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Use of essential oil mixture to improve antioxidant capacity and concentrations of cecum short-chain fatty acids in Turkish domestic geese (*Anser anser*)

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Abstract: The aim of this study is to investigate the effect of essential oil mixture supplemented in drinking water on antioxidant capacity and intestinal health in geese. One hundred eight chicks (which were 3 days old) were randomly allocated to 3 groups and each group was allocated to 6 subgroups. Research groups have been as follows: C (Control; without supplementation); E1 (0.4 ml/L essential oil mixture supplementation) and E2 (0.8 ml/L essential oil mixture supplementation). The duration of the experiment was 13 weeks. In the first 4 weeks of the trial, the animals were fed for the chick period. In the last 9 weeks of the trial, geese were fed in the pasture under the conditions of Kars province. In the 4th week and at the end of the experiment, GSH exhibited a linear response (P=0.008 and P=0.004, respectively). However, MDA, GSH, SOD, GPx, CAT, nitric oxide, ceruloplasmin, albumin, total protein and globulin were not affected. At the end of the experiment, acetic acid, butyric acid, isocaproic acid and total short-chain fatty acid concentrations were linearly affected with the gradued level of essential oil mixture. There were no significant differences in propionic acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid and BCFA concentrations. In conclusion, water containing essential oil mixture in geese can be used to improve antioxidant capacity and intestinal health.

Keywords: Antioxidant capacity, cecum short-chain fatty acid concentrations, essential oil mixture, Turkish domestic goose (*Anser Anser*).

Türk yerli kazlarında (*Anser anser*) uçucu yağ karışımının antioksidan kapasitesi ve sekum kısa zincirli yağ asidi konsantrasyonlarının iyileştirilmesi için kullanımı

Özet: Bu çalışmanın amacı, içme suyuna eklenen uçucu yağ karışımının kazlarda antioksidan kapasite ve bağırsak sağlığı üzerindeki etkisini araştırmaktır. Yüz sekiz civciv (3 günlük) rastgele 3 gruba ve her bir grup 6 alt gruba ayrılmıştır. Araştırma grupları şu şekildedir: C (Kontrol; Katkı takviyesiz); E1 (0,4 ml / L uçucu yağ karışımı takviyeli) ve E2 (0,8 ml / L uçucu yağ karışımı takviyeli). Deneme süresi 13 haftadır. Denemenin ilk 4 haftasında hayvanlar civciv dönemi besin madde ihtiyaçlarına gore beslenmiştir. Denemenin son 9 haftasında Kars ili şartlarında merada beslenmiştir. Denemenin 4. haftası ve bitiminde, GSH linear bir artış göstermiştir (sırasıyla P=0,008 ve P=0,004). Ancak, MDA, GSH, SOD, GPx, CAT, nitrik oksit, seruloplazmin, albümin, toplam protein ve globulin konsantrasyonları muameleleren önemli düzeyde etkilenmemiştir. Deneme sonunda asetik asit, butirik asit, izokaproik asit ve toplam kısa zincirli yağ asidi konsantrasyonları dereceli uçucu yağ karışımı ile doğrusal olarak etkilenmiştir. Propiyonik asit, izobütirik asit, valerik asit, izovalerik asit, kaproik asit ve BCFA konsantrasyonları açısından önemli bir farklılık bulunmamıştır. Sonuç olarak, kazlarda içme sularında bulunan uçucu yağ karışımı, antioksidan kapasite ve bağırsak sağlığını iyileştirmede kullanılabilir.

Anahtar sözcükler: Antioksidan kapasite, sekum kısa zincirli yağ asidi konsantrasyonları, uçucu yağ karışımı, Türk yerli kazı (*Anser Anser*).

Introduction

The prolonged use of antibiotics as growth factor has prevented the growth of beneficial microorganisms in digestive tract of animals as well as pathogenic microorganisms (20). Use of antibiotics in feeds has promoted development of resistance against bacteria. In addition, residues in animal products have become a risk to human health (1). For this negative reasons, antibiotics have been prohibited as growth factor in animal feed. After this ban probiotics, prebiotics, enzymes, organic acids and some product, such as essential oils, started to be used as an alternative feed additive to antibiotics (7, 15, 41).

Rosemary is in the *Lamiaceae* family (5, 34). Its leaves contain strong antioxidants such as *carnosol, rosmarinic acid* and *carnosic acid*. Mint is in the *Labiatae* family. Its active compounds are flavones, r*osmaniric acid*, *chlorogenic acid* and triterpenic substances (30). Juniper is in the *Cupressaceae* family (16). In juniper; there are inositis, flavonoids, glycosides, resin, invert sugar, katesin, organic acids, volatile oil, terpenic acids, lacoanthocyanidine substances (22). Oregano is in the *Lamiaceae* family (containing carvacrol and phenolic monoterpenoids) (9, 29). These plant have appetite-enhancing, digestive- stimulanting, anticoccidial, antihelmintic, antiviral, antimicrobial and antioxidant effect (19).

Recent studies have focused on the use of essential oil blends and aromatic herbs in animal nutrition. In this study, it was aimed to investigate the effect of essential oil mixture on blood antioxidant capacity and intestinal health in geese.

Materials and Methods

Animals, experimental design and feed: Ethical approval for this study was obtained from the Kafkas University Animal Experiments Local Ethics Committee (Decision No: KAU-HAYDEK /2018-054/2019-001). One hundred eight chicks (which were 3 days old) were randomly allocated to 3 groups and these groups were allocated to 6 replicate pens (100x100 cm) (6 chicks in each subgroup). The animals were fed with a basal diet based on corn and soybean meal (Table 1). All diets were determined according to NRC standards (28). Nutrient analyses of the feed were performed according to AOAC (3). The duration of the experiment was 13 weeks. In the first 4 weeks of the trial, the animals were fed for the chick period. Each subgroup was equipped with manual feeders and automatic nipple drinkers. Water and feed were given *ad libitum*. In the last 9 weeks of the trial, geese were fed in the pasture under the conditions of Kars province. Goose breeding widely takes place in Kars province because of the suitable climatic and geographic conditions (10). The geese were exposed to natural daylight and kept with a room temperature of 24±3°C. The animals were taken to the pasture between 8-12 am and 13-18 pm. Research groups have been designed as follows: C (Control; without supplementation); E1 (0.4 ml/L essential oil mixture supplementation in drinking water) and E2 (0.8 ml/L essential oil mixture supplementation in drinking water). The essential oil mixture (Mintofarm®) used in the research was obtained from a private company (FARMAVET A.Ş.). Composition of Mintofarm used in the study is shown in Table 2.

Table 1. Composition of basal diets used in experiment (%)[1].

Feed materials	**%**
Corn	56.35
Soyben meal (CP, 46%)	36.10
Corn gluten (CP, 60%)	4.35
Limestone	1.45
Dicalciumphosphate	1.00
DL- Methionine	0.08
L-Lysine Hydrochloride	0.07
Vitamin- mineral premix	0.40
Salt	0.20
Total	100.00
The calculated value	
Crude protein, %	23.00
ME (kcal/kg)	2909.33
Ca, %	0.90
Total P, %	0.59
Analysis Values	
ME (kcal/kg)	2915.25
Crude protein, %	23.11
Ca, %	1.01
Total P, %	0.49

[1] As-fed basis
[2] Vitamin-mineral premix provided per kg diet: Vit. A 8000 IU, Vit. D3 1000 IU, Vit. E 20 IU, Vit. K 0.5 mg, Vit. B1 3 mg, Vit. B2 9 mg, Vit. B6 7 mg, Vit. B12 0.03 mg, niacin 35 mg, D-pantothenic acid 10 mg, folic acid 0.55 mg, biotin 0.18 mg, Fe 100 mg, Cu 8 mg, Zn 100 mg, Mn 120 mg, I 0.7 mg, and Se 0.3 mg.

Table 2. Chemical composition essential oil mixture used in experiment (%).

Product Composition*	**%**
Mint oil	2
Juniper Oil	2
Rosemary Oil	2
Oregano Vulgare oil	2
Surfactants and Stabilizers	15
Water (transporter)	77

*Mintofarm.

Blood antioxidant capacity: Blood samples were taken from the wing veins of the animals into anticoagulant (EDTA) tubes at the 4th week and at the end of the experiment. After a sufficient amount of blood sample was separated as whole blood, plasma of the remaining blood was obtained. The samples taken were centrifuged at 3000 rpm for 15 minutes and stored at -20 ºC until analysis. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzyme activities in plasma were determined by ELISA device (Epoch, Biotek, USA) using commercial kits (Cayman Chemical Company, USA). Whole blood reduced glutathione (GSH) analysis was determined colorimetrically (Epoch, Biotek, USA) according to the method of Beutler et al. (4). The malondialdehyde (MDA) in plasma was determined by the method of Yoshoiko et al.(42), ceruloplasmin by the method of Colombo and Ricterich and albumin and total protein levels by a commercial test kit (Biolabo, Maizy, France) (8). Globulin value was determined by subtracting albumin from total protein (11). Nitric oxide levels in serum Miranda et al. (26) was determined according to the method they reported.

Cecal short-chain fatty acid concentrations: The cecal digesta that was obtained after sacrificing the animals, was used for the determination of acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic and isocaproic acid with a gas chromatography (Shimadzu GC, Shimadzu Co., Kyoto, Japan), a flame ionization detector **(**FID) and colons (Teknokroma; TR-151035, TRB-FFAP 30m×0.53 mm×0.50 µm). At the end of the study, the cecum content were stored at -18ºC and then were dissolved at + 4ºC before analysis. The contents were centrifuged at 4000 rpm for 15 min at +4°C for homogenization. The supernatant was taken into an Eppendorf tube and mixed with 0.2 mL ice-cold 25% metaphosphoric acid solution. After that, the tubes were kept in ice for 30 min to ensure the collapse of proteins. Subsequently, tubes were centrifuged for 10 min at 11000 rpm at + 4°C. Supernatants were analyzed using GC. The analysis was performed according to Zhang et al. (43). Helium was used for the carrier gas and the colon temperature was programmed so that it was increased stepwise from 110°C to 180°C. Also, the FID and injector block temperature was set to 250°C.

Statistical Analysis: The one-way analysis of varience (ANOVA) method was used for the statistical calculations of the groups and polynomial contrast test was used to determine the dose effect of the essential oil mixture used at different levels in the groups. Statistical differences and tendence analysis were considered significant at P≤0.05. The statistical analysis was done with the SPSS software package (35).

Results

Blood antioxidant capacity: In the 4th week and at the end of the experiment, the increase by essential oil mixture, GSH exhibited a linear response (P=0.008 and P=0.004, respectively). However, MDA, GSH, SOD, GPx, CAT, nitric oxide, ceruloplasmin, albumin, total protein and globulin were not affected by essential oil mixture added. The blood antioxidant capacity parameters of the study at the 4th week and at the end of the experiment are shown in Table 3 and Table 4.

Table 3. Influence of essential oil mixture on antioxidant capacity in the 4th week of the experiment.

	Groups					
Blood Parameters	**C**	**E1**	**E2**	**SEM**	**Significance**	
	x̄	**x̄**	**x̄**		**L**	**Q**
MDA (µmol/L)	7.11	7.27	7.34	0.22	0.699	0.936
Nitric oxide (µmol/L)	31.72	33.58	35.15	1.82	0.475	0.973
GSH (mg/dL)	18.73	24.11	26.51	1.25	0.008	0.513
SOD (U/mL)	47.27	52.60	53.33	8.27	0.226	0.588
CAT (nmol/min/mL)	1.19	1.20	1.23	0.02	0.529	0.875
GPx (nmol/min/mL)	261.48	286.73	288.86	6.22	0.073	0.362
Ceruloplasmin (mg/dL)	19.27	19.31	19.28	0.58	0.991	0.980
Albumin (g/dL)	2.76	2.72	2.71	0.08	0.838	0.957
Total protein (g/dL)	5.91	5.90	5.87	0.10	0.907	0.969
Globulin (g/dL)	3.15	3.17	3.16	0.11	0.970	0.942

[1] Data represent mean values of 6 replicates per treatment,
[2] Groups; C: Control without supplementation drinking water, E1: 0.4 mL essential oil mixture supplementation in drinking water and E2: 0.8 mL essential oil mixture supplementation in drinking water,
[3] Polynomial contrasts: L=linear and Q=quadratic effect of supplemental essential oil mixture.

Table 4. Influence of essential oil mixture on antioxidant capacity at the end of the experiment.

	Groups					
Blood Parameters	**C**	**E1**	**E2**	**SEM**	**Significance**	
	x̄	**x̄**	**x̄**		**L**	**Q**
MDA (µmol/L)	7.19	7.30	7.37	0.18	0.717	0.951
Nitric oxide (µmol/L)	32.41	34.17	34.93	1.19	0.418	0.852
GSH (mg/dL)	20.04	24.58	27.36	1.09	0.004	0.640
SOD (U/mL)	49.14	51.65	54.72	2.09	0.308	0.953
CAT (nmol/min/mL)	1.20	1.21	1.22	0.02	0.691	0.914
GPx (nmol/min/mL)	267.41	289.03	291.59	9.42	0.322	0.648
Ceruloplasmin (mg/dL)	19.51	19.53	19.48	0.54	0.985	0.977
Albumin (g/dL)	2.74	2.72	2.71	0.03	0.721	0.990
Total protein (g/dL)	5.89	5.89	5.87	0.09	0.949	0.971
Globulin (g/dL)	3.14	3.16	3.16	0.10	0.967	0.976

[1] Data represent mean values of 6 replicates per treatment,
[2] Groups; C: Control without supplementation drinking water, E1: 0.4 mL essential oil mixture supplementation in drinking water and E2: 0.8 mL essential oil mixture supplementation in drinking water,
[3] Polynomial contrasts: L=linear and Q=quadratic effect of supplemental essential oil mixture.

Table 5. Effect of essential oil mixture on cecal short-chain fatty acid concentrations at the end of the experiment (µmol/g).

	Groups					
SCFA Parameters	**C**	**E1**	**E2**	**SEM**	**Significance**	
	x̄	**x̄**	**x̄**		**L**	**Q**
Acetic acid	50.09	58.68	72.06	3.70	0.012	0.732
Propionic acid	21.88	20.62	26.30	1.32	0.167	0.219
Isobutyric acid	0.86	1.01	1.07	0.07	0.258	0.774
Butyric acid	9.46	15.97	17.30	1.35	0.013	0.317
Isovaleric acid	1.07	1.17	1.27	0.08	0.355	0.996
Valeric acid	1.47	1.49	1.74	0.09	0.270	0.602
Isocaproic acid	0.04	0.10	0.09	0.00	0.011	0.042
Caproic acid	0.08	0.08	0.08	0.00	0.317	0.946
BCFA	3.41	3.68	4.09	0.23	0.265	0.898
Total SCFA	84.86	98.96	119.76	5.66	0.008	0.750

[1] Data represent mean values of 6 replicates per treatment,
[2] Groups; C: Control without supplementation drinking water, E1: 0.4mL essential oil mixture supplementation in drinking water and E2: 0.8 mL essential oil mixture supplementation in drinking water,
[3] Polynomial contrasts: L=linear and Q=quadratic effect of supplemental essential oil mixture.
[4] BCFA (Branched Chain Fatty Acids): isobutyric acid+isovaleric acid+valeric acid.
[5] Total SCFA (Short Chain Fatty Acids): acetic acid+propionic acid+isobutyric acid + butyric acid+isovaleric acid+valeric acid.

Cecal short-chain fatty acid concentrations: Cecal short-chain fatty acid concentrations (µmol/g) measured at the end of the experiment are given in Table 5. Acetic acid, butyric acid, isocaproic acid and SCFA were linearly affected by the gradued level of essential oil mixture (P=0.012, P=0.013, P=0.011 and P=0.008, respectively). There were no significant differences in propionic acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid and BCFA concentrations.

Discussion and Conclusion

The sources of natural antioxidant compounds are plants. Plants show antioxidant properties with the phenolic compounds they contain (25). Free oxygen radicals damage the organism and this situation is kept under control by antioxidant systems. In pathological conditions, the oxidant and antioxidant balance changes. Major phenolic antioxidants prevent cell death under oxidative stress (31). Phenolic compounds in the plant exhibit antioxidant effects, especially due to their redox properties. Therefore, redox agents act as hydrogen donors, oxygen inhibitors, and metal chelators (36). Oxidative stress and balance between antioxidant capacity organ or organ determines the susceptibility of their systems to oxidative stress. GSH, antioxidant vitamins, antioxidant enzymes cells against oxidative damage plays an important role in protection (33). GSH is used for therapeutic purposes in preserving antioxidant capacity (2). In our study, GSH exhibited a linear response with the increase of the levels of essential oil mixture. Due to the insufficient number of studies using aromatic plants and extracts in goose, in the discussion has also been benefited

from studies in other different breeds and species. Chen et al. (6) showed that flaxseed improved the antioxidant status in the 1-day-old gosling (Huoyan Geese). The use of aromatic plant oil mixture in quail breeders' drinking water has been protective against oxidative stress (12). In a study, the use of thyme oil in Tuj lamb improved blood oxidant-antioxidant balance (13). In another study, Habibi et al. (17) reported that serum total antioxidant capacity was affected the addition of ginger essential oil in broiler. In a study using phytogenic feed additives in broilers, antioxidant capacities increased in liver and jejunum and decreased liver lipid peroxidase level (27). Overall, these results show that essential oil mixture could be considered as strong natural antioxidants in poultry diets.

Short chain fatty acids are formed by bacterial fermentation. Short-chain fatty acids stimulate cell growth and differentiation in the gut, thereby improving intestinal integrity, as well as preventing the growth of pathogenic microorganisms by lowering the digestive system pH (21). Researchers reported that there is a close relationship between the composition of cecum microflora and SCFA concentration (24). Increased SCFA concentrations have been shown to have beneficial effects on energy, metabolism, microflora and immune responses (37). The contribution of the use of barley in goose rations to the metabolizable energy of SCFA formation in secum is 2.7 kJ g^{-1} (18). Weng et al. (40) reported that the value of isocaproic acid decreases in the investigation of the relationship of metabolic and microbiota variables with diet in the case of intestinal inflammation. The decrease in isocaproic acid value shows that pH acidity in the intestine is active and pathogenic microorganisms are active in inflammation. In the light of these studies, the increase in secum short chain fatty acids can be interpreted as having a positive effect on intestinal health. Among SCFAs, butyric acid is a primary energy source for enterocytes. In cellular differentiation and takes part in proliferation in the intestinal mucosa (32). In our study, acetic acid, butyric acid, isocaproic acid and SCFA were linearly affected with the gradued level of by essential oil mixture. There were no significant differences in propionic acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid and BCFA concentrations. The number of studies investigating cecum short-chain fatty acids in intestinal health in poultry is very limited. Moreover, in the literature search, no articles investigating geese or SCFAs were found. In one study, the addition of bilberry to rat diets enriched cecum SCFAs (23). The presence of green tea extract and black tea extract in rat rations stimulates cecum SCFA production (38). In a different study, limonene caused significant changes in short-chain fatty acids in mice (39). On the other hand, use of thyme and black cumin oil in broiler rations did not affect cecum short chain fatty acids (14). The differences in the results of these studies can be explained by the type and dosage of the plant extracts added, the ratio of volatile fatty acids and active ingredients, house conditions and the influence of environmental factors.

In conlusion, the addition of essential oil mixture to drinking water has been found to be effective in protecting geese against oxidative stress and improving gut health according to the blood antioxidant capacity and cecum SCFA results. Further studies are needed to illuminate the investigated parameters.

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Ethical Statement

Ethical approval for this study was obtained from the Kafkas University Animal Experiments Local Ethics Committee (Decision No: KAU-HAYDEK /2018-054/2019-001).

Conflict of interest

The authors declared that there is no conflict of interest.

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