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RESEARCH ARTICLE

Effect of Dietary Essential Oils and/or Humic Acids on Broiler Performance, Microbial Population of Intestinal Content and Antibody Titres in the Summer Season

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Summary

This study was conducted to observe the effect of essantial oils and/or humic acids in broilers reared farm condition (without suitable techical equipment) during summer season. A total of two hundred male chicks broilers (Ross-308) aged one day were weighed and randomly allocated to five treatment groups each containing 40 chicks. Each grop was divided into four replicate. Chicks were assigned to the basal diet (CON) and the basal diet supplemented with 250 ppm antibiotic (ANTI), 1000 ppm essential oils (EO), 1500 ppm humic acids (H) and combination of same levels of EO plus humic acids (EO+H) diet until 42 days of age, respectively. The colony forming units (CFU) of *Escherichia coli* in the digesta of birds fed either the diet supplemented antibiotic and the diet supplemented with essential oils and/or humic acids were significantly lower than in those given control. However, inclusion of essential oil and/or humic acids increased the CFU of *Lactobacilli* compare to those antibiotic supplementing. Antibody titres against Newcastle Disease Virus (NDV) were tending to numerically increase in all supplemental diets. At the end of the experiment, essential oils and/or humic acids did not show favorable effect in animal performance. Supplements did not alter the carcass traits.

Keywords: *Broiler, Summer Season, Essential Oils, Humic acids, Performance*

Etçi Piliçlerde Esansiyel Yağlar ve/veya Humatın Yaz Sezonunda Performans, İnce Bağırsak Mikrobiyel Populasyonu ve Antikor Titreleri Üzerine Etkisi

Özet

Bu araştırma, sıcak yaz aylarında uygun teknik donanım desteğinin olmadığı çiftlik şartlarında beslenen etçi piliç rasyonlarında esansiyel yağ ve/veya humik asit ilavesinin etkilerini gözlemlemek amacıyla yapıldı. Araştırmada, bir günlük yaşta 200 adet (Roos-308) civciv her birinde 40 civciv bulunacak şekilde rasgele dağıltılarak 5 gruba ayrıldı. Her grup dört tekrar grubundan oluşturuldu. Civcivlere temel diyet (kontrol), temel diyete ilave edilen 250 ppm antibiyotik (ANTI), 1000 ppm esansiyel yağ karışımı (EO), 1500 ppm humik asit (H) ve 1000 ppm esansiyel yağ karışımı +1500 ppm humik asit (EO+H) şeklinde oluşturulan yemler 42 gün süreyle yedirildi. Kontrol grubuna göre tüm katkılı gruplardaki piliçlerin bağırsak içeriğinde, *Escherichia coli* koloni oluşturma birimi (CFU) önemli derecede azaldı. Bununla birlikte, *Lactobacilli* koloni oluşturma birimi (CFU) bakımından, antibiyotik tüketen tüketen piliçlerin bağırsak içeriğinde, önemli derecede düşüş gözlenirken; esansiyel yağ ve/veya humik asit tüketen gruplarla kontrol grubu arasında bir farklılık gözlenmedi. Newcastle Virusuna (NDV) karşı antikor titresi, katkılı gruplarda kontrole göre artma eğilimi gösterdi. Araştırma sonunda esansiyel yağ ve/veya humik asit ilavesi hayvansal performans ve karkas özellikleri bakımından olumlu bir etki yapmadı.

Anahtar sözcükler: *Broiler, Yaz sezonu, Esansiyel yağ, Humik asit, Performans*

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INTRODUCTION

Thermoneutral requirements of birds have an important role in keeping their high performance. The optimum temperature for performance is likely to be 18 to 22°C for growing broilers [1]. Unfavorable changes may occur in bacterial microflora, when normal environment temperature (averaging 25°C) raise up to high (averaging 30°C). In summer season, it is difficult to control on farm temperature especially, without suitable technical equipment. High ambient temperature may induce unfavorable changes in intestinal microbiota and by this may have adverse effect on growth performance and feed efficiency [2]. Feed additives such as antibiotic, probiotics, prebiotics may be need to diminish adverse effect of high environmental temperature to maintenance for normal microbial balance in intestines of the birds reared in summer season. The ban on the use of antibiotics as growth promotors has stimulated the search for alternative feed supplements in animal production. Herbs, spices, and various plant extract have received increased attention as possible antibiotic growth promotors replacement. It is clear that controlling the microflora could positively influence birds' performance and dietary feed additives such as probiotics [3], prebiotics [4] humic acids [5] and essential oils [6] may enrich the diversity of *Lactobacillus*.

Lactobacillus species may repress pathogenic microorganism as *aerobic bacteria, coliform, E.coli, Enterococcus* in chicken jejunum and caecum, by this the microbial balance may be restored and the natural stability of jejunal and caecal microbiota of broiler chicken having suffered from heat stress may be maintained [2,7]. EO extracted from herb and spices are a complex mixture of various compounds, which consists of aromatic and volatile substances. EO basically consist of two classes of compound, the terpens and phenylpropenes. Terpens and phenylpropenes are synthesized by the mevalonic and shimic pathway, respectively. Thymol and carvacrol are classified as mono-terpenoids or isoprenoids [8]. EO have long been recognized because of their anti-microbial activity. It has been suggested that their lipolitic property [9] and chemical structure [10,11] could play a role.

Humic acids, a part of fertilizers, are derived from plant matter decomposed by bacteria and contain humus, humic acid, fulvic acid, ulmic acid, and some microelements [12]. The humic acids to come into existence such as phenols, carbohydrates and amino acids transform into much more complex compounds [13]. Humic acids stimulate the immune system receptors in the gut lining to protect against pathogens and stabilizes. It was also stated humic acids would be an alternative to antibiotic growth promoters in broiler diets [14].

However, there are no published reports related to the using of present additives on broiler performance during summer season as we know. Therefore, the aim of this study was to investigate the applicability of essential oils and/or humic acids in broilers reared farm condition (without suitable technical equipment) during summer season.

MATERIAL and METHODS

A total of two hundred male chicks broilers (Ross-308) aged one day were weighed and randomly allocated to five treatment groups each containing 40 chicks. Each group was divided into four replicate. All birds were fed a starter diet until 21 d of age and a finisher diet until 42 d of age. Diets were formulated according to NRC [15] and analyzed by the AOAC [16]. The ingredients and calculated composition of commercial basal diets are given *Table 1*. Feeding programmes were as follows; 1) chicks did not receive any additives (control) diets, 2) chicks received 250 ppm flavomycine (ANTI), 3) chicks received 1000 ppm essential oils (EO), 4) chicks received 1500 ppm humic acids (H), and 5) chicks received combined 1000 ppm essential oils plus 1500 ppm humic acids (EO+H) diets. Feed and water were offered for ad libitum consumption, and lighting was continuous throughout experimental period. Experiment was carried out during the summer season (July-August) on the Application and Research Farm of the Veterinary Faculty, Mustafa Kemal University, without suitable technical equipment. The ambient temperature and humidity were daily recorded throughout the experimental period *(Table 2)*.

A commercial blend of EO, *Fitococci*™ contained 463 g active ingredients, basically including carvacrol and tyhmol from thyme oil, origanium oil, garlic oil, anise oil and, fennel oil. *Farmagulator-Dry Humic acids* ™, each kilogram of humic acids contained 160 mg polymeric polyhydroxy acids (humic, fulvic, ulmic, and humatomelanic acids), 663.3 mg SiO_2, and other minerals (Mn, 50 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.5 mg; and Al, Na, K, Mg, and P in trace amounts) were provided Farmavet International Inc., Kocaeli, 41400, Turkey.

Table 1. *Ingredient composition of the experimental diets*
Tablo 1. *Deneme rasyonlarının bileşimi*

PARAMETERS	Control		ANTI		EO		H		EO+H	
	0-3 wk	4-6 wk	0-3 wk	4-6 wk	0-3 wk	4-6 wk	0-3 wk	4-6 wk	0-3 wk	4-6 wk
Ingredients, %										
Maize	45.0	45.0	45.0	45.0	45.0	45.0	45.0	45.0	45.0	45.0
Wheat	4.0	6.0	4.0	6.0	4.0	6.0	4.0	6.0	4.0	6.0
Bran	4.0	7.0	4.0	7.0	4.0	7.0	4.0	7.0	4.0	7.0
Soybean meal	24	20	24	20	24	20	24	20	24	20
Cotton seed meal	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Fish meal	7.0	5.0	7.0	5.0	7.0	5.0	7.0	5.0	7.0	5.0
Sunflower meal	7.0	8.0	7.0	8.0	7.0	8.0	7.0	8.0	7.0	8.0
Limestone	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
DCP	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Salt	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vit-Min. premix *	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Flavomycine	--	--	*0.025*	*0.025*	--	--	--	--	--	--
Fitococci™	--	--	--	--	*0.1*	*0.1*	--	--	*0.1*	*0.1*
Farmagulator ™	--	--	--	--	--	--	*0.15*	*0.15*	*0.15*	*0.15*
Chemical analysis, %										
Crude matter	90	89.5	90	89.5	90	89.5	90	89.5	90	89.5
Crude protein	23	20.0	23	20.0	23	20.0	23	20.0	23	20.0
Crude ash	5.6	6.0	5.6	6.0	5.6	6.0	5.6	6.0	5.6	6.0
Ether extract	6.7	9.8	6.7	9.8	6.7	9.8	6.7	9.8	6.7	9.8
Calculated analysis										
ME, MJ/kg	12.97	13.38	12.97	13.38	12.97	13.38	12.97	13.38	12.97	13.38
Ca, %	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8
P, %	0.6	0.7	0.6	0.7	0.6	0.7	0.6	0.7	0.6	0.7
Methi+Cystine, %	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8
Lysine, %	1.2	1.1	1.2	1.1	1.2	1.1	1.2	1.1	1.2	1.1

***:** *Supplied per kilogram of diet:* Vitamin A 15.000 IU; cholecalsiferol 1.500 ICU; vitamin E, 30 IU; menadion, 5.0 mg; thiamin, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; pantothenic acid, 8.0 mg, pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B12, 15 µg; Mn, 80.0 mg; Zn, 60 mg; Fe, 30.0 mg; Cu, 5.0 mg; I, 2.0 mg; Se, 0.15 mg

Table 2. *Temperature and relative humidity levels of research farm throughout experiment*
Tablo 2. *Deneme boyunca araştırma çiftliğinin sıcaklık ve nem seviyeleri*

Week	Temperature (°C)	Humidity (%)
1	32.7	65.5
2	31.3	62.1
3	31.6	60.7
4	32.1	64.5
5	30.7	62.8
6	32.2	61.2

At 42 days of age, digest content samples from the small intestine (from the distal end of the duodenum to the ileo-cecal junction) and cecum of 6 birds from each replicate were collected into glass containers at slaughter. Samples put on ice till they were transported to the laboratory for enumeration of microbial populations.

Representative 1 g digest contents were homogenized with 9 ml of sterile 0.1% peptone in a stomacher. Decimal dilutions were prepared in 0.1% peptone water. The Escherichia coli was enumerated on TBX Medium (Oxoid CM945) at 30°C for 4 h then 44°C for 18 h. (Blue-green colonies was determined *E. Coli*). Lactobacillus spp. were enumerated on Rogosa Agar (Oxoid CM627) after anaerobic incubation at 30°C for 5 days.

At 7 days of age, all chicks were vaccinated with Newcastle Disease Virus [(Nobilis Ma5 + Clone30 (6.0 log10 EID50)] via drinking water. Blood samples from the wing veins of 6 birds from each replicate were collected into tubes at 21 days of age to determine maximal antibody titres. Tubes were centrifuged for 15 min at 1000 rpm to obtain serum which was inactivated at 56°C for 30 min and was stored at 20°C till the hemagglutination inhibition (HI) titres were determined. HI titres were measured by using procedure of micro plate HI test [17]. All titres were recorded as log2 of the reciprocal of the endpoint dilution.

Body weights (BWs) of broilers in each pen were individually measured on a weekly basis. At the same days, feed intake was recorded and feed conversion ratio (FCR) was calculated on a pen basis (kg feed/kg gain). Mortality was recorded as it occurred. At the end of the experiment, 3 birds per each replicate, whose body weights were nearest

to average body weight of their own groups, slaughtered to determine carcass traits. Some parts of carcass (thigh, wing, back-breast, neck and waist) of each bird were weighed and recorded.

Statistical analysis

The data between groups for performance parameters and all other values were analyzed by one-factor ANOVA using the general linear models procedure of SAS software [18] for the main effect of treatments. Multiple-comparison procedure [19] was used when the F-test was significant (P<0.05).

RESULTS

Effects of dietary supplementation on intestinal microbial CFU are presented in *Table 3*. The inclusion of either antibiotic or essential oils and/or humic acids reduced (P<0.05) the CFU of *E. coli* compared to those fed the basal diet

Table 3. *Microbial enumeration (log10 cfu/g) in ileo-cecal digesta and antibody titres (log2) against Newcastle virus of broilers fed experimental diets*
Tablo 3. *Deneme rasyonlarını tüketen etçi piliçlerin ileo-sekal mikroorganizma sayısı (log10 cfu/g) ve Newcastle virusuna karşı anitikor titreleri (log2)*

Parameters	Diets [1]					*F*
	Control	ANTI	EO	H	EO+H	
E. Coli	4.1±0.14 [a]	2.9±0.28 [c]	3.4±0.11 [b]	3.6±0.27 [b]	3.5±0.13 [b]	19.14 **
Lactobacilli	4.0±0.18 [ab]	3.4±0.22 [c]	4.3±0,14 [a]	4.2±0.17 [ab]	4.1±0.24 [ab]	11.79 **
Antibody titres	5.02±0.27 [b]	5.31±0.16 [ab]	5.63±0.26 [a]	5.51±0.19 [a]	5.53±0.32 [a]	1.01 -

Means represent from 24 birds per treatment
a,b Means values within a row having differing superscripts are significantly different by least significant differences test (P<0.05)
[1] Flavomycine 250 ppm (ANTI); 1000 ppm essential oil extracts from Fitococci ™ (EO); 1500 ppm Humic acids from Farmagulatör DRY™ (H) ; 1000 ppm essential oil extracts plus 1500 ppm Humic acids (EO+H)

Table 4. *Performance parameters of broilers fed experimental diets*
Tablo 4. *Deneme rasyonlarını tüketen etçi piliçlerin performans değerleri*

Parameters	Diets [1]					*F*
	Control	ANTI	EO	H	EO+H	
Body Weight, g						
1. d	41±0.61	41±0.55	42±0.53	40±0.74	40.3±0.51	1.50 -
7.d	121±2.99 [b]	141±3.49 [a]	124±3.26 [b]	115±3.13 [b]	116±3.71 [b]	10.70 **
14.d	297±8.63 [b]	349±7.49 [a]	304±9.06 [b]	290±10.09 [b]	279±8.58 [b]	9.53 **
21. d	600±15.54 [bc]	695±15.54 [a]	617±15.43 [b]	576±16.59 [bc]	568±15.18 [c]	10.83 *
28. d	1008±23.26 [b]	1117±28.69 [a]	1023±21.53 [b]	1007±33.65 [b]	972±21.50 [b]	3.23 *
35. d	1422±37.74 [b]	1590±29.73 [a]	1439±33.37 [b]	1452±39.58 [b]	1404±33.64 [b]	3.65 **
42. d	1847±39.74 [b]	1955±39.29 [a]	1868±44.58 [b]	1834±46.91 [b]	1748±44.58 [c]	2.45 *
Feed Intake g/birds						
1to7 d	102 ±4.14 [b]	116±1.47 [a]	108±2.52 [ab]	106±6.29 [ab]	97±4.41 [b]	3.23 *
8 to14 d	258±10.72	269±2.59	264±5.72	262±11.06	248±21.18	0.41 -
15 to 21 d	552±15.89	543±39.97	563±14.80	539±21.71	507±19.04	0.77
22 to 28 d	751±20.01	733±30.71	777±13.42	730±18.86	720±13.65	1.17 -
29 to35 d	859±16.70	818±28.10	884±30.25	812±24.81	861±10.24	1.74 -
36 to 42 d	938±13.27 [ab]	899±47.76 [b]	872±43.89 [b]	877±20.48 [b]	1001±20.30 [a]	2.76 *
1 to 42 d	3460±33.89	3379±39.74	3468±43.52	3327±52.71	3434±37.93	0.70 -
Feed:gain, g:g						
1 to7 d	1.30±0.35 [ab]	1.17±0.36 [b]	1.35±0.38 [a]	1.40±0.80 [a]	1.29±0.16 [ab]	3.30 *
8 to14 d	1.46±0.11 [ab]	1.29±0.01 [b]	1.47±0.52 [ab]	1.51±0.50 [a]	1.51±0.61 [ab]	1.93
15 to 21 d	1.83±0.06 [ab]	1.58±0.13 [b]	1.69±0.05 [ab]	1.90±0.11 [a]	1.76±0.03 [ab]	1.86
22 to 28 d	1.84±0.06	1.74±0.08	1.92±0.05	1.71±0.09	1.79±0.06	1.40 -
29 to 35 d	2.08±0.35	1.74±0.10	2.13±0.17	1.82±0.10	1.99±0.24	0.87 -
36-42 d	2.41±0.16	2.46±0.17	2.20±0.29	2.42±0.36	2.91±0.31	0.80 -
1 to 42 d	1.92±0.06 [ab]	1.77±0.06 [b]	1.91±0.04 [ab]	1.87±0.10 [ab]	2.01±0.05 [a]	1.58

a,b Means values within a row having differing superscripts are significantly different by least significant differences test (P<0.05)
[1] Flavomycine 250 ppm (ANTI); 1000 ppm essential oil extracts from Fitococci ™ (EO); 1500 ppm Humic acids from Farmagulatör DRY™ (H) ; 1000 ppm essential oil extracts plus 1500 ppm Humic acids (EO+H)

(control). On the other hand, the CFU of Lactobacilli was significantly higher in birds fed the diet containing essential oils than those given either the control or the diet with others dietary supplementations (P<0.01). The values for antibody titres against NDV were not significantly affected by dietary supplementation (*Table 3)*.

The values for feed intake and feed efficiency were not significantly affected by dietary treatments for 42 days period. On contrary, body weight was higher (P<0.05) in birds fed the diet containing antibiotic than those given either control or the diet with essential oils and/or humic acids *(Table 4)*. No differences in carcass traits were observed among the dietary treatments *(Table 5)*.

The observed effects of essential oils and/or humate on growth performance in chickens satisfied thermoneutral requirements are either positive [6,24,25] or non-significant [5,26]

The inclusion level of present supplements were sufficiently high according to the manufacturer's recommendation. It was expected that adding to diet commercial preparations of essential oils/ humic acids would stimulate growth performance of broilers in summer season by decreasing adverse effects of high ambient temperature. But, the supplements could not reflect their favorable antimicrobial effect in the growth performance *(Table 4)*. The values for feed intake and feed efficiency were not significantly affected by dietary

Table 5. *Some carcass traits of broilers fed experimental diets*
Tablo 5. *Deneme rasyonlarını tüketen etçi piliçlerin bazı karkas özellikleri*

Items, g	Diets [1]					*F*
	Control	ANTI	EO	H	EO+H	
Thigh	419±12.34	428±12.34	419±12.34	410±12.34	428.±12.34	0.847 -
Wing	155±5.91	162±5.91	157±5.91	147±5.91	153±5.91	0.438 -
Breast	537±15.89	574±15.89	547±15.89	537±15.89	542±15.89	0.440 -
Neck	59±2.54	61±2.54	58±2.54	61±2.54	60±2.54	0.953 -
Waist	152±4.94	157±4.94	168±4.94	151±4.94	151±4.94	0.095 -

Means represent from 12 birds per treatment
[1] Flavomycine 250 ppm (ANTI); 1000 ppm essential oil extracts from Fitococci ™ (EO); 1500 ppm Humic acids from Farmagulatör DRY™ (H) ; 1000 ppm essential oil extracts plus 1500 ppm Humic acids (EO+H)

DISCUSSION

Essential oils have long been recognized because of their anti-microbial activity [20,21]. Due to this property, essential oils have gained much attention in investigations on their potential as alternatives to antibiotics for therapeutic purposes. It has been suggested that their lipolitic property [9] and chemical structure [10,11] could play a role on their anti-microbial activity.

In this study, the supplementation of essential oils and/or decreased the colony unit forming of *E. coli* and increased the colony unit forming of *Lactobacilli* (P<0.01). This was in accordance with finding of some researchers who mentioned that EO [22] and humic acids [16] restrain pathogen bacteria as *E. coli* by increasing non-pathogens. Jang et al.[22] found that, CFU of *E. coli* decreased and CFU of *Lactobacilli* increased by addition of essential oils at the level of 50 ppm. Similarly, a blend of capsicum, cinnamaldehyde and carvacrol lowered the number of *E. coli* and *Clostridium perfiringens* in ceca [23].

treatments for 42 days period. On contrary, body weight was higher (P<0.05) in birds fed the diet containing antibiotic than those given either control or the diet with essential oils and/or humic acids. The efficacy of EO on growth-related parameters of broilers are not consistent as it was supplemented to diets at levels of 20 - 200 mg/kg diet [22].

In agreement with the present study, Jang et al.[22] found that mean body weight, feed intake, and feed efficiency of broilers were not affected by the adding of essential oils at the level of 25 and 50 mg/kg although favorable change occured in intestine. On the other hand, Karaoglu et al.[27] indicated that diets containing 0.1, 0.2 and 0.3% humic acid did not significantly affect growth performance of broilers. Contrary to the finding in the present study, Simsek et al.[28] reported that diet containing 400 mg/kg anise oils improve body weight at 42th days in normal environmental temperature, but others two levels (100 and 200 mg/kg anise oil) did not affect the body weight.

No differences in carcass traits were observed among the dietary treatments. Similarly, some

researchers found that dietary feeding EO 8 and/or humic acids [27,29,30] did not improved carcass weight, and carcass traits in broiler reared under well-nourished and comfort ambient temperature. Karaoglu et al.[27] observed that three different level of humic acids (0.1, 0.2 and 0.3%) did not affect the wing, thing, breast and leck weights of birds reared in normal ambient temperature.

The competence of alternative additives to antibiotics on broiler performance are strongly depending on environmental factors such as less hygienic conditions, less digestible diet, hot environmental temperature. Our chickens were exposed hot environment temperature (averaging 30.7-32.7°C) during experiment. Presumable, the observed lack of a growth-promoting effect could be attributed to increased environmental temperature. It is well know that a hot environment is one of the most important stressors which depress the animal performance. It is concluded that a blend of EO and /or humic acids showed a beneficial result such as decrease in *E. Coli* CFU and increase in *Lactobacilli* CFU in intestinal microflora. But this favorable action did not reflect to the broiler performance during summer season.

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