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The Effects of Dietary Clove Extract On Carcass Characteristics, Digestive Organ Size And Total Coliform Counts of Small Intestine in Broilers*

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This study was aimed to determine the effect of different levels of clove extract supplementation in diets on carcass characteristics, digestive organ size and total coliform counts of small intestine in broilers and to determine whether it could be alternative to antibiotic feed additives or not. Three hundred 3-day-old commercial broiler chicks (Ross–308) were divided into groups of 60 birds in each and randomly assigned to the five treatment diets with three replicate. Experimental groups were; consuming the basal diet (Control group) or the basal diet supplemented with 100, 200, 400 ppm of clove extract 10 ppm avilamycin. Experiment was continued 42 days. Except abdominal fat ratio ($P<0.05$), carcass characteristic and digestive organ weights were similar in all groups ($P>0.05$). Supplementing antibiotic and 400 ppm clove extract have been decreased the total coliform microorganism counts at 21st and 42nd days of the experiment ($P<0.001$). Clove extract has the positive effects on microbial population of gut and fat deposition of organism and it is natural and safety feed additive so that supplementation of 400 ppm clove extract to diets can be considered as an alternative natural growth promoter for poultry instead of antibiotics.

Keywords: *Clove extract, antibiotic, carcass characteristics, coliform microorganism, broiler.*

Karanfil Ekstraktının Etlik Piliçlerde Karkas Özellikleri, Sindirim Organları Ağırlığı ve İnce Bağırsaklardaki Toplam Koliform Bakteri Sayısı Üzerine Etkisi

Bu araştırmada, temel rasyona farklı dozlarda ilave edilen karanfil ekstraktının etlik piliçlerin karkas özelliklerini, sindirim sistemi organ ağırlığını ve bağırsaklardaki toplam koliform bakteri sayısını ne ölçüde etkileyeceği ve antibiyotik yem katkılarına alternatif olup olamayacağının tespit edilmesi amaçlanmıştır. Araştırmada, her grupta 60 adet olmak üzere beş grupta toplam 300 adet 3 günlük yaşta ticari etlik civciv (Ross–308) kullanılmıştır. Ayrıca grupların her biri 20'şer adet civciv içeren 3'er alt gruba ayrılmıştır. Rasyonlara katılan karanfil ekstraktı ve antibiyotik deneme gruplarını oluşturmuştur. Deneme grupları temel rasyona 100, 200, 400 ppm karanfil ekstraktı ve 10 ppm antibiyotik (Avilamisin) katılarak oluşturulmuştur. Karkas özellikleri ve sindirim sistemi organ ağırlıkları bakımından, karın yağı oransal değeri ($P<0.05$) haricinde gruplar arasında istatistiksel olarak farklılık tespit edilmemiştir. Rasyona ilave edilen antibiyotik ve 400 ppm düzeyindeki karanfil ekstraktı hem 21 hem de 42. günlerde ince bağırsak toplam koliform mikroorganizma sayısını önemli ölçüde düşürmüştür ($P<0.001$). Sonuç olarak; karanfil ekstraktının ince bağırsak mikrobiyel populasyonu ve vücutta yağ birikimi üzerine olan olumlu etkisi, doğal ve güvenilir olması nedeni ile antibiyotiklere alternatif olarak etlik piliç rasyonlarında 400 ppm dozda kullanılabileceği kanaatine varılmıştır.

Anahtar kelimeler: *Karanfil ekstraktı, antibiyotik, karkas özellikleri, koliform bakteri, etlik piliç.*

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Introduction

The practice of feeding livestock with subtherapeutic levels of antibiotics has been in use for over fifty years. Antibiotic usage is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (1). This acquired resistance occurs not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations. At slaughter, resistant strains from the gut may contaminate poultry carcasses and as a result poultry meats are often associated with multiresistant microorganisms. Hence, antimicrobial resistant faecal microorganism from poultry can infect humans both directly and via food. Although rare, these resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora. Therefore, the use of antibiotic growth promoters has been banned in many countries, especially in the European Union (1, 2). However, antibiotic growth promoters have been banned by Ministry of Agriculture in Turkey with an announcement that published in 2006 (3). As a result, new commercial additives of plant origin, considered to be natural products that consumers would

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accept, have been proposed to livestock producers. Herbs, spices, and various plant extracts have received increased attention as possible antibiotic growth promoter replacements. In this view, aromatic plants and essential oils extracted from these plants became interesting due to their antimicrobial (4), antioxidant (5) effects and their stimulating effects on animal performance (6-9) and digestive enzymes (10).

Eugenol is a major component of clove extract and exhibits a wide range of antimicrobial activity in vitro (11, 12). Additionally dietary antibiotics have been shown to lower intestinal weight (13). Thus, it can be suggested that spice extracts may be more effective in improving growth performance, balancing intestinal microflora and lowering intestinal weight.

Clove and its extract have been used extensively for many years in food products, perfumery, and dental and oral products due to their different medicinal properties. In addition, antiseptic, appetite and digestion stimulant (14), strong antimicrobial and antifungal (11), analgesic and anti-inflammatory (15), anesthetic (16), anti-inflammatory and anticarcinogenic (17), antiparasitic (18) and antioxidant (5) activities of clove and its ingredients have been reported. Clove extract has been studied very few as a performance enhancer. Therefore, we evaluated the effects of the different level of clove extract supplementation as growth enhancer and in vivo antimicrobial agents on carcass characteristics, digestive organ size and total coliform counts of small intestine in broilers.

Materials and Methods

Animals, Diets and Experimental Design: Three hundred 3-day old broilers (Ross-308, unsexed) obtained from a local hatchery were randomly divided into five treatment groups of 60 birds in each group that varied according to their diets. Each treatment group was further sub-divided into three regular replicates in a way to equal live weight and gender. Birds were fed a basal diet (Control), or basal diet supplemented with 10 ppm antibiotic (Avilamycin, Kartal chem., TURKEY), 100, 200 and 400 ppm clove extract (*Syzygium aromaticum*) (Ozdrog Co., Hatay, TURKEY)... Clove extract was dissolved in vegetable oil and then gently added to the standard diets for preparing clove groups. The diets were prepared freshly each day. Research rations were prepared according to NRC (19) standards and the ingredients and chemical composition of the diets are shown in Table 1. Fresh feed and water were provided daily at 08.00 h and were available ad libitum. Experiment was continued 42 days (from 3 to 45 d of age).

At the end of study (42nd day), five male and five female chicken that body weights close to group average were selected from per groups and were slaughtered for carcass characteristics. These chickens were removed from feathers, head and legs than inner organs (except from kidneys and lungs) were taken off and weighted. Carcasses were chilled for 24 h at +4 $^{\circ}$C. After than according to Institute of Turkish Standarts rules (20), rumps (from articulatio coxa), breast (from articulatio sternocostalis), wings (from articulatio humeri), neck and back were removed from carcass and these pieces were weighted together with skin.

The small intestine was immediately exposed, and the contents of the lower half of the ileum were collected into sterile stomacher bag to total coliform counting. The ileum was defined as that portion of small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileocecal junction. The weights of the proventriculus, gizzard, small and large intestines without content, pancreas and liver without gall bladder were measured individually. The same applications were carried out at 21st day of the experiment on five chicks.

The small intestine total coliform microorganism counts were determined on Violet Red Bile agar (Merck) by the methods of Arda (21) at the 21st and 42nd days of the experiment.

Chemical Analysis: Chemical composition of feed ingredients and feces samples (dry matter, crude protein, ash and ether extract) were analyzed according to the AOAC (22) procedures and crude fiber was determined by the methods of Crampton and Maynard (23).

Statistical Analysis: After tests of normality, data were subjected to analysis of variance, and when significant differences were obtained, means were further subjected to Duncan's multiple range tests by using SPSS for Windows: 11.5, SPSS inc. (24). The results were considered as significant when P values were less than 0.05 and 0.01.

Results

The effects of dietary clove extract and antibiotic on carcass characteristics of broilers are shown in Table 2. Relative weight (%BW) of selected digestive organs of broilers at 21st and 42nd days of the experiment is shown in Table 3 and 4, respectively. Total coliform microorganism counts of small intestine of broilers at 21st and 42nd days of the experiment are shown in Table 5.

Table 1. Ingredient and chemical composition of standard diets (%).

Ingredients, %	1 to 21	22 to 42
Corn	55.71	60.86
Soybean Meal (48% CP)	30.50	31.00
Vegetable Oil	4.80	4.63
Fish Meal (Anchovy, 64% CP)	5.80	-
Dicalcium Phosphate	1.40	1.40
Ground Limestone	0.90	1.20
Salt	0.25	0.33
DL-Methionine	0.13	0.07
L-Lysine	0.01	0.01
Vitamin Premix*	0.25	0.25
Mineral Premix**	0.25	0.25
Total	100	100
Analysis+Calculated, %		
Dry matter	89.71	90.06
Crude protein	23.00	19.90
Crude fiber	3.55	4.38
Crude ash	6.35	5.67
Ether extract	6.89	6.75
Calcium	0.99	0.90
Available phosphorus	0.45	0.35
Methionine+ Cystine	0.90	0.72
Lysine	1.35	1.08
ME, kcal/kg	3209	3225

*Vitamin premix supplied per 2 kg; vitamin A 12.000.000 IU; vitamin D_3 3.000.000 IU; vitamin E 50.000 IU; vitamin K_3 5.000 mg; vitamin B_1 3.000 mg; vitamin B_2 6.000mg; niacin 45.000mg; Calcium d-pantothenat 10.000 mg; vitamin B_6 7.500 mg; vitamin B_{12} 30 mg; folic acid 1000 mg; d-biotin 150 mg.
**Mineral premix supplied per 1 kg; Mn 100.000 mg; Fe 60.000 mg; Zn 60.000 mg; Cu 5.000 mg; Co 300 mg; I 1.000 mg; Se 350 mg.

Table 2. Effect of dietary antibiotic and clove extract on carcass characteristics of broilers, (Mean±SEM)[1]

			Clove extract, ppm			
Ratio (%)	Control	Antibiotic	100	200	400	F
Body weight, g	2690.50±86.71	2722.00±62.47	2692.50±86.83	2712.50±77.79	2743.00±38.96	0.09^{-}
Hot Carcass (%BW)	71.88±0.37	72.07±0.43	71.62±0.50	71.91±0.35	72.03±0.44	0.58^{-}
Cold Carcass (%BW)	71.30±0.36	71.58±0.46	71.10±0.51	71.62±0.38	71.64±0.48	0.50^{-}
Legs (%CW)	40.87±0.42	41.03±0.17	41.13±0.18	41.40±0.15	41.47±0.47	2.11^{-}
Breast (%CW)	37.21±0.36	37.29±0.45	37.49±0.32	37.48±0.44	37.48±0.40	1.32^{-}
Wings (%CW)	8.70±0.09	8.66±0.17	8.70±0.26	8.68±0.25	8.63±0.16	1.43^{-}
Back+Neck (%CW)	11.60±0.24	11.54±0.51	11.45±0.37	11.36±0.39	11.37±0.31	1.96^{-}
Abdominal fat (%CW)	1.62±0.23^{a}	1.48±0.10^{ab}	1.23±0.11^{ab}	1.08±0.12^{b}	1.06±0.11^{b}	2.90*
Heart (%BW)	0.46±0.01	0.45±0.02	0.46±0.02	0.46±0.02	0.45±0.02	0.23^{-}
Liver (%BW)	2.03±0.07	2.03±0.03	2.17±0.06	2.18±0.06	2.15±0.12	1.28^{-}
Spleen (%BW)	0.07±0.00	0.08±0.00	0.08±0.01	0.08±0.01	0.08±0.00	0.34^{-}

$^{-}$: P>0.05
*: P<0.05
$^{a, b,}$: Mean values within a row having different superscripts are significantly different.
[1]: Means represent 10 chicks of 3 pens.
BW: Slaughter body weight, **CW**: Cold carcass weight

Table 3. Effect of dietary antibiotic and clove extract on relative weight (%BW) of selected digestive organs of broilers at 21st day of the experiment, (Mean±SEM)[1]

			Clove extract, ppm			
Ratio (%BW)	Control	Antibiotic	100	200	400	F
Liver	2.77±0.12	2.70±0.14	2.87±0.08	2.82±0.13	2.73±0.12	0.08-
Pancreas	0.24±0.02	0.22±0.01	0.23±0.01	0.21±0.01	0.22±0.01	1.00-
Gizzard	1.30±0.04	1.30±0.06	1.31±0.04	1.26±0.03	1.20±0.05	1.00-
Proventriculus	0.44±0.03	0.44±0.02	0.43±0.02	0.43±0.01	0.43±0.01	0.09-
Small intestine	4.07±0.14	3.90±0.12	4.06±0.13	3.99±0.10	3.84±0.09	0.74-
Large intestine	0.96±0.03	0.95±0.02	0.95±0.02	0.95±0.01	0.93±0.01	0.31-

-: P>0.05
1: Means represent 5 chicks of 3 pens
BW: Slaughter body weight.

Table 4. Effect of dietary antibiotic and clove extract on relative weight (%BW) of selected digestive organs of broilers at 42nd day of the experiment, (Mean±SEM)[1*]

			Clove extract, ppm			
Ratio (%BW)	Control	Antibiotic	100	200	400	F
Pancreas	0.19±0.01	0.18±0.01	0.19±0.01	0.19±0.01	0.18±0.01	0.05-
Gizzard	1.26±0.06	1.39±0.05	1.29±0.06	1.29±0.03	1.28±0.03	1.16-
Proventriculus	0.34±0.02	0.33±0.01	0.34±0.01	0.33±0.01	0.33±0.01	0.17-
Small intestine	2.45±0.04	2.33±0.03	2.45±0.04	2.40±0.04	2.35±0.03	1.77-
Large intestine	1.19±0.03	1.19±0.04	1.24±0.04	1.21±0.05	1.18±0.04	0.27-

-: P>0.05
1: Means represent 10 chicks of 3 pens
*: Liver relative weights represented at Table 3
BW: Slaughter body weight.

Table 5. Effect of dietary antibiotic and clove extract on total coliform microorganism counts of small intestine of broilers, log_{10} cfu/g, (Mean±SEM)[1]

			Clove extract, ppm			
	Control	Antibiotic	100	200	400	F
21st day	5.70±0.27[a]	4.53±0.16[b]	5.72±0.54[a]	5.14±0.11[a]	4.51±0.20[b]	8.38***
42nd day	5.40±0.27[a]	2.91±0.19[b]	4.99±0.15[a]	4.39±0.63[a]	3.08±0.44[b]	8.74***

***: P<0.001.
a, b, c: Mean values within a row having different superscripts are significantly different.
1: Means represent 5 chicks of 3 pens;

Discussion

Carcass characteristics: Except abdominal fat ratio ($P<0.05$), carcass characteristic means were similar in all groups ($P>0.05$). The active ingredients of spice extracts have positive effects on digestive enzymes and digestibility so dietary supplements of them enhance growth and carcass yield (14). Contrary to our study, Simsek et al. (25) reported that adding of plant extracts to ration had positive effects on the carcass yield. Besides Guler et al. (8) reported that dietary antibiotic (0,1 %) and black cumin seeds (one of five levels 0, 0.5, 1, 2 and 3 %) were fed to broiler chickens, except heart ratio means, liver, abdominal fat, legs, wings, breast and back+neck ratio means had been positively affected with black cumin seeds supplementation especially the level of 1% black cumin.

The authors explained the lack of effect by pointing out that the birds' performance was already superior, leaving no room for growth enhancing effects of the additives. This statement could be in line with studies of Coates et al. (2) and Hill et al. (26) who demonstrated that well-nourished healthy chicks responded less to antibiotic supplements when they were housed in a carefully cleaned and disinfected place. Also the same results with our study have been reported by Simsek et al. (27) in broilers fed with dietary essential oil mix (thyme, clove and anise).

Lipidemic effect: Spices and their extracts have lipotrope effects. Several active ingredients of spices influence lipid metabolism predominantly by mobilization of fatty acids, increase preferential utilization of fats and lower perirenal adipose weight (28). In our study clove extract had also lowered perirenal adipose tissue weights and this effect was dose depended. Abdominal fat relative weights of clove extract groups were 24.07 and 16.89 percent in C–100 group, 33.33 and 27.03 percent in C-200 group, 34.57 and 28.38 percent lower than control and antibiotic groups, respectively. Similarly, several studies accomplished in this area have the same results with our study (8, 9, 25).

Digestive Organ Size: Hill et al. (26) indicated that dietary inclusion of antibiotics, given as growth promoters, reduced intestine weight by thinning the intestinal wall and shortening the gut, but in our study this effect was not noticed for antibiotic group. Small intestine relative weights were not statistically but numerically affected from dietary supplements in our study. It may be related to well-nourished healthy chicks responded less to antibiotic supplements when they were housed in a carefully cleaned and disinfected place. Similarly, Hernandez et al. (29) reported that no differences were noticed for proventriculus, gizzard, liver, pancreas and large or small intestine weights in broilers fed with dietary essential oil at 21st and 42nd days of age.

The liver relative weights were not statistically but numerically higher in clove extract groups than the others. It may be related to the active ingredients of spice extracts enhance liver metabolism and increase liver weight (30). The same result has been reported by Simsek et al. (27) in broilers consumed dietary anise oil.

In vivo antimicrobial action: In general, clove extract and antibiotic supplementation decreased total coliform microorganism counts of small intestine of broilers at 21st and 42nd days of the experiment (Table 5). This study proved in vivo antimicrobial effect of clove extract with dose dependent. And the effective dose was found to be minimum 400 ppm for balancing gut microflora.

At 21st day of the experiment the coliform microorganism counts of 1 g small intestine content in C-400 group were 20.88 and 0.44 percent lower than control and antibiotic groups, respectively. However, at 42nd day of the experiment the lowest coliform microorganism population was found in antibiotic group. Fortunately, the difference between C-400 and antibiotic groups was only 0.06 percent. Small intestine coliform microorganism counts of C-400 group were 42.96 percent lower than negative control group. The results of the other clove groups were better than control group at 42nd day of the experiment.

A wide range of in vitro antimicrobial activity of spice extracts has been proved by previous studies (11, 12, 14).

Guler et al. (4) investigated whether a mixture of essential oil from thyme and anise with 0, 100, 200 and 400 ppm levels and antibiotic with 10 ppm level supplementation could have effects on the number of cecal coliform microorganism in broilers fed with corn-soy meal based rations. Chicks fed the diets containing essential oil mixture and antibiotic showed a reduced cecal coliform microorganism counts when compared to the negative control group. And they reported that the antimicrobial activity of essential oils has increased related to its dose.

A field study conducted by Köhler (31) with a commercial preparation of essential oils showed a reduction of colony forming units of Clostridium perfringens as compared to the positive control diet containing zinc bacitracin at the level of 20 ppm. Similarly, a blend of capsicum, cinnamaldehyde and carvacrol lowered the number of Escherichia coli and Clostridium perfringens in ceca (32). These results were in accordance with our results for antimicrobial activity of spice extracts in broilers.

In conclusion clove extract improved the carcass charecteritics slightly and have shown strong antimicrobial effect on total coliforms of small intestine. Our results justify the possibility use of 400 ppm clove extract as an alternative natural antimicrobial matter and growth promoter for poultry instead of antibiotics.

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