin HCL/ kg). LB1: diet contained 1 percent LB seed, LB2: diet contained 2 percent LB seed, LB3: diet contained 3 percent LB seed.

Table 3 Diet formulation and composition during the finisher period (25-42 d).

Item	B	BA	LB1	LB2	LB3
Ingredient (%)					
Corn	66.88	66.88	64.52	62.15	60.56
Soybean meal	24.31	24.31	26.13	27.95	28.21
gluten	2.92	2.92	1.61	0.3	0.0
Corn oil	2.39	2.39	3.33	4.27	4.9
LB	0.0	0.0	1.0	2.0	3.0
Dicalcium Phosphate	1.51	1.51	1.48	1.46	1.45
Calcium carbonate	0.75	0.75	0.73	0.72	0.71
Salt	0.31	0.31	0.31	0.31	0.32
Sodium bicarbonate	0.08	0.08	0.07	0.07	0.07
Vitamin–mineral premix	0.25	0.25	0.25	0.25	0.25
Mineral premix	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.16	0.16	0.17	0.17	0.18
L-lysine HCl	0.19	0.19	0.15	0.10	0.10
L-Threonine	0.0	0.0	0.0	0.0	0.0
Calculated Nutrient Content					
AME (Kcal/Kg)	3100	3100	3100	3100	3100
Crude protein%	18.00	18.00	18.00	18.00	18.00
Available phosphorus%	0.38	0.38	0.38	0.38	0.38
Calcium%	0.76	0.76	0.76	0.76	0.76
Na%	0.15	0.15	0.15	0.15	0.15
DCAB meq/Kg	221	221	221	221	221
Lys (dig)%	0.99	0.99	0.99	0.99	0.99
Met (dig)%	0.45	0.45	0.45	0.45	0.45
Met + Cys (dig)%	0.77	0.77	0.77	0.77	0.77
Thr (dig)%	0.66	0.66	0.66	0.66	0.66

B: BD, BA: BD + 250 ppm dietary antibiotic (Licodan contained 8.8 g Lincomycin HCL/ kg). LB1: diet contained 1 percent LB seed, LB2: diet contained 2 percent LB seed, LB3: diet contained 3 percent LB seed.

Hemoglobin and white Blood Cells

On days 22 and 42, wing vein blood samples of four birds from each experimental group were used to evaluate the concentration of hemoglobin, white blood cells, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, and heterophil/ lymphocyte ratio of the broilers according to Ghiță *et al.* [26].

Biomarkers of Liver and Kidney

On day 42, wing vein blood samples of three birds in each replicate and placed into laboratory tubes. Following coagulation, the samples were centrifuged at 3,500 rpm for 15 minutes to separate the serum. The calorimetric activities of ALT and AST, as well as the levels of uric acid, and creatinine were measured using a spectrophotometer along with commercial kits [27].

Carcass Characteristics

At 42 days of age, after a six-hour fasting period, three birds from each experimental unit were slaughtered, and the carcass yield was determined by calculating the carcass weight (excluding the head, feet, neck, and viscera) about the live weight of the broilers. Additionally, the yields of the breast and thigh plus drumstick (leg) were assessed as the relative weights of these parts compared to the eviscerated carcass weight. The percentages of internal organs, including abdominal fat, liver, pancreas, and bursa of Fabricius, were calculated as a percentage of the live weight [28].

Intestinal Bacteria Population

On day 42, following slaughter, the ileal contents were collected into sterile tubes and transported on ice to the microbiology laboratory for the assessment of *Lactobacillus* and *E. coli* counts according to Basit et al. [29]. Briefly, MRS agar was employed for the cultivation of *Lactobacillus* species, with plates incubated under microaerophilic conditions at 37 °C for 48 hours. For *E. coli*, MacConkey agar plates were used and incubated aerobically at 37 °C for 24 hours.

Intestinal Morphology

On day 42, three birds with body weights similar to those in the corresponding experimental unit were slaughtered by cervical dislocation. Immediately afterward, jejunum samples were collected. The samples were rinsed with normal saline, and 3 cm segments from the center of the jejunum were isolated for the study of jejunal morphological parameters [30].

Table 4 Effect of the LB on the growth performance of broilers during 1 to 42 d of age.

	Treatments1							
1-42 d	B	BA	LB1	LB2	LB3	SD	SEM	P-Values
BWG (g/bird/d)	58.50 ± 4.70	56.00 ± 4.70	52.57 ± 4.70	52.25 ± 4.70	52.00 ± 4.70	4.70	2.35	0.230
FI (g/bird/d)	153.49 ± 13.14 a	153.74 ± 13.14 a	138.62 ± 13.14 ab	130.82 ± 13.14 b	133.52 ± 13.14 b	13.14	6.57	0.031
FCR (g/g)	2.62 ± 0.024 b	2.74 ± 0.024 a	2.63 ± 0.024 b	2.50 ± 0.024 d	2.56 ± 0.024 c	0.024	0.012	0.012
FC/WG**	51502 ± 3243.6 a	51065 ± 3243.6 a	45714 ± 3243.6 b	51280 ± 3243.6 a	54587 ± 3243.6 a	3243.6	1621.8	0.047
ROI***	1.07 ± 0.048 b	1.00 ± 0.048 b	1.13 ± 0.048 a	0.78 ± 0.048 c	0.52 ± 0.048 d	0.048	0.024	0.031

*Means within each row with no common superscript differ (P ≤ 0.05). B: BD, BA: BD + 250 ppm dietary antibiotic (Licodan contained 8.8 g Lincomycin HCL/ kg). LB1: diet contained 1 percent LB seed, LB2: diet contained 2 percent LB seed, LB3: diet contained 3 percent LB seed. **FC/WG (Tomans); ROI= return on investment.

Statistical Analysis

The data were considered as a CRD model analyzed using the generalized linear model procedure of SAS software. Differences among treatments were assessed using Duncan's multiple-range tests.

RESULTS

Growth Performance

As shown in Table 4, birds fed diets containing 2% or 3% Lemon balm (LB) had significantly lower feed intake (FI) and feed conversion ratio (FCR) compared to the control and antibiotic groups (P<0.05, different superscripts). The lowest feed cost per kg weight gain (FC/WG) and the highest return on investment (ROI) were observed in the 1% LB group (P<0.05, different superscripts).

Humoral Immunity

As shown in Table 5, dietary inclusion of Lemon balm (LB) significantly increased antibody titers against Newcastle disease (ND), with the highest ND titer observed in the 3% LB group (P<0.05, different superscripts). Additionally, the 3% LB group had the highest antibody titer against avian influenza (AI), which was significantly higher than the control and antibiotic groups.

Hemoglobin and white Blood Cells

As shown in Table 6, dietary supplementation with Lemon balm (LB) had no significant effect on hemoglobin concentration, white blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, or heterophil/lymphocyte ratio at either 22 or 42 days of age (P > 0.05).

Hepatic and Renal Biomarkers

As shown in Table 7, dietary supplementation with Lemon balm (LB) significantly reduced serum AST, uric acid, and creatinine levels compared to the control group (P<0.05, different superscripts), while ALT levels were not significantly affected.

Carcass Characteristics

The different experimental treatments did not affect carcass yield, breast, thigh + drumstick, abdominal fat, liver, and bursa Fabricius percentage of broilers on day 42 (Table 8, P>0.05). However, using two or three percent LB increased the pancreas percentage compared to antibiotic treatment (P≤0.05, Table 8).

Table 5 Effects of the LB on antibody titers against ND and AI in broilers.

Treatments	ND	AI
B	3.00 c	3.25 b
BA	3.45 c	3.33 b
LB1	4.00 b	3.50 b
LB2	4.15 b	4.00 ab
LB3	4.75 a	4.67 a
SEM	0.182	0.321
P-Values	0.021	0.213

*Means within each row with no common superscript differ (P ≤ 0.05). B: BD, BA: BD + 250 ppm dietary antibiotic (Licodan contained 8.8 g Lincomycin HCL/ kg). LB1: diet contained 1 percent LB seed, LB2: diet contained 2 percent LB seed, LB3: diet contained 3 percent LB seed.

Table 6 Effect of the different diets on the Hemoglobin (Hb), White Blood Cell (WBC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Heterophil/ lymphocyte ratio (H/L) of the broilers.

	Treatments[1]						
22 days	B	BA	LB1	LB2	LB3	SEM	P-values
Hb (g/d)	9.41	11.83	10.65	11.33	10.80	0.338	0.19
WBC (x10^9/L)	137.85	96.18	145.45	136.25	134.60	5.898	0.07
MCH (pg)	47.75	47.23	50.25	49.25	49.30	0.441	0.17
MCHC (g/dL)	34.82	35.53	37.63	36.58	37.25	0.365	0.06
H/L	0.08	0.04	0.12	0.07	0.07	0.01	0.15
42 days							
Hb (g/d)	12.78	12.15	13.03	12.65	12.10	0.328	0.90
WBC (x10^9/L)	126.26	110.18	128.93	136.01	129.60	3.238	0.11
MCH (pg)	48.12	49.05	510.20	51.10	50.30	0.532	0.21
MCHC (g/dL)	35.38	34.36	35.85	36.84	35.96	0.286	0.07
H/L	0.05	0.03	0.05	0.07	0.07	0.005	0.17

B: BD, BA: BD + 250 ppm dietary antibiotic (Licodan contained 8.8 g Lincomycin HCL/ kg). LB1: diet contained 1 percent LB seed, LB2: diet contained 2 percent LB seed, LB3: diet contained 3 percent LB seed.

Intestinal Bacteria Population

Different treatments did not affect the ileal *Lactobacillus* count of the broilers (Table 9). The use of antibiotics or different levels of LB decreased the ileal *E. coli* count of the broilers (P≤0.05). There was no difference among the ileal *E. coli* of the birds fed different levels of LB. So, usage of 1% LB is enough to decrease ileal *E. coli*.

Table 7 Effect of the different diets on the hepatic and renal biomarkers of broilers.

	Treatments1						
	B	BA	LB1	LB2	LB3	SEM	P-values
ALT (U/L)	24.4	24.1	23.5	22.7	22.9	0.687	0.234
AST (U/L)	21.2 a	19.1ab	17.0 b	17.3 b	16.7 b	1.056	0.015
Uric acid (mg/dL)	5.48 a	4.92 a	4.26 b	3.75 b	3.84 b	0.314	0.027
Creatinine (mg/dL)	0.687a	0.615ab	0.531b	0.521b	0.518b	0.051	0.018

*Means within each row with no common superscript differ (P ≤ 0.05). B: BD, BA: BD + 250 ppm dietary antibiotic (Licodan contained 8.8 g Lincomycin HCL/ kg). LB1: diet contained 1 percent LB seed, LB2: diet contained 2 percent LB seed, LB3: diet contained 3 percent LB seed.

Table 8 Effects of the LB on carcass characteristics (%) of broilers.

Treatments	carcass	breast	Thigh + drumstick	abdominal fat	liver	pancreas	Bursa Fabricius
B	75.86	42.18	28.27	2.07	2.1	0.23 [ab]	0.14
BA	74.95	42.48	27.67	3.25	2.2	0.18 [b]	0.15
LB1	74.57	40.32	28.11	2.09	2.6	0.20 [b]	0.16
LB2	73.10	43.06	29.19	1.70	2.1	0.30 [a]	0.17
LB3	76.40	43.15	27.33	2.27	2.4	0.25 [a]	0.16
SEM	0.353	0.400	0.341	0.207	0.171	0.014	0.011
P-values	0.221	0.163	0.684	0.153	0.714	0.024	0.142

*Means within each row with no common superscript differ (P ≤ 0.05). B: BD, BA: BD + 250 ppm dietary antibiotic (Licodan contained 8.8 g Lincomycin HCL/ kg). LB1: diet contained 1 percent LB seed, LB2: diet contained 2 percent LB seed, LB3: diet contained 3 percent LB seed.

Table 9 Effect of the different diets on the ileal microflora in broilers (log CFU/g).

	Treatments1						
	B	BA	LB1	LB2	LB3	SEM	P-Values
Lactobacillus	7.950	8.320	8.168	8.210	8.287	0.280	0.915
E. coli	7.955 a	7.149 b	7.260 b	7.215 b	7.208 b	0.210	0.710

*Means within each row with no common superscript differ (P ≤ 0.05). B: BD, BA: BD + 250 ppm dietary antibiotic (Licodan contained 8.8 g Lincomycin HCL/ kg). LB1: diet contained 1 percent LB seed, LB2: diet contained 2 percent LB seed, LB3: diet contained 3 percent LB seed.

Intestinal Morphology

The dietary treatments had no significant influence on muscle layer thickness, villus width (VW), and crypt depth (CD) of the broilers on day 42 (Table 10). The birds fed with 3 percent LB had lower VH compared to control group. Also, the birds fed with 3 percent LB had lower VH/ CD ratio compared to the group that received antibiotics (P≤0.05). Thus, usage of 3 percent LB had negative effect on jejunum morphology of the broilers.

Table 10 Effects of the LB on the jejunum morphology of broilers.

Treatments	Muscle layer thickness (µ)	VH (µ)	VW (µ)	CD (µ)	VH/ CD
B	191.18	951.93 a	121.14	145.17	6.557 ab
BA	201.53	956.41 a	132.11	142.11	6.730 a
LB1	196.94	949.97 a	124.78	144.67	6.566 ab
LB2	195.45	935.05 ab	119.34	146.12	6.399 ab
LB3	189.10	908.49 b	117.23	149.14	6.091 b
SEM	8.155	10.76	8.15	4.78	0.217
P-values	0.719	0.688	0.563	0.342	0.468

*Means within each row with no common superscript differ (P ≤ 0.05). B: BD, BA: BD + 250 ppm dietary antibiotic (Licodan contained 8.8 g Lincomycin HCL/ kg). LB1: diet contained 1 percent LB seed, LB2: diet contained 2 percent LB seed, LB3: diet contained 3 percent LB seed.

DISCUSSION

Growth Performance

Previous studies have demonstrated that phytogenics can influence broiler growth performance through multiple mechanisms. First, their strong antibacterial properties help reduce pathogenic microflora in the gastrointestinal tract, which in turn decreases mortality rates during the rearing period [31]. Second, phytogenics improve the flavor and aroma of feed, leading to increased FI [32]. Finally, these compounds stimulate appetite and enhance nutrient digestion, further supporting growth [33]. Broilers-fed diets containing 2% or 3% lemon balm (LB) exhibited reduced feed intake (FI) and feed conversion ratio (FCR). Nonetheless, previous studies have identified LB as a highly palatable herb that can enhance nutrient intake in poultry [32]. In the present study, dietary inclusion of LB up to 3% did not significantly affect body weight gain in broilers. This finding aligns with Petrovic et al. [34], who reported no significant differences in live body weight among the broilers supplemented with LB. Conversely, Kwiecień and Winiarska-Mieczan [35] demonstrated that broilers fed 2% dried LB achieved higher live body weights at 42 days compared to those receiving flavomycin or 2% grass silage, indicating a beneficial effect of LB on growth performance. Variations in geographical location, seasonal changes, climate conditions, harvesting time, processing methods, dosage of active compounds, broiler strain, housing conditions, and management practices are key factors contributing to the inconsistent results reported in the literature. In the present study, supplementation with 2% or 3% lemon balm (LB) significantly reduced the feed conversion ratio (FCR) of broilers. Consistent with these findings, Poorghasemi et al. [21] reported that inclusion of LB extract as a natural feed additive improved broilers' performance during both the grower and finisher phases. Similarly, Wang et al. [36] demonstrated that herbal feed additives positively influenced broiler FCR by stimulating immune responses in the ileum. Furthermore, supplementation with 1% LB reduced feed costs in broiler production. Supporting this, Sapsuha et al. [37] found that the use of herbal substances in broiler diets effectively decreased overall feed expenses.

Humoral Immunity

The inclusion of lemon balm (LB) in broilers' diets positively influenced antibody titers against Newcastle disease (ND). Specifically, broilers fed a diet containing 3% LB exhibited the highest antibody titers against avian influenza. Consistent with these findings, Nobakht et al. [38] reported that supplementation with 0.5% LB improved the humoral immunity in laying hens by alleviating stress. Herbal substances modulate immune function through diverse physiological mechanisms that affect both innate and adaptive immunity. These mechanisms include increasing serum immunoglobulin levels and antibody production; expanding populations of T-helper ($CD4^+$) and T-suppressor ($CD8^+$) lymphocytes; enhancing neutrophil adhesion and phagocytic activity; activating macrophages to improve pathogen clearance; increasing natural killer (NK) cell numbers and nonspecific cellular immunity; inhibiting C3 convertase in the classical complement pathway to mitigate inflammatory tissue damage; stimulating the secretion of tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) to coordinate antiviral and inflammatory responses; elevating nonspecific immunity mediators such as acute-phase proteins and antimicrobial peptides; and promoting lymphoid cell proliferation and overall cellular immune function [39].

Hepatic and Renal Biomarkers

The inclusion of lemon balm (LB) in broilers' diets significantly reduced serum levels of aspartate aminotransferase (AST), uric acid, and creatinine, suggesting an improvement in liver and kidney function. These results align with previous studies reporting similar protective effects [40, 41]. Zead et al. [40] observed that LB supplementation decreased AST activity in broilers exposed to paracetamol-induced hepatotoxicity. Furthermore, Li et al. [42] demonstrated that bioactive herbal compounds, such as flavonoids, effectively lower AST and alanine aminotransferase (ALT) levels, thereby enhancing hepatic cell health.

Carcass Characteristics

Different inclusion levels of lemon balm (LB) did not affect carcass yield, breast, thigh plus drumstick, abdominal fat, liver, or bursa of Fabricius percentages in broilers. These results corroborate findings by Poorghasemi et al. [21] who also reported that LB did not influence carcass traits of the broilers. In contrast, Marcinčakova et al. [43] observed that supplementation with 2% LB powder increased the relative weights of leg and breast muscles. Similarly, Shahbazi and Heidari [44] demonstrated that adding 0.3% LB extract to drinking water improved carcass yield and reduced abdominal fat. Notably, dietary inclusion of 2% or 3% LB increased pancreas percentage compared to antibiotic treatment, which may be attributed to LB’s stimulatory effects on enzyme secretion and nutrient digestibility. Petrovic et al. [34] also reported no significant effect of clove and LB supplementation on broilers' carcass yield. The observed discrepancies among studies may result from differences in diet composition, herbal dosage and duration, as well as poultry management practices.

Intestinal Bacteria Population

The use of antibiotics or varying levels of lemon balm (LB) significantly reduced the ileal *E. coli* counts in broilers. Elbaz et al. [45] similarly reported that bioactive herbal compounds decrease intestinal *E. coli* populations in broilers. Furthermore, Elbaz et al. [45] highlighted that essential oils can serve as effective antibiotic alternatives by modulating gut microbiota and enhancing intestinal health. Supporting this, Park and Kim [46] and Su et al. [47] demonstrated that supplementation with essential oils improves gut health through their beneficial effects on intestinal microbial communities. Phyto biotics are believed to exert their influence on broiler microbiota via multiple mechanisms: disrupting the membranes of pathogenic microorganisms, altering cell surface properties to reduce virulence, and stimulating the immune system by activating lymphocytes, macrophages, and natural killer (NK) cells. Additionally, phytobiotics protect the intestinal mucosa from colonization by harmful bacteria and promote the proliferation of beneficial microbes, thereby fostering a healthier gut environment [48].

Intestinal Morphology

No significant differences were observed in intestinal morphology among broilers fed the control diet, antibiotic-supplemented diet, or diets containing 1% or 2% lemon balm (LB). However, broilers receiving 3% LB exhibited reduced villus height (VH) and villus height to crypt depth ratio (VH/CD). Ahsan et al. [49] reported that supplementation with the phytobiotic Digestarom® increased VH, villus diameter, and goblet cell number while reducing crypt depth and muscularis thickness. Previous studies have demonstrated that herbal feed additives can enhance intestinal morphology in poultry

[50], likely due to their stimulatory effects on cellular growth and anti-inflammatory properties [51]. Conversely, Zhang et al. [52] found that plant extract supplementation did not affect intestinal morphology. These conflicting results suggest that the impact of phytogenics on intestinal morphology is variable and depends on factors such as the type and dosage of active compounds, birds' age and strain, feed ingredient composition and quality, as well as other dietary additives. Notably, high doses of phytogenics may adversely affect intestinal cell development, as evidenced in the present study by the negative effect of 3% LB inclusion on jejunal villus height. Excessive levels of phytogenic compounds may induce intestinal irritation or damage, leading to accelerated epithelial cell turnover and heightened immune responses. Consequently, energy resources are redirected from growth-related processes toward tissue repair, which can result in shortened villi and compromised nutrient absorption efficiency [53, 54].

CONCLUSION

In conclusion, the inclusion of 1% lemon balm (LB) powder in broilers' diets from 1 to 42 days of age positively influenced feed conversion per weight gain (FC/WG) and return on investment (ROI), serving as important economic indicators. Additionally, it enhanced humoral immunity and reduced ileal *E. coli* counts. Conversely, supplementation with 3% LB is not recommended due to its association with a decreased ROI. Despite the promising findings regarding the effects of dietary lemon balm on broiler growth performance, humoral immunity, and intestinal health, this study has some limitations that should be acknowledged. The most notable limitation is the absence of a long-term evaluation, which restricts the ability to fully assess the sustained efficacy and potential cumulative effects of lemon balm supplementation over extended production cycles. Therefore, further research involving prolonged feeding trials under commercial conditions, as well as comprehensive assessments of physiological and economic impacts, is warranted to validate and expand upon these findings.

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