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Original Research Article

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

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ABSTRACT

The aim of the study was to determine the effects of dietary organic acid blend (OAB) and oregano essential oil (OEO) combination (Lunacompacid® Herbex Dry) on the performance and Cp count in the ileum of broiler chickens, and establish connections between performance and Cp proliferation. A total of 200 one day-old Avian Farm male broiler chicks were randomly divided into 2 equal groups (not supplemented control and birds receiving dietary addition of Lunacompacid® Herbex Dry (2g/kg of food) for 6 weeks. Body weights, body weight gains, food intake and food efficiency were weekly evaluated in the present study. Hot carcass weight and yield, and the ileum Ph and Cp count in the ileum content of broiler chickens were determined at the end of the experiment. In the present study, the combination of dietary OAB and OEO significantly decreased ileum pH (*P<0.05)* and Cp count (*P<0.001)* in the ileum content, but did not influence growth and carcass performance of broiler chickens. It was concluded that the lack of significant effects on broiler performance could be related to ideal rearing condition of our experiment. It is possible that the use of OAB and OEO combination in diets of broiler housed under suboptimal conditions may keep down Cp proliferation in the chicken gut and therefore may improve performance in this way.

Key Words: Broiler Chicken, Organic Acid, Oregano Essential Oil, Performance, *Clostridium perfringens*

Etlik Piliç Yemlerine Katılan Organik Asit Karışımı ve Oregano Esansiyel Yağı Kombinasyonunun (Lunacompacid® Herbex Dry) Performans ile İleumdaki *Clostridium Perfringens* Proliferasyonu Üzerine Etkileri

ÖZET

Bu çalışma; etlik piliç yemlerine katılan organik asit karışımı ve oregano esansiyel yağı kombinasyonunun (Lunacompacid® Herbex Dry) performans ile ileumdaki *Clostridium perfringens* sayısı üzerindeki etkilerini belirlemek ve böylece performans ile *Clostridium perfringens* proliferasyonu arasında bağlantı kurmak amacıyla yapılmıştır. Araştırmada toplam 200 adet günlük yaşta Avian Farm broyler erkek civciv kullanılmıştır. Civcivler rastgele her biri eşit sayıda hayvan içeren kontrol ve deneme grupları şeklinde 2 ana gruba ayrılmıştır. Kontrol grubunun yemine herhangi bir katkı yapılmazken, deneme grubundaki hayvanların yemine araştırma süresince 2g/kg düzeyinde Lunacompacid® Herbex Dry ilave edilmiştir. Araştırmada; gruplara ait canlı ağırlık, canlı ağırlık kazancı, yem tüketimi ve yemden yararlanma oranları haftalık olarak saptanmıştır. Sıcak karkas ağırlığı ve verimi ile ileum içeriğinin pH'sı ve ileumdaki *Clostridium perfringens* sayısı ise araştırmanın sonunda belirlenmiştir. Lunacompacid® Herbex Dry katkısı; deneme grubundaki hayvanların ileum pH'sı (*P<0.05)* ve *Clostridium perfringens* sayısını (*P<0.001)* önemli düzeyde azaltırken, büyüme ve karkas performansı üzerinde etkili olmamıştır. Performans üzerinde önemli bir etkinin gözlenmemesinin nedeni denemedeki ideal bakım koşullarına bağlanmıştır. Sonuç olarak; organik asit karışımı ve oregano esansiyel yağı kombinasyonunun, suboptimal koşullarda bakılan etlik piliçlerin sindirim kanalındaki *Clostridium perfringens* proliferasyonunu kontrol altında tutmak suretiyle performansı iyileştirebileceği kanısına varılmıştır.

Anahtar Kelimeler: Etlik Piliç, Organik Asit, Oregano Esansiyel Yağı, Performans, *Clostridium perfringens*

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INTRODUCTION

In recent years, natural alternatives for substituting the prohibited antibiotic growth promoters with organic acids and their salts, essential oils, probiotics, prebiotics and oligosaccharides have received much attention due to their inhibiting activity on the growth and development of pathogens in the gastrointestinal tract of poultry **(**Wald, 2004; Bauermann, 2006).

Organic acids have shown positive results in poultry production, by reducing the intestinal pH and bacterial growth intolerant to pH changes (Ao *et al.,* 2009; Pirgozliev *et al.,* 2008), thus providing better intestinal health for the bird to obtain maximum nutrient absorption. The antibacterial activity of organic acids is related to the reduction of pH, as well as their ability to dissociate, which is determined by the pKa-value of the respective acid, and the pH of the surrounding milieu (Davidson, 2001). The magnitude of their antimicrobial effects varies from one acid to another and is dependent on concentration and pH (Chaveerach *et al.,* 2002). It has been traditionally assumed that blends of organic acids having different pKa values have a broader spectrum of action throughout the intestine, where different pH values are encountered when the feed moves toward the large intestine.

For many years aromatic plants and their essential oils have been used as pharmaceuticals in alternative medicine and as a natural therapy (Mitscher *et al.,* 1987). Oregano (*Origanum vulgare* L.) is an aromatic plant with a wide distribution throughout the Mediterranean area and Asia (Vokou *et al.,* 1993). The essential oil obtained from oregano plant by a steam distillation process comprises more than 20 ingredients and, most of which are phenolic compounds (Vekiari *et al.,* 1993). The major components of oregano oil are carvacrol and thymol that constitute about 78 to 82% of the total oil (Adam *et al.,* 1998). It has been suggested that the oregano essential oil (OEO) have antimicrobial (Sivropoulou *et al.,* 1996; Lambert *et al.,* 2001), antifungal (Thompson, 1989), insecticidal (Karpouhtsis *et al.,* 1998) and antioxidant (Botsoglou *et al.,* 2002) properties.

Poultry are susceptible to potentially pathogenic microorganism such as *Escherichia coli, Salmonella, Campylobacter* and *Clostridium ssp.* Pathogenic microorganism in the small intestine compete with the host for nutrients and also reduce the digestion of fat and fat soluble vitamins due to deconjugating effects of bile acids (Engberg *et al.,* 2000). The bacterium *Clostridium perfringens* (Cp) is considered an opportunistic pathogen **(**Craven *et al.,* 2001) and commonly found in the gastrointestinal tract of poultry; leads to the development of necrotic lesions in the intestinal wall, resulting in mortality of poultry (Paulus and Ruckebusch, 1996). The subclinical form of Cp associated necrotic enteritis causes a reduction in performance and overall health of poultry (Kaldhusdal and Lovland, 2000). It is reported that the incidence of necrotic enteritis caused by Cp has recently increased because of the withdrawal of in-feed antibiotic growth promoters with anti-clostridial activity (Van Immerseel *et al.,* 2004; Williams, 2005; Knarreborg *et al.,* 2002).

A number of studies have suggested that dietary supplementation of organic acids reduces proliferation of pathogens in the gastrointestinal tract of poultry (Hinton and Linton, 1988; Izat *et al.,* 1990; Mchan and Shotts, 1992; Byrd *et al.,* 2001; Chaveerach *et al.,* 2004). Additionally, it is reported that some essential oils such as thymol, cinnamaldehyde and eucalyptol have a strong antimicrobial activity against Cp (Candan *et al*., 2003; Mitsch *et al*., 2004; Jujena and Friedman, 2007). It may be possible that natural alternatives for substituting the prohibited antibiotic improve the performance by reducing proliferation of Cp in the chicken gut. Thus, the present study was conducted to determine the effects of a combination of dietary organic acid blend (OAB) and OEO (Lunacompacid® Herbex Dry) on the performance and Cp count in the ileum of broiler chickens, and establish connections between performance and Cp proliferation.

MATERIALS AND METHODS

Birds, management and protocol design

A total of 200 one day-old Avian Farm male broiler chicks were obtained from a commercial hatchery. The chicks were individually weighed and randomly divided into 2 groups (control and treated group), each of them being constituted by 5 replicate subgroups of 20 birds. Water and feed were provided *ad libitum* throughout the

experiment. Birds were exposed to 23 hours of light and 1 hour of darkness per day. Wood shavings were used as litter. Ventilation and heat were provided and adjusted as necessary to maintain bird comfort. Birds were vaccinated against Newcastle disease on the 6th and 19th days and against the Gumboro disease on the 12th day of the experiment. The vaccinations were administrated via drinking water. Mortality was recorded as it occurred. Experiment lasted for 41 days. All birds were managed and cared according to the University of Uludag Ethical Committee recommendations. Experimental diets were prepared as mash and formulated to be isoenergetic and isonitrogenous which are necessary to meet the minimum nutrient requirements of broiler chickens, as recommended by the NRC (1994). The ingredients and analysed nutrient composition of the broiler starter (0 to 21 days), grower (22 to 35 days) and finisher diets (36 to 41 days) are shown in Table 1. Whereas the control group received the basal diets (starter, grower and finisher), chickens in the treatment group were fed with basal diets supplemented with Lunacompacid® Herbex Dry **(**Luna Kimya Ltd. Sti., Tuzla, Istanbul, TURKEY**)** at the dose of 2g/kg of food. Per 1.4 kg Lunacompacid® Herbex Dry used in the experiment contains formic acid (30%), propionic acid (10%), ammonium formate (10%), lactic acid (15%), fumaric acid (15%), citric acid (1%) and oregano (*Origanum vulgare* L.) essential oil (5%).

Table 1. Ingredients and analysed nutrient composition of the broiler starter, grower and finisher diets (as fed basis).

Ingredients, % of diet	Starter	Grower	Finisher
Maize	31.10	30.24	21.31
Wheat	17.50	25.00	35.00
Full fat Soy	27.50	23.00	25.00
Soybean meal, 44% CP	17.50	10.50	7.00
Poultry by-product, 58% CP	-	3.50	3.50
Meat and bone meal, 35% CP	2.50	3.50	2.50
Vegetable oil	1.70	2.70	3.30
Limestone	0.70	0.52	0.70
Salt	0.30	0.20	0.17
Dicalcium phosphate	0.75	0.14	0.69
DL-Methionine	0.27	0.31	0.33
L-Lysine	0.02	0.23	0.34
VMP [a]	0.10	0.10	0.10
Antioxidant [b]	0.01	0.01	0.01
Multienzyme [c]	0.05	0.05	0.05
Total	100	100	100
Analysed composition			
Dry matter, %	89.85	90.25	90.10
Crude protein, %	22.14	20.54	20.65
Ether extract, %	7.82	9.34	10.02
Crude ash, %	7.05	6.61	6.30
Starch, %	38.67	37.04	36.96
Sucrose, %	5.12	4.51	4.51
Calcium, %	1.44	1.02	0.92
Total phosphorus, %	0.63	0.55	0.43
Lysine [d], %	1.30	1.10	1.00
Methionine/Cystein [d], %	0.98	0.87	0.84
Metabolisable energy [e], (kcal/kg)	3164	3145	3202

[a]VMP:Vitamin Mineral Premix providing per kg of diet:12 000 IU Vitamin A, 1 500 IU Vitamin D_3, 30 mg Vitamin E, 5 mg Vitamin K_3, 3 mg Vitamin B_1, 6 mg Vitamin B_2, 5 mg Vitamin B_6, 0.03 mg Vitamin B_{12}, 40 mg Nicotinamide, 10 mg Calcium-D-Pantothenate, 0.75 mg Folic acid, 0.075 mg D-Biotin, 375 mg Cholin chloride, 80 mg Manganese, 40 mg Iron, 60 mg Zinc, 5 mg Copper, 0,4 mg Iodide, 0.1 mg Cobalt, 0.15 mg Selenium, 10 mg Antioxidant; [b]Providing per kg of diet: 2.5 mg BHA, 3.125 mg Etoxyquine, 2.5 mg Citric acid, 2.5 mg Orthophosphoric acid, 2.5 mg Monodiaglyceride fatty acids; [c]Providing per kg of diet: 2100 U cellulase, 3750 U Xylanase, 350 U β-glucanase, 350 U α-amylase, 100 U Phytase, 25 U Pectinase; [d]Calculated values; [e]Metabolisable energy values for the experimental diets were calculated using the equation of Hartel (1977) as follows: ME (kcal/kg) = 239x ((Crude Fat % x 0.3431) + (Crude Protein % x 0.1551) + (Saccharose % x 0.1301) + (Starch % x 0.1669)).

Measurements and analyses

Diets were chemically analyzed according to the standard procedures of the AOAC (1994). Metabolisable energy of experimental diets were calculated using the equation of Hartel (1977).

All chicks were individually weighed at the beginning of the experiment (1-day-old) then weekly for the whole experimental period. Food intake was determined on a pen basis and the average bird weight gains and food conversion ratios adjusted for mortality, were determined weekly. On day 41, all birds were slaughtered in a commercial slaughterhouse and hot carcasses (without neck, giblets, and feet) were weighed in order to determine hot carcass weight and yield.

On day 41, twenty broilers from each main group (5 pens of 4 chicks per treatment) were randomly selected and euthanized to determine each of ileum pH and Cp count. The carcasses of euthanized broilers were subsequently opened and the entire gastrointestinal tract was removed aseptically. Ileum (from Meckel's diverticulum to the cecal junction) was ligated at both sides with light twine before separating the content from the ileum. To determine the pH, 10 g of intestinal content from ileum were collected aseptically in 90 ml sterilized physiological saline (1:10 dilution) (Al-Natour and Alshawabkeh, 2005) and pH was measured. Cp count of ileum was determined by TUBITAK Marmara Research Center (Gebze, Kocaeli, TURKEY). Contents of ileum were transfered into sterile plastic bags and samples immediately transported in cold chain to the laboratory. One gram of each sample was diluted 1:9 (wt/vol) in sterile saline. All samples were subjected to 10 sequential dilutions 1:9 (vol/vol), and 0.1 mL of each sample was plated as duplicates by using tryptose sulfite-cycloserine (TSC) agar (Oxoid CM587, Basingstoke, Hampshire, England). The samples were incubated for 22 ± 2 h at 37 ºC. Incubation procedure was conducted under anaerobic conditions using the AnaeroGen Atmosphere Generation System (Oxoid, Basingstoke, UK). After incubation, typical black colonies were counted. Results were expressed as $\log_{10}$ colony-forming units per gr of ileal digesta ($\log_{10}$ CFU/g).

Statistical analysis

Data were tested for determine normal distribution by F-test. Body weight, hot carcass weight, and ileum pH and Cp count between groups were analyzed by independent sample T-test. The statistical analyses for body weight gain, food intake and food efficiency were performed using the Mann-Whitney test. Chi-square test was used for hot carcass yield and mortality rate.

Differences were considered significant at a probability level of $P<0.05$ in all analyses. All statistical analysis was performed with SPSS software (version 10.0, SPSS Inc, USA).

RESULTS AND DISCUSSION

The effects of dietary OAB and OEO combination (Lunacompacid® Herbex Dry) on growth and carcass performance of broiler chickens are summarized in Tables 2 and 3, respectively. There were no significant effects of dietary OAB and OEO combination on growth (body weight, body weight gain, food intake, food conversion ratio) and carcass (hot carcass weight and hot carcass yield) performance, and mortality of broilers. Reports on the effects of dietary organic acid and essential oil supplementation on the performance of broiler chickens are conflicting. A significant improvement on growth performance of broilers fed diets supplemented with propionic or fumaric acids was observed by Waldroup *et al.* (1995). Positive effects on growth performance of broilers have also been reported for fumaric, propionic, sorbic and tartaric acids (Vogt *et al*., 1981; 1982). However, growth performance of broiler chickens was not affected by supplementation of Lunacompacid® Herbex Dry containing OAB in the present study (Table 2). These results are in agreement with many investigators **(**Izat *et al.,* 1990; Kaniawati *et al.,* 1992; Hernandez *et al.,* 2006; Houshmand *et al.,* 2012), who did not find any positive effects of organic acids on growth performance of broiler chickens. Significant improvement in carcass performance of broilers fed diets supplemented with citric, propionic or fumaric acids were reported by some investigators (Snow *et al.* 2004, Abd El-Hakim *et al.* 2009). In contrast, Thirumeignanam *et al.* (2006) and Adil *et al.* (2011) did not determine any effect of organic acids on the carcass performance of broilers, as it was observed in this study (Table 3). Dibner and Buttin (2002) suggested that the buffering capacity of the diet, presence of other antimicrobial compounds, acid type and concentration, composition of diet

and environment of the experiment could be considered as responsible factors for inconsistency in results. In agreement with this study, Botsoglou *et al.* (2002) reported that inclusion of 50 and 100 mg/kg OEO could not exert any growth promoting action in experimental broilers. Similarly, Lewis *et al.* (2003) reported that performance of growing broilers was not affected by using an oregano-based supplement. On the other hand, positive effects of essential oils have been obtained from the several field studies (Bassett, 2000; Langhout, 2000).

Table 2. Effects of Lunacompacid® Herbex Dry supplementation on growth performance and mortality of broiler chickens.

	Groups		***P***
	Control	**+Lunacompacid® Herbex Dry**	
Body weight, g			
Week 0	42.80 ± 0.32	43.64 ± 0.37	NS
Week 1	132.37 ± 1.62	133.88 ± 1.41	NS
Week 2	271.09 ± 4.19	265.21 ± 3.64	NS
Week 3	543.05 ± 9.26	522.66 ± 8.03	NS
Week 4	965.05 ± 38.13	942.79 ± 14.68	NS
Week 5	1589.39 ± 24.15	1565.72 ± 22.84	NS
Week 6	2087.73 ± 31.19	2078.71 ± 28.93	NS
Body Weight Gain, g			
BWG0-1	89.51 ± 1.94	90.24 ± 1.48	NS
BWG0-2	228.19 ± 6.09	221.57 ± 5.85	NS
BWG0-3	499.43 ± 10.65	479.02 ± 3.63	NS
BWG0-4	920.66 ± 21.59	899.28 ± 13.99	NS
BWG0-5	1543.99 ± 32.75	1523,13 ± 27.59	NS
BWG0-6	2042.00 ± 41.75	2035.61 ± 19.05	NS
Food Intake, g			
FI0-1	117.08 ± 2.12	119.12 ± 2.09	NS
FI0-2	330.94 ± 5.22	325.16 ± 4.60	NS
FI0-3	771.77 ± 16.07	762.17 ± 8.82	NS
FI0-4	1545.69 ± 31.40	1505.84 ± 23.34	NS
FI0-5	2717.68 ± 47.27	2694.36 ± 57.13	NS
FI0-6	3744.44 ± 76.78	3769.30 ± 38.18	NS
Food Conversion Ratio, g/g			
FCR0-1	1.31 ± 0.03	1.33 ± 0.02	NS
FCR0-2	1.45 ± 0.04	1.47 ± 0.03	NS
FCR0-3	1.55 ± 0.01	1.59 ± 0.01	NS
FCR0-4	1.68 ± 0.01	1.68 ± 0.01	NS
FCR0-5	1.76 ± 0.01	1.77 ± 0.01	NS
FCR0-6	1.83 ± 0.02	1.85 ± 0.02	NS
Mortality, %	5.0	7.0	NS

BWG0-i: Body weight gain calculated for a period of i weeks; FI0-i: Food intkae measured for a period of i weeks; FCR0-i: Food conversion ratio (Food intake / Body weight gain) calculated for a period of i weeks.

NS: Not significant.

Results are expressed as mean ± SEM of 5 pens of 20 chicks per treatment.

Table 3. Effects of Lunacompacid® Herbex Dry supplementation on carcass performance of broiler chickens.

	Groups		*P*
	Control	**+Lunacompacid® Herbex Dry**	
Pre-slaughter body weight, g	2087.73 ± 31.19	2078.71 ± 28.93	NS
Hot carcass weight, g	1504.27 ± 25.77	1505.60 ± 24.28	NS
Hot carcass yield, %	72.05 ± 0.72	72.43 ± 0.60	NS

NS: Not significant.
Results are expressed as mean ± SEM of 5 pens of 20 chicks per treatment.

Table 4. Effects of Lunacompacid® Herbex Dry supplementation on the ileum pH and *Clostridium perfringens* count in the ileum content of broiler chickens.

	Groups		*P*
	Control	**+Lunacompacid® Herbex Dry**	
Ileum pH	6.38 ± 0.23	6.10 ± 0.24	<0.05
Clostridium perfringens count (log_{10} cfu/g)	12.70 ± 1.68	0.40 ± 0.22	<0.001

NS: Not significant.
Results are expressed as mean ± SEM of 5 pens of 4 chicks per treatment. Each value is based on means of twenty experiments in duplicate analysis.

In this study, a significant reduction in ileum pH (*P < 0.05)* was observed in broiler chickens fed diet supplemented with Lunacompacid® Herbex Dry (Table 4). The reason for the significant reduction in ileum pH of broiler chickens is probably due to the effect of OAB in Lunacompacid® Herbex Dry. Abdel-Fattah *et al.* (2008) reported that the pH values in different gastro-intestinal tract segments were decreased with supplementation of organic acids irrespective of type and dose used. A similar result was observed by Thirumeignanam *et al.* (2006) that the dietary supplementation of organic acids caused a significant reduction in pH of crop, gizzard and duodenal contents of broiler chickens. Cp count in the ileum content of broiler chickens fed diet supplemented with Lunacompacid® Herbex Dry has also significantly decreased (*P< 0.001*) in the present study (Table 4). It was concluded that decreased Cp count in the ileum content may be due to the combined antibacterial effects of OAB and OEO on pathogen microorganism. Lunacompacid® Herbex Dry used in this study contains some short-chain organic acids such as formic, propionic, lactic and citric acids. Dibner and Butin (2002) reported that the short-chain organic acids such as formic, acetic, propionic, butyric, lactic, malic, tartaric, citric acids have specific antimicrobial activity. Undissociated forms of the organic acids, at a pH below the pKa of the acid, work much better as antimicrobials than the dissociated forms; it takes 10 or 20 times the amount of dissociated acids to get the same antimicrobial effect as the undissociated forms of the acids (Presser *et al.*, 1997). It has been hypothesized that the undissociated form of short-chain organic acids can easily penetrate the lipid membrane of the bacterial cell (Eklund, 1983). Once internalized into the neutral pH of the cell cytoplasm dissociate into anions and protons (Cherrington *et al.*, 1991; Davidson, 2001). This causes the cytoplasm of the bacterial cell to utilize excessive energy to maintain a near neutral pH, which may result in depletion of cellular (Davidson, 2001). The antibacterial effect of short-chain organic acids on the pathogen microorganism has been reported by many researchers. Hinton and Linton (1988), Izat *et al.* (1990), Mchan and Shotts (1992), Byrd *et al.* (2001) and Chaveerach *et al.* (2004) who reported that the addition of lactic, formic and propionic acids to the diet and/or water effectively reduced proliferation of *Escherichia coli*, coliforms, *Salmonella* and *Campylobacter* in the gastrointestinal tract of poultry. Furthermore, numerous reports exist about the *in vitro* antibacterial effects of different plant extracts (*Origanum vulgare*, *Piper nigrum*, *Syzygium aromaticum*, *Thymus vulgaris*) and their essential oil components (thymol, carvacrol, eugenol) against *Clostridium sporogenes* (Paster *et al.*, 1990; Dorman and Deans, 2000), and other pathogen bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* (Juven *et al.*, 1994; Cosentino *et al.*, 1999). Howewer, there are limited numbers of *in vivo* studies about the antibacterial effect of essential oils on

the pathogen microorganism in the gastrointestinal tract of broiler chickens. Mitsch *et al.* (2004) studied the effect of a commercial blend containing thymol and carvacrol essential oils on the presence of Cp in the feces and intestines of broilers. They determined that the addition of thymol and carvacrol essential oils to the feed caused a significant reduction of Cp count in the jejunum on day 14 and 30, and in the cloaca on day 14. The exact antimicrobial mechanism of essential oils is poorly understood. However, it has been suggested this effect is mainly due to the lipophilic character of the active principles (Conner, 1993), which permeate the cell membranes and mitochondria of the microorganisms and inhibit, among others, the membrane bound electron flow and therewith the energy metabolism. This leads to a collapse of the proton pump and draining of the ATP pool. High concentrations of essential oils also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Helander *et al.*, 1998).

CONCLUSIONS

It was demonstrated in the present study that the combination of dietary OAB and OEO (Lunacompacid® Herbex Dry) significantly decreased ileum pH and Cp count in the ileum content, but did not influence performance parameters. We concluded that the lack of significant effects on broiler performance could be related to ideal rearing condition of our experiment, because growth promoting effects of antimicrobial agents will become apparent under suboptimal conditions, for instance poor hygiene condition or the feeding of low digestible diets. Increasing numbers of Cp under suboptimal conditions cause to lower performance in the broiler chickens with decreasing growth rate and food efficiency **(**Stutz and Lawton, 1984; Kaldhusdal and Hofshagen, 1992). It is possible that the use of OAB and OEO combination in diets of broiler housed under suboptimal conditions may keep down pathogen microorganism proliferation such as Cp in the chicken gut and therefore may improve performance in this way.

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