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REGULAR ARTICLES

Effects of essential oil mixtures on expression of genes involved in lipogenesis and fatty acid oxidation in geese (*Anser anser*)

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Abstract

The use of essential oils has recently increased in the poultry sector. The aim of this study was to investigate the effects of essential oil mixture (juniper, mint, oregano and rosemary oil) on fatty acid oxidation and lipogenic gene expression in geese. Research groups were formed as C (control; no additives), EK1 (0.4 ml/l essential oil mixture supplemented) and EK2 (0.8 ml/l essential oil mixture supplemented). Relative expression levels of genes included in lipogenesis (ACCα, ChREBP, FASN, LXRα and SREBP-1) expression levels of genes included in fatty acid oxidation (ACOX1, CPT1, CPT1A, PPARα and PPARγ) were measured using RT-qPCR. Group EK1 upregulates the mRNA expression levels of genes involved in lipogenesis such as ACCα, ChREBP and SREBP-1, while it downregulates the mRNA expression in levels of all genes involved in fatty acid oxidation. Group EK2 increases the mRNA expression levels of genes involved in lipogenesis such as ACCα, FASN and SREBP-1, while it decreased mRNA expression at the levels of all genes involved in fatty acid oxidation, as in the other group. In the study, adding an essential oil mixture to drinking water is predicted to increase fatty liver because it upregulates genes related to fat synthesis (lipogenesis) and downregulates genes related to fat degradation (fatty acid oxidation).

Keywords Geese · Gene expression · Essential oil · Lipogenesis · Fatty acid oxidation

Introduction

New spices and aromatic plants and their essential oils have been used as an alternative to antibiotics in livestock and have increased poultry health and productivity as a result (Kishawy et al., 2022). Essential oils are widely used in the food industry and alternative medicine (Bartonkova and Dvorak, 2018). With this information, essential oil supplementation to feed or water is a novel strategy for improving animal health (Sabino et al., 2018). Essential oils usually used in poultry rations include juniper, oregano, mint and rosemary (Abd El-Hack et al., 2022). Previously, a study has reported that adding an essential oil mixture to water can be used to improve antioxidant capacity and intestinal health and may also have a protective effect against oxidative stress (Durna Aydin et al., 2022).

The regulatory mechanisms of lipid metabolism have been the focus of research by nutritionists. The liver is the organ that is the main regulator of fatty acid metabolism (Han et al., 2015). The liver of goose is an excellent model for understanding the molecular mechanisms of lipogenesis (Tang et al., 2018). Lipogenesis is a metabolic process in which fatty acids and glycerol are converted into fats. Lipogenesis involves both fatty acid and triglyceride synthesis (Kersten, 2001). The transcription factor sterol regulatory element-binding protein 1 (SREBP-1) plays a major role in

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hepatic fatty acid metabolism by regulating the expression of various genes that conduce to fatty acid synthesis. Liver X receptor α (LXRα) has been found to be a central nuclear transcription factor and is an important regulator of lipid expression of this lipogenic transcription factor (Han et al., 2009). SREBP-1 and LXRα lead to the improved plasma triglycerides and fatty liver (Han et al., 2009). For the liver of animals, LXRα activation was described to increase of genes involved in lipogenesis, such as SREBP-1, fatty acid synthase (FASN), carbohydrate response element-binding protein (ChREBP) and acetyl-CoA carboxylase (ACCα) (Han et al., 2009).

Fatty acid oxidation is a biochemical duration in which fatty acid molecules are crack up (Houten and Wanders, 2010). In eukaryotes, the process takes place in the mitochondria and leads to the production of acetyl coenzyme A to be used in the Krebs cycle and nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2) coenzymes used in the electron transport system (Houten and Wanders, 2010). As a result, very long-chain fatty acids are oxidized in peroxisomes, releasing CO_2 and ketone bodies. Peroxisome proliferator-activated receptors (PPARs) are in the nuclear receptor family of ligand-activated transcription factors (Chinetti et al., 2000). In particular, PPARα and PPARγ play an essential role in the metabolic adaptation of the liver to fasting situations by inducing gene expression of the rate-limiting enzymes for mitochondrial and peroxisomal fatty acid oxidation, such as carnitine palmitoyltransferase 1 (CPT1), carnitine palmitoyltransferase 1A (CPT1A) and acyl-coenzyme A oxidase 1 (ACOX1) in mammals (Houten and Wanders, 2010).

Fat concentration is established by the balance between fat synthesis (lipogenesis) and fat breakdown (fatty acid oxidation) (Kersten, 2001).

In this study, we examined the effects of the addition of different proportions of essential oil mixture to drinking water on expression of gene involved lipogenesis and fatty acid oxidation in geese.

Material and methods

Animals, experimental design and feed

Ethical confirmation for study was obtained from the Animal Experiments Local Ethics Committee of Kafkas University (No: KAU-HAYDEK/2019–128). One hundred eight chicks (3 days old) were randomly divided into 3 groups, and these groups were separated to 6 replicate pens (100 × 100 cm) (6 chicks chosen in each group). The animals were fed with a basal diet based on Table 1. All diets were specified according to NRC standards (NRC, 1994). Nutrient analyses of the feed were applied according to AOAC (AOAC, 2000). The duration of the experiment was 13 weeks. During the first 4 weeks of the experiment, chick period feeds were given to the animals. Feed and water were given ad libitum. In the last 9 weeks of the experiment, the geese were fed in the pasture. The research groups were designed as follows: C (control; no additives); EK1 (0.4 ml/l essential oil blend supplement in drinking water) and EK2 (0.8 ml/l essential oil blend supplement in drinking water). The essential oil mixture (Mintofarm®) used in the study was obtained from a private company. The formulation of Mintofarm® used in the study is shown in Table 2. Rosemary is in the Lamiaceae family. Its leaves contain strong antioxidants

Table 1 Composition of diets in experiment (%)

Feed materials	%[1]
Corn	56.35
Soybean meal (CP, 46%)	36.10
Corn gluten (CP, 60%)	4.35
Limestone	1.45
Dicalcium phosphate	1.00
DL-Methione	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 Formulation of essential oil mixture used in the study (%)

Product combination*	%
Juniper oil	2
Mint oil	2
Oregano oil	2
Rosemary oil	2
Surfactants and stabilizers	15
Water (transporter)	77

*Mintofarm®

such as carnosol, rosmarinic acid and carnosic acid (Sevim et al., 2020). Mint is in the Labiatae family. Its active compounds are flavones, rosmarinic acid, chlorogenic acid and triterpenic substances (Öztürk et al., 2002). Juniper is in the Cupressaceae family. In juniper, there are inositis, flavonoids, glycosides, resin, invert sugar, katesin, organic acids, volatile oil, terpenic acids and leucoanthocyanidin substances (Gauquelin et al., 2002). Oregano is in the Lamiaceae family (containing carvacrol and phenolic monoterpenoids) (d'Antuono et al., 2000).

RNA isolation and cDNA synthesis

Total RNA isolation and quality control from liver tissue samples were as applied (Kurar et al., 2010). A total of 30 mg of liver sample was cut with a scalpel and homogenized in 1 mL of TRIzol with the help of a mechanical homogenizer. Two hundred microlitre of chloroform was added to the 1000 µl suspension taken into the Eppendorf tube and mixed by the vortex. The samples, which were kept at room temperature for 8–10 min, were centrifuged for 15 min at a speed of 13,000 g in a cooled centrifuge at + 4˚C. The resulting supernatant was separated into another Eppendorf tube without disturbing the phases. Five hundred microlitre isopropanol (Sigma-Aldrich) was added to 350–400 µl supernatant; the mixture was mixed by turning it upside down, and it was kept at room temperature for 10 min, and then, the RNA pellet formed was precipitated at the bottom by centrifugation at 13,000 g speed for 10 min in a cooled centrifuge. After removing the supernatant part, washing with cold ethanol 3 times (70%, 70% and 95%, respectively) was done at + 4˚C at 7500 g speed for 5 min. After the RNA pellet was dried sufficiently, it was poured into 50 µl diethyl pyrocarbonate-treated water (DEPC-dH_2O) which has been resolved. The quantity and quality of the RNA samples were determined using the spectrophotometer. In order to determine the quality of the RNA samples, the quality of the RNA bands stained with ethidium bromide (EtBr) after 1% agarose gel electrophoresis by placing 5 µl of 6X loading dye on RNA at a concentration of 1 µg/10 µl was checked visually on a UV transluminator and in the imaging system. DNAse-I enzyme reaction was applied to eliminate possible gDNA contamination. In order to stop the enzymatic reaction, it was incubated for 10 min at 65˚C with the addition of 1 µl of EDTA. A total of 1–2 µg of RNA was converted to cDNA using commercial kit (I-Script, BioRAD, USA) by reverse transcriptase enzyme (Hitit et al., 2021).

Gene expression by quantitative polymerase chain reaction (qPCR)

The sequences of the lipogenesis gene (SREBP-1, LXRα, FASN, ACCα and ChREBP) primers used in our study, as forward and reverse primers, are given in tables (Table 3). The sequences of the fatty acid oxidation gene (PPARα, PPARγ, CPT1, CPT1A and ACOX1) primers used in our study, as forward and reverse primers, are given in table (Table 4). The genes to be used and 1 reference gene (βactin) were designed according to the criteria reported by Wang and Seed (2007) using sequence information identified through primers (http://www.http://eu.idtdna.com/site).

According to the kit protocol, 10 µl of Sso Advanced SYBR Green PCR Master Mix (Biorad, USA) and 5 pMol of primer and ddH2O were added to 4 µl cDNA converted from 1 to 2 µg RNA, and the total volume was completed to 20 µl. RT-qPCR was performed with Rotor-Gene Q (QIAGEN). RT-qPCR analysis was performed for 40 cycles at 94 °C for 15 s, at 60 °C for 30 s and at 70 °C for 30 s after incubation at 95 °C for 15 min. Melting curve analysis demonstrates specific binding of the primer, 1 min at 95 °C and then increased by 0.8 °C per second from 60 to 95 °C. The expression profiles of the obtained cDNAs were monitored using RT-qPCR (Atli et al., 2010). Data from RT-qPCR were recorded as Cq. The same amount of mix without template (ddH_2O) was used as negative control.

Table 3 Primer sequences for real-time PCR in lipogenesis genes

Gene name	Forward primer (5′-3′)	Reverse primer	Product size (bp)	Accession number	Reference
SREBP-1	CGAGTACATCCGCTTCCTGC	TGAGGGACTTGCTCTTCTGC	92	EU333990	Han et al. (2015)
FASN	TGGGAGTAACACTGATGGC	TCCAGGCTTGATACCACA	109	EU770327	Han et al. (2015)
ACCα	TGCCTCCGAGAACCCTAA	AAGACCACTGCCACTCCA	163	EF990142	Han et al. (2015)
LXRα	CCCAGCCCTTCCCACAAACT	CTGCCTCGCTTCACGGTTATTAG	156	HM138512	Han et al. (2015)
ChREBP	AAGAAGCGGCTCCGAAAG	TGGTGGGTGCTGGGTGT	236	GW342987.1	Han et al. (2015)
β-Actin[a]	CAACGAGCGGTTCAGGTGT	TGGAGTTGAAGGTGGTCTCG	92	M26111.1	Han et al. (2015)
18S[a]	TTGGTGGAGCGATTTGTC	ATCTCGGGTGGCTGAACG	129	L211170.1	Han et al. (2015)

[a]Reference gene

Table 4 Primer sequences for real-time PCR in fatty acid oxidation genes

Gene name	Forward primer (5′-3′)	Reverse primer	Product size (bp)	Accession number	Reference
PPARα	ATCTATCCCTGGCTTCTCCA	AGCATCCCATCCTTGTTCATT	117	AF481797	Han et al. (2015)
CPT1A	TCCAGCATGTGAAAGACAGC	GCCTGAGGCTCTGAATCATC	151	XM013195075.1	Zhao et al. (2022)
CPT1	GTCTCCAAGGCTCCGACAA	GAAGACCCGAATGAAAGTA	193	GW342945	Han et al. (2015)
ACOX1	ACAGAAAGAGCAAGGAGGAT	GCACGAGGTCAACAGAAGT	51	KC424582	Han et al. (2015)
PPARγ	CCTCCTTCCCCACCCTATT	CTTGTCCCCACACACACGA	108	AF481798.1	Han et al. (2015)
β-Actin[a]	CAACGAGCGGTTCAGGTGT	TGGAGTTGAAGGTGGTCTCG	92	M26111.1	Han et al. (2015)
18S[a]	TTGGTGGAGCGATTTGTC	ATCTCGGGTGGCTGAACG	129	L211170.1	Han et al. (2015)

[a]Reference gene

RT-q(PCR) data analysis

At least two housekeeping genes were used in the normalization of the data as reported by Kayis et al. (2011). The primary efficacy was calculated using five data points from the kinetic curve graph of the exponential phase of the graph of fluorescence values log transformed according to the method defined by Schefe et al. (2006). If the efficiency was found to be 100%, mRNA RT-qPCR data were normalized with the method of Livak and Schmittgen (2001). If the efficiency was not 100%, normalization was performed using the determined efficiency coefficient.

Statistical analysis

RNA RT-qPCR relative gene expression was confirmed by assay design (*F*-test) analysis methods. mRNA RT-qPCR gene expression data were statistically analysed with SPSS (SPSS 23.0 for

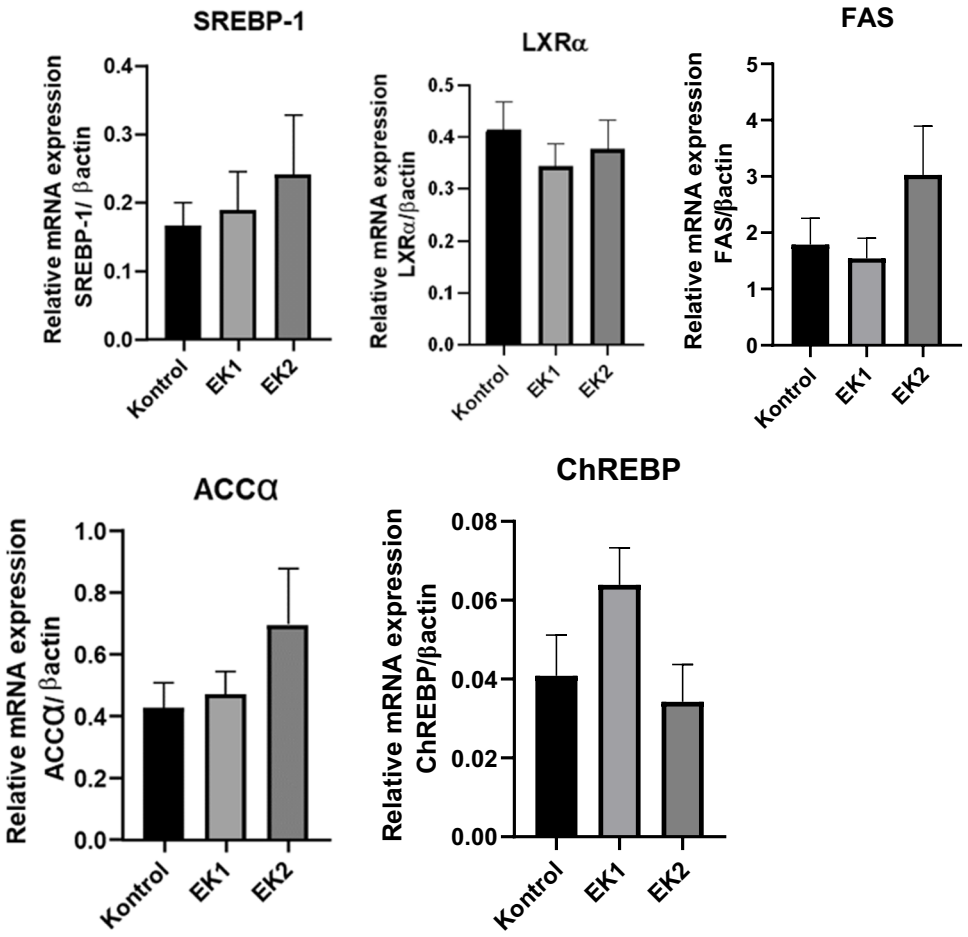

Fig. 1 Expression levels of genes included in lipogenesis (ACCα, ChREBP, FASN, LXRα and SREBP-1) mRNA in control, EK1 and EK2. Data was presented as mean ± standard error of means (SEM)

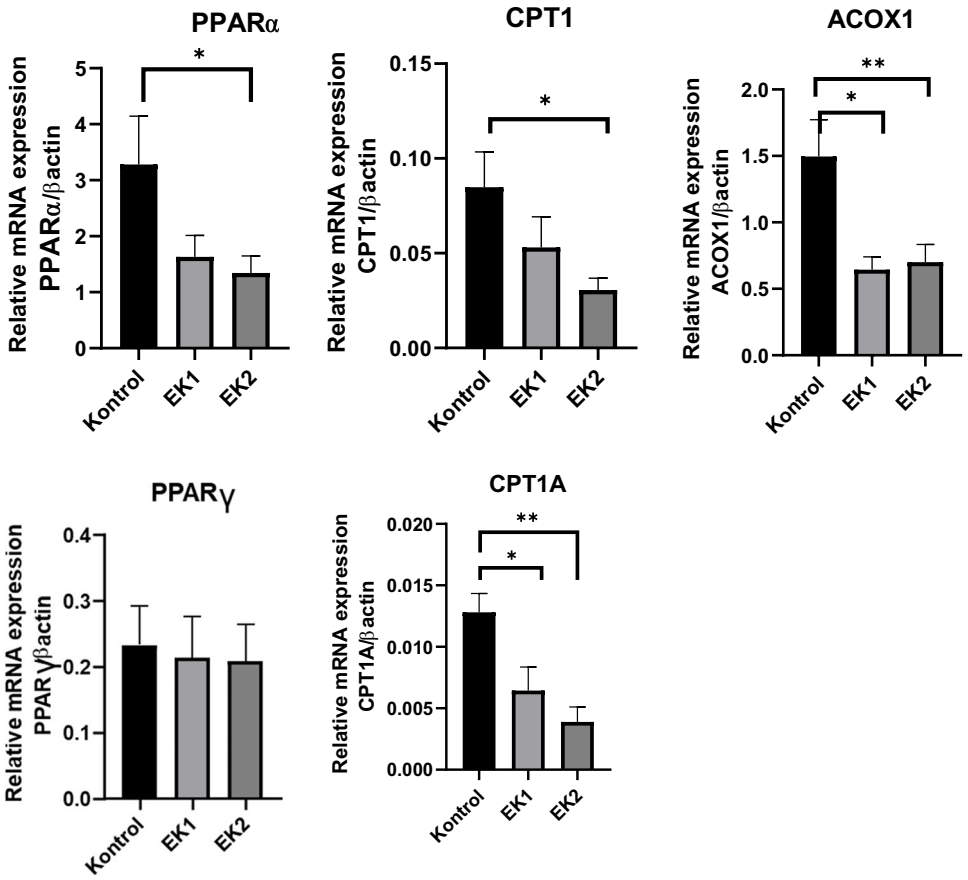

Fig. 2 Expression levels of genes included in fatty acid oxidation (ACOX1, CPT1, CPT1A, PPARα and PPARγ) mRNA in control, EK1 and EK2. $^{*}P < 0.05$ and $^{**}P < 0.01$. Data was presented as mean ± standard error of means (SEM)

Windows; Armonk, NY, USA) software using one-way analysis of variance (ANOVA) method Tukey test (SPSS, 2015).

Results

Lipogenesis involved gene expression

In our study, involved lipogenesis and fatty acid oxidation mRNA gene expression were investigated in control, EK1 and EK2 groups, but the increases and decreases in the regulation in the lipogenesis gene group are not statistically significant ($P > 0.05$) (Fig. 1). Expression levels of genes included in lipogenesis increased in SREBP1, ACCα and ChREBP genes in the EK1 group compared to the control group (Fig. 1). In the EK2 group, ChREBP gene was downregulated in contrast to other lipogenesis genes (Fig. 1). LXRα and FASN genes were decreased in the EK1 group compared to the control group, and FASN gene expression was at a very high level in the EK2 group (Fig. 1).

Fatty acid oxidation involved gene expression

Expression levels of genes involved in fatty acid oxidation were downregulated in EK1 and EK2 compared to the control group (Fig. 2). This decrease was statistically significant in all other genes (PPARα, CPT1, CPT1A and ACOX1) excluding PPARγ gene ($P < 0.05^{*}$, $P < 0.01^{**}$) (Fig. 2). On the other hand, an increase in the ACOX1 gene was observed in the EK2 group compared to the EK1 group (Fig. 2).

Discussion

Foie gras is an ideal model for exploring the molecular mechanisms of lipogenesis and fatty acid oxidation. This study was planned to research the effect on lipogenesis and fatty acid oxidation in geese with different ratios of essential oil added to drinking water. One hundred eight local Turkish geese were divided into 3 groups with basal diet (Table 1), with no essential oil added to drinking water (control), 0.4 ml/l essential oil mixture added (EK1) and 0.8 ml/l essential oil mixture added (EK2).

Fatty acid synthetase (FASN) is found generally in the liver and plays a crucial role in lipid metabolism by controlling the synthesis of fatty acids in vivo (Liu et al., 2021). It has been observed that an increase in liver size increases the level of FASN gene expression in geese (Liu et al., 2021). 0.4 ml of essential oil mixture was downregulated in the group, whereas FASN gene was upregulated in the group

to which 0.8 ml was added. This situation was not statistically significant. The FASN gene was upregulated EK2 group. In contrast, in broilers, it showed that FASN gene was downregulated when essential oils from different plants were added to the diet (Kishawy et al., 2022). The different regulations of the genes associated with lipogenesis can be explained by the difference in the type of essential oil used and the animal species. Our study was the first to examine liver gene expressions of adding essential oil to drinking water in geese.

In our study, it was observed that the ACCα and SREBP-1 genes associated with lipogenesis in the liver of drinking water supplemented with essential oil in geese were upregulated in direct proportion in the EK1 and EK2 groups. In a study using soya oil as a feed additive in broiler chickens, no change in SREBP-1 gene expression was observed (Hassanabadi et al., 2015). The opposite of our result, in a study about broilers, showed that ACCα gene was downregulated when essential oils from different plants were added to the diet (Kishawy et al., 2022). However, while the ChREBP gene was upregulated at the addition of 0.4 ml/L essential oil mixture to drinking water, it was downregulated when more (0.8 ml/L) essential oil mixture was added.

LXRα (liver X receptor) is an important factor regulating lipid metabolism (Han et al., 2010). In our study, LXRα gene was downregulated in a non-significant manner in the EC1 group. In the EK2 group, LXRα continued to be downregulated. In other studies, the LXRα gene in the liver was upregulated (Feng et al., 2020). This may suggest that the addition of essential oil to feed or drinking water may show different results between species.

Feeding a diet supplemented with essential oil in pigs has been shown to increase the level of CPT1 in the liver (Huang et al., 2022). In a study conducted in geese with palmitic acid, it was found to upregulate CPT1 and PPARα gene expression even at low concentrations (Pan et al., 2011). In contrast to these studies, all the genes (ACOX1, CPT1, CPT1A, PPARα and PPARγ) associated with fatty acid oxidation were downregulated. This statistically significant result may be associated with the result that essential oil reduces fatty acid oxidation in the liver in geese. This can cause fat accumulation in the liver.

FASN and ACCα, important rate-limiting enzymes for fatty acid synthesis, are regulated by SREBP1 and ChREBP in the liver (Qin et al., 2018). It has been reported that when fat synthesis (lipogenesis) is greater than fat secretion (fatty acid oxidation), fatty liver occurs in geese or ducks (Zhu et al., 2011). In our study, essential oils affected the increase of lipogenesis and decrease of fatty acid oxidation in the liver. According to this result, our study suggests that the addition of essential oil to water is important for the development of fatty delicious goose liver.

Lipogenesis is high sensible to changes in the nutrition. (Tang et al., 2018). Polyunsaturated fatty acids reduce lipogenesis by depressing gene expression in the liver, including that of fatty acid synthase and stearoyl-CoA desaturase (Kersten, 2001). Inversely, a diet plenty in carbohydrates encourages lipogenesis in the liver (Kersten, 2001). In our study, we have shown that the addition of essential oils to the diet increases lipogenesis.

In conclusion, our data indicate that the EK1 and EK2 groups have a marked effect on the regulation of genes and pathways involved in lipogenesis and fatty acid oxidation compared to C. This situation resulted in an increase in genes that cause lipogenesis, while the genes leading to fatty acid oxidation were downstream. Additionally, as our study is the first in this area, it provides new insights into the breeding of geese with high-quality fatty liver.\

Author contribution O.D.A., G.Y. and P.S. designed the research, and O.D.A., M.H., O.K. and O.M. conducted the experiments. M.H. and Z.U. analysed data, and O.D.A., M.H. and Z.U. wrote the manuscript. All authors read and approved the final manuscript.

Data availability Data will be made available on request.

Declarations

Ethics approval In 108 animals of Kars region in Turkey local goose breed, 3 groups (control, EK1 and EK2) will be studied, regardless of gender, with the samples obtained as a result of storing the liver samples taken in the study with the approval number 2018–54 of the Kafkas University Animal Experiments Local Ethics Committee. In addition, the authors complied with Directive 2010/63/EU.

Conflict of interest The authors declare no competing interests.

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