Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2013, 4 (10):47-51
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Effects of lemon essential oil on gastrointestinal tract, blood parameter and immune responses in broilers

[1]Sayed Abdoullah Hosseini, [1]Mohammad Nasari, [1]Abolfazel Zarai, [1]Houshang Lotfollahian, [2]Seyyed Reza Riyazi and [3]Amir Meimandipour

[1]Animal Science Research Institute of Iran, Karaj, Iran
[2]Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran
[3]Animal and Marine Biotechnology Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

---

ABSTRACT

The present study was designed to investigate the effects of lemon essential oil on gastrointestinal tract, blood parameter and immune responses. A total of one thousnds and twenty hundred one day old chicks were assigned in 5 treatments including Control, Control diet + 0.1 g/Kg Protexin, Control diet + 0.1 g/kg Virginomycin, control diet + 0.1 and 0.2 g/kg Lemon essential oil with 4 replicates and 60 chicks in each replicate. Digestive organs weight such as liver, gizzard, and the parts of intestine (duodenum, jejunum and ileum) and the length of intestine different parts were not affected by any treatment. Serum metabolite, SRBC response and immunoglobulin G and M were not affected by treatments. Finally, using up to 0.2 g/kg lemon essential oil could be suggested for using in broiler chickens diet.

Key words: Lemon essential oil, gastrointestinal tract, Blood parameter, Immune responses, Broilers

---

INTRODUCTION

Antimicrobial compounds produced by microorganisms have been used in poultry diets to increase performance and decrease morbidity particularly in broiler chickens for decades. However, consumer pressure related to the potential development of antibiotic-resistant bacteria has resulted in the development of non-antibiotic feed additives that may also improve broiler performance [5]. Many studies have been performed to find alternatives to antibiotics, with probiotics and plant extracts.

The action of probiotics can be explained by their production of antimicrobial substances that protect villi and absorptive surfaces against toxins produced by the pathogens, improvement of immunity stimulation, and ability to increase volatile fatty acids [19].

Aromatic plants and their extracts have received attention as growth and health promoters recently. It is known that most of their properties are due to the essential oils and other secondary plant metabolites. Essential oil enhance production of digestive secretions, stimulates blood circulation, exerts antioxidant properties, reduce levels of pathogenic bacteria and may enhance immune status [5].

Lemon is an important medicinal plant of the family Rutaceae. The main bioactive compound of lemon peel essential oil are Limonene, Terpinene, Pinene and Geranial.These compouds are classified as Terpenoids. They play

a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic and other pharmaceutical functions.

Furthermore, Studies showed that fruits of the Citrus family (particularly orange and lemon), are rich in Phenolic compounds [2]. Phenolic compounds exhibit considerable antimicrobial activity. Their antimicrobial ability may modulate the gut ecosystem to affect feed efficacy.

Antibacterial potential of lemon oil against some bacterial strains has been reported.Smith-Palmer et al., [20] studied antibacterial properties of some plant essential oil and found that lemon oil has slight inhibitory effect against five food-borne pathogens.In other study, Prabuseenivasan et al., [16] showed that lemon oil has moderate inhibitory effects against 6 bacterial species.Both gram-positive and gram-negative bacteria were sensitive to this essential oil. Ferdes and Ungureanu [8] confirm that Essential oils from aromatic plants such as lemon, mint, juniper and rosemary, possess antimicrobial and antifungal activity. However, there have only been limited studies conducted to investigate the effects of Lemon oil on growth performance and immune responses in broiler chickens. Orange (*Citrus aurantium*) and lemon (*Citrus limon*) peel are commonby-products of the food and juice extraction industryand the most widely consumed citrus in the world [10].They are also available at low cost in most seasons in some countries like Iran, and currently there is no information available about feeding orange and lemon peel extracts to broiler chickens under heat stress conditions [2].

Thus, the present study was conducted to compare the effects of substitution oflemon essential oil and probiotic as alternatives to virginamycin on gastrointestinal tract, blood parameters and the antibody responses to sheep red blood cell in growing broiler chickens.

MATERIALS AND METHODS

A total of 1200 1-d-old male Cobb broiler chicks, which obtained from a local hatchery, were used in the experiment. The birds were randomly allocatedon the weight basis to five dietary treatments with four replicate and 60 chicks in each replicate. The five dietary treatments consisted of a maize-soybean meal control diet and four test diets containing: 100 ppm of virginamycin; 100 ppm a commercial probiotic; 100 and 200 ppm of lemon essential oil. The diets were prepared in mash form and formulated to be adequate in all nutrients according to the Cobb recommendation (Table 1). Food and water were provided ad libitum. The chicks were subjected to artificial fluorescent illumination for 23 h/d.The experimental diets were offered from 1 to 42 d of age, when the experiment was finished.

Table 1: The nutritional composition of dietary treatments

Ingredients	Diets			
	A	B	C	D
Corn	55.60	59.67	65.3	70
Soybean meal (44%)	39	35.20	29.60	24.80
Concentrate A[1]	2.50	2.00	-	-
Concentrate B[2]	-	-	2.00	2.00
Calcium Carbonate	1.40	1.20	1.20	1.15
Soybean oil	1.20	1.60	1.60	1.75
Salt	0.30	0.33	0.30	0.30
Calculated Composition				
ME (kcal kg^{-1})	2930	3000	3060	3120
Crude protein (%)	22.3	21	19	17.2
Threonine (%)	0.87	0.80	0.71	0.64
Calcium (%)	1.05	0.90	0.86	0.83
Av. Phosphorus (%)	0.50	0.50	0.43	0.41
Lysine (%)	1.21	1.1	0.92	0.80
Meth+cyst (%)	0.93	0.84	0.74	0.66

[1]Supplied per kilogram of diet: Me, 3650 (kcal kg^{-1}); Protein, 18.4 (%); Calcium, 16.9 (%); Av. Phosphorus, 15.26 (%); Lysine, 5.26 (%); Meth+cyst, 10.66 (%); Threonine, 1.04 (%); Sodium, 1.16 (%).

[2]Supplied per kilogram of diet: Me, 3620 (kcal kg^{-1}); Protein, 19.4 (%); Calcium, 16.63 (%); Av. Phosphorus, 14.18 (%); Lysine, 3.11 (%); Meth+cyst, 8.57 (%); Threonine, 1.28 (%); Sodium, 1.47 (%).

Traits:

Intestinal organ assay:

At the age of 42 days, birds were killed and different parts of the abdominal viscera, including small intestine, gizzard, heart, liver, duodenum (pylorus to entrance of bile ducts), jejunum (bile duct entrance to Meckel's diverticulum), ileum (Meckel's diverticulum to the ileo-cecal junction) and cecum were weighed. Afterward, the

duodenum, jejunum, ileum and caecum were resected and laid out in a straight line without loops and without stretching and the lengths were measured. Afterward, the length of the small intestine, duodenum, jejunum, ileum and cecum were measured using the method described by Leopol [15].

Serum biochemical studies:
At the 35 days of age, 5 ml of blood was collected fromthe brachial vein of 10 birds in each dietary treatment. Subsequently,the concentrations of total protein, calcium, phosphorous, albumin, globulin, triglyceride and total cholesterolin serum samples were analyzed by an automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, NY) using colorimetric methods, following the instructions of the manufacturer of the corresponding reagent kit (Zhongsheng Biochemical Co., Ltd., Beijing, China).

SRBC:
At 28 days of age, 15 birds in eachdietary group were inoculated with 0.2 mL of a 7%suspension of sheep red blood cells (SRBC). 7 days post inoculation, SRBC-inoculated birds were bledthrough the brachial vein to measure the antibodytiter (log 2) by the micro titer hemmagglutination procedure [22]. The reciprocal of the highest dilution where there was complete agglutination was taken as titer.

To determine the antibody response to SRBC, a direct hemagglutination assay was used. A direct hemagglutination assay was performed to measure the total antibody (IgM and IgG) response to SRBC in serum. Briefly, serum samples were incubated at 56°C for 30 min to inactivate the complement. Fifty microliters of PBS containing 0.05% BSA was dispensed into each well of a round-bottomed 96-well microplate. Serum samples (50 µl) were then added and serially double diluted in the wells from columns 2 to 12. The first column (PBS only) of wells was considered blank. Then, 50 µl of 1% SRBC in PBS was added to all wells to make a 100µl final volume. Subsequently, the plates were shaken for 1 min and incubated for 24 h at 37°C to determine agglutination titers. A positive result was recorded when at least 50% SRBC agglutination was observed. To measure anti-SRBC IgG and IgM antibodies, serum samples were treated with 0.2 M 2-mercaptoethanol (2-ME) for 30 min at 37°C. This treatment inactivates IgM, and as a result, hemagglutination observed after treatment with 2-ME is due mostly to the presence of IgG antibodies. The difference between total antibody and IgG titers determines the IgM titer.

Statistical analysis:
Data from all response variables were subjected to one way analysisof variance applying SAS program [17] using general liner model GLM. Significant difference among treatment means were separated using Duncan's multiple range procedure [7] at 1 and 5% probability.

RESULTS AND DISCUSSION

The weights of selected internal organs (liver, gizzard and small intestines), abdominal fat and small intestinal length were measured individually (Table 2). The weights of these internal organs were expressed as percentages of live body weight.

Table 2: Relative weights (%BW) of visceral organs of broilers fed the control, antibiotic, probiotic and lemon essential oils in 42 day of age

	Items					
Treatment	Carcass yield (%)	Abdominal fat (%)	Brest (%)	Thigh (%)	Liver (%)	Gizzard (%)
Control	71.49	1.07	24.51	18.59	2.22	2.62
Virginamycin	71.71	1.71	23.19	19.31	2.23	2.84
Protexin	70.56	1.14	22.91	19.04	2.27	2.84
LEO (100 mg/kg)	67.67	1.31	22.96	19.05	2.51	2.71
LEO (200 mg/kg)	70.80	1.28	22.94	19.91	2.12	2.91
SE	0.50	0.12	0.38	0.22	0.04	0.22

**LEO, lemon essential oil*

Carcass yield, breast, thigh, abdominal fat and relative weights of the liver, gizzard as well as small intestine length were not significantly affected by supplementation of lemon to diets.

These finding are in agreement with the results of Cabuk et al., [6] who indicated that some intestinal organ weights such as liver, pancreas, and gizzard were not affected by addition of essential oil mixture to the diet. Similar results were observed by Hernandez et al., [9] who found no difference in weight of organs of broiler chickens fed diets containing an extract from thyme and oregano. The bioactive components in natural medicines are quite complex and their mode of action in unclear, though they may serve to provide animals with nutrients and bioactive components such as anti-microbial activity, immune enhancement and stress reduction [18].

Table 3: Relative weights and lengths (100g live weight) of intestinal segments of broilers fed the control, antibiotic, probiotic and lemon essential oils in 42 day of age

	Treatments*					
Items	1	2	3	4	5	SE
Duodenum length (cm/100g)	1.22	1.06	1.23	1.17	1.12	0.03
Duodenum weight (g/100g)	1.17	0.97	1.05	1.42	1.03	0.05
Juojenum length (cm/100g)	2.79	2.77	2.75	2.93	2.87	0.05
Juojenom weight (g/100g)	1.82	1.41	1.64	2.04	1.71	0.08
Ileum length (cm/100g)	3.11	2.99	3.22	3.35	3.13	0.07
Ileum weight (g/100g)	2.53	2.28	2.48	2.99	2.24	0.11

**1= control, 2, 3, 4, 5 describe diets containing 100 mg kg^{-1} Virginamycin, 100 mg kg^{-1} Probiotic, 100 mg kg^{-1} Lemon essential oil, and 200 mg kg^{-1} Lemon essential oil, respectively.*

The results for relative weight and length of intestinal segments are presented in Table 3. Dietary addition of essential oils of medical herbs in this experiment did not affect the relative weight and length of intestinal segments. Total serum cholesterol and triglycerides showed no significant difference among groups. Also, no significant difference was detected in serum total protein, albumin, globulins, calcium and phosphorus in the biochemical analysis.

These results were similar to those found by Lee et al., [13,14] and Bampidis et al., [3] in studies with broiler chickens and turkeys using different active compounds (carvacrol, cinnamaldehyde, thymol) and essential oils (oregano and CRINA Poultry).

No significant differences were observed between groups in the serum levels of total protein, albumin, and globulins. These results were similar to those found by Traesel et al., [21] and Bampidis et al., [3] in studies with broiler chickens and turkeys using different active compounds (carvacrol, cinnamaldehyde, thymol) and essential oils (oregano and CRINA Poultry). The obtained results, as normal serum albumin levels, exclude the presence of hepatic insufficiency in our study (but not exclude hepatic disease), since albumin synthesis occurs in the liver [12]. Similar results were found in a study by Abd El-Hakim et al., [1] with broilers fed herbs and/or organic acid. These authors also suggested that the effects of plant extracts on plasma proteins are species-specific. The results obtained by Ghazalah and Ali [11] showed significant increases in total protein and globulins fractions serum values when broilers were fed diets with 0.5% rosemary dried leaf meal.

Table 4: Effects of different treatments on blood biochemical parameters

	Treatments*					
Items	1	2	3	4	5	SE
Total protein (g/dL)	4.05	4.21	4.30	4.47	4.42	0.10
Albumin (g/dL)	1.60	1.73	1.53	1.62	1.62	0.02
Globulin (g/dL)	2.45	2.48	2.76	2.84	2.80	0.09
Cholesterol (mg/dL)	162	158.17	157	160.57	161.57	3.13
Triglyceride (mg/dL)	94.28	75.50	78.50	62.14	62.14	5.47
Calcium (mg/dL)	10.75	10.82	10.78	10.95	10.95	0.13
Phosphorus(mg/dL)	5.85	5.10	5.86	5.08	5.08	0.17

**1= control, 2, 3, 4, 5 describe diets containing 100 mg kg^{-1} Virginamycin, 100 mg kg^{-1} Probiotic, 100 mg kg^{-1} Lemon essential oil, and 200 mg kg^{-1} Lemon essential oil, respectively.*

Table 5: Effects of different treatments on immune response of broilers

	Treatments*					
Items	1	2	3	4	5	SE
SRBC antibody titer (log_2)	7.37	7.87	7.71	7.71	6.55	0.21
Immunoglobulin G (mg/dL)	4.87	4.87	5.28	5.57	4.66	0.19
Immunoglobulin M (mg/dL)	2.50	3.00	2.42	2.14	1.88	0.17

**1= control, 2, 3, 4, 5 describe diets containing 100 mg kg^{-1} Virginamycin, 100 mg kg^{-1} Probiotic, 100 mg kg^{-1} Lemon essential oil, and 200 mg kg^{-1} Lemon essential oil, respectively.*

This experiment resulted in no observed effects of either antibiotic or essential oil on SRBCs antibody titer, IgG and IgM levels in all treatments. Basmacioğlu Malayoğlu et al., [4] also concluded that dietary supplementation with oregano showed no significant influence on immune response of broilers.

CONCLUSION

Under the conditions of this study, even though the addition of lemon essential oil did not have a significant effect on chickens blood parameters, gut morphology and immune responses but it can be stated that this essential oil up to 200 mg kg^{-1} feed could be an option to use in broiler chickens diets. However, more experiments are needed to explain whether essential oils can be used as a growth promoter or as an alternative to antibiotics in poultry diets.

REFERENCES

[1] Abd El-Hakim, A.S., Cherian, G., and Ali, M.N, *Int. J. Poult. Sci*, **2009**, 8, 14–20.
[2] Akbarian, A., Golian, A., Kermanshahi, H., Farhoosh, R., Raji, R., De Smet, S., And Michiels, J, *Spanish Journal of Agricultural Research*, **2013**, 11(1), 109-119.
[3] Bampidis, V.A., Christodoulou, V., Florou-Paneri, P., Christaki. E,, Chatzopoulou. P.S., Tsiligianni, T., and Spais, A.B, *Br. Poult. Sci*, **2005**, 46, 595–601.
[4] Basmacioglu Malayoglu, H., Baysal, S., Misirlioglu, Z., Polat, M., Yilmaz, H., and Turan, N, *Br. Poult. Sci*, **2010**, 51, 67–80.
[5] Brenes, A., and Roura., E, *Anim. Feed Sci. Technol*, **2010**, 158, 1–14.
[6] Cabuk M., Bozkurt, M., Alcicek, A., Akbas, Y., and Kücükyılmaz, K, *South Afr. J. Anim. Sci*, **2006**, 36**,** 135-141.
[7] Duncan, D.B, *Biometrics*, **1955**, 11, 1-42.
[8] Ferdes, M., and Ungureanu, C, U.P.B. *Sci. Bull*, **2012**, Series B, 74(2), 87-98.
[9] Hernandez, F., Madrid, J., Garcia, V., Orengo, J., and Megias, M.D, *Poult. Sci*, **2004**, 83, 169–174.
[10] Ghasemi, K., Ghasemi, Y., and Ebrahimzadeh, M.A, *Pak. J. Pharm. Sci*, **2009**, 22, 277-281.
[11] Ghazalah, A.A., and Ali, A.M, *Int. J. Poult. Sci*, **2008**, 7, 234–239.
[12] González, F.H.D., and Silva, S.C, Perfil bioquímico sangüíneo.González FHD, Silva SC (eds) Introdução à Bioquímica Clínica Veterinária, UFRGS, Porto Alegre, **2006**, 2nd edn. pp 313–358.
[13] Lee, K.W., Everts, H., Kappert, H.J., Frehner, M., Losa, R., and Beynen, A.C, *Br. Poult. Sci*, **2003**, 44, 450–457.
[14] Lee, K.W., Everts, H., Kappert, H.J., Van Der Kuilen, J., Lemmens, A.G., Frehner, M., and Beynen, A.C, *Int. J. Poult. Scie*, **2004**, 3, 613–618.
[15] Leopold, A.S, *J. Wild Manage*, **1953**, 17, 197-203.
[16] Prabuseenivasan, S., Jayakumar, M., and Ignacimuthu, S, BMC. *Complement. Altern. Med*, **2006**, 6, 39.
[17] SAS, **2001**, SAS user's Guide: Statistic.Version 8.2. SAS Institute lnc., CARY, NC, USA.
[18] Safamehr, A., Mirahmadi, M., and Nobakht, A, *Iran. J. Anim. Sci*, **2012**, 3(4), 721-728.
[19] Shams Sharg, M., Dastar, B., Zarehdaran, S., Khomeiri, M., and Moradi, A, *J. Appl. Poult. Res*, **2012**, 21, 201-208.
[20] Smith-Palmer, A., Stewart, J., And Fyfe, L, *Letters in Applied Microbiology*, **1998**, 26, 118-122.
[21] Traesel, C.K., Wolkmer, P., Schmidt, C., Silva, C.B., Paim, F.C., Rosa, A.P.,Alves, S.H., Santurio, J.M., and Lopes, S.T.A, *Comp. Clin. Path*, **2010**, 20, 453-460.
[22] Wegmann, T.G., and Smithies, O.A, *Transfusion*, **1966**. 6, 67-73.