



ARAŞTIRMA

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The Effects of Clove Extract Supplementation on Performance And Digestibility of Nutrients in Broilers*

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This study was aimed to determine the effect of different levels of clove extract supplementation in diets on performance and nutrient digestibility in broilers and to determine whether it could be alternative to antibiotics 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. Birds were fed basal diet or the basal diet supplemented with 100, 200, 400 ppm of clove extract and 10 ppm avilamycin. Experiment was continued 42 days. There were differences in body weight and daily body weight gain at 7. and 21. days measurements in 400 ppm clove extract and antibiotic supplemented groups but differences of the other periods were not statistically important. There was difference in daily feed intake in 400 ppm clove extract and antibiotic supplemented groups at 4. week of the experiment but differences of the other weeks were not statistically important. The best feed conversion ratio was in 400 ppm clove supplemented group and there were statistically important differences in feed conversion ratio among groups. The best digestibility degrees were in 400 ppm clove supplemented and antibiotic groups, then 200, 100 and Control groups followed them respectively. Clove extract has the positive effects on performance and digestion process and it is natural and safety feed additive so that 400 ppm supplementation of clove extract to diets can be considered as an alternative natural growth promoter for poultry instead of antibiotics.

Keywords: Clove extract, antibiotic, performance, digestibility, broiler.

Karanfil Ekstraktının Etlik Piliçlerde Performans ve Ham Besin Maddelerinin Sindirilme Derecesi Üzerine Etkisi

Bu araştırmada, temel rasyona farklı dozlarda ilave edilen karanfil ekstraktının etlik piliçlerin performansını ve ham besin maddelerinin sindirilme derecesini ne ölçüde etkileyeceği ve antibiyotik yem katkılarına alternatif olup olamayacağını 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. Canlı ağırlık ve günlük canlı ağırlık artışları bakımından gruplar arasında 7. ve 21. gün tartımlarında 400 ppm karanfil ekstraktı ve antibiyotik gruplarında iyileşme tespit edilirken, diğer dönemlerde elde edilen farklılıklar istatistiksel olarak önemli bulunmamıştır. Yem tüketimi bakımından 4. haftada 400 ppm karanfil ekstraktı ve antibiyotik gruplarında iyileşme tespit edilirken, diğer haftalarda gruplar arasında istatistiksel olarak bir farklılık gözlenmemiştir. En iyi yemden yararlanma oranı 400 ppm karanfil ekstraktı grubunda tespit edilmiş olup, 1., 3., 4. ve 1-6. haftalarda gruplar arasındaki fark istatistiksel olarak önemli bulunmuştur. Kuru madde, ham protein ve ham yağ sindirilebilirliği bakımından gruplar arası farklılık istatistiksel olarak önemli bulunmuştur. En iyi sindirilme dereceleri 400 ppm karanfil ekstraktı ve antibiyotik ilave edilen gruplarda tespit edilirken, bunları 200, 100 ppm karanfil ekstraktı ilave edilen ve kontrol grupları izlemiştir. Sonuç olarak; karanfil ekstraktının performans ve sindirim 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, performans, sindirilebilirlik, etlik piliç.*

Introduction

The practice of feeding livestock with subtherapeutic levels of antibiotics has been in use for over fifty years. Antibiotics affect microflora by influencing on the metabolism of the microorganisms, and suppress pathogen microbial growth in the gut (1). Usage of antibiotics has negative effects on animals and production such as residues in tissues, withdrawal period, and development of resistance in microorganisms. Therefore, the use of antibiotic growth promoters has been banned in many countries, especially in the European Union (1).

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However, antibiotic growth promoters have been banned by Ministry of Agriculture in Turkey with an announcement that published in 2006 (2). As a result, new commercial additives of plant origin, considered to be natural products that consumers would 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 (3), antioxidant (4) effects and their stimulating effects on animal performance (5-8) and digestive enzymes (9).

The observed effects of aromatic plants and their extracts on growth performance in chickens are either positive (5-8, 10) or non-significant (11-14). When the effect was positive, weight gain and feed intake were increased whereas the feed:gain ratio was lowered when compared to control. Dietary essential oils can also improve digestion. A number of studies have reported the positive effect of spices or their active components on digestion process. They have been shown to stimulate bile salt secretion and digestive enzyme activities of intestinal mucosa and of pancreas (10, 11, 15).

Clove extract is commonly used in the food industry because of its special aroma and natural safety. In addition, the essential oil from clove also exhibited strong antibacterial properties. Antiseptic, appetite and digestion stimulant (10), strong antimicrobial and antifungal (16), analgesic and anti-inflammatory (17), anesthetic (18), anti-inflammatory and anticarcinogenic (19), antiparasitic (20) and antioxidant (4) activities of clove and its ingredients have been reported. Clove extract has been studied very few as a performance enhancer. In this study, we aimed the use of clove extract in animal nutrition as a natural growth promoting substance instead of antibiotics. For this purpose, the different level of clove extract were added in standard diet, and studied to determine of effect on performance compared to control and antibiotic groups.

Materials and Methods

Animals, Diets and Experimental Design: Three hundred 3-day old broilers (Ross-308) 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 (21) 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).

Body weights were recorded weekly. Feed intake per pen was measured throughout the experiment and the feed:gain ratio was calculated on a pen weight basis. Mortality and BW of dead birds were recorded daily.

Digestibility of nutrients was determined by method of acid-insoluble ash (as natural indicator). During the last 7 d (35 to 42 d) of the experiment, excreta from each cage were collected quantitatively and daily. Excreta were dried at 80°C until constant weight, homogenized, and sampled by cage. Diet and excreta were ground to pass through a 1-mm screen.

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, acid-insoluble ash was determined by the methods of Vogtmann et al. (23) and crude fiber was determined by the methods of Crampton and Maynard (24).

Statistical Analysis

Data were subjected to analysis of variance, and when significant differences were obtained, means were further subjected to Duncan's multiple range test by using SPSS for Windows: 11.5, SPSS inc. (25). Mortality rates were subjected to Chi-square test at the Crosstabs model of SPSS. The results were considered as significant when P values were less than 0.05 and 0.01.

Results

In general, no differences in body weights, body weight gain, feed intake or mortality rate were observed in broilers fed with different diets (Table 2). From 0 to 21 d of experiment, broilers fed the 400 ppm clove extract and antibiotic diet grew faster than the broilers fed the control. But, differences were statistically important in feed:gain ratio from 0 to 21 d and 0 to 42 d of experiment. The best performance levels were observed from the broiler fed the 400 ppm clove extract diet and antibiotic, 200 ppm clove extract, 100 ppm clove extract and control groups were followed respectively. The 3 to 6 weeks of the trial, no differences in BW, BW gain, feed intake, or feed:gain ratio were observed.

Antibiotic and plant extract supplementation improved apparent whole-tract digestibility of the nutrients (Table 3). Antibiotic and 400 ppm clove extract supplementation improved apparent fecal digestibility of dry matter ($P < 0.05$), crude protein ($P < 0.05$) and ether extract ($P < 0.01$). Thus, increased digestibility of the nutrients with dose dependent of clove extract supplementation was observed.

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 D₃ 3.000.000 IU; vitamin E 50.000 IU; vitamin K₃ 5.000 mg; vitamin B₁ 3.000 mg; vitamin B₂ 6.000mg; niacin 45.000mg; Calcium d-pantothenat 10.000 mg; vitamin B₆ 7.500 mg; vitamin B₁₂ 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 antibiotic and clove extract on performance of broilers, (Mean±SEM)¹

Weeks	Control	Antibiotic	Clove Extract, ppm			F
			100	200	400	
Body weight						
			(g)			
0	61.20±0.71	61.30±0.69	61.20±0.71	61.20±0.71	61.20±0.71	0.00 [~]
1	206.65±4.42 ^{abc}	211.00±3.89 ^{ab}	199.05±4.19 ^c	201.52±3.64 ^{bc}	213.60±3.17 ^a	2.49*
2	503.20±9.04	512.20±8.69	490.42±8.16	501.42±7.51	521.33±6.20	2.14 [~]
3	959.00±16.10 ^b	982.73±14.50 ^{ab}	954.05±12.55 ^b	969.93±12.37 ^{ab}	1007.17±9.49 ^a	2.61*
4	1523.40±27.22	1539.73±23.34	1521.75±20.16	1528.91±19.97	1562.42±12.99	0.62 [~]
5	2119.91±34.90	2139.11±34.44	2124.27±29.78	2132.33±28.52	2161.64±20.17	0.34 [~]
6	2692.73±39.13	2724.55±36.16	2697.84±32.93	2715.55±27.66	2745.46±21.81	0.44 [~]
Weight gain						
			(g/d)			
1-3	42.75±0.74 ^b	43.88±0.66 ^{ab}	42.52±0.57 ^b	43.27±0.56 ^b	45.05±0.42 ^a	2.86*
3-6	82.48±1.04	82.88±0.92	83.07±0.89	83.20±0.82	82.75±0.54	0.60 [~]
1-6	62.63±0.92	63.41±0.85	62.78±0.68	63.20±0.77	63.91±0.50	0.45 [~]
Feed intake						
			(g/d)			
1-3	59.79±1.25	59.87±1.04	59.90±0.46	59.98±0.42	60.58±0.47	0.16 [~]
3-6	161.18±3.07	160.89±4.19	163.97±1.29	162.73±0.52	159.69±0.09	0.48 [~]
1-6	110.49±2.17	110.39±2.33	111.93±0.83	111.30±0.61	110.14±0.60	0.24 [~]
Feed:gain						
			(g:g)			
1-3	1.38±0.02 ^{ab}	1.35±0.01 ^b	1.40±0.00 ^a	1.38±0.01 ^{ab}	1.32±0.01 ^c	10.42***
3-6	1.95±0.01	1.94±0.03	1.97±0.03	1.96±0.03	1.93±0.04	0.35 [~]
1-6	1.76±0.01 ^{ab}	1.74±0.01 ^{bc}	1.78±0.00 ^a	1.76±0.01 ^{ab}	1.72±0.01 ^c	8.61**
Mortality ²						
			n			
1	-	-	-	-	-	
2	-	1	-	-	-	
3	-	-	-	-	-	
4	-	-	1	-	-	
5	-	-	-	-	-	
6	-	-	-	-	-	
Mortality rate, %	0.00	1.67	1.67	0.00	0.00	X ² : 3.020, P>0.05
Live rate, %	100	98.33	98.33	100	100	

[~]: P>0.05, * : P<0.05, ** : P<0.01, *** : P<0.001, ^{a, b, c}: Mean values within a row having different superscripts are significantly different.

¹: Means represent 3 pens.

²: means of mortality rate were subjected to Chi-square test.

Table 3. Effect of antibiotic and clove extract on digestibility of diets of broilers, (Mean±SEM)¹

	Control	Antibiotic	Clove extract, ppm			F
			100	200	400	
Dry matter	67.08±3.88 ^b	73.06±1.21 ^a	67.86±1.14 ^b	68.99±1.22 ^b	73.57±1.23 ^a	4.32*
Crude protein	69.11±2.74 ^b	72.21±1.85 ^a	69.81±1.65 ^b	70.79±1.90 ^b	72.17±1.87 ^a	3.21*
Ether extract	82.62±1.67 ^c	84.16±0.35 ^{ab}	82.76±0.32 ^c	83.49±0.41 ^b	86.17±0.29 ^a	6.31**

*: P<0.05, **: P<0.01, ^{a, b, c}: Mean values within a row having different superscripts are significantly different.

¹: Means represent 3 pens of 10 chicks per treatment.

Discussion

Plant extracts fed to broilers gave live performance levels similar to those of the antibiotic growth promoter, results that agree with Jamroz and Kamel (15) who observed improvements of 8.1% in daily gain and 7.7% in feed conversion ratios in 17-d-old poult fed a diet supplemented with a plant extract containing capsaicin, cinnamaldehyde, and carvacrol at 300 ppm. In contrast, Botsoglou et al. (14), showed that oregano oil exerted no growth-promoting effect when administered at 50 or 100 mg/kg of feed. In the present study, a little growth promoter effect of additives was observed, but none of the treatments caused significant effects. The trial was conducted at ideal conditions of experimentation, which could affect the degree of growth promotion (Table 2). Plant extract effects may be due to the greater efficiency in the utilization of feed, resulting in enhanced growth. There is evidence to suggest that herbs, spices, and various plant extracts have appetite- and digestion-stimulating properties and antimicrobial effects (10). Clove extract contain different molecules (mainly eugenol) that have intrinsic bio-activities on animal physiology and metabolism.

The mechanisms by which these products influence the gut microflora and growth performance of poultry are not known properly. As antibiotics, plant extracts could control and limit the growth and colonization of numerous

pathogenic and nonpathogenic species of bacteria in the gut. The plant extracts clearly demonstrate antibacterial properties, although the mechanistic processes are poorly understood (9, 10, 15). Likewise, recent studies on supplementation of plant extracts to the broiler diets have supported our study with the similar results (5-7, 11).

We conclude that clove extract improved the digestibility of the feeds for broilers. The effect of different additives on digestibility improved the performance slightly, but this effect was statistically nonsignificant. Our results justify further research in this area to determine the optimal dietary inclusion level and the mode of action of these and other plant products to achieve optimal growth performance and digestion and this study justify the possibility use of plant extracts especially 400 ppm as an alternative natural growth promoter for poultry instead of antibiotics.

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