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Effects of Adding Fennel (*Foeniculum vulgare*) In Rations on Fattening Performance and Blood Oxidant-Antioxidant Balance in Rams

Sibel ALAPALA [1*], Yasemin SUNUCU KARAFAKIOĞLU [2], Salih SEZER [3]
Funda KARABAĞ [4]
[1] Uşak University, Faculty of Agriculture, Department of Animal Breeding, Uşak
[2] Uşak University, Faculty of Education, Department of Science Education, Uşak
[3] Uşak University, Eşme Vocational College, Uşak
[4] Uşak University, Faculty of Science and Literature, Department of Molecular Biology and Genetics, Uşak
[*]Corresponding author: yasemin.sunucu@usak.edu.tr

Abstract
This study aimed to determine the effects of adding fennel seeds *(Foeniculum vulgare*) to the diet on the biochemical parameters and live weight gains in rams. Thirty male rams of the Eşme breed, with an average live weight of 39 kg, were used, divided into two groups, each containing 15 rams. The control group received a ration without any additional supplementation, while the treatment group had fennel seeds added to their rations at a rate of 1.5 % per animal per day for 60 days. At the end of the experiment, blood samples were collected from the sheep to analyze malondialdehyde (MDA), protein carbonyl concentrations (PCO), superoxide dismutase (SOD), and catalase (CAT) enzyme activities. The study found a statistically significant difference between the groups in terms of daily live weight gain, with the control group showing 0.300 kg and the fennel group showing 0.330 kg. The MDA, SOD, protein, and catalase values were examined in the collected blood samples, revealing significant differences in MDA, SOD, and catalase values, while no significant difference was observed in PCO values. The addition of fennel seeds showed an increase in antioxidant levels and a decrease in lipid peroxidation, which could effectively protect rams from oxidative stress that reduces their productivity and resilience. Moreover, the study concluded that the use of fennel seeds in ram rations did not have any harmful effects.

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1. Introduction

The general aim of livestock enterprises is for the producer to obtain the highest level of efficiency from the animals he/she raises and reproduces in his/her enterprise. Genetic and environmental factors significantly affect the productivity of animals. Nutrition, in other words, feed, has an important place among environmental factors. Roughages are of great importance in the feeding of ruminant animals in terms of both feeding physiology and business economics. However, as the vegetation period and duration of each region are different as a natural result of different climatic conditions, animals can naturally meet some of their green fodder needs only during certain periods of the year. Especially due to limiting factors in the winter season, the use of roughage comes to the fore. The use of these roughages causes significant economic losses. For this reason, productivity differences can be seen depending on the season. These undesirable productivity losses in livestock enterprises can be prevented by using silages obtained from different sources (Kurtoğlu, 1998; Çiftçi et al., 2005). Due to the geographical features of Turkey, many medicinal and aromatic plants can be grown and they are collected from nature, as in many parts of the world. While some of the medicinal and aromatic plants in Turkey (laurel, sage, linden blossom, licorice root, rosemary, juniper bark, etc.) are collected from nature, some of them (coriander, thyme, cumin, anise, fenugreek, mint, fennel) are cultivated (Özhatay and Koyuncu, 1998). Fennel is one of the 300 genera in the Apiaceae family (Davis, 1978). It is grown mostly in European countries, India, Egypt, Turkey, China, Argentina, Indonesia and Pakistan in the world. It is known to be grown in the provinces of Istanbul, Zonguldak, Kastamonu, Artvin, Bursa, Çanakkale, Kırklareli, Kocaeli, Samsun, Sinop and Trabzon in the north of Turkey and in the region where Amanos Mountains in Hatay province are located in the south (Koru, 2019). Fennel is cultivated in the provinces of Antalya (150 kg da^{-1}), Burdur (120 kg da^{-1}) and Konya (150 kg da^{-1}) (Anonymus, 2019). The chemical composition of fennel includes essential oil, fatty acids, phenylpropanoids, monoterpenes, sesquiterpenes and coumarin. It also contains triterpene, tannin, flavonoid, cardiac glycosides and saponin. Fennel contains approximately 20 % fatty acids. Petroselinic acid is a characteristic fatty acid of fennel oil. Petroselinic acid level can be between 70 % and 80 % (He and Huang, 2011). Fennel has been reported to contain 6.3 % moisture, 9.5 % protein, 10 % fat, 13.4 % minerals, 18.5 % fibre and 42.3 % carbohydrates. Minerals and vitamins found in Fennel are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C (Rather et al., 2016). This study was conducted to determine the effects of adding the antioxidant fennel plant (*Foeniculum vulgare*) to the diets of sheep of the Eşme breed on live weight gain and biochemical parameters.

2. Materials and Methods

The study was conducted in a private enterprise in Eşme district of Uşak province, following the approval of the Uşak University Animal Experiments Local Ethics Committee (USAKHADYEK 2023/01). The animal material of the study consisted of 30 Eşme breed sheep, approximately 7-8 months old, with an average live weight of 39 kg. The animals were divided into two groups, one control and one experimental, with 15 sheep in each group. Care was taken to ensure that the animals in the groups were with similar birth dates and weights, and the groups were placed in separate pens so that they could not come into contact with each other. The animals were fed with feed prepared to meet the daily dry matter and nutrient needs reported by NRC (2007) for sheep. In the study, the animals were placed in the pens in their usual conditions in such a way as to prevent them from reaching each other's feed. In the control group, no changes were made in the feeding and water consumption patterns, and only 1.5% fennel seed per day was added to the rations of the animals in the experimental group. Following a seven-day eating acclimatization period, they were fed with the food allocated to them for a total of 60 days of the main study period.

Feeding was done in two meals a day, morning and evening, and roughage and mixed feed were given together in the same meal. At the end of the study, blood samples were taken before the morning feeding. Adequate blood samples were taken from the jugular vein of the sheep into anticoagulant tubes and brought to the Uşak University Central Research Laboratory for biochemical parameters. Plasma and serum samples and erythrocyte packets obtained by centrifugation (Nüve, NF 1000R) (1500 g, 4 ^{o}C, 15 min) were placed in eppendorf tubes and stored in the freezer at -20 ^{o}C until analysis. Preparation of erythrocytes was carried out according to the method reported by Witterbourn et al. (1975). MDA (Malondialdehyde) levels in the blood samples were determined according to the method reported by Draper and Hardley (1990), PCO levels were determined according to the method reported by Levine et al. (1990), and SOD and CAT enzyme activities in erythrocyte packages were determined according to the methods reported by Sun et al. (1998) and Luck (1995) using a spectrophotometer device (Shimadzu UV 1601). Independent samples t test was used for statistical calculations on the data of the groups obtained in the study and to determine the significance of the differences between the mean values of the groups. The level of significance was taken as $p<0.05$. All the analyses were conducted in SPSS 23.0 statistical program package. In the study, the sheep were fed with diets containing 14.4 % crude protein and a metabolic energy of 2760 kcal kg^{-1}. The raw material and calculated nutrient compositions of the treatment rations are given in Table 1.

Table 1. Raw material and nutrient compositions of intensive feed mix

Raw materials	Quantity, kg	Nutrients	Quantity, %
Barley	400.0	Dry Matter	88.7
Wheat	300.0	Crude Protein	14.4
Bran	84.6	Raw Fat	3.7
Cotton Seed Meal	92.6	Crude Cellulose	7.0
Full Fat Soy	83.4	Ash	4.9
Vegetable Oil	17.0	Calcium	0.7
Marble Dust	13.4	Phosphorus	0.5
Salt	7.0	Nitrogen-Free Extracts	58.6
Vit.-Min. premix*	1.0	Metabolic Energy kcal/kg	2760.0

The roughage and concentrate feeds used in the ration were mixed with each other and given to the sheep as complete rations. Live weights of the sheep were determined by weighing them on an empty stomach at the beginning of fattening and at the end of the 60th day of fattening. Feed and water were given ad libitum throughout the treatment period. In this study, which lasted a total of 60 days and investigated the effects of fennel supplementation on live weight gain and blood oxidant-antioxidant balance in sheep, data on live weight gains are given in Table 2.

Table 2. Mean live weight increases as a result of fennel supplementation

	Control		Fennel				
	X	Sx	X	Sx	t	F	P
Mean Live Weight per livestock, kg.	39.8640	1.2851	39.3993	1.3960	0.948	0.012	P>0.05
Mean Live Weight at the end of fattening, kg.	57.8987	1.2439	59.2247	1.8019	-2.345	2.274	P>0.05
Total Live Weight increase	18.0347	0.5320	19.8253	0.6997	0.027	1.797	P<0.05
Daily Live Weight increase, kg	0.3000	)84	3307	)116	.889	)72	:0.05

At the end of the study, as a result of the addition of 1.5 % fennel in the treatment group, the daily live weight gain was found to be 0.330± 0.01 kg, and in the control group it was

found 0.300±0.08 kg. Total live weight gain was measured as 19.82 ± 0.6 kg in the fennel supplemented group and 18.03±0.5 kg in the control group. A statistical difference was detected between the groups ($p<0.05$). The effects on MDA (Malondialdehyde), protein levels, SOD (Superoxide dismutase) and CAT (Catalase) activities are given in Table 3.

Table 3. Effects of fennel supplementation on MDA, protein, SOD and CAT levels in lambs.

	Control		Fennel				
	Average	Standard deviation	Average	Standard deviation	t	F	P
MDA mg/dl	39.8640	1.2851	39.3993	1.3960	0.948	0.012	P>0.05
SOD mg/dl	57.8987	1.2439	59.2247	1.8019	-2.345	2.274	P>0.05
Protein mg/dl	18.0347	0.5320	19.8253	0.6997	0.027	1.797	P<0.05
CAT mg/dl	0.3000	0.084	0.3307	0.0116	-7.889	2.072	P<0.05

Some biochemical parameters were examined in blood samples taken from the jugular vein at the beginning and end of the treatment. While a statistical difference ($p<0.05$) was detected between the groups in terms of MDA, SOD and CAT activities, which reveal the antioxidant- oxidant status of the biochemical values measured, it was found that the difference between the protein carbonyl levels of the control and 1.5% fennel group is not significant.

3. Discussion

In this study, which was carried out to determine whether fennel, which is among the feed additives used to increase the utilization of the feed given to animals, increase the quality of the products obtained, ensure that the animals are raised in a healthy way and reduce the cost of the obtained product, can be an alternative, the sheep used as animal material were fed with two different rations, the control and the treatment group with 1.5 % fennel added to the feed, during the study, which lasted a total of 2 months. Fennel seed addition to the feed was performed by selecting amounts close to the level used in previous studies (Hajalizadeh et al., 2019). In order for natural herbal products to be used as feed additives, they must not adversely affect both the productivity and health of the animal (Rochfort et al., 2008). In the current study, total live weight gains and daily live weight gains were found to be high in the group which were feed with feed supplemented with fennel seeds, which is consistent with the study conducted by Hajalizadeh et al. (2019). In the study of Hassan and Hassan (2009), in which they added black cumin, rosemary and probiotics to the rations of Karadi sheep, the increase in live weight gain is compatible with the study. Shahsavari et al. (2021) found that total live weight gain and daily live weight gain were significant ($p<0.05$) in Kermani lambs supplemented with fennel in their rations, which is consistent with the current study. Liu et al. (2021), in their study investigating the effect of fennel seed powder supplementation on feed intake and carcass in Cobb chickens, determined that fennel supports the growth and development of chickens, but found that there was no significant difference ($P > 0.05$) in the growth performance of broiler chickens. The results they obtained differ from the current study in terms of fattening performance. Recent research on antioxidants has focused on naturally occurring molecules to reduce consumer concerns about the safety and toxicity of their synthetic counterparts (Gharaghani et al., 2015). It is recommended to use dietary antioxidants in animal feed to reduce lipid peroxidation and protect the quality of animal products (Wood and Enser 1997). It is known that fennel seed is a potential source of natural antioxidants (Oktay et al., 2003). Free radicals produced during the function of body cells cause oxidative stress and damage cells. Cells have enzymatic and non-enzymatic antioxidant defence systems to protect themselves from the damage of free radicals (İnce et al., 2010). In the current study, a statistically significant difference ($p<0.05$)

was determined between the groups in terms of the lipid peroxidation product MDA levels, which are indicators of oxidative stress, and SOD, an enzymatic antioxidant, and CAT enzyme activity levels (Table 3). This finding shows the effect of fennel, which has antioxidant properties (Marino et al., 2007; Parejo et al., 2004; Ruberto et al., 2000; Shahat et al., 2011), on blood oxidant- antioxidant balance when added at the rate of 1.5% to the feed of sheep. SOD enzyme reduces superoxide levels within the cell by converting superoxide radical into hydrogen peroxide and molecular oxygen, thus it shows antioxidant properties. SOD is one of the most important antioxidant systems in the organism, together with catalase and glutathione redox system (Repine et al., 1997). SOD measurement can give an idea about the antioxidant capacity of the organism. Aydin et al. (2020) studied the effect of using thyme oil (*Origanum vulgare*) on fattening performance and blood oxidant-antioxidant balance in weaned Tuj breed lambs, and the effect of adding thyme oil to the ration was found to be insignificant on fattening performance, which is different from the current study, but its effect on SOD and GPx values, two of the blood oxidant-antioxidant parameters, was found to be significant ($p<0.05$). Studies on fennel have reported that the presence of trans-anethole as an active ingredient at levels of 38-82 % gives fennel antioxidant properties (Gulfraz et al., 2008; Shahat et al., 2011, Zoubiri et al., 2014). However, Cengiz (2018) suggested that the addition of rosemary and fennel essential oil mixture to the quail diet reduced the MDA level in breast meat stored at +4 ^{0}C under refrigerator conditions. They also found that the addition of fennel to the feed of quail was found to affect liver MDA, GSH, Vit C levels and GPx and CAT activities. In the current study, MDA and CAT activities were also found to be significant. The current study is consistent with the study (Sheweita et al. 2016), in which they stated that administering fennel essential oils to mice significantly increased CAT activities, one of the endogenous antioxidant enzymes, and did not induce lipid peroxidation. Choi and Hwang (2004) reported that oral administration of fennel fruit methanolic extract had antioxidant effects by reducing plasma MDA level and increasing CAT activity in an experimental model of acute and subacute inflammatory diseases and type IV allergic reaction in rats. Koppula and Kumar (2013) studied the lipid peroxidation of fennel in liver and brain tissues against stress in rats, and found that the fennel group had lower liver MDA levels compared to the control group. Kazak et al. (2020) determined that the addition of 1% ground fennel seed in quail diets reduced liver MDA level and GPx activity and increased Vit C levels ($p<0.05$). The significant decrease in MDA level is compatible with the current study. Ghiasvand et al. (2021) studied the effects of adding fennel essential oil to the diet on growth performance and serum biochemistry in broiler chickens and found that it had no effect on serum MDA concentrations, but these results are different from the results of the current study. PCO (Protein carbonyl) products are formed as a result of damage to many amino acid residues and peptide backbones as a result of the interaction of ROS (reactive oxygen species) with proteins. The most commonly measured product of protein oxidation is protein carbonyls. Detection of PCO levels is a sensitive method for determining oxidative protein damage (Evans et al. 1999). No literature data could be found regarding the examination of PCO levels in studies conducted with the addition of fennel, and this study is the first in this regard. It has been found that adding fennel, which has antioxidant properties, to the feed of sheep at a level of up to 1.5% has no effect on PCO levels.

4. Conclusion

Along with the increasing demands for livestock, the increasing presence of foodborne diseases reveals the necessity of ensuring food safety and developing new strategies to raise healthy farm animals. In recent years, as consumers have turned to healthy and safe food consumption, the use of medicinal and aromatic plants, especially as productivity enhancers, in the production of animal

products has come to the fore. When fennel seeds are added to feed, they make the aroma of the feed more delicious and cause sheep to consume it more willingly. In the current study, fennel supplementation increased feed consumption in sheep. The mean weight gain achieved at the end of 60 days and the higher daily live weight gain have revealed that fennel will provide more economic profit for livestock enterprises, considering its low cost. The fact that the addition of fennel to the diet increases blood antioxidant levels and decreases lipid peroxidation may be effective in protecting sheep from oxidative stress, which reduces productivity and resistance in sheep. With this study, it can be concluded that the use of fennel seed in the diets of sheep does not have a harmful effect. It was concluded that fennel can be used as an alternative feed additive to synthetic products to meet consumer demand in animal products, but this potential should be confirmed with different ruminant species and long-term studies. If the data obtained as a result of such studies support the findings of the current study, it will be possible for fennel to be used especially by organizations that produce feed additives and animal breeders, and thus the use of fennel in ruminant animals will become widespread.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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