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The impact of feeding clove essential oils and organic acids on immunity, gut health and economics of broiler production

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Abstract
In this experiment the effect of dietary supplementation of clove essential oil and organic acids alone or in combinations on immunity and gut health of broilers was investigated. A total of 420 'Vencobb-400' broilers were divided into 8 dietary treatments with 3 replicates of 20 chicks each. The control group (A) received basal diet and the dietary different treatment group B received basal diet supplemented with clove essential oil at 200 mg/kg, whereas, groups C, D and E received basal diet supplemented with sorbic acid, fumaric acid and propionic acid at a dose of 1%, and groups F, G and H received basal diet with combination of clove essential oil at 200 mg/kg along with sorbic acid, fumaric acid and propionic acid at a dose of 1%, respectively. The different dietary treatment found to significantly ($P< 0.01$) influence the immune responses against ND at 6th week of age. The total viable count was reduced significantly ($P< 0.01$) in all treatment groups compared control. The *E. coli* count was significantly ($P< 0.01$) reduced in treatment group G, H and F offered blend of organic acid and clove essential oil compared to control and other treatment groups. The study suggests that modulation of broiler gut microbiota composition and enhance immunity through the administration of combination of essential oil and organic acids offers an effective means for improving economics of broiler production. Furthermore, the combination of clove essential oil with fumaric acid gave the best results in this experiment.

Keywords: Organic acids, Clove essential oil, immunity, gut health

Introduction
The intestinal health of broiler chickens depends on microbota balance, management, water quality [1]. The disturbance in the gastrointestinal tract can affect digestive, absorptive, metabolic, and immunological functions in birds which in turn lead to economic losses.
Antibiotic growth promoters have been used in poultry production to promote growth Antibiotics used as growth promoters in poultry feeds have been banned recently due to development of antibiotic resistant to human pathogenic bacteria [2]. In order to find substitutes for AGP, different natural additives have been evaluated [3]. Currently, such alternatives are the addition of organic acids and essential oil in poultry diets.
Organic acids are naturally found in the intestinal tract of animals, being originated from microbial fermentation organic acids have shown positive results in poultry production, for reducing the intestinal pH and bacterial growth intolerant to pH changes [4, 5]. One of the characteristics of these acids is that they do not dissociate completely in water and are related to the inhibition of bacterial growth [6]. The essential oil extracted from aromatic plants has been shown antifungal, anticoccidial, antibacterial [7], and antioxidant [8] activities.
Although, there are several studies showing the effects of organic acids and essential oils on intestinal health and growth performance of broiler chickens [1, 9]. Therefore, the objective of the present study was to evaluate, combining the effects of organic acids and essential oils as an alternatives option to the use of antibiotics in broiler diets, with possible complementary effect. Therefore, the purpose of this experiment was to investigate the effects of feeding blends of different organic acids and clove essential oil on the, immunity and the gut health of broiler chickens and economics of production.

Materials and Methods
The experiment was conducted on four hundred eighty Vencobb-400 strain *straight run* broilers for the period of six weeks to study the effect of clove essential oil and organic acids on performance of broilers. The day-old broiler chicks, immediately after arrival, were randomly divided into eight equal groups of 60 birds each *viz*., A to H having three replicates of 20 birds each. The birds in control group (A) offered basal diet adequate in all nutrients as per BIS (2007) [10]. The birds in dietary treatment groups B were offered diets containing clove

essential oil at dose rate of 200mg/kg. The birds in dietary treatment groups C, D and E were offered different organic acids *viz*. 1% sorbic acid, 1% fumaric acid and 1% propionic acid, respectively in diet of broilers. Birds in groups F, G and H were offered diet containing clove essential oil at dose rate of 200mg/kg in combination with different organic acids *viz*. sorbic acid, fumaric acid and propionic acid at 1% level, respectively. All the diets were isocaloric and isonitrogenous.

The birds from the experimental trials were assessed for the antibody titer against the Newcastle Disease (ND) at 21st and 42nd days of age. Two birds from each replicate were randomly selected and the blood samples were collected via wing vein. The serum was separated by centrifugation at 3000 RPM for 20 minutes and stored at -20^{0}C. The Haemagglutination Inhibition (HI) test was performed as per O.I.E. (1992) [11]. Two fold serial dilutions of antigen and serum was used as antigen for HI test. The HI titer was expressed as $\log_2$ value of the highest dilution of serum causing complete inhibition of 8 HA unit of antigen. The initial ND titers were also assed at 0 day of age.

The total viable count and *E. coli* count in all the treatment groups was studied for gut health at 21st day age following standard laboratory procedures. At 21st day for determination of total viable count and *E. coli* count, one bird from each replicate was scarified. Large intestine was opened immediately after sacrificing and weighed, 1 g of ceacal content was collected in sterile glass vial diluted in 9 ml Normal saline and then 2 serial dilutions were made for inoculation. Then, 10µl content from the last test tube was poured on the EMB agar plates and Nutrient agar plates and kept for incubation for 24 hrs at 37 ^{0}C. After incubation, total bacterial colonies were counted as colony forming units (CFU /per gram) of samples [12] (DIFCO, 1977). The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to ISO (1995) [13]. The results of the total bacterial count were expressed as the number of organism of colony forming units per gram (CFU/gm) of cecal samples. The initial total viable count and *E. coli* count at "0" day of age in experimental chicks was determined.

The economics of broiler production was worked out by considering the prevailing prices of inputs and sale price of broilers in local market. The data was analyzed following the Complete Randomized Design as described by Snedecor and Cochran [14].

Results and Discussions

Immune Response

The immune response was judged by employing HI test to detect the antibody titer against New Castle Disease (ND) at 3rd and 6th weeks. The results for antibody titer against New Castle Disease ($\log_2$ values) at 3rd and 6th weeks are presented in Table 1. The analysis of variance for the antibody titers against ND ($\log_2$ values) revealed non-significant differences in all treatment groups at 3rd weeks of age. The antibody titers against ND ($\log_2$ values) were numerically higher in treatment groups receiving supplementation of clove essential oil and organic acids singly or in combinations compared to control, but statistical difference was non-significant.

The different dietary treatment found to significantly ($P<$ 0.01) influence the immune responses against ND at 6th week of age. The antibody titers against ND were improved in birds fed either essential oil or organic acids independently. Moreover, the combinations of essential oil with organic acids have further enhanced the immune response in broilers.

Table 1: Antibody titers against ND ($\log_2$ values) at 3rd and 6th weeks in broilers fed clove essential oil and organic acids

Treatment Groups	ND titers ($\log_2$ values) 3rd week	ND titers($\log_2$ values) 6th week
A	2.83± 0.31	3.33^{c}±0.33
B	4.83±0.70	5.67^{ab}± 0.33
C	4.33±0.33	4.67^{b}±0.21
D	4.00±0.58	5.00^{ab}±0.52
E	4.67±0.61	5.17^{ab}± 0.31
F	4.83±0.65	5.83^{ab}± 0.31
G	5.33±0.71	6.17^{a}± 0.60
H	4.33±0.76	5.50^{ab}± 0.56
CD	NS	1.600**
CV %	33.71	19.83

Means bearing different superscripts within a column differ significantly. ***P*< 0.01. NS-Non-significant.

Table 2: Analysis of variance for Antibody titers against ND ($\log_2$ values) at 3rd and 6th weeks in broilers fed clove essential oil and organic acids

Source	df	3rd week ND titers		6th week ND titers	
		MSS	'F' value	MSS	'F' value
Treatments	7	3.378	1.538^{NS}	4.667	4.444**
Error	40	2.196	-	1.050	-

***P*< 0.01, NS-Non-significant.

The findings are in accordance with researchers who revealed that the essential oils promote the production of antibodies, thus enhancing the efficacy of vaccination [15, 16, 17]. Similarly, Youdim and Deans [18], reported that the better performance might be due to the antioxidant properties of essential oil utilized by the cells, thus having a sparing effect on the intracellular antioxidant systems. Acamovic and Brooker [19] and Silveira *et al*. [20] reported that herbs rich in flavonoids such as thyme and carvacrol could improve the immune functions through acting as antioxidants and extending the activity of vitamin C. Hashemipour *et al*. [21] reported that feeding birds with diets contained carvacrol plus thymol linearly increased the primary and secondary response against SRBC antigen and IgG. Similarly, Saleh *et al*. [22] revealed that supplementing diets with thyme and ginger oils at 100 and 200 mg/kg, respectively, in chicken diet increased antibody production. Serum immunoglobulin levels in birds was significantly increased [23]. Furthermore, Ozek *et al*. [24] reported that essential oil enhanced ND Ab titre in laying hens. It has been observed by several researchers that addition of some phytogenic additives or their products such as cold pressed oil, essential oil or extracts to animal and poultry diets improved immune response [25, 26].

Several studies demonstrated that organic acids could stimulate the natural immune response in poultry. Dehghani and Jahanian [27] reported that addition of organic acid in the broiler diet significantly increased antibody titers against New Castle disease virus at 12th day post vaccine in chicks. This finding is advocated by Abdel-Fattah *et al*. [28] and Rahmani and Speer [29], who reported that the addition of organic acid to broiler diets increased immunity of birds.

Khan and Nagra [30] reported that level of antibody titer against New Castle Disease vaccination was statistically similar in both conventional premix and organic acid group in broilers.

Gut Health

The ceacal contents were examined for determination of Total viable count and *E. coli* count at the 21st day of age and presented in (Table3) and Analysis of variance of Total viable count and *E. coli* count presented in (Table 4).

Table 3: Bacterial count log_{10} (CFU/gm) of Broiler Chicken Intestine at 21st day fed clove essential oil and organic acids

Treatment group	TVC	Coliform
A	8.30^{a} ±0.05	7.09^{a} ±0.06
B	7.77^{bc} ±0.05	6.40^{d}±0.07
C	7.85^{bc}±0.09	6.85^{b}±0.06
D	7.88^{b}±0.03	6.62^{c}±0.04
E	7.82^{bc}± 0.03	6.90^{b}±0.05
F	7.71^{bc}±0.05	6.27^{de}±0.02
G	7.64^{c}±0.10	6.23^{e} ±0.05
H	7.67^{c}±0.10	6.28^{de} ±0.03
CD	0.286**	0.208 **
CV %	1.53	1.32

Means bearing different superscripts within a column differ significantly. ***P*< 0.01.

Table 4: Analysis of variance for Bacterial count log 10 (CFU/gm) of Broiler Chicken Intestine at 21st day fed clove essential oil and organic acids

Source	df	Total viable count		Coliform count	
		MSS	'F' value	MSS	'F' value
Treatments	7	0.132	9.161**	0.333	43.956**
Error	16	0.014	-	0.008	-

***P*< 0.01.

The total viable count was reduced significantly (*P*< 0.01) in all treatment groups compared control. The *E. coli* count was significantly (*P*< 0.01) reduced in treatment group G, H and F offered blend of organic acid and clove essential oil compared to control and other treatment groups. Treatment group G offered blend of clove essential oil and fumaric acid showed numerically lower *E. coli* count among all treatment groups.

The structure of essential oils is one of the central factors determining the efficacy of essential oils as antimicrobial and antioxidant activities [31]. Clove oil contains about 831 g/kg of eugenol [32]. The suppression of harmful microorganisms resulted in better growth and metabolism of beneficial microbes, which may have improved the growth performance observed in this study.

The present findings are in the line of many investigations where the antimicrobial properties of plant essential oils have been confirmed [33, 34]. Many researchers reported that essential oils derived from oregano, clove and anise have been posse's antimicrobial properties [35, 36]. Similarly, Hashemipour *et al.* [37] studied the effect of EO (thymol and carvacrol mixture) on ileal microbial population in broiler chickens and observed that coli population was significantly decreased in birds fed 100 and 200 mg/kg EO. Valero and Salmeron [38], noted that essential oil molecules from nutmeg, peppermint, clove, cinnamon and thyme have a positive effect against Bacillus.

The findings are also corroborate with Hussein *et al.* [39] revealed that the intestinal bacterial population, coliforms, Escherichia coli, and Salmonella spp. in the ileal content were lower in groups treated with cold-pressed clove oil (1.5 ml/kg) compare to control group. Nunez and Aquino [32] reported that the main compositions of clove are eugenol (831 g/kg), β caryophyllene (68.8 g/ kg), α-humulene (24.8 g/kg), oxicaryophylene (35.9 g/kg) and eugenyl acetate (24.1 g/kg). Eugenol is primarily responsible for bacteriocidal/ bacteriostatic properties [40, 41].

The findings in the present study are in analogy with reports of Arzu *et al.* [42] revealed that dietary organic acid blend and oregano essential oil combination significantly decreased ileum pH and Clostridium perfringens count in the ileum content. As per our findings Gunal *et al.* [43] also revealed that the use of organic acid mixture in broiler chicken diet significantly decreased the total bacterial and gram negative bacterial counts. Moharrery and Mahzonieh [44] recorded that broiler chicken fed diet with malic acid decrease in *E.Coli* population in the intestines. Some researchers reported that the feed supplementation of some materials, such as organic acids and essential oils have been used to inhibit bacterial growth [45, 46] and to reduce the bacterial load of carcasses [47]. The suppression of harmful microorganisms resulted in better growth and metabolism of beneficial microbes, which may have improved the growth performance observed in this study.

Results of presents study are in agreement with results of studies in which different essential oils or organic acids were added to poultry diets. Aksit *et al.* [48] reported that supplementation of organic acid combination (for each kg of the diet, 200 mg lactic acid, 250 mg formic acid, and 80 mg propionic acid) or essential oil (for each kg of the diet 15 mg Origanum unites) could be beneficial to reduce microbiological load thus preventing food poisoning and early spoilage of chicken meat.

Economics of broiler production

The economics of broiler production of all the treatment groups was worked out by considering the prices of inputs prevalent in the market at the time of experiment. The broilers were sold at the rate of Rs. 80/kg on live body weight basis. Other expenses like labour, electricity and miscellaneous expenses were also considered as uniform for all the treatment groups. The details of the same are presented in Table 5. From the table, it is observed that cost of production (Rs. /bird) was 155.43, 154.34, 167.46, 163.91, 161.17, 168.79, 163.90 and 161.65 for treatment groups A, B, C, D, E, F, G and H, respectively. Whereas, the cost of production (Rs. /kg live weigh) in treatment groups A to H was 71.60, 70.02, 77.04, 70.52, 69.95, 76.28, 67.45 and 70.15, respectively.

By considering the selling price of broilers at Rs.80 per kg on live body weight basis, the net profit (Rs./ bird) were 8.40, 9.98, 2.96, 9.48, 10.05, 3.72, 12.55 and 9.85 for the treatment groups A, B, C, D, E, F, G and H, respectively.

The result on economics of broiler production revealed that broiler offered diet with blend of clove essential oil and fumaric acid proved to be economically most beneficial.

Among different dietary treatments, the lowest feed cost per kg live weight gain for total period was observed in treatment G which might be due to better FCR in this group.

Present findings corroborate with Borazjanizadeh *et al.* [49] who evaluated the economic value of clove and oregano on broiler chickens diets. Treatments were 0, 0.5 and 1% of clove and oregano that showed significant effects on feed intake and the cost of 1 kg feed and cost of 1 kg meat production. Nath *et al.* [50] reported tulsi (*Vitex negundo*), black pepper (*Piper nigrum*) and clove (*Curcuma longa*) extract is economic and safe in broiler production. Singh *et al.* [51] found highest benefit: cost ratio in cinnamon at 0.5 groups, and it was lowest in cinnamon at 1.0% and cinnamon at 1.5% groups. Mustafa and Mukhtar [52] the addition of 200g/kg Mixture of EO (Anise, Clove, Caraway; 1:1:1) recorded economic benefits. Singh *et al.* [53] lowest feed cost/kg body weight gain was observed in broilers fed diet supplemented with calcium sorbate @ 3% with better economics.

Table 5: Economics of broilers fed Clove essential oil and different organic acids

Sr. No.	Parameter	Dietary Treatment Groups							
		A	B	C	D	E	F	G	H
1	Chick cost (Rs.)	38	38	38	38	38	38	38	38
2	Feed intake (g)								
	a) Pre-starter	114.10	112.57	116.48	114.02	112.93	108.80	114.95	115.22
	Starter	756.43	754.16	749.59	793.48	772.55	764.56	851.56	766.59
	b) Finisher	2894.72	2813.53	2805.60	2984.32	2879.93	2798.39	2876.91	2854.90
	Total Feed Intake (g)	3765.26	3680.25	3671.67	3891.82	3765.41	3671.74	3843.42	3736.71
3	Feed price per kg (Rs.)								
	a) Pre-starter	28.83	29.19	32.83	30.08	30.35	33.19	30.44	30.71
	b) Starter	29.00	29.36	33.00	30.25	30.52	33.36	30.61	30.88
	c) Finisher	28.40	28.76	32.40	29.65	29.92	32.76	30.01	30.28
4	Feed cost per bird (Rs.)								
	a) Pre-starter	3.29	3.29	3.82	3.43	3.43	3.61	3.50	3.54
	b) Starter	21.94	22.14	24.74	24.00	23.58	25.50	26.07	23.67
	c) Finisher	82.21	80.91	90.90	88.48	86.16	91.67	86.33	86.44
5	Total feed cost per bird (Rs.)	107.43	106.34	119.46	115.91	113.17	120.79	115.90	113.65
6	Miscellaneous cost per bird (Rs.)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
7	Net cost of production per bird (Rs.)	155.43	154.34	167.46	163.91	161.17	168.79	163.90	161.65
8	Cost of production per kg live weight (Rs.)	71.60	70.02	77.04	70.52	69.95	76.28	67.45	70.15
9	Live body weight at the end of 6th week (g)	2170.81	2204.15	2173.66	2324.41	2304.05	2212.67	2429.89	2304.43
10	Returns on sale @ Rs. 80 per kg live body weight	173.66	176.33	173.89	185.95	184.32	177.01	194.39	184.35
11	Net profit per bird (Rs.)	18.23	21.99	6.44	22.04	23.16	8.23	30.49	22.70
12	Net profit per kg(Rs.)	8.40	9.98	2.96	9.48	10.05	3.72	12.55	9.85

Conclusions

Essential oil combined with organic acids administration in feed displayed synergistic modulation effects on immunity and gut health which is very important for the exploration of new alternatives for antibiotic growth promoters in broiler production. Thus, the present study shows that administration of combination of clove essential oil and organic acids exerts a positive effect on gut health and enhancement of immunity with a concomitant enhancement in economics of broiler production.

References

1. Oviedo-Rondón EO. Holistic view of intestinal health in poultry. Animal Feed Science Technology. 2019; 250:1–8.
2. Phillips I. Withdrawal of growth-promoting antibiotics in Europe and its effects in relation to human health. International Journal Antimicrobial Agents. 2007; 30:101–7.
3. Sethiya NK. Review on natural growth promoters available for improving gut health of poultry: an alternative to antibiotic growth promoters. Asian Journal of Poultry Science. 2016; 10:1–29.
4. Pirgozliev V, Murphy T, Owens B, George J, McCann ME. Fumaric and sorbic acid as additives in broiler feed. Research in Veterinary Science Livestock Research for Rural Development. 2008; 84 (3):387-394.
5. Ao T, Cantor AH, Pescatore AJ, Ford MJ, Pierce JL, Dawson KA. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. Poultry Science. 2009; 88: 111-117.
6. Khan SH, Iqbal J. Recent advances in the role of organic acids in poultry nutrition. Journal Applied Animal Research. 2016; 44:359–69
7. Giannenas I, Florou-Paneri P, Papazahariadou M, Christaki E, Botsoglou, Spais AB. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with Eimeria tenella. Archives pet food. 2003; 57:99-106.
8. Botsoglou NA, Christaki E, Florou–Paneri P, Giannenas I, Papageorgiou G, Spais AB. The effect of a mixture of herbal essential oils or α–tocopheryl acetate on performance parameters and oxidation of body lipid in broilers. South African Journal Animal Science. 2004; 34:52–61.
9. Gole MA, Manwar SJ, Kuralkar SV, Kawitkar SB, Waghmare SP, Chaudhary SP *et al*. Studies on supplementation of peppermint essential oil and organic acids on performance and gut health of broilers. Journal of Entomology and Zoology Studies. 2018; 6(6):453-459
10. BIS. Bureau of Indian Standards, Poultry Feeds Specification (5th Revision). IS: 1374, Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi-11, 2007.
11. OIE, Manual of standards for diagnostic test and vaccines, off, Int. Epizootics, Paris, 1992.
12. DIFCO Manual of microbiological culture media. 1977; 2:646-647.
13. ISO Recommendation of the meeting of the subcommittee, International Organization for Standardization, on meat and meat products. ISO/TC-36/Sc-6. The Netherlands, 1995, 10-18.
14. Snedecor GW, Cochran WG. Statistical Methods. 8th Edn IOWA State University Press, Ames, USA, 1994.
15. Awaad MHH, Abdel-Alim GA, Sayed KS, Ahmed KA, Nada AA, Met-alii ASZ *et al*. Immunostimulant effects of essential oils of peppermint and eucalyptus in chickens. Pakistan Veterinary Journal. 2010; 2:61–66.
16. Barbour EK, Saade MF, Nour AMA, Kayali G, Kodess S, Ghannam R, Shaib H. Evaluation of essential oils in the treatment of broilers co-infected with multiple respiratory etiologic agents. International Journal of Applied Research Veterinary Medicine. 2011; 4:317–333.
17. Faramarzi S, Bozorgmehrifard MH, Khaki A, Moomivand H, Ezati MS, Ra-so ulinezhad S *et al*. Study on the effect of Thymus vulgaris essential oil on humoral immunity and performance of broiler chickens after La Sota vaccination. Ann. Biol. Res. 2013; 6:290–294.

18. Youdim KA, Deans SG. Dietary supplementation of oregano (*Thymus vulgaris* L.) essential oil during the lifetime of the rat: its effects on the antioxidant status in liver, kidney and heart tissues. Mech Ageing Dev. 1999; 109:163-175.
19. Acamovic T, Brooker JD. Biochemistry of plant secondary metabolites and their eff ects in animals. Proc. Nutrition. Society. 2005; 64:403–412.
20. Silveira SRC, Andrade LN, Sousa DP. A Review on Anti-Inflammatory Activity of Monoterpenes. Molecules. 2013; 18:1227-1254.
21. Hashemipour H, Kermanshahi H, Golian A, Veldkamp T. Eff ect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. Poultry Science. 2013; 92(8):2059-69.
22. Saleh N, Allam T, El-latif AA, Ghazy E. The Effects of Dietary Supplementation of Different Levels of Thyme (Thymus vulgaris) and Ginger (*Zingiber officinale*) Essential Oils on Performance, Hematological, Biochemical and Immunological Parameters of Broiler Chickens. Global Veterinaria. 2014; 12(6):736-744.
23. Sangoh P, Min RC, Byung-sung P, Jong H. The meat quality and growth performance in broiler chickens fed diet with cinnamon powder. Journal Environment Biology. 2013; 34:127-133.
24. Ozek K, Wellmann KT, Ertekin B, Tarım B. Effects of dietary herbal essential oil mixture and organic acid preparation on laying traits, gastrointestinal tract characteristics, blood parameters and immune response of laying hens in a hot summer season. Journal of Animal Feed Science. 2011; 20:575–586.
25. (25) Ashour EA, Alagawany M, Reda FM, ME Abd El-Hack. Eff ect of supplementation of yucca schidigera extract to growing rabbit diets on growth performance, carcass characteristics, serum biochemistry and liver oxidative status. Asian Journal Animal Veterinary Adv. 2014; 9:732-742.
26. Alagawany MM, Farag MR, Dhama K, Abd El-Hack ME, Tiwari R, Alam GM. Mechanisms and Beneficial Applications of Resveratrol as Feed Additive in Animal and Poultry Nutrition: A Review. International Journal of Pharmacology. 2015; 11:213-221.
27. Dehghani N, Jahanian R. Effects of dietary organic acid supplementation on immune responses and some blood parameters of broilers fed diets with different protein levels. World's Poultry Science Journal. 2012; 39:569–575.
28. Abdel-Fattah SA, Sanhoury MHE, Mednay NME, Abdul-Azeem F. Thyroid activity of broiler chicks fed supplemental organic acids. International Journal of Poultry Science. 2008; 7:215- 222.
29. Rahmani HR, Speer W. Natural additives influence the performance and humoral immunity of broilers. International Journal of Poultry Science. 2005; 4:713-717.
30. Khan Asma, Nagra SS. Effects of organic feed supplements on live performance, biochemical profile and immune response of broiler chicks. Ind. Journal Poultry Science. 2008; 43(1):45-48.
31. Patra AK. An overview of antimicrobial properties of different classes of phytochemicals. In Dietary Phytochemicals and Microbes, Springer, The Netherlands. 2012; 1:32.
32. Nunez L, Aquino MD. Microbicide activity of clove essential oil (*Eugenia caryophyllata*). Braz. Journal Microbiology. 2012; 43:1255–1260.
33. Lis-Balchin M, Deans SG. Studies on the potential usage of mixtures of plant essential oils as synergistic antibacterial agents in foods. Phytotherapy Research. 1998; 12:472-475.
34. Juven BJ, Kanner J, Schved F, Weisslowicz H. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. Journal of Applied Bacteriology. 1994; 76:626-631.
35. Dorman HJD, Deans SG. Antimicrobial agents from plants: Antimicrobial activity of plant volatile oils. Journal Applied Microbiology. 2000; 88:308-316.
36. Singh G, Kapoor IP, Pandey SK, Singh UK, Singh RK. Studies on essential oils: Part 10; antibacterial activity of volatile oils of some spices. Phytotheropy Research. 2002; 16:680-682.
37. Hashemipour H, Kermanshahi H, Golian A, Veldkamp T. Eff ect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. Poultry Science. 2013; 92(8):2059-69.
38. Valero M, Salmeron MC. Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. International Journal of Food Microbiology. 2003; 85:73-81.
39. Hussein Mohamed MA, Mohamed E, Abd El-Hack, Mahgoub SA, Saadeldin IM, Swelum AA. Effects of clove (*Syzygium aromaticum*) oil on quail growth, carcass traits, blood components, meat quality, and intestinal microbiota. Poultry Science. 2019; 98:319-329.
40. Walsh SE, Maillard JY, Russell AD, Catrenich CE, Charbonneau DL, Bartola RG. Activity and mechanisms of action of selected biocidal agents on Gram-positive and −negative bacteria. Journal Applied Microbiology. 2003; 94:240–247.
41. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food Cont. 2010; 21:1199–1221.
42. Arzu A, Deniz G,Onnan A, Gencoglu H, Kara cagada. Effects of a combination of dietary organic acid blend and oregano essential oil (Lunacompacid® Herbex dry) on the performance and Clostridium perfringens proliferation in the ileum of broiler chickens. Journal of Biology and Environmental Science. 2014; (22):61-65.
43. Gunal M, Yayli G, Kaya O, Karahan N, Sulak O. The effect of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. International Journal of Poultry Science. 2006; 5(2):149-155.
44. Moharrery A, Mahzonieh M. Effect of malic acid on visceral characteristics and coliform counts in small intestine in the broiler and layer chickens. International Journal of Poultry Science. 2005; 4(10):761-764.
45. Teissedre PL, Waterhouse AL. Inhibition of oxidation of low-density lipoproteins by phenolic substances in different essential oils varieties. Journal of Agricultural and Food Chemistry. 2000; 48:3605-3801.
46. Lambert RJW, Skandamis PN, Coote PJ, GJE Ny-Chas. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thy-mol, and carvacrol. Journal of Applied Microbiology. 2001; 91:453-462.

47. Izat AL, Tidwell NM, Thomas RA, Reiber MA, Adams MH, Colberg M *et al.* Effects of a buffered propionic acid in diets on the performance of broiler chickens and on microflora of the intestine and carcass. Poultry Science. 1990; 69:818-826.
48. Aksit M, Goksoy E, Filiz Kok D Ozdemi, Ozdoan M. The impacts of organic acid and essential oil supplementations to diets on the microbiological quality of chicken carcasses Arch. Geflugelk. 2006; 70(4):168–173.
49. Borazjanizadeh M, Eslami M, Bojarpour M, Chaji M, Fayazi J. The effect of clove and oregano on economic value of broiler Chickens diet under hot weather of Khuzesta. Journal of Animal and Veterinary Advances. 2011; 10(2):169-173.
50. Nath DD, Rahman MM, Akter F, Mostofa M. Effects of Tulsi, Black pepper and Cloves extract as a growth promoterr in broiler. Bangladesh Journal of Veterinary Medicine. 2012; 10(1and 2):33-39.
51. Singh J, Sethi APS, Sikka SS, Chatli MK, Kumar P. Effect of cinnamon (*Cinnamomum cassia*) powder as a phytobiotic growth promoter in commercial broiler chickens. Animal Nutrition and Feed Technology. 2014; 14:471-479.
52. Mustafa DBM, MA Mukhtar. Effect of mixture of three herbal essential oils on performance, carcass yield and blood serum constituents of broiler chicks. World Journal of Pharmacy and Pharmaceutical Science. 2016; 5(2):63-72.
53. Singh Tomar, Sunil Nayak, Baghel RPS, Malapure CD, Kumar Govil, Dinesh Thakur. Organic acids supplementation in the diets and performance of broiler chicken. Indian Journal of Animal Nutrition. 2017; 34(4):458-462.