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Research Article

The Impact of an Essential Oil Mixture on Growth Performance and Intestinal Histology in Native Turkish Geese *(Anser anser)*

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Abstract

This study investigated the impact of an essential oil (EO) mixture (Biomin) on growth performance, carcass yield, visceral organ weights, and duodenal crypt depth in native Turkish geese *(Anser anser)*. Sixty 1-day-old goslings constituted the animal material of the trial. Goslings were divided into three treatment groups, each consisting of four subgroups having five animals each. Animals were homogenously distributed among the groups after being weighed and identified. The three treatment groups received the essential oil mixture at rates of 0.0% (without EO), 0.1% and 0.2%, respectively. The animals were given concentrate feed (21.93% CP and 3010 kcal/kg ME) for the first 2 weeks, and were both grazed and provided with barley meal for the next 8 weeks. The trial was continued for a period of 10 weeks. Results showed that the essential oil mixture had no significant effect on live weight gain, feed intake, feed conversion rate, carcass yield, and heart-liver-gizzard weights (P>0.05). On the other hand, the crypt depths of the EO 0.1% and EO 0.2% groups were greater than that of the EO 0.0% group (P<0.05). This study demonstrated that EO supplementation had no impact on performance, but increased duodenal crypt depth in native Turkish geese.

Keywords: *Crypt depth, Essential oil, Native Turkish goose, Performance*

Yerli Türk Kazlarında *(Anser anser)* Esansiyel Yağ Karışımının Büyüme Performansı ve Bağırsak Histolojisi Üzerine Etkisi

Öz

Bu çalışmada esansiyel yağ (EY) karışımının (Biomin) yerli Türk kazlarında besi performansı, karkas randımanı, bazı organ ağırlıkları ve duodenum kript derinliği üzerine etkisi incelenmiştir. Hayvan materyali olarak 60 adet 1 günlük Yerli Türk kazı *(Anser anser)* kullanılmıştır. Kazlar üç deneme grubuna ve her birinde beş hayvan bulunan dört alt gruba ayrılmıştır. Hayvanlar tartılarak ve ayaklarından numaralandırılarak homojen dağıtılmıştır. Esansiyel yağ, gruplara sırasıyla; %0.0 (EY'siz), %0.1 ve %0.2 düzeylerinde verilmiştir. Hayvanlar iki hafta konsantre yemle (%21.93 HP ve 3010 kcal/kg ME) sekiz hafta mera+arpa kırmasıyla beslenmiştir. Deneme 10 hafta sürmüştür. Araştırmada esansiyel yağ karışımının canlı ağırlık artışı, yem tüketimi, yem dönüşüm oranı, karkas randımanı, kalp, karaciğer, taşlık ağırlıkları üzerine anlamlı bir etkisi olmamıştır (P>0.05). Diğer yandan EY %0.1 ve %0.2 gruplarındaki hayvanlara ait kript derinlikleri, EY %0.0 grubuna göre daha yüksek çıkmıştır (P<0.05). Yapılan bu araştırma yerli Türk kazlarında EY karışımının hayvanların performansına herhangi bir etki yapmazken, duodenum kript derinliğini artırdığını göstermiştir.

Anahtar sözcükler: *Esansiyel yağ, Kript derinliği, Performans, Yerli Türk kazı*

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INTRODUCTION

The intensive and indiscriminate use of antibiotics in the poultry sector has led to antibiotic residues in food products of animal origin, and the emergence of cross-resistant bacteria. These risks, which are both directly and indirectly linked to human health, have impelled researchers to investigate alternative growth factors [1]. Since the ban of the use of antibiotic growth promoters (AGPs) in food animals in the European Union (EU) complying with EU law, aromatic herbs and herbal products (oil, powder) have attracted the attention of the poultry sector as feed additives alternative to antibiotics [2-5]. Essential oils (EOs) are natural and non-residual alternative feed additives, which are obtained from aromatic herbs by various methods and are used as flavourings, appetite-stimulants, digestive stimulants and performance enhancers [6]. The type and level of phenolic compounds found in essential oils vary with the aromatic herbs the oils are derived from, and elicit various activities (i.e. antioxidant, antimicrobial, antifungal) [7-9]. Dietary supplementation with essential oils increases the productive capacity of poultry species by increasing the activity of digestive enzymes and eliminating pathogenic microorganisms [10,11]. Previous research has shown that carvacrol essential oils increase villus height and the villus height/crypt depth ratio, which in return improves digestive capacity [12,13]. Furthermore, rosemary oil essential oils have also been reported to increase the digestion of fat and fat-soluble vitamins by enabling the binding of bile acids to substances, and thereby, to improve the feed conversion rate [14]. Thus, it is considered that the combined use of synergistic essential oils, which are derived from different aromatic herbs and show different effects, even at low but appropriate doses, could improve animal production [15].

People have increasingly demanded poultry meat as an alternative source of animal protein [16]. Furthermore, goose meat is healthy, wholesome, and contains low cholesterol levels. Therefore, compared to other poultry species, geese are well adapted to cold climate conditions, more resistant to diseases, and do not require to be housed in costly pens. Although geese are classified as waterfowl, they are capable of living in drylands. Geese make good use of pasture plants and can be fed by grazing alone [17].

This study was aimed at the investigation of the impact of two different doses of an EO mixture on performance and intestinal histomorphology in native Turkish geese.

MATERIAL and METHODS

Ethical Approval

This study was conducted according to the approval (KAU-HADYEK/2019-118) granted by the Local Ethics Board for Experimental Animals of Kafkas University.

Animals and Treatment Design

The study was carried out at the premises of the Research Farm of Kafkas University. Sixty 1-day-old native Turkish goslings *(Anser anser)* of both sexes constituted the animal material. After hatching, the goslings were weighed, identified by unique numbers, and assigned to three groups. Each 20 animals in groups were divided into four subgroups containing five animals. The animals were homogeneously distributed among the groups by weight (32.76±0.01 g). The essential oil mixture was given to the three treatment groups at concentrations of 0%, 0.1% and 0.2%, respectively, in drinking water. During the first two weeks of the study, the animals were housed in cages under favourable environmental conditions and were fed on commercial goose starter ration containing 21.93% of crude protein (CP) and 3010 kcal/kg of metabolizable energy (ME) [18]. As from the third week, the animals were raised on the floor and acclimatized to grazing. The minimum area provided to each animal raised on the floor was 0.5 m^2. As of the third week, the animals were not only grazed but also provided with a barley meal (BM). Feed and water were provided *ad libitum*. The study was continued for ten weeks. The nutrient and chemical compositions of the starter ration, barley meal and pasture plants that the geese were fed on are presented in *Table 1*. The flora of the pasture consists of 64% Gramineae, 23% Leguminosae, and 13% other species. The treatment groups received different doses of essential oil and aromatic herb mixture containing peppermint oil, garlic oil, aniseed oil, fennel oil, cinnamon and cumin. The mixture was a commercial product supplied from Austria (BIOMIN GmbH).

Performance

Starting from the day of hatching to the day of slaughter, the animals were weighed individually on a weekly basis to determine their live weight gain. The feed intake (first two weeks concentrate feed-next eight weeks barley meal) of the animals was also calculated on a weekly basis. Feed conversion rates (FCR) were determined by calculating the ratio of feed intake to live weight gain.

Carcass Traits

Ten-week-old geese were fasted for 12 h prior to slaughter. Then, six animals, representative of the live weight of the groups, were selected from each group and were sacrificed by slaughtering the head at the occipital-atlantoaxial articulation. The birds were bled for 10-15 min and defeathered with machines. Following evisceration, the liver, gizzard and heart were weighed individually. The hot carcass weights of the unchilled carcasses were determined.

Histological Procedure

The duodenal tissue samples taken from the geese were firstly fixed in 10% formalin solution for 24 h. Then routine

Table 1. *Composition and nutrient ingredients of the feeds*

Ingredients (starter ration)		**%**
Corn, yellow		64.40
Soybean meal, 48% CP		21.46
Fish meal, 64% CP		7.00
Sunflower meal, 32% CP		4.80
Vegetable oil		0.55
Limestone		0.65
Dicalcium phosphate		0.35
DL-methionine		0.08
L-Lysine HCl		0.15
L-Threonine		0.06
Salt		0.25
Vit-Min Mix*		0.25
Nutrient Values		
Dry matter (%)		89.72
Crude protein (%)		21.93
Ca (%)		0.87
Available P (%)		0.44
Na (%)		0.16
Met+Cys (%)		0.90
Lysine (%)		1.34
Threonine (%)		0.91
Tryptophan (%)		0.26
Metabolic Energy, (kcal/kg)		3010
Chemical Analysis		**%**
Items	**Barley Meal**	**Pasture**
Dry matter (%)	88.11	94.55
Crude protein (%)	12.28	7.96
Crude fat (%)	2.18	1.65
Crude ash (%)	3.73	7.43
Crude fiber (%)	5.41	42.88
Neutral detergent fiber (%)	23.23	60.30
Acid detergent fiber (%)	6.35	48.27
Lignin (%)	0.57	9.62
Non-protein nitrogen (%)	76.41	40.09
Non-fiber carbohydrate (%)	58.59	22.67
Hemicellulose (%)	16.88	12.04
Starch (%)	55.23	-
Metabolic Energy Poultry kcal/kg	3097.05	-

** Vit-Min mix: Vit A: 10.000IU, Vit D_3: 4.000IU, Fe (iron sulfate monohydrate): 30 mg; I (calcium iodine anhydride): 1.5 mg, Co (cobalt carbonate monohydrate): 0.5 mg, Cu (copper sulfate pentahydrate): 5 mg, Mn (manganese oxide): 80 mg, Zn (zinc oxide): 80 mg, Se (selenium selenite): 0.3 mg*

tissue processing was applied and embedded in paraffin. Five-micron-thick sections were cut from the paraffin blocks and applied Mallory's modified triple staining (Triple) with a view to demonstrate the general structure of the duodenal tissue. The sections were examined with a light microscope (Olympus BX51, Japan). The crypt depths of the geese in all groups were measured using the ImageJ (LOCI, University of Wisconsin) software.

Table 2. *Effects of EO on performance parameters of geese (Mean±SEM; n=20)*

Periods	**EO Groups**	**LWG (g)**	**FI (g)**	**FCR**
0-2. weeks	0.0%	33.91±0.98	49.93±0.27	1.48±0.04
	0.1%	33.18±1.53	49.59±0.24	1.50±0.07
	0.2%	32.32±0.17	49.53±0.28	1.53±0.01
P		0.584	0.531	0.680
2-4. weeks	0.0%	51.51±0.79	121.33±0.28	2.36±0.04
	0.1%	51.16±0.26	121.53±0.39	2.38±0.02
	0.2%	51.90±0.86	121.88±0.38	2.35±0.04
P		0.755	0.559	0.688
4-6. weeks	0.0%	45.47±0.78	171.05±0.48	3.76±0.06
	0.1%	45.81±0.67	168.55±0.57	3.68±0.05
	0.2%	49.49±1.17	169.18±0.45	3.67±0.05
P		0.787	0.403	0.423
6-8. weeks	0.0%	49.10±0.69	201.24±0.50	4.27±0.09
	0.1%	50.03±0.71	201.21±0.45	4.13±0.11
	0.2%	48.55±0.66	201.58±0.61	4.08±0.11
P		0.342	0.858	0.424
8-10. weeks	0.0%	48.87±0.32	228.60±0.34	4.66±0.03
	0.1%	47.52±0.85	228.61±0.64	4.82±0.08
	0.2%	48.05±0.70	228.28±0.44	4.75±0.07
P		0.385	0.376	0.279
0-10. weeks	0.0%	45.36±0.25	154.53±0.11	3.40±0.02
	0.1%	45.09±0.40	153.90±0.08	3.41±0.03
	0.2%	45.59±0.17	154.09±0.05	3.29±0.04
P		0.534	0.059	0.635

EO: *Essential oil;* ***LWG:*** *Live weight gain;* ***FI:*** *Feed intake;* ***FCR:*** *Feed conversion rate.* ***SEM:*** *Standart error of the mean*

Statistical Analysis

The differences between the groups for performance, carcass traits, and histological parameters were analysed using the Statistical Package (SPSS portable PASW 18) software by one-way analysis of variance (ANOVA). The paired comparison of the data was performed with Duncan's test. The significance level was accepted as $P<0.05$.

RESULTS

The effects of the EO mixture on growth performance are shown in *Table 2*. The results obtained in the present study demonstrated that the EO mixture had no effect on live weight gain, feed intake and feed conversion rate ($P>0.05$).

Values pertaining to the slaughter and carcass traits of the study groups are presented in *Table 3*. Accordingly, it was

Table 3. *Effects of EO on carcass traits of geese (Mean±SEM; n=6)*

EO Groups	Slaughter Weight (g)	Carcass Yield (%)	Liver (g)	Gizzard (g)	Heart (g)
0.0%	3263.4±22.64	71.66±0.51	161.60±2.25	187.60±10.69	24.80±1.36
0.1%	3236.2±52.66	71.22±0.33	161.60±2.94	178.40±6.55	25.80±1.74
0.2%	3173.3±34.84	71.38±0.30	161.40±2.11	170.80±4.90	25.00±1.97
P	0.279	0.724	0.998	0.343	0.909

EO: *Essential oil;* ***SEM:*** *Standart error of the mean*

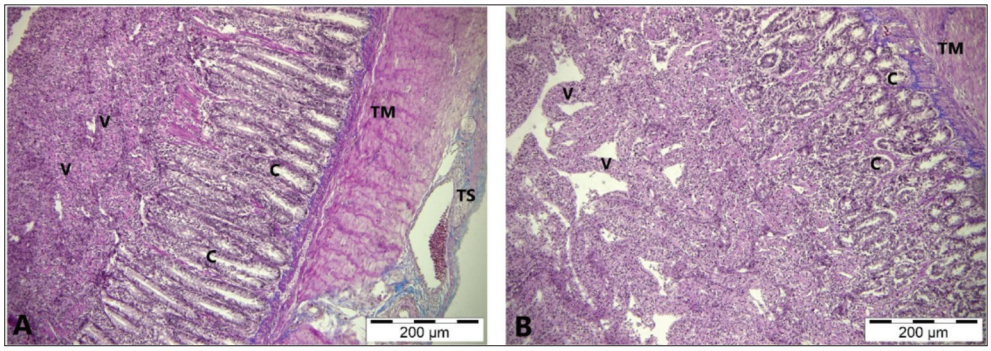

Fig 1. Goose duodenum tissue. **A:** 0.1% EO group, **B:** 0.2% EO group. **V:** Intestinal villi, **C:** Crypts, **TM:** Tunica muscularis, **TS:** Tunica serosa; Triple staining

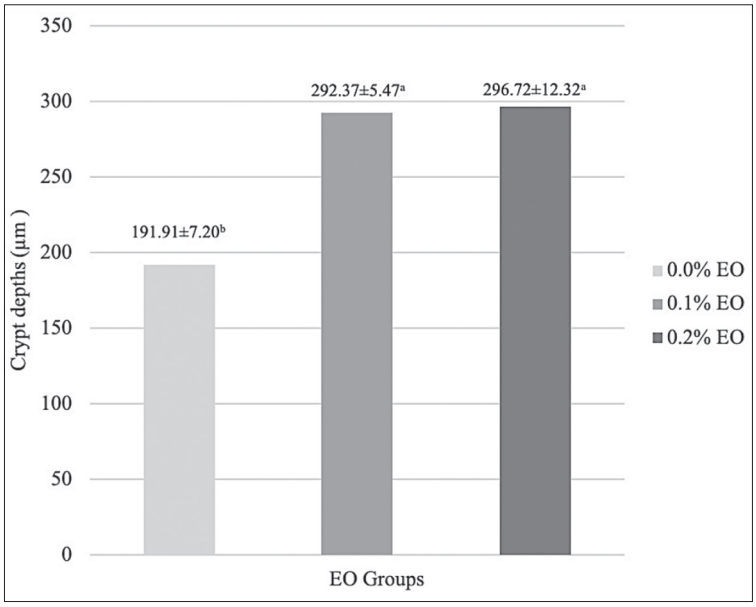

Fig 2. Effects of EO on duodenal crypt depths (µm), (Mean±SEM; n=6); [a,b] Values in the same column with no common superscript differ significantly (P<0.05); **EO:** Essential oil; **SEM:** Standart error of the mean

determined that the groups did not differ for the weight at slaughter and carcass yield of the animals (P>0.05). Similarly, no significant difference was determined between the groups for liver, gizzard and heart weights (P>0.05). The average weight of the liver, which is one of the most valuable products of geese, was determined to be 161.60 g, 161.60 g and 161.40 g, respectively, in the groups that were given 0%, 0.1% and 0.2% EO.

In all groups, the duodenal tissue of the geese was observed to be composed of the innermost tunica mucosa, tunica muscularis consist of smooth muscle cells and the outermost tunica serosa consist of connective tissue layers. The tunica mucosa formed the intestinal villi and crypts *(Fig. 1, Fig. 2)*.

The statistical analysis of the intestinal crypt depths of all groups demonstrated that the 0.0% EO group significantly differed from the 0.1% and 0.2% EO groups (P<0.05).

DISCUSSION

No statistically significant difference existed between the EO treatment groups (0.0%, 0.1% and 0.2%) for the live weight gain values between weeks 0-10 of the study. Generally, it is considered that herbal extracts produce favourable results in poultry. However, there are very few studies conducted on the use of aromatic herbs in geese. In these limited number of studies, it has been suggested that, in geese, performance values are not affected by supplementation with aromatic herbs [19,20]. The results of

the present study are in agreement with these previous poultry studies reporting unaffected performance [21-23]. Researchers have also reported that improved environmental conditions and reduced pathogen burden decrease the effects expected from the use of essential oils. Jamroz et al.[24] contrary to reported that 100 mg of an essential oil mixture containing carvacrol, cinnamaldehyde and capsicum increased live weight parameters in broiler chickens. This increase was attributed to cinnamaldehyde, which is the main active substance of cinnamon, increasing the secretion of pancreatic and intestinal enzymes, and capsicum increasing the production of hepatic enzymes, bile, pancreatic enzymes and intestinal lipase, resulting in an increased absorption of nutrients [24,25]. The results of the present study do not agree with some literature reports [26-28]. Some studies suggest that essential oil supplementation shows adverse effects on the performance of poultry [29]. Differences between the results of the present study and previous research have been attributed to different essential oil components having been used and tested, the essential oils found in the composition of mixtures interacting with each other, and EOs having been used at different levels. Furthermore, it is considered that geographical conditions, the timing of harvest, as well as animal- and environment-related factors may also contribute to discrepancies in study results.

Throughout the study period, no significant difference was observed between the groups for weekly feed intake and feed conversion rate values. In previous studies conducted by Baowei et al.[19] and Yaman et al.[20] in geese, it was reported that neither feed intake nor feed conversion rate was affected. Similarly, while Fascina et al.[30] indicated that essential oil mixtures had no effect on growth performance, feed intake and feed conversion rate, Nobakht and Mehmannavaz [31] reported that different fat resources did not cause any change in average feed intake values in broiler chickens. Furthermore, Khaksar et al.[32] reported that thymol essential oil had no effect on feed intake and feed conversion rate in quails. These findings are in agreement with previous studies, which were conducted by Mathlouthi et al.[33], Küçükyılmaz et al.[34], and suggested that essential oil mixtures did not affect feed intake and feed conversion rate.

The effects of essential oils arise from the active molecules found in their structure. The results of the present study are similar to those reported by Lee et al.[35] and Günal et al.[36], who suggested that herbal extracts do not affect feed intake in broiler chickens, but contradict with the report of Tekeli et al.[37], who suggested that herbal extracts increase feed intake in broiler chickens. Jamroz et al.[24] reported that the supplementation of maize- and wheat-based rations with an essential oil mixture containing carvacrol, cinnamon oil and black pepper oil increased the feed conversion rate of broiler chickens by 4%. On the other hand, Al-Kasie [38] indicated that supplementation with an essential oil mixture containing carvacrol and cinnamon oil decreased the feed conversion rate, when given at a dose of 200 ppm ($P<0.05$).

The end-study carcass traits and visceral organ weights of the groups are presented in *Table 3*. Accordingly, no statistically significant difference was observed between the groups for slaughter weight, carcass yield and heart, liver and gizzard weights ($P>0.05$).

The findings obtained in the present study comply with those reported by Muhl and Liebert [39], who suggested that the supplementation of broiler grower rations with phytogenic extracts (5% carvacrol, 3% cinnamaldehyde and 2% *Capsicum oleoresin)* had no effect on carcass yield, and also agree with the report of Şimsek et al.[40], who indicated that mixtures of carvacrol, clove oil and aniseed oil do not have any effect on carcass yield and characteristics in broiler chickens. Furthermore, similar to the present study, upon investigating the effects of different protein sources in geese, Şahin et al.[41] reported to have not detected any difference between the study groups for carcass traits. The similarities between the results of these different studies are attributed to the similarity of the management conditions the animals were exposed to.

The results obtained for carcass yields in the present study differ not only from those reported by Fascina et al.[30] who indicated a significant increase in carcass yield with the supplementation of broiler rations with phytogenic extracts, but also from those reported by Al-Kasie et al.[38] who pointed out to significantly increased carcass yields with the supplementation of broiler rations with essential oil mixtures containing carvacrol and cinnamon oil ($P<0.01$).

Literature reports have been published, which suggest that herbal feed additives improve slaughter and carcass traits [40,42]. Researchers attribute this positive effect to the appetizing, enzyme-secretagogue and antimicrobial activity of essential oils [43].

The results of the present study are similar to those reported by Criste et al.[44] for gizzard and hearth weights, by Kim et al.[45] for liver weight, by Yeganeparast et al.[22] for hearth weights, and by Salehifar et al.[46] for liver and gizzard weights. Çelik and Şahin [47] determined that an essential oil mixture, which was administered in drinking water and contained peppermint oil, carvacrol, cade oil and rosemary oil did not affect heart, liver and gizzard weights. The visceral organ weight parameters determined in the present study differ from the results of some other previous studies conducted on essential oil supplementation [42,48].

Low crypt depth is one of the main indicators of intestinal health. Peng et al.[49] reported that the supplementation of broiler rations with oregano essential oil reduced the

depth of intestinal crypts, and thereby, increased growth performance. Some other studies have reported that essential oil supplementation does not affect duodenal crypt depth in broiler chickens [50,51]. In agreement with the present study, Yang et al.[52] reported that cinnamon oil, when supplemented at a high dose (800 mg/kg), increased duodenal crypt depth, but had no effect on performance parameters. In fact, increased crypt depth was observed not to show any adverse effect on performance parameters in geese.

In conclusion, it has been determined that, in geese, supplementation with different doses of essential oil mixtures increases intestinal crypt depth, but this increase does not affect live weight gain. In view of the limited number of studies in geese, it is considered that further studies need to be carried out in poultry, and particularly in geese, such that different doses of various essential oils are investigated in different slaughtered age.

Conflict of Interest

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

Author Contributions

Experimental design, feeding trial and article writing; MÖ and TŞ, Performance parameters calculation; ÖK, Histological analysis; EKS, Statistical Analysis; SAI and TK, Interpretation and editing of results; MAY.

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