

# Effect of *Lipia citridora* leaves powder on growth performance, carcass traits, blood metabolites and meat quality of broilers

Mehrparvar, M.<sup>1</sup>, Mazhari, M.<sup>1\*</sup>, Esmacilipour, O.<sup>1</sup>, Sami, M.<sup>2</sup>

<sup>1</sup>Department of Animal Sciences, Faculty of Agriculture, University of Jiroft, Kerman, Iran

<sup>2</sup>Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Bahonar University, Kerman, Iran

## Key words:

broiler, blood metabolites, growth performance, *Lipia citridora*, meat quality

## Correspondence

Mazhari, M.  
Department of Animal Sciences,  
Faculty of Agriculture, University of Jiroft, Kerman, Iran  
Tel: +98(34) 43347061  
Fax: +98(34) 43347060  
Email: mozhgan.mazhari@gmail.com

Received: 9 May 2016

Accepted: 26 July 2016

## Abstract:

**BACKGROUND:** Since the prohibition of antibiotic growth promoter in poultry ration by the European Union, poultry producers have been trying to replace them by other growth promoters like medicinal herbs. *Lipia citridora* is a medicinal herb enriched by several powerful phenolic compounds and with antioxidant and antimicrobial properties which lead to enhancing appetite and growth performance of broiler. **OBJECTIVES:** The aim of this study was to investigate the effect of *Lipia citridora* leaves powder (LCLP) on growth performance, carcass traits, blood metabolites, and meat quality of broilers. **METHODS:** The experiment was carried out with 160 one-day-old male Ross 308 broiler chickens in a completely randomized design. The chicks were allocated to four diets including basal diet and treatment diets which were supplemented with 3 levels of LCLP (0.25, 0.5 and 1.0% of diet) in 16-floor pens with 10 chickens each. At the end of the experiment, 2 chicks/pen were selected and the assigned parameters were evaluated. **RESULTS:** All levels of LCLP improved ( $p < 0.01$ ) feed intake, body weight gain and feed conversion ratio in all periods except for starter phase. Also, 1% LCLP improved FI (1.49%), BWG (4.20%) and FCR (2.59%) compared to control group during the whole period. Birds fed herbal additive had a significantly higher relative weight of carcass, breast, bursa of fabricius and spleen in 1% LCLP group compared with the control group. Addition of 1% of LCLP decreased ( $p < 0.05$ ) LDL (15.85%), cholesterol (8.73%), triglyceride (8.82%), and increased ( $p < 0.01$ ) white blood cells (8.04). Meat quality enhanced via an intense reduction ( $p < 0.01$ ) in thiobarbituric acid (50.15%) and cooking loss (14.16%). **CONCLUSIONS:** Our finding showed that the highest level of LCLP in this study (1%) performed the best as improved growth performance, ameliorated serum lipids, increased white blood cells and enhanced the meat quality.

## Introduction

Increasing growth performance in broilers needs more research trials on nutrition

requirements. In this line the growth promoters are the main purpose of nutrition studies. Antibiotic growth promoters (AGP) have been used for reducing the spread of

diseases and as growth promoter to enhance broiler performance. However, many AGP have already been restricted by animal farms in the European Union and some parts of Asia because of their residual effects on poultry products and harmful effects on human health (Lillehoj and Lee, 2012). Consequently, nutritionists and production managers have to find alternatives that have potential to alleviate the problems related to AGP. Therefore, herbal additives including herbs, spices and various plant extracts have received increased attention as possible AGP replacements due to their effects on microflora. Scientific evidence suggests that these herbal additives have appetite and digestion stimulating properties and promote the growth of beneficial bacteria and inhibit the growth of unfavorable bacteria in the gut (Windisch et al., 2008).

*Lippia citriodora* with the common names of lemon verbena and lemon beebrush, is a species of flowering plant in the verbena family Verbenaceae. *Lippia citriodora* (LC) is a perennial shrub or sub-shrub with pointed and slightly rough to touch leaves and emits a powerful scent reminiscent of lemon when bruised. *Lippia citriodora* leaves are used to add a lemony flavor in many culinary purposes such as fish and poultry dishes, vegetable marinades, salad dressings, jams, puddings, and beverages. It is also used to make herbal teas and refreshing sorbets (Funes et al., 2009). Therefore, LC products and their compounds can be considered in the food category. This plant is mainly used as a spice and as a medicinal plant. This medicinal herb has already proven its antimicrobial effect and stimulating growth performance in lambs (Casamassima et al., 2013). It has been reported that the most phenolic compounds in this herb

include Apigenin, 7-glucuronide, Luteolin-7-diglucuronide, Verbascoside, Isoverbascoside, and Martinoside, of which the major one is Verbascoside (Bilia et al., 2008). Verbascoside has a high potency of antioxidant, is antibacterial and antitumor and promotes the growth performance. *Lippia citriodora* contains mucilage, tannin and flavonoids as well, and is alkaloids-free (Bilia et al., 2008). There are not enough findings about using this herb in broiler ration, thus, the aim of this study was to evaluate the effect of *Lippia citriodora* leaves powder on growth performance, carcass traits, blood metabolites and meat quality of broilers.

## Materials and Methods

**Birds, diets and management:** The experimental protocols were approved by the Animal Care Committee of University of Jiroft, Iran. A total number of 160 day-old male Ross 308 broiler chicks (average weight of 45 g) were obtained from Mahan commercial hatchery (Mahan, Kerman, Iran). The birds were fed to match the requirements recommended by the Ross 308 recommendations (Aviagen, 2007) during the starter (1-10 d), grower (11-25 d) and finisher (26-42 d) periods (Table 1). During the whole experiment the chicks were allocated to four diets including control (basal) diet and treatment diets which were supplemented with 3 levels of *Lippia citriodora* leaves powder (0.25, 0.5 and 1.0%) in 16-floor pens with 10 chickens each in a completely randomized design. *Lippia citriodora* leaves were prepared from university farm (University of Jiroft, Jiroft, Iran), rinsed and chopped by hand and then dried at room temperature. It was added

to each treatment diet daily. All diets were provided isocaloric and isonitrogenous. No antimicrobials or anticoccidials were added into the diets. Each pen was equipped with a feeding trough and nipple drinkers and birds had free access to feed and water. The pen (1×1.5 m<sup>2</sup>) floor was covered with wood-shaving litter. Broilers were vaccinated against common diseases of the area based on Jiroft University Veterinary Protocol. Birds received 24 h light in first week of age and were then subjected to 23-h light: 1-h darkness until 42 d of age. Temperature was initially set at 32°C for the first 3 d of age and decreased by 3°C per week. The humidity was 50-60% during the experiment.

**Sample collection and measurements:** Body weight gain of birds was measured per pen at the end of each period. Feed intake of the birds was measured on a pen basis for the same periods. Mortality and the weight of birds that died or were culled during the trial were recorded. Feed conversion ratio was calculated for these periods. At day 42 of age, two birds per replicate whose weight were close to the average weight of each pen, were selected and killed by cervical dislocation, and blood samples were collected from the brachial vein in two tubes to obtain serum and plasma. One tube of blood samples was immediately centrifuged at 1000 × g for 15 min at 4 °C for analyzing serum glucose, total cholesterol, triglyceride, HDL, LDL by an autoanalyser using the protocol provided by the kit manufacturer (Zist-Shimi, Tehran, Iran). Heparin-containing tubes were used to avoid blood clot formation. Blood smears were prepared on slides and painted by Gimsa method. The white blood cells (WBC) and red blood cells (RBC) counts and the hemoglobin (Hb) values were determined by Sys-

mexK-1000 apparatus (Japan).

The blood-collected birds mentioned above were considered to determine the carcass traits. Carcass yield was determined as the carcass weight in relation to live BW and expressed as percentage of BW, whereas other carcass cuts including breast, leg, liver, Bursa fabricius, spleen and ventral fat were expressed as percentage of carcass weight.

Muscle from the right side of breast was used to determine objective thiobarbituric acid, water-holding capacity, cooking loss, drip loss and pH.

The concentration of thiobarbituric acid (TBA) in the sausage was measured photometrically according to Bruna et al., (2001). One gram of the sausage homogenate was minced in 10 ml of TBA (20%) for 2 min. After the addition of 0.5 ml of butylated hydroxytoluene (0.19 M) and centrifugation for 6 min at 3,000 × g (Hermle Z383 K, Hermle GmbH, Wehingen, Germany), the solution was filtered through filter paper (MN 613, Macherey-Nagel GmbH, Dueren, Germany). To 0.7 mL of the filtrate, the same volume of TBA (0.02 M) was added and heated for 30 min at 100°C (LAT GmbH, Garbsen, Germany). After cooling for 10 min, the TBA concentration was determined at 532 nm (Helios β, Unicam Chromatography GmbH, Kassel, Germany). All experiments were performed in triplicate, and results were expressed as micrograms of malondialdehyde per gram of sample.

For measuring Water-holding capacity, one gram of minced breast meat sample was placed on a round plastic plate with small holes. The plate with meat sample on it was then fitted into a 2-mL plastic tube. This tube was centrifuged at 920 × g for 10 min. The released water content was measured

and calculated as a percentage of the initial weight (Castellini et al., 2002).

To obtain Cooking loss, one fillet from each whole breast was individually weighed and cooked in a convection oven on aluminum trays at 180°C until the core temperature reached 80°C. The fillet was then allowed to equilibrate to room temperature and reweighed, and cooking loss was determined as the percentage of weight lost by the sample (Bertrama et al., 2003).

For determining drip loss, breast fillet was individually weighed, sealed in plastic bags, stored at 4°C for 24 h, and then reweighed (Saenmahayak et al., 2010).

The pH value was determined at 24 h (ultimate pH) postmortem in the breast muscle at a 2-cm depth (Jaturasitha et al., 2008), using a portable pH meter (IQ150, IQ Scientific Instruments Inc., Carlsbad, CA) equipped with a stainless electrode (pH57-SS).

**Statistical analysis:** Prior to analysis, all percentage data were normalized by subjecting to arc sine transformation. As the pen represented the experimental unit, the effect of LCLP on performance, carcass traits, serum parameters, immunity and meat quality of broilers was statistically analyzed in a completely randomized design using the GLM procedure of SAS 9.1 (2001). Treatment means were compared using Tukey's multiple range test.

## Results

**Growth performance:** The effect of *Lippia citriodora* leaves powder (LCLP) on feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of broilers at different periods is shown in Table 2. Dietary supplementation of LCLP increased

( $p < 0.01$ ) FI and BWG during the grower, finisher and whole periods compared with the control group. Also, 1% LCLP improved FI (1.49%), BWG (4.20%) and FCR (2.59%) compared to control group during the whole period ( $p < 0.01$ ). However, none of the three levels of LCLP had significant effect on performance during the starter period.

**Carcass traits:** Birds fed different levels of LCLP had a significantly heavier relative weight of carcass and breast, spleen and bursa of fabricius, whereas significant difference was not detected on relative weight of thigh, liver and abdominal fat by treatments in this study (Table 3).

**Serum metabolites:** Serum glucose level was not affected in birds fed LCLP in this study (Table 4), but by increasing the level of LCLP in diet the hypo-effect of this herb on serum lipids was more pronounced. All levels of LCLP decreased ( $p < 0.05$ ) cholesterol, triglyceride and LDL, compared to the control group. Adding LCLP to the diet increased ( $p < 0.01$ ) white blood cells, while red blood cells counts, hemocrite and hemoglobin values were not changed among the control and treatment groups.

**Meat quality:** The effect of LCLP on meat quality of broilers at 42 d of age is shown in Tables 5. Data showed when LCLP was introduced into the diet, the meat quality was improved ( $p < 0.05$ ) via an intense reduction ( $p < 0.01$ ) in TBA and cooking loss. Although meat quality was enhanced ( $p > 0.05$ ) in terms of increase in water holding capacity (WHC) and pH and decrease in drip loss, these changes were not significant.

## Discussion

**Growth performance:** According to our

Table 1. Composition of starter, grower, and finisher diets. <sup>1</sup>Vitamin mix provided the following per kilogram of diet: vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 215 IU; vitamin E (DL- $\alpha$ -tocopheryl acetate), 18 IU; vitamin K (menadione), 2 mg; thiamine, 18 mg; riboflavin, 6.6 mg; pyridoxine, 3 mg; vitamin B12 (cyanocobalamin), 0.015 mg; niacin, 10 mg; pantothenic acid (D-calcium pantothenate), 4.8 mg; folic acid, 1 mg; biotin, 0.15 mg; choline chloride, 500 mg; ethoxyquin (antioxidant), 1 mg. <sup>2</sup>Mineral mix provided the following per kilogram of diet: Fe, 50 mg; Mn, 100 mg; Zn, 7.84 mg; Cu, 10 mg; I, 1 mg; Se, 0.2 mg.

Ingredient (%)	Starter (1-10 d)	Grower (11-25)	Finisher (26-42)
Corn	53.38	54.81	60.63
Soybean meal	38.45	36.23	30.85
Vegetable oil	3.49	5.00	4.85
Limestone	1.54	1.35	1.30
Dicalcium Phosphate	1.64	1.29	1.21
Vitamin mix1	0.25	0.25	0.25
Mineral mix2	0.25	0.25	0.25
Salt (NaCl)	0.29	0.29	0.29
L-Lysine	0.33	0.23	0.13
DL-Methionine	0.39	0.29	0.25
Calculated nutrients			
ME (kcal/kg)	3025	3150	3200
Crude protein (%)	22	21	19
Lysine (%)	1.43	1.30	1.09
Methionine+Cystine (%)	1.07	0.95	0.86
Threonine (%)	0.94	0.90	0.85
Calcium (%)	1.05	0.90	0.85
Available phosphorus (%)	0.50	0.45	0.42

Table 2. The effect of *Lipia citridora* leaves powder (LCLP) on performance of broilers at different periods. IFI = feed intake, BWG = body weight gain and FCR = feed conversion ratio. Means within the same column with uncommon superscript differ significantly ( $p < 0.05$ ).

Parameters	Treatments				SEM	p-Value
	Control	0.25% LCLP	0.5% LCLP	1% LCLP		
FI (g/b)1						
1-10 <sup>d</sup>	189.6	191.4	191.6	191.9	1.43	0.68
11-25 <sup>d</sup>	760.6	771.1	765.9	761.1	5.39	0.50
26-42 <sup>d</sup>	2071.8 <sup>b</sup>	2100.5 <sup>ab</sup>	2105.9 <sup>a</sup>	2115.3 <sup>a</sup>	7.89	0.01
Overall	3127.6 <sup>b</sup>	3170.0 <sup>a</sup>	3169.8 <sup>a</sup>	3174.1 <sup>a</sup>	8.02	0.004
BWG (g/b)1						
0-10 <sup>d</sup>	143.8	144.5	145.4	145.5	1.17	0.73
11-25 <sup>d</sup>	428.6 <sup>b</sup>	440.7 <sup>a</sup>	441.4 <sup>a</sup>	450.6 <sup>a</sup>	2.43	0.0003
26-42 <sup>d</sup>	1124.3 <sup>b</sup>	1155.0 <sup>a</sup>	1160.9 <sup>a</sup>	1171.9 <sup>a</sup>	6.01	0.0008
Overall	1760.4 <sup>c</sup>	1805.3 <sup>b</sup>	1812.9 <sup>ab</sup>	1834.3 <sup>a</sup>	6.63	0.0001
FCR1						
0-10 <sup>d</sup>	1.33	1.32	1.32	1.32	0.008	0.89
11-25 <sup>d</sup>	1.77 <sup>a</sup>	1.75 <sup>a</sup>	1.74 <sup>ab</sup>	1.69 <sup>b</sup>	0.01	0.006
26-42 <sup>d</sup>	1.84 <sup>a</sup>	1.82 <sup>b</sup>	1.81 <sup>b</sup>	1.80 <sup>b</sup>	0.005	0.002
Overall	1.78 <sup>a</sup>	1.76 <sup>ab</sup>	1.75 <sup>bc</sup>	1.73 <sup>c</sup>	0.005	0.0004

data, addition of *Lipia citridora* leaves powder (LCLP) improved growth performance



Table 3. The effect of *Lipia citridora* leaves powder (LCLP) on relative weight of carcass (relative weight: g/100 g of live weight), and carcass cuts (relative weight: g/100 g of carcass weight) of broilers at 42 d of age. <sup>a,b</sup>Means within the same column with uncommon superscript differ significantly ( $p < 0.05$ ).

Parameters	Treatments				SEM	p-Value
	Control	0.25% LCLP	0.5% LCLP	1% LCLP		
Carcass	83.72 <sup>b</sup>	84.62 <sup>ab</sup>	85.10 <sup>ab</sup>	86.45 <sup>a</sup>	0.523	0.02
Breast	26.54 <sup>b</sup>	26.76 <sup>b</sup>	27.68 <sup>a</sup>	27.96 <sup>a</sup>	0.195	0.0005
Thigh	23.38	23.54	23.82	23.89	0.509	0.87
Liver	2.58	2.74	2.77	2.76	0.118	0.66
Spleen	0.050 <sup>b</sup>	0.059 <sup>ab</sup>	0.069 <sup>ab</sup>	0.076 <sup>a</sup>	0.005	0.027
Bursa	0.11 <sup>b</sup>	0.13 <sup>ab</sup>	0.13 <sup>ab</sup>	0.14 <sup>a</sup>	0.005	0.014
Abdominal fat	1.47	1.42	1.37	1.31	0.078	0.56

Table 4. The effect of *Lipia citridora* leaves powder (LCLP) on Blood parameters (mg/dl) of broilers at 42 d of age. 1HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein. 2WBC: White Blood Cells; RBC: Red Blood Cells. Hb: Hemoglobin. HCT: Hematocrit. Means within the same column with uncommon superscript differ significantly ( $p < 0.05$ ).

Parameters	Treatments				SEM	p-Value
	Control	0.25% LCLP	0.5% LCLP	1% LCLP		
Serum metabolites						
Glucose (mg/dl)	208.00	204.50	203.25	202.75	1.990	0.28
Triglyceride(mg/dl)	110.50 <sup>a</sup>	104.50 <sup>ab</sup>	102.75 <sup>ab</sup>	100.75 <sup>b</sup>	2.010	0.03
Cholesterol (mg/dl)	114.50 <sup>a</sup>	110.25 <sup>ab</sup>	105.50 <sup>b</sup>	104.50 <sup>b</sup>	1.840	0.008
HDL1 (mg/dl)	72.50	73.75	75.75	76.50	1.550	0.330
LDL1 (mg/dl)	20.50 <sup>a</sup>	18.25 <sup>b</sup>	18.00 <sup>b</sup>	17.25 <sup>b</sup>	0.420	0.0009
Blood cells <sup>2</sup>						
WBC ( $\times 10^3/\mu\text{l}$ )	21.55 <sup>c</sup>	22.44 <sup>b</sup>	23.15 <sup>ab</sup>	23.28 <sup>a</sup>	0.191	0.0001
RBC ( $\times 10^6/\mu\text{l}$ )	2.20	2.24	2.26	2.26	0.030	0.690
Hb (g/dl)	9.32	9.40	9.60	9.62	0.130	0.340
HCT (%)	33042	33.55	35.60	35.82	0.62	0.06

during the grower, finisher and whole periods; just there was not an improvement at starter period. The best growth performance was observed by addition of 0.5 and 1% LCLP. In a study by Rafiee et al. (2016), using 0.5 and 1% *Lipia citridora* in broiler ration from 25 to 42 days of age and in heat stress condition resulted in increasing average weight gain and feed intake by 5.81 and 3.29%, and reducing feed conversion ratio by 2.59% by 1% *Lipia citridora* compared to control group. In line with our study, Casamassima et al. (2013) investigated the influence of 2.5 and 5 mg/kg *Lipia citridora* extract on lambs and showed that both levels of extract increased FI and average daily gain and decreased FCR. In another exper-

iment the levels of 5 and 10 mg/kg *Lipia citridora* extract on pigs was assessed and only the higher level improved FI, BWG and FCR as compared to the control group (Pastorelli et al., 2012). Administration of herbal additives to the poultry diets has a positive effect on the food flavor that results in a higher feed consumption by modulating appetite and secretion of gastrointestinal digestive enzymes in birds. Also, Casamassima et al. (2013) reported that the pure extract of phenylpropanoid at the rate of 1 and 2 mg/kg increased weight gain of rats by 6.9 and 7.8%, respectively. Phenylpropanoids (especially verbascoside) are the main active components existing in *Lipia citridora* extract which act as a digestibil-

Table 5. The effect of *Lipia citridora* leaves powder (LCLP) on meat quality of broilers at 42 d of age. 1TBA: Thiobarbituric Acid, ( $\mu\text{g}$  MDA/g meat), MDA: Malondialdehyde; WHC: Water Holding Capacity. Means within the same column with uncommon superscript differ significantly ( $p < 0.05$ ).

Parameters	Treatments				SEM	P-Value
	Control	0.25% LCLP	0.5% LCLP	1% LCLP		
TBA1	0.27 <sup>a</sup>	0.17 <sup>bc</sup>	0.19 <sup>b</sup>	0.14 <sup>c</sup>	0.008	<0.0001
WHC1 (%)	74.33	74.93	75.12	77.30	0.900	0.16
Cooking loss (%)	35.85 <sup>a</sup>	32.65 <sup>b</sup>	30.80 <sup>b</sup>	30.77 <sup>b</sup>	0.750	0.001
Dripping loss (%)	44.00	41.50	39.75	40.50	1.830	0.420
pH	5.81	5.89	6.00	5.92	0.070	0.310

ity enhancer, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes, and thus improving growth performance in poultry (Newall et al., 1996).

**Carcass traits:** Data showed that addition of LCLP increased the relative weight of carcass and breast. The higher breast yields of birds fed diet with 1% LCLP might be associated with higher activity of digestive enzymes and more efficient uptake of nutrients (specifically proteins) which led to better growth rate (Lewis et al., 2003). Parallel to our study, the relative weight of breast increased in broiler fed with 1% *Lipia citridora* (Rafiee et al., 2016). The results expressed that inclusion of LCLP to diet elevated the relative weight of spleen and bursa of fabricius in treated birds but the relative weight of abdominal fat (AF) did not change among treatments. Increased weight of these two lymphoid organs may indicate a higher immunity in *Lipia citridora*-treated birds which could be justified by the antimicrobial activity of phenylpropanoids and flavonoids in this herb. Demir et al. (2003) reported that herbal additives had no significant effect on relative weight of AF, but Kamisoyama et al. (2008) observed that 1 and 2% flavonoid oil suppressed the relative weight of AF in rats fed diet with high fat content. It was attributed to the increased activity of acetyl-CoA carboxylase and de-

creased activity of fatty acid synthase by flavonoid oil supplementation.

**Serum metabolites:** The present study revealed that the higher levels of LCLP were supplemented to the diets, a more hypocholesterolemic effect was seen in birds. Similar results were found in an investigation by Pastorelli et al. (2012) with pigs fed 5 and 10 mg/kg *Lipia citridora* extract. These authors suggested that feeding 10 mg/kg *Lipia citridora* extract suppressed serum LDL in pigs by 17%. One mechanism involved in the overall hypocholesterolemic effect of *Lipia citridora* supplementation might be attributed to the active components of this plant as the natural antioxidants. Phenolic compounds (flavonoids, tannins, phenolic acids, terpenes) are the molecules responsible for antioxidant activity of herbal additives. These components can adhere to LDL, inhibit the free radicals and in turn decrease cholesterol absorption to the blood (Lee et al., 2004). The other mechanism might be attributed to Verbascosides in *Lipia citridora* that are known to decrease cholesterol biosynthesis by inhibiting key enzymes, that is, 3-hydroxyl-3-methylglutaryl-coenzyme A reductase in poultry (Liu et al., 2003). Biochemical analysis of serum is very important for explanation of clinical findings. Biochemical analysis is used to support a diagnosis, to determine a cure for the disease, or to control the applied cure,

to evaluate and to interpret the results of the research.

Serum Cholesterol, triglyceride and LDL levels showed a decreasing pattern when *Lipia citridora* was supplemented to the diet but glucose and HDL did not differ significantly. Pastorelli et al., (2012) showed decreasing serum LDL by 17% in pigs fed 10 mg/kg lemon verbena extract. Hypercholesterolemia is associated with an increased risk of coronary heart disease and one of the several efforts in lowering the blood cholesterol is supplementing animal and avian diets with medicinal plants or natural feed additives with Hypocholesterolemic activity (Rojhan, 2000). Adding LCLP to the diet increased white blood cells (WBC). It may be due to the effect of phenolic compounds of *Lipia citridora* on different parts of WBC, including lymphocytes. Rafiee et al. (2016) revealed that addition of *Lipia citridora* to broiler diets increased lymphocytes by 4.51% compared to control group. White blood cells also called leukocytes, are an important part of the immune system. These cells help fight infections by attacking bacteria, viruses, and germs that invade the body. Lee et al. (2004) concluded that the flavonoid-rich herbs resulted in improved immunity of birds due to their antimicrobial activity. Pastorelli et al. (2012) showed that the supplementation of 5 and 10 mg/kg *Lipia citridora* extract in broilers elevated the IgA and IgG. These results validate the responsibility of herbs that are rich in flavonoids such as *Lipia citridora* as antioxidants and may therefore enhance the immune functions.

**Meat quality:** Meat quality was improved in this study via decreasing ( $p < 0.05$ ) TBA and cooking loss and good modification ( $p > 0.05$ ) of WHC, drip loss and

pH. Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes. The destruction can lead to cell death and also to the production of toxic and reactive aldehyde metabolites, known as free radicals. Among these free radicals, malondialdehyde (MDA) is the most important and main final product of lipid peroxidation that has often been used for determining oxidative damage (Paradis et al., 1997). Overall, the amount of thiobarbituric acid (TBA) is considered as the indicator of MDA level. Medicinal herbs have strong antioxidant activity and active components of *Lipia citridora* are reported to inhibit lipid peroxidation (Casamassima et al., 2013). The results obtained in the present study imply that the active substances of the *Lipia citridora* may improve the antioxidant status of broilers due to the antioxidant property of Verbascoside by reducing the level of TBA. It was suggested that the high antioxidant activity of Verbascoside is due to the presence of phenolic OH groups which act as hydrogen donors to the proxy radicals produced during the first step in lipid oxidation, thus retarding the hydroxyl peroxide formation (Funes et al., 2009). Based on these findings, we state that *Lipia citridora* might play an important role as an exogenous antioxidant and could also be applicable as a protective agent against tissue damage. Several studies have been conducted on the improving effect of medicinal herbs on meat quality. The inclusion of Verbascoside to diets depressed the lipid peroxidation in muscles of rats significantly (Gao et al., 1999). Choiem Hwang (2005) demonstrated that medicinal plants elevated the antioxidant enzymes activity and reduced the MDA level in rats. Natural antioxidant-fed birds showed a lower meat oxidation degree



than those that did not receive natural antioxidant in their diet (Sarraga and Regueiro, 1999). Considering that MDA is produced as a result of PUFA oxidation, Pallazo et al. (2015) reported that the intake of *Lipia citridora* resulted in a decrease in saturated fatty acids level and a higher level of PUFA in rabbit meat. No influence on carcass characteristics and the chemical composition of meat was observed. A positive effect on alpha-tocopherol tissue content and on thiobarbituric reactive substance (TBARS) values was observed in their study. These researches concluded that the *Lipia citridora* extract had the potential to improve rabbit meat without altering the chemical and physical characteristics of meat. The pH is an important index of meat quality. Decreased pH after slaughter is due to the proteins denaturation which results in lower WHC and lighter color meat (Briskey and Wismer-Pedersen, 1961). Jang et al. (2008) reported that a blend of herbal extracts increased pH of broiler meat and Mirshekar et al. (2009) showed a decrease on TBA of meat. The WHC of meat products is a very important quality attribute which has an influence on product yield, which in turn has economic implications, but is also important in terms of eating quality. A number of pre-and post-mortem factors influence the WHC of meat. Proteins denaturation and reduction of pH in meat cause the contraction of myofibrils of muscle and decrease the WHC and nutritive value of meat (Castellini et al., 2002). The other factor influencing the WHC is a proteolytic enzyme called  $\mu$ -calpain which is activated after slaughter, resulting in higher WHC and which leads to meat tenderness. Although the oxidation and lower pH stop the activity of this enzyme, the medicinal herbs promote the

proteolytic activity of  $\mu$ -calpain and improve the meat quality (Huff-Lonergan and Lonergan, 2005). The more WHC there is in meat, the lower the occurrence of cooking loss and drip loss (Warris, 2000).

**Conclusion:** The results showed that the *Lipia citridora* supplementation served as a good growth promoter and immune enhancer in this study, since it improved growth performance, ameliorated serum lipids, increased white blood cells and enhanced the meat quality. The best results were observed by the highest level of *Lipia citridora* (1%), however, the results obtained in this study justify further research in this area.

### Acknowledgements

The authors greatly acknowledge the financial support of University of Jiroft, Iran and the staff of the Animal Science Department for their technical assistance.

### References

1. Aviagen. (2007) Ross 308: Broiler Nutrition Specification. Aviagen Inc., Huntsville, AL, USA.
2. Bertrama, H.C., Andersena, H.J., Korlssona, A.H. (2003) Prediction of technological quality (cooking loss) of pork based on fresh meat characteristics. *Meat Sci.* 65: 707-712.
3. Bilia, A.R., Giomi, M., Innocenti, M., Gallo-ri, S., Vincieri, F.F. (2008) HPLC/DAD-ESI-MS analysis of the constituents of aqueous preparations of verbena and lemon verbena and evaluation of the antioxidant activity. *J Pharm Biomed Anal.* 46: 463-470.
4. Briskey, E.J., Wismer-Pedersen, J. (1961) Biochemistry of pork muscle structure. Rate of anaerobic glycolysis and temperature change versus the apparent structure of

- muscle tissue. *J Food Sci.* 26: 297-305.
5. Bruna, J.M., Ordonez, J.A., Fernandez, M., Herranz, B., dela Hoz, L. (2001) Microbial and physico-chemical changes during the ripening of dry fermented sausages superficially inoculated with or having added an intracellular cell-free extract of *Penicillium aurantiogriseum*. *Meat Sci.* 59: 87-96.
  6. Casamassima, D., Palazzo, M., D'Alessandro, A.G., Colella, G.E., Vizzarri, F., Corino, C. (2013) The effects of lemon verbena (*Lippia citriformis*) verbascoside on the productive performance, plasma oxidative status, and some blood metabolites in suckling lambs. *J Anim Feed Sci.* 22: 204-212.
  7. Castellini, C., Mugnai, C., Dal Bosco, A. (2002). Effect of organic production system on broiler carcass and meat quality. *Meat Sci.* 60: 219-225.
  8. Choiem Hwang, J.K. (2005) Effect of some medicinal plants on plasma antioxidant system and lipid levels in rats. *Phytother Res.* 19: 382-395.
  9. Demir, E., Sarica Ozcan, M.A., Suicmez, M. (2003) The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. *Br Poult Sci.* 44: 44-45.
  10. Funes, L., Fernandez, A.S, Laporta, O., Pons, A., Roche, E., Segura, C.A., Fernandez, G.A. (2009) Correlation between plasma antioxidant capacity and verbascoside levels in rats after oral administration of lemon verbena extract. *Food Chem.* 17: 589-598.
  11. Gao, J.J., Igalashi, K., Nukina, M. (1999) Radical scavenging activity of phenylpropanoid glycosides in *Caryopteris incana*. *Biosci Biotechnol Biochem.* 63: 983-988.
  12. Huff-Lonergan, E., Lonergan, S.M. (2005) Mechanisms of water-holding capacity of meat: The role postmortem biochemical and structural changes. *Meat Sci.* 71: 194-204.
  13. Jang, A., Liu, X.D., Shin, M.H., Lee, B.D., Lee, S.K., Lee, J.H., Jo, C. (2008) Antioxidative potential of raw breast meat from broiler chicks fed a dietary medicinal herb extract mix. *Poult Sci.* 87: 2382-2389.
  14. Jaturasitha, S., Srikanchai, T., Kreuzer, M., Wicke, M. (2008) Differences in carcass and meat characteristics between chicken indigenous to northern Thailand (Black-Boned and Thai Native) and imported extensive breeds (Bresse and Rhode Island Red). *Poult Sci.* 87: 160-169.
  15. Kamisoyama, H., Honda, K., Tominaga, Y., Yokota, S., Hasegawa, S. (2008) Investigation of the anti-obesity action of licorice flavonoid oil in diet-Induced obese rats. *Biosci Biotechnol Biochem.* 72: 3225-3231.
  16. Lee, K.W., Everts, H., Beynen, A.C. (2004) Essential Oils in Broiler Nutrition. *Int J Poult Sci.* 3: 738-752.
  17. Lewis, M.R., Rose, S.P., MacKenzie, A.M., Tucker, L.A. (2003) Effects of dietary inclusion of plant extracts on the growth performance of male broiler chickens. *Br Poult Sci.* 44: 42-43.
  18. Lillehoj, H.S., Lee, K.W. (2012) Immune modulation of innate immunity as alternatives-to-antibiotics strategies to mitigate the use of drugs in poultry production. *Poult Sci.* 91: 1286-1291.
  19. Liu, M.J., Li, J.X., Guo, H.Z., Lee, K.M., Qin, L., Chan, K.M. (2003) The effects of verbascoside on plasma lipid peroxidation level and erythrocyte membrane fluidity during immobilization in rabbits: a time course study. *Life Sci.* 73: 883-892.
  20. Mirshekar, R., Dastar, B., Shabanpour, B. (2009) Effect of Rosemary, Echinacea, Green tea Extracts and ascorbic acid on broiler meat quality. *Pak J Biol Sci.* 12: 1069-1074.
  21. Newall, C.A., Anderson, L.A., Phillipson, J.D. (1996) Herbal Medicines -A Guide for

- Healthcare Professionals. The Pharmaceutical Press, London, UK.
22. Palazzo, M., Vizzarri, F., Nardoia, M., Ratti, S., Pastorelli, G., Casamassima, D. (2015) Dietary *Lippia citriodora* extract in rabbit feeding: effects on quality of carcass and meat. Arch Anim Breed. 58: 355-364.
  23. Paradis, V., Kollinger, M., Farbe, M., Holstage, A., Poynard, T., Bedossa, P. (1997) In situ detection of lipid peroxidation by-product in chronic liver diseases. Hepatology. 26: 135-143.
  24. Pastorelli, G., Rossi, R., Corino, C. (2012) Influence of *Lippia citriodora* verbascoside on growth performance, antioxidant status, and serum immune globulins content in piglets. Czech J Anim Sci. 57: 312-322.
  25. Rafiee, F., Mazhari, M., Ghoreishi, M., Esmailipour, O. (2016) Effect of lemon verbena powder and vitamin C on performance, and immunity of heat stressed broilers. J Anim Physiol Anim Nutr. doi: 10.1111/jpn.12457.
  26. Rojhan, M. (2000) Medicine and medicinal cure. (1<sup>st</sup> ed.) Alavi Press. Tehran, Iran.
  27. Saenmahayak, B., Bilgili, S.F., Hess, J.B., Singh, M. (2010) Live and processing performance of broiler chickens fed diets supplemented with complexed zinc. J Appl Poult Res. 19: 334-340.
  28. Sarraga, C., Regueiro, J.A. (1999) Membrane lipid oxidation and proteolytic activity in thigh muscles from broilers fed different diets. Meat Sci. 52: 213-219.
  29. Warriss, P.D. (2000) Meat Science. An Introductory Text. CABI Pub. Inc. New York, USA.
  30. Windisch, J., Schedle, W., Plitzner, C., Krommayr, A. (2008) Use of phytogenic products as feed additives for swine and poultry. J Anim Sci. 86: 140-148.

## اثر پودر برگ به لیمو بر عملکرد رشد، خصوصیات لاشه، متابولیت‌های خونی و کیفیت گوشت جوجه‌های گوشتی

مرضیه مهرپرور<sup>۱</sup> مژگان مظهری<sup>۱\*</sup> امیدعلی اسماعیلی پور<sup>۱</sup> مسعود سامی<sup>۲</sup>

(۱) گروه علوم دامی، دانشکده کشاورزی، دانشگاه جیرفت، کرمان، ایران

(۲) گروه بهداشت مواد غذایی، دانشکده دامپزشکی، دانشگاه شهید باهنر کرمان، کرمان، ایران

(دریافت مقاله: ۲۰ اردیبهشت ماه ۱۳۹۵، پذیرش نهایی: ۵ مرداد ماه ۱۳۹۵)

### چکیده

**زمینه مطالعه:** از زمان محدودیت استفاده از آنتی بیوتیک‌ها به عنوان محرک رشد در جیره طیور، پرورش دهندگان به دنبال جایگزین کردن آنها با دیگر عوامل محرک رشد نظیر گیاهان دارویی هستند. گیاه به لیمو، یک گیاه دارویی غنی از ترکیبات فنولی و با خواص آنتی اکسیدانی و آنتی میکروبی قوی است که منجر به تحریک اشتها و بهبود عملکرد رشد طیور می‌گردد. هدف: این مطالعه به منظور بررسی اثر استفاده از پودر برگ به لیمو بر عملکرد رشد، متابولیت‌های خونی، خصوصیات لاشه و کیفیت گوشت جوجه‌های گوشتی طراحی شد. روش کار: این آزمایش روی ۱۶۰ قطعه جوجه گوشتی نر یک روزه سویه راس ۳۰۸ در قالب طرح کاملاً تصادفی انجام شد. پرندگان به چهار تیمار آزمایشی شامل: جیره پایه و تیمارهای مکمل شده با سه سطح پودر برگ به لیمو (۰/۲۵، ۰/۵ و ۱٪ جیره) در ۱۶ پن و هر کدام شامل ۱۰ پرنده اختصاص داده شدند. در پایان آزمایش دو پرنده از هر پن انتخاب و پارامترهای مورد نظر اندازه گیری شد. نتایج: تمام سطوح پودر برگ به لیمو منجر به بهبود مصرف خوراک، افزایش وزن روزانه و ضریب تبدیل غذایی در همه دوره ها به جز دوره آغازین شدند. همچنین مصرف یک درصد پودر برگ به لیمو، منجر به بهبود مصرف خوراک (۱/۴۹٪)، افزایش وزن روزانه (۴/۲۰٪) و ضریب تبدیل غذایی (۲/۵۹٪) در کل دوره آزمایش در مقایسه با گروه کنترل شد. پرندگان تغذیه شده با ۱٪ پودر برگ به لیمو، دارای وزن نسبی لاشه، سینه، بورس و طحال بالاتری در مقایسه با گروه کنترل بودند. افزودن ۱٪ پودر برگ به لیمو، منجر به کاهش معنی‌دار ( $P < 0/05$ ) سطح کلسترول (۸/۷۳٪)، تری گلیسرید (۸/۸۲٪)، لیپوپروتئین با دانسیته کم (۱۵/۸۵٪) و افزایش گلبول‌های سفید خون (۸/۰۴٪) شد. پودر برگ به لیمو بر کیفیت گوشت تأثیر مثبتی داشت و باعث کاهش تیوباربیتوریکی اسید (۵۰/۱۵٪) و افت پخت (۱۵/۱۴٪) شد. نتایج این آزمایش نشان داد که افزودن پودر برگ به لیمو به میزان یک درصد در جیره می‌تواند منجر به بهبود عملکرد رشد، بهبود متابولیت‌های سرم خون، افزایش گلبول‌های سفید خون و کیفیت گوشت جوجه‌های گوشتی شود.

**واژه‌های کلیدی:** جوجه‌های گوشتی، متابولیت‌های خونی، عملکرد رشد، به لیمو، کیفیت گوشت

\* نویسنده مسؤول: تلفن: ۴۳۳۴۷۰۶۱ (۳۴) +۹۸، شماره: ۴۳۳۴۷۰۶۰ (۳۴) +۹۸، Email: mozhgan.mazhari@gmail.com