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Research Article

Effects of the Supplementation of Essential Oil Isolated from Orange Peel *(Citrus sinensis L.)* to Broiler Diets on the Performance[#]

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Abstract

In this study, the effects of essential oil isolated from orange peel (OEO) added to broiler diets on the performance were examined. In the research, 432 broiler chickens were used and the experiment lasted for six weeks. In the experiment, 0 (1st group), 50 (2nd group), 100 (3rd group) and 150 mg/kg (4th group) of OEO (*Citrus sinensis L*.) were added to broiler diets and 4 different diets were used. The effect of OEO added to broiler diets on the live weights were found significant (P<0.05), except for 1st and 5th weeks. The more the doses of the OEO were the more the live weights increased, the dose of 150 mg/kg had the maximum increase. Feed intake was found significant (P<0.05), except for the 2nd week. The addition of 150 mg/kg of OEO improved the feed efficiency (P<0.05). Carcass weight, carcass yield, things, breast, back, wing, abdominal fat and heart weights significantly increased (P<0.05) as OEO dose added to diets increased.

Key words: Orange essential oil (*Citrus sinensis L*.), broiler, performance

Portakal Kabuğundan (*Citrus sinensis* L.) İzole Edilen Esansiyel Yağın Broyler Diyetlerine Eklenmesinin Performans Üzerine Etkileri

Özet

Bu araştırmada, etlik piliç karmalarına portakal kabuğu (*Citrus sinensis* L.) uçucu yağı (PKUY) ilavesinin broyler performansı üzerine olan etkisi incelenmiştir. Araştırmada, 432 adet etlik civciv kullanılmış ve deneme 6 hafta sürdürülmüştür. Etlik piliç karma yemlerine kontrol (1. Grup), 50 mg/kg (2. Grup), 100 mg/kg (3. Grup) ve 150 mg/kg (4. Grup) PKUY ilave edilmiş ve 4 farklı rasyon kullanılmıştır. Etlik piliç karmalarına PKUY ilavesinin canlı ağırlığa etkileri 1. ve 5. haftalar hariç, önemli (P<0.05) bulunmuştur. PKUY dozları arttıkça canlı ağırlık artmış ve 150 mg/kg'lık doz en yüksek ağırlığı sağlamıştır. Yem tüketimleri bakımından farklılıklar 2. hafta hariç, önemli (P<0.05) bulunmuştur. Karmalara 150 mg/kg PKUY ilavesi, yemden yararlanmayı önemli derecede (P<0.05) iyileştirmiştir. Karkas ağırlığı, karkas randımanı, but, sırt, kanat, göğüs, abdominal yağ ve kalp ağırlıkları PKUY dozları arttıkça önemli (P<0.05) düzeyde artmıştır.

Anahtar kelimeler: Portakal kabuğu uçucu yağı (*Citrus sinensis* L.), etlik piliç, performans

Introduction

Effective protection of farm animals from various pathogens is widely used in synthetic semi-synthetic antimicrobials. But it also raises the risk of developing resistance against these exploitation pathogens (Bach Knudsen, 2001; Botsoglou et al., 2002; Inci et al., 2016; Inci et al., 2016a). One of these is antibiotics and has been used for years, especially as a growth factor. It has been shown that antibiotic resistance can be transferred from a bacterial pathway through other bacterial conjugation. Increasing frequency of resistant strains of bacteria in recent years, and these strains have raised concerns that antibiotics used in humans to treat disease can also cross-resist (Ceylan et al., 2003; Sogut et al. 2012). As a result, the use of

#Summarized from a part of Ahmet AYDIN's Ph.D thesis.

antibiotics has been banned since 2006, when the European Union decided in 2002. There has been a considerable increase in the work on the development of alternative feed additives (Mellor, 2000; Şengezer and Güngör 2008; Sogut et al., 2011). These alternative feed additives; Enzymes, organic acids, probiotics, prebiotics, immunostimulating products and natural plant extracts (Çetin and Yıldız 2004). Turkey is extremely rich in plants containing essential oils and about 1/3 of its flora consists of medicinal and aromatic plants (Başer, 1995). Since ancient times, it has been used by people as a germicide, digestive stimulant, antidiarrheal, painkiller, sedative, breath-releasing, anti parasite, urine enhancer, gastrointestinal diseases inhibiting intestinal worms (Cowan, 1999; Alçiçek, 2008) Examples of important medicinal substances used are linalool, limonene, 1,8-octanediol isolated from coriander (Coriandrum sativum L.), cumin (Carum carvi L.), lavender (Lavandula stoechas L.) and orange peel (Citrus sinensis L.) substances such as cineol and carvol can be shown.. In a study in which the chemical composition of essential oils was determined; The highest lemonen rate was found to be orange peel oil with 93.4% (Alçiçek et al., 2009).

The fruit group which is the most cultivated in the world with a total of 115,650,545 tons today is citrus fruit. 55.26% of world citrus production is oranges, the largest orange producers; USA, Brazil, Mexico, Spain, Italy, India, Israel, Egypt, Argentina and Turkey (Kafa et al., 2009). The areas grown orange in Turkey are Mediterranean coast borders and southern coasts of the Aegean region. It is also cultivated on the eastern shores of the Black Sea Region The most grown orange is mainly Antalya, Adana, Mersin, Hatay, Aydın and Muğla. The most important orange species produced are Washington, Jaffa and Valencia (Anonymous, 2010).

Studies on the use of volatile oils obtained from medical plants as growth factors have been reported to significantly improve parameters such as live weight gain, feed consumption, feed utilization, egg yield and carcass yield. Examples from these studies are given below.

The live weight gain and feed efficiency rates of broiler chickens on the 21st and 42nd days were significantly higher ($p < 0.01$) added to chicken diets the mixture of essential oil (thyme, laurel, fennel, sage, myrtle leaf and orange peel oil) and organic acid mixture alone or in combination (Bozkurt et al., 2007).

In the broiler chicks, 5 different feeds (control, 2500 mg / kg organic acid, 1000 mg / kg probiotic, 36 and 48 mg / kg volatile oil) were used and the live weights in the groups were 1909, 1937, 2015, 2063 and 2060 g; the feed intakes 3942, 3993, 4045, 4078 and 4037 g; the feed efficiency 2.07, 2.06, 2.01, 1.97 and 1.96 g and the carcass yield 73.9, 73.9, 74.3, 74.3 and 75.2 % respectively. It has been reported that essential oil and probiotics have a significant effect ($p < 0.05$), but no difference in terms of abdominal fat and mortality (Alçiçek et al., 2004). In a study conducted by adding 24 and 48 mg / kg vegetable essential oil mixture to the feed of chickens, 21-42. No significant change in live weight was observed on days, but there was an improvement in feed efficiency ($P < 0.05$) (Çabuk et al., 2006).

It was determined that there was a significant ($P < 0.01$) positive effect on live weight gain, feed intake and feed efficiency at the end of the study by adding antibiotic, prebiotic, mannan oligosaccharide, oregano volatile oil, hops plant extract and oregano + hops mixture to broiler diets (Bozkurt et al., 2009a).

In this study, the effects of essential oil isolated from orange peel (OEO) added to broiler diets on the performance were examined.

Materials and Methods

Material

Animal Material

The animal material of the study consisted of 432 coob-500 strain male and female chicks a day. The chicks were obtained from the incubation unit of a private poultry company (Bandirma Bozlar Poultry and Feed Industry).

Feed Material

The feed mixes used in the experiment were prepared in a special feed factory (Bozlar Tavukçuluk and Feed Industry). The orange peel essential oil added to the broiler chicks diets was obtained by aqueous distillation method. Orange peel essential oil in a private company (İzmir Türer Tarım AS) with zeolite impregnated as a carrier with the help of micro mixer and then mixed at the diets 50, 100 and 150 mg / kg levels.

The composition of the intensive feed mixes used in the experiment and the nutrient content are given in Table 1. The values recommended by the NRC (Anonymous, 1987) are based on the preparation of the rations. Essential chemical compositions of essential oil of orange peel used in the experiment are given in Table 2.

Table 1. Ingredient composition of the experimental diets

Ingredients %	Starter period (0-3 wk.) (For 2000 kg)	Ingredients %	Finisher period (3-6 wk.) (For 2000 kg)
Maize	829.2	Maize	972.6
Soybean meal	460.0	Soybean meal	332.0
Whole soybean	416.0	Whole soybean	290.0
Broken rice	200.0	Broken rice	150.0
Acid oil	15.0	DDCS *	70.0
Marble dust	26.8	Chicken flour	40.0
Salt	6.0	Acid oil	40.0
carbonate	4.5	Frost oil	40.0
BroilerVit.	5.0	Marble dust	22.7
MCP	25.6	Salt	5.0
L-Lysin	3.3	Carbonate	4.2
Antikoksidial	1.1	BroilerVit.	5.0
L-Trionin	1.0	MCP	18.9
Fitaz 500	0.2	L-Lysin	2.9
Liquid methionin	6.3	Antikoksidial	1.1
-	-	L-Trionin	0.8
-	-	Fitaz 500	0.2
-	-	liquid methionin	4.6
Total	2000.0	Total	2000.0
Crude protein (%)	21.0	Crude protein (%)	19.0
(%) ME (kcal/kg)	2988.0	ME (kcal/kg)	3176.0

DDCS: Corn by-product

Table 2. Essential chemical compositions of essential oil of orange peel used in the experiment

Plant material used in oil	Essential components in oil	%
Orange peel (*Citrussinesis L.*)	Limonen	93.6
	Mirsen	1.3

Method

Experimental design

This study was carried out Carried out in chicken coops of private company (Bozlar Poultry and Feed Industry, Bandırma) All chicken wing banded and weighted then total 432 chicken assigned to 4 groups with 6 replicates containing 18 chicken each randomly. Ad libitum feeding and continuous illumination were applied during the six week trial period. The study was conducted according to the design of random parcels.

The experiment was organized into 4 groups. The first group was formed by the control group, the second group (50 mg / kg), the third group (100 mg / kg), and the fourth group (150 mg / kg) by the addition of orange peel essential oil (OPEO).

Diets containing 21% HP and 2999kcal / kg ME in the first period (0-3 weeks) and 19% HP and 3164kcal / kg ME in the second period (3-6 weeks) were fed to the animals. The trial was continued for 42 days (6 weeks).

Execution of the experiment

During the trial; Weekly weight averages of the groups were determined by weighing the animals on the same day and hour each week. At the same time, the feeds were weighed and the feed intake and feed efficiency rates of the animals were also determined.

Slaughter and carcass characteristics

At the end of the trial, a total of 120 animals, 5 animals from each subgroup, were selected to be of equal number of male and female, 60 females and 60 male animals were slaughtered and the hot carcasses separated into pieces. Carcass pieces and organs were weighed to determine carcass characteristics Carcass yields were also determined by dividing the carcass weights by the slaughter weights.

While the carcass was divided, it was divided into 4 parts as thigh, wing, breastand back, and each piece was weighed separately. The liver, heart, gizzard and abdominal fat were also weighed and evaluated.

Feed analysis

The analysis values of the feed mixes and the raw materials involved in the mix were calculated according to the Weende analysis method (Bulgurlu and Ergül 1978). The chemical analysis and antimicrobial characteristic of the orange peel essential oil added to the diets were made at the Anadolu University Medical and Aromatic Plant and Drug Research Center (MPRC). Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 6358), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter aerogenes* (NRLL 3567), *Proteus vulgaris* (NRRLB 123), *Salmonella typhimurium* NRRLB 4420) and *Aspergillus niger* (ATCC 10549) were used as test microorganisms in this research center.

Microdilution method Koneman et al (1997) was used for evaluation of essential oil as antimicrobial, and chloramphenicol (Sigma, Germany) was used as a standard antibacterial agent. Agar diffusion method (20) was used to determine antifungal activity and ketoconazole (Sigma, Germany) was used as the standard antifungal agent. The results are given as (MIC = Minimum Inhibitory Concentration) values (mg / mL). The chemical composition of essential oils was determined by gas chromatography (GC, Shimadzu GC-9A, CR4A integrator) and gas chromatography-mass spectroscopy (GC / MS, Hewlett Packard GCD).

Evaluation of results

The evaluation of the results was made using the SPSS package program according to the trial design established in the random parcels (Özdamar, 1999). The following mathematical model has been used for this purpose.

$Y_{ijkl} : \mu + a_i + b_j + ijkl$, Y_{ijkl}: l. sex j. Feed group k. Essential oil and the observation value obtained from the first replicate, μ : Expected average of population, a_i: i. Fixed effect of sex, b_j: j. Fixed effect of the feed group, c_k: k. Fixed effect of essential oil, e_{ijkl}: Chance-related error

Comparisons of group-to-group statistical features were made using the One Way-Anova analysis method and the Duncan test (38) of group-by-group significance controls.

Result and Discussion

The calculated analysis results of feed raw materials used in this study are given in Table 3. The minimum inhibitor concentration (MIC, mg / mL) of essential oil used in the trial is given in Table 4.

Table 3. Crude nutrient analysis results of diets used in the experiment

Ingredients	First Stage	Second Stage
Dry matter (%)	90.65	90.12
Crude protein (%)	21.46	19.57
Crude oil (%)	8.51	10.44
Crude cellulose (%)	3.96	3.55
Starch (%)	36.42	38.54
Sugar (%)	1.56	1.31
Crude ash (%)	6.25	5.60
M.E. (kcal/kg)	2999.30	3164.47

Table 4. Minimum inhibitor concentration (mic) values for essential oil, mg / mL

Microorganism	**Source**	**Essential oil tested**	**Standard agent**
		Orange peel oil	
Bacteria			Chloram phenicol
Escherichia coli	ATCC 25922	**125**	62.5
Staphylococcus aureus	ATCC 6358	**125**	7.81
Pseudomonas aeruginosa	ATCC 27853	**500**	250.0
Enterobacter aerogenes	NRLL 3567	**250**	125.0
Proteu svulgaris	NRRLB 123	**250**	31.25
Salmonella typhimurium	NRRLB 4420	**250**	62.5
Fungi			ketoconazole
Aspergillus niger	ATCC 10549	**1000>**	62.5
Candida albicans	OGU	**250**	125.0

Live weight

The effect of the addition of orange peel essential oil (OPEO) on the live weight of broiler chicks is given in Table 4. The highest weight was seen in the group supplemented with 2646.16 g and 150 mg / kg PKUY, while the lowest weight was observed in the control group with 2555.38 g. Differences were statistically significant (P <0.05).

Feed intake

When feed consumption is examined (Table 5); According to the control group, feed consumption was significantly (P<0.05). Lower in the group with 150 mg / kg OPEO supplement. The other groups were similar to the control group.

Feed Efficiency

The effect of feed efficiency from OPEO diet on broiler chickens is given in Table 4. When compared with the control group, significant improvement (P> 0.05) was achieved in the group containing 150 mg / kg of OPEO. The other groups were similar to the control group

Vitality

The differences between the groups were not significant when looking at vitality.

Table 5. Some development performance results in end of the Trail (6th week)

Groups	N	L.W. (g)	F.I. (g)	F.E.(g)	V (%)
1 (Control)	103	2555.38 ^{b}	4727.17^{a}	1.85^{a}	95.4
2 (50 mg/kg)	100	2565.05 ^{ab}	4611.00 ^{ab}	1.80^{ab}	92.6
3 (100mg/kg)	105	2578.14 ^{ab}	4618.67 ^{ab}	1.79^{ab}	97.2
4 (150mg/kg)	103	2646.16 ^{a}	4546.67 ^{b}	1.72^{b}	95.4

$^{a, b,}$: Differences between averages with different letters are important (P<0.05).
LW: Live Weight, F.I.: Feed Intake, F.E.: Feed Efficiency, V: Vitality.

Slaughter and carcass characteristics

The effects of diets OPEO additions on carcass characteristics are given in Table 6 and the effects on some other carcass parts are given in Table 7.

The differences in carcass weights were significant (P <0.05), with the highest weight being 1834.97 g in the 4th group with 150 mg / kg dose and 1752.60 g in the lowest weight control group.

The differences between the groups in terms of efficiency ratios were significant (P<0.05). The highest rate was 70.35% in the group with 150 mg / kg PKUY (Table 5).

The differences between the groups were significant (P <0.05) in terms of breast, thigh, back and wing weights (Table 6), and weights increased as the PKUY dose level increased.

Table 6. Carcass characteristics

Groups	N	Carcass (g)	Yield (%)	Breast (g)	Thigh (g)	Back (g)	Wing (g)
1 (Control)	30	1752.60 ^{b}	69.01 ^{b}	676.23 ^{b}	467.43 ^{c}	303.00 ^{c}	164.80 ^{c}
2 (50 mg/kg)	30	1768.20 ^{b}	69.37 ^{ab}	682.43 ^{b}	496.63 ^{b}	320.37 ^{b}	181.90 ^{b}
3 (100mg/kg)	30	1778.33 ^{b}	69.69 ^{a}	686.53 ^{b}	501.37 ^{b}	327.20 ^{b}	188.80 ^{b}
4 (150mg/kg)	30	1834.97 ^{a}	70.35 ^{a}	714.23 ^{a}	535.20 ^{a}	350.47 ^{a}	232.33 ^{a}

$^{a, b, c,}$: Differences between averages with different letters are important (P<0.05).

The differences between the groups in terms of gizzard and liver weights were not significant (P> 0.05) and the differences between heart and abdominal fat were significant (P <0.05) (Table 7).

Table 7. Other some part of carcass

Groups	N	Gizzard (g)	Heart (g)	Liver (g)	Abdominal fat (g)
1 (Control)	30	44.90	14.77 ^{c}	41.17	30.70 ^{b}
2 (50 mg/kg)	30	43.53	16.87 ^{b}	47.97	32.87 ^{ab}
3 (100mg/kg)	30	43.50	17.23 ^{b}	49.83	30.07 ^{b}
4 (150mg/kg)	30	44.37	19.83 ^{a}	51.20	35.50 ^{a}

$^{a, b, ,}$: Differences between averages with different letters are important (P<0.05).

Conclusion

It was determined that the addition of 50, 100 and 150 mg / kg of essential oil (*Citrus sinensis L.*) to broiler chicks had significant effects on broiler's live weight values, feed intake, feed efficiency, carcass characteristics.

As the amount of orange peel essential oil (OPEO) added to the diets increased, the differences in live weight among the groups also increased (P <0.05). However, when we look at the first three weeks we talked about in the starter period; The doses of OPEO added to the diets did not have a significant effect on live weights when compared to the control group. These results were obtained from Çabuk et al (2006), Gemci (2006), Simsek et al. (2005) are parallel to the results of this study. Alçiçek et al. (2003) predict that the OPEO may have been affected later.than other essential oils. When we look at the finisher period (3-6th weeks) in general, it is seen that 150mg / kg of OPEO added to the feeds gives a higher live weight than the control group (P <0.05). It has been reported that this increase in live weight is achieved by promoting endogenous enzyme activity as well as the antimicrobial activity that OPEO has. Bozkurt et al (2007), Babaoğlan et al (2009), Erener et al (2007) reported that this increase in weight was due to increased enzyme activity of OPEO, regulation of microflora and increased nutrient absorption by reducing the activities of pathogenic bacteria.

When we look at feed intake in general; A decrease in feed consumption was observed as the OPEO dose level increased. Due to the aromatic properties of essential oils, the effects of increasing appetite and feed consumption did not appear in this study. The fact that the experiment was conducted in the warmer months may have caused the intake of feed to be low. Gemci (2006) reported that they predict that the intake of food will be low due to the temperature. Compared to the 150 mg / kg dosing control group except the first two weeks, it provided a significantly lower feed consumption (P <0.05). Alçiçek et al (2003) found that the 24 mg / kg essential oil dose on day 21 did not cause a difference in feed consumption compared to the control group, but doses of 48 and 72 mg / kg reduced feed consumption significantly (P<0.05). The results of decreasing feed intake with increasing doses were consistent with the results obtained in this study. Çabuk et al (2006) The results reported by the OPEO doses of 24 and 48 mg / kg significantly reduced feed consumption compared to the control group support the results obtained in this study. Mikulski et al (2008) reported that the addition of 500 and 1000 mg / kg of a mixture containing OPEO significantly increased feed consumption. Bozkurt et al. (2009b), Tekeli et al. (2006), Suk et al. (2003) determined that the addition of the essential oil mixture had no effect on broiler feed consumption.

With the exception of week 2, the differences between the feed efficiency values of the groups throughout the whole trial were significant (P <0.05). Bozkurt et al. (2009a), Bozkurt et al. (2009b), El-Ghousein and Al-Beitawi, (2009), Çabuk et al. (2006), Alçiçek et al. (2004), Alçiçek et al (2003) have reported that benefiting from the diet by adding volatile oil mixture is positively affected.

The differences in carcass weight among the groups were significant (P <0.05), while the weights were 1834.97 g in group 4 (150mg / kg) and 1. Group (control) was 17752.60 g. The 150 mg / kg OPEO supplement group gave significantly more carcass weight (P <0.05) than the control group. It is seen that as the dose level increases, the carcass weight increases. Mazmanoglu (2008) found that a mixture of 200 mg / kg of essential oil with 300 mg / kg essential oil finishing feed increased the carcass weight significantly (P <0.05) group The results is similar to the results in this study.

When the carcass yields were examined, the differences between the groups were significant (P <0.05). The highest carcass yield was found in the group supplemented with PKUY from 70.35% to 150 mg / kg. Efficiency ratios also increased as dose levels increased. The lowest rate was achieved in the control group. Şimşek et al. (2007a) supplemented with 100, 200 and 400 mg / kg anise essential oil resulted in 71.89% of the cold carcass ratio in the control group and 73.06% in the 400 mg / kg essential oil supplement group The difference between the two values was significant (P <0.05) and the results are consistent with this study. Efficiency ratios also increased as dose levels increased. The lowest rate was achieved in the control group. Yeşilbaş et al. (2009); Al-Kassie (2009); The results of Alçiçek et al. (2004) are consistent with the results of this study.

The differences between the groups in terms of breast, thigh, back and wing weights were significant (P <0.05). All treatment groups (50, 100 and 150 mg / kg OPEO) received significantly more weight (P <0.05) than control. El-Ghousein and Al-Beitawi (2009) reported the back, wing and breast weights, Simsek et al. (2005,2007b) breast weights, Simsek et al. (2007a) wing weights found significant differences (P <0.05) between the groups. The results are similar to this study. When the gizzard and liver weights were examined, the differences between the groups were not significant (P> 0.05) and heart weights were found

to be significant. Yıldız (2007); Çabuk et al. (2006); Gemci (2006) found the differences to be insignificant.

Abdominal fat weights were found to be significant (P <0.05) 150 mg / kg OPEO supplemented groups gained more weight (P <0.05) than control group. Al-Kassie (2009) and Babaoğlan et al. (2009) support the results of this study, which confirms that the addition of different doses of essential oil to diets increased abdominal fat weight significantly (P <0.05).

If we will make a general evaluation; according to the results obtained without trial; it has been determined that orange peel essential oil, which is considered as an alternative growth factor, has significant effects on carcass characteristics, general performance criteria such as live weight, feed consumption and feed efficiency in broiler chickens. Considering the whole of the experiment, the addition of 150 mg / kg of OPEO to broiler mixed feeds significantly improved the live weight compared to the control group. As a result, at the end of the trial, the orange peel volatile oil addition at 150 mg / kg provided 91 g more live weight per chick than the control group.. A difference at this level means that OPEO.

As a result, we found that orange peel, obtained by distillation from orange peel which is a citrus growing in our country, can be used for poultry feeding, and that the addition of 150 mg / kg orange peel volatile oil to broiler chicks showed the best effect, but it can be said that there is a need for further research in this area, given the scarcity of scientific studies on this subject.

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