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The effect of cumin essential oil on the fermentation quality, aerobic stability, and *in vitro* digestibility of vetch-oat silages

Kimyon uçucu yağının fiğ-yulaf silajlarının fermantasyon kalitesi, aerobik stabilitesi ve i*n vitro* sindirilebilirlik üzerine etkisi

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ABSTRACT

Objective: This study has been conducted in order to determine the effect of cumin essential oil on the fermentation quality, aerobic stability, *in vitro* metabolic energy contents and relative feed value of vetch-oat silages.

Material and Methods: Vetch and oat were harvested in May and were wilted for approximately 3 hours. Cumin essential oil was added to silages at CMN2:200 mg/kg, CMN3:300 mg/kg and CMN5:500 mg/kg levels, while no addition was made to the control (CON) group. Vetch-oat mixture was placed in plastic bags, and these bags were vacuumed and were stored at 8±2 °C, at laboratory conditions. In the following 70 days after the ensilage, three packages from each group were opened, and silages were analysed in terms of physical, chemical and microbiological analysis. Aerobic stability test was conducted for five days to silages, which were opened at the end of the ensilage period. Moreover, enzyme soluble organic matter (ESOM) amount, metabolic energy (ME) contents, and relative feed value (RFV) were determined.

Results: The addition of essential oil prevented crude protein (CP) degradation, and found out to be higher in experimental groups in comparison to the control group (CMN2: %10.04, CMN3: %10.25, CMN5: % 10.25) ($P<0.05$). The ammonia nitrogen levels (NH_3-N) have also decreased depending upon the increase in the amount of the added essential oil ($P<0.05$). It was also found out that neutral detergent fiber (NDF), acid detergent fiber (ADF) and cellulose contents were low in CMN3 and CMN5 groups ($P<0.05$). It was also determined that with the addition of essential oil, the amount of lactic acid bacteria (LAB) increased ($P<0.05$), and the amount of yeast and mould decreased both at anaerobic and aerobic periods.

Conclusion: Cumin essential oil increased the fermentation qualities of vetch-oat silages. Moreover, it affected the relative feed value positively.

Keywords: Aerobic stability, cumin volatile oil, vetch-oat silage, silage fermantation

ÖZ

Amaç: Bu araştırma kimyon uçucu yağının, fiğ-yulaf silajlarının fermantasyon kalitesi, aerobik stabilitesi, *in vitro* metabolik enerji içerikleri ve nispi yem değeri üzerine etkilerini belirlemek amacı ile düzenlenmiştir.

Materyal ve Yöntem: Fiğ- yulaf, mayıs ayında çiçeklenme başlangıcında hasad edilmiş ve yaklaşık 3 saat süreyle soldurulmuştur. Kimyon uçucu yağı silajlara Km2:200 mg/kg, Km3:300 mg/kg ve Km5:500 mg/kg düzeyinde katılmıştır. Kontrol (Kont) grubuna hiçbir katkı maddesi ilavesi yapılmamıştır. Fiğ-yulaf, plastik torbalarda silolanmıştır. Paketler laboratuvar koşullarında 8±2 °C'de depolanmışlardır. Silolamadan sonraki 70. günde her bir gruptan 3 paket açılarak silajlarda fiziksel, kimyasal ve mikrobiyolojik analizler yapılmıştır. Silolama döneminin sonunda açılan silajlara 5 gün süre ile aerobik stabilite testi uygulanmıştır. Ayrıca, enzimde çözünen organik madde miktarı, metabolik enerji içerikleri ve nispi yem değeri belirlenmiştir.

Araştırma Bulguları: Uçucu yağ ilavesi ham proteinin (HP) parçalanmasını önlemiş, kontrole göre (Kont:% 9.73) muamele gruplarında (Km2:%10.04, Km3:%10.25, Km5: %10.25) yüksek olduğu bulunmuştur ($P<0.05$). Amonyak azotu düzeyleri de (NH_3-N) uçucu yağın ilave edilen miktarındaki artışa bağlı olarak düşmüştür ($P<0.05$). Fiğ-yulaf silajlarının nötr deterjanda çözünmeyen lif (NDF), asit deterjanda çözünmeyen lif (ADF) ve selüloz içeriklerinin, Km3 ve Km5 düzeylerinde düşük olduğu bulunmuştur ($P<0.05$). Lactic acid bakterileri (LAB) sayıları uçucu yağ ilavesiyle artmış ($P<0.05$), maya ve küf sayıları ise hem anaerobik hem de aerobik dönemde düşmüştür.

Sonuç: Kimyon uçucu yağı fiğ-yulaf silajlarının fermantasyon özelliklerini artırmıştır. Ayrıca nispi yem değerini olumlu yönde etkilemiştir.

Anahtar sözcükler: Aerobik stabilite, kimyon uçucu yağı, fiğ-yulaf silajı, silaj fermantasyonu,

INTRODUCTION

Vetch (*Vicia sp.*), which is an annual plant, is almost everywhere in our country planted both in summer and winter, and is used in animal nutrition as grain, green or hay, and as silage and straw. Especially in recent years, vetch is planted together with crops such as barley and oat, and is used for grazing, as green plant or hay, or as silage (Ergül, 2002). Oat is an annual, cold climate plant, which is used for both human and animal nutrition. More than 75% of the oat planted fields consist of *Avena sativa*. Therefore, oat is usually known as *Avena sativa* (Butt et al., 2008).Oat is therefore known to be quite suitable for intercropping with forage legumes. The widest area of usage of oat is animal nutrition. However, it is also used in human nutrition, pharmaceutical, and cosmetic industries. Oat grains are also known to be a very good source of feed for all farm animals as well as for buffalo, sheep, and horses (Schipper et al. 1991). Recently, research has also been conducted for the use of oat in poultry nutrition (Macleod et al. 2008; Kheravii et al. 2018).Today the use and areas of usage of oat in human nutrition are also increasing. The nutritional value of oat is dependent primarily on its oil content. While the oil content of wheat, barley, and rye, is 1.5-2%, the oil content of oat is 4.58% (Schipper et al., 1991).

Oat straw and oat silage are the most common feeds in ruminant nutrition (Mahouachi et al. 2003). It was reported that the active ingredient called scopoletin, which is found in oat straw has antibacterial, antiseptic, bronchial relieving and cancer preventing effects (Saraçoğlu, 2003). The crude protein content of oat silage is between 8% and 10%. This level is too low to provide optimal microbial growth in the rumen and adequate digestible protein from the small intestine. In a study conducted by Mahouachi et al. (2003), it was revealed that the yield performance usually decreased when oat and other silages were used alone in ruminant nutrition. Therefore, it is preferred to use these through intercropping with legumes such as vetch in animal nutrition.

In a study conducted, it was concluded that in order to have high yield and high quality in grass production, the vetch-wheat intercropping should necessarily be done in autumn, and that the vetch-wheat intercropping would be more appropriate for the leaf / stem ratio, which is the determinant of grass quality, and that for a better grass production, the intercropped crops should be harvested during the milk stage of wheat. In addition, it was also reported in another study that due to its high leaf / stem ratio and crude protein yield, the vetch-wheat intercropping should be done at the rate of 70-30%, whereas it would be more suitable to intercrop vetch-wheat at the rate of 80-20% for high crude cellulose and ash ratio (Taş, 2010). Moreover, high quality silages can also be obtained through additives (Ergül, 2002).

Anatolian people have used plants for the treatment since paleolithic age. Today, it is used for medical purposes at least 500 plants in Turkey (Sancaktaroğlu and Bayram, 2011). *Cuminum cyminum L*. (cumin), the homeland of which is Egypt, is grown in countries having a coast on the Mediterranean Sea, and in the Middle Anatolian region of Turkey. Cumin being one of the most important exportation goods, the production amount of cumin changes by years; while the recent amount of production in recent years has been reported as 19,175 tons (TUIK, 2018). Cumin fruits include 2.5-6% essential oil, 10-23% fixed oil, 15-25% protein, tannin, flavonoid, resin and glue (Ceylan et al. 2003).

In a study conducted by Turan and Soycan-Önenç (2018), the addition of 300 mg/kg of cumin essential oil into alfalfa silage stimulated the enzyme activities which break the cell wall down, thus causing the cell wall to break down. It also promoted LAB growing, and increased the number and enhanced its efficiency. Correspondingly, it was also found out that the transformation of sugar into lactic acid (LA) increased, the high level of LA in the environment inhibited the protein degrading enzymes by decreasing the pH, and also decreased the degradation of proteins into ammonia. Moreover, it was also determined that it increased the enzyme soluble organic matter, and concordantly provided the increase of ME content. Similarly, the relative feed value, as well as the dry matter intake has also increased.

This study has been planned in order to determine the effect of cumin essential oil on the fermentation quality, aerobic stability, *in vitro* metabolic energy contents, and relative feed value of vetch-oat silage.

MATERIAL and METHODS

Material

The study material consisted of vetch-oat, which was sown at 75-25% ratio, and cumin (*Cuminum cyminum L.*) essential oil. The essential oil, which had been obtained through water steam distillation, was provided from an export firm. The chemical analysis results of cumin essential oil (Table1) are given below.

Table 1. The chemical composition of cumin essential oil, %

Çizelge 1. *Kimyon uçucu yağının kimyasal bileşimi, %*

Compouns	Value
Cuminaldehyde	44.47
Carvacrol	12.12
Para Cymen	8.82
Safranal	6.57
Gamma Terpinen	5.64
Beta-pinen	4.93
(-) Alpha Cedren	4.48
Others	9.86
Unknown	3.11
Total	100

Method

Vetch and oat were cut in May at the beginning of the blooming period for vetch, and at the beginning of milk stage for oat. Cumin essential oil was added after cutting the plants at 1.5-2.0 cm sized-pieces at the silage machine. The study was conducted with 4 groups including one control group (CON) without the addition of cumin essential oil, and with groups of 200 mg/kg (CMN2), 300 mg/kg (CMN3) and 500 mg/kg (CMN5) cumin essential oil addition. Approximately 2 kg of sample was put into plastic bags, and were vacuumed. The plastic bags were then wrapped with stretch film for about 10-12 times, and finally were strapped with packaging tape. For every group, 3 packages were prepared, which were in total 12 packages, and were stored at a closed storage (18±4 °C) for 70 days for fermentation. At the day of opening the packages (70th day), silages were scored by three observers in terms of colour, smell, and structure. Flieg score of the silages were calculated after the determination of their dry matter and pH values (Kılıç, 1986). The pH values of silages were determined via a digital pH meter, and the Buffer capacity (Bc) through the statements of Playne and McDonald (1966), LA was determined through spectrophotometric method according to Barker and Summerson, (1941).

The NH_3-N and water-soluble carbohydrate (WSC) contents were found out according to the methods given by Anonymous (1986), and the aerobic stability test was carried out according to the method developed by Ashbell et al. (1991). Microbiological (LAB, yeast, mould) analysis were carried out according to the methods developed by Seale et al. (1990), whereas total mesophilic aerobic bacteria (TMAB) count was done according to the method given in Anonymous (2014). The TMAB, LAB, enterobacter, yeast and mould counts were transformed into logarithm coliform unit (cfu/g).

The contents of crude nutrients, some of which are CP, ether extract (EE), crude ash (CA), and crude fiber (CF) were determined through Weende analysis method (Bulgurlu and Ergül, 1978). Nitrogene-free extract (NFE) was determined through differential method. The cell wall components of feeds, NDF, ADF, and acid detergent insoluble lignin (ADL) contents were determined according to the method of Van Soest et al. (1991). In addition, hemicellulose and cellulose were found out through calculating method (Close and Menke 1986).

The solubility levels of organic matters (OM) in silages were determined through cellulose method (De Boever et al. 1986, Naumann and Bassler 1993). The enzyme soluble organic matter (ESOM) amounts were calculated according to the equation given below:

ESOM, % = DM - CA – G.

Chemical components of the essential oils were detected via gas chromatography-mass spectrophotometer (GC / MS, HP 6890 GC / 5973 MSD) at the Ege University Center R&D and Pharmacokinetic Applications-Environmental & Food Analysis Laboratories-Food Control Laboratory (Bornova, Izmir, Turkey) according to the United State Pharmacopeia National Formularty.

Metabolizable energy and relative forage value estimating

The *in vitro* metabolic energies (ME) of silages were calculated according to the below given equations by using the crude nutrients and cell walls, which were obtained through chemical analysis (Anonymous, 1991).

ME_{CNC} kcal/kg OM= 3260 + (0.455 x CP* + 3.517 x EE*) – 4.037 x CF* (Anonymous, 1991), (*in OM g/kg).

ME_{NDF}, kcal/kg DM= 3381.9-19.98 x NDF* (Kirchgessner et al. 1977).

ME_{ADF}, MJ/kg DM= 14.70-0.150 x ADF* (Kirchgessner and Kellner, 1981).

ME_{ADL}, kcal/kg DM= 2764.4-102.73 x ADL* (Kirchgessner et al. 1977).

***** NDF, ADF and ADL in %, ME contents were translated into kilocalories.

ME_{ESOM}, MJ/kg DM=0.54+0.001987 CP*+0.01537 ESOM*+0.000706 EE* x EE*-0.00001262 ESOM* x CA*-0.00003517 ESOM* x CP* (Jeroch et al. 1999).

*(CP, EE, CA g/kg; ESOM in g/kg DM).

The equations developed by Van Dyke and Anderson (2000), which are given below, were used for the determination of relative feed value. In the first phase, digestible dry matter (%DDM) was calculated by the use of ADF content of the feed.

DDM, % of DM **=** 88.9 – (0.779 x % ADF), DMI (as a % body weight) **=** 120 / % NDF

RFV **=** %, DDM x % DMI x 0.775.

The data, which were obtained at the end of the research, were evaluated through variance analysis at SPSS v.18 (SPSS, 2009) packaged software. Duncan test was used in the comparison of group average (Efe, 2000).

RESULTS

It was determined that the colour of the vetch-oat silages at the day of unwrapping them was light yellow-green. It was also observed that the there was a strong sour smell at the CON group, while the other three groups had a nice and mild acidic smell; and it was also determined that the structure of the stems and leaves were not deteriorated (Table 2), and that the quality category was satisfactory. It was also found out that the best result according to Flieg score evaluations was at CMN3 group (CON: 82.77, CMN2: 84.05, CMN3: 88.48, CMN5: 86.87).

Table 2. The effects of cumin essential oil on silage qualities

Çizelge 2. *Kimyon uçucu yağının silaj kalitesine etkisi*

Treatments	Smell	Structure	Colours	DLG point	Quality	Flieg Point	Quality
CON	8.0	4.0	1.0	13	Moderate	82.77	Excellent
CMN2	12.0	4.0	2.0	18	Excellent	84.05	Excellent
CMN3	12.0	4.0	2.0	18	Excellent	88.48	Excellent
CMN5	12.0	4.0	2.0	18	Excellent	86.87	Excellent

The crude nutrient contents of vetch-oat silages are given in Table 3. The OM amount in all groups was found out to be close to the control group. CA amount was determined to be slightly lower in CMN2 and CMN3 than in the CON group. This difference, in terms of OM, results from the ash content being high. When crude protein content was compared to the CON group, it was found out that it increased, and that this increase was statistically significant (P<0.05) (CON: % 9.73, CMN 2: % 10.04, CMN 3: % 10.25, CMN: % 10.25).

Ether extract values of vetch-oat silages of CON, CMN2, CMN3 and CMN5 were found out to be 1.94, 2.23, 2.45 ve 2.68 respectively (P<0.05). Crude cellulose amount in the CON group, CMN2, CMN3 and CMN5 groups was found out to be 41.51%, 38.93%, 37.65%, and 39.89% respectively, and it was determined that the difference among the groups was significant (P<0.05). The NFE contents in CMN3, in comparison to the CON group and the other two experimental groups was found out to increase significantly (P<0.05). It was also determined CA content in CON: 13.42, CMN2: 13.20%, CMN3: 13.32%, CMN5: 13.52%. The CA value of CMN5 being high may be caused by the contamination of the sample with stone or soil. The NDF contents of vetch-oat silages were determined to be 64.25%, 64.88%, 63.39 % and 63.72% for the CON group, CMN2, CMN3 and CMN5 respectively. The cumin essential oil decreased the NDF, ADF, and cellulose contents of vetch-oat silage compared to CON at CMN3 and CMN5 levels (P<0.05). For the ADL level; however, the level of use of cumin had no effect (P>0.05).

The chemical analysis results of vetch-oat silages are given in Table 4. While the DM content of silages was determined as the highest in CMN3 with 30.41%, the lowest was found out to be in CMN2 group (P<0.05). The addition of cumin essential oil did not affect pH level (P>0.05), yet decreased the WSC level (P<0.05). This effect became more evident with the increase of the essential oil (pH: CON: 4.46, CMN2: 4.43, CMN3: 4.43, CMN5: 4.43, WSC: CON: 14.08 g/kg CMN2: 9.09, CMN3: 11.85 g/kg KM, CMN5: 11.20 g/kg KM).

The NH3-N concentration of silages were affected by the use of cumin essential oil CMN2 (54.24 g/kg TN), CMN3 (48.58 g/kg TN) and CMN5 (48.46 g/kg TN), and decreased significantly (P<0.05) in comparison to the CON group (56.54 g/kg TN).

In the study conducted, the WL of CON, CMN2, CMN3 and CMN5 groups were found out to be 1.14%, 1.09%, 0.94% and 1.08% respectively (P>0.05).

On the day of opening the silages (Table 5), enterobacteria count was determined as 0.85 log10 cfu/g, while it was found out to be 0.06 log10 cfu/g in CMN2, CMN3 and CMN5 (P<0.05). LAB count revealed a significant increase in comparison to the CON group. This increase was determined as the highest in CMN2 group with 6.30 log10 cfu/g (P<0.05). When compared to the CON group, yeast and mould counts decreased in CMN2, CMN3 and CMN5 (P<0.05). The results of aerobic stability test applied to the silages for 5 days are given in Table 6.

Table 3. Chemical composition of fresh vetch-oat and silages ensiled for 70th days (DM %)

***Çizelge 3.** Fiğ-yulaf ve 70. gün silajların kimyasal bileşimi, % KM'de*

Treatments	OM	CP	EE	CF	NFE	CA	NDF	ADF	ADL	Hemicellulose	Cellulose
FM	87.97	9.61	2.12	42.21	34.03	12.03	60.76	40.45	6.02	20.31	34.43
CON	86.58±0.09^{ab}	9.73±0.01^{b}	1.94±0.06^{c}	41.51±0.06^{a}	33.40±0.10^{c}	13.42±0.09^{ab}	64.25±0.09^{b}	43.16±0.18^{a}	6.66±0.20	21.09±0.26^{c}	36.50±0.13^{a}
CMN2	86.80±0.07^{a}	10.04±0.10^{a}	2.23±0.09^{b}	38.93±0.07^{c}	35.59±0.15^{b}	13.20±0.07^{b}	64.88±0.05^{a}	40.73±0.19^{c}	6.69±0.02	24.15±0.21^{a}	34.05±0.20^{c}
CMN3	86.68±0.11^{ab}	10.25±0.08^{a}	2.45±0.11^{ab}	37,65±0.07^{d}	36.33±0.10^{a}	13.32±0.11^{ab}	63.39±0.02^{d}	40.99±0.09^{c}	6.61±0.05	22.40±0.11^{b}	34.39±0.14^{c}
CMN5	86.48±0.02^{c}	10.25±0.11^{a}	2.68±0.03^{a}	39.89±0.20^{b}	33.65±0.19^{c}	13.52±0.02^{a}	63.72±0.03^{c}	41.65±0.19^{b}	6.64±0.17	22.07±0.21^{b}	35.01±0.23^{b}
P	0.088	0.007	0.001	0.001	0.001	0.088	0.001	0.001	0.978	0.001	0.001

FM: Fresh material, CON: Control, CMN2: Cumin essential oil 200 mg/kg,CMN3: Cumin essential oil 300 mg/kg. CMN5: Cumin essential oil 500 mg/kg. OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen-free extract, CA: Crude ash, NDF: Neutral detergent fiber, ADF:Acid detergent fiber, ADL:Acid detergent lignin.

$^{abc:}$ Means with different letters in the same column are statistically significant ($P<0.05$).

Table 4. Fermentation quality of vetch-oat silage ensiled for 70th days

***Çizelge 4.** Fiğ-Yulaf ve 70.gün silajlarının fermantasyon kalitesi*

Grup	DM (%)	pH	WSC (g/kg DM)	LA (g/kg DM)	NH_3-N (g/kg TN)	WL (%)
FM	31.24	6.2	40.73	-	-	-
CON	28.22±0.08^{c}	4.46±0.07	14.08±0.34^{a}	33.58±0.13^{d}	56.54±0.08^{a}	1.14±0.17
CMN2	28.19±0.08^{c}	4.43±0.03	9.09±0.44^{c}	40.39±0.21^{a}	54.24±0.03^{b}	1.09±0.13
CMN3	30.41±0.12^{a}	4.43±0.03	11.85±0.19^{b}	38.75±0.02^{b}	48.58±0.08^{c}	0.94±0.01
CMN5	29.60±0.09^{b}	4.43±0.03	11.20±0.23^{b}	34.43±0.32^{c}	48.46±0.03^{c}	1.08±0.02
P	0.001	0.931	0.001	0.001	0.001	0.624

FM: Fresh material, CON: Control, CMN2: Cumin essential oil 200 mg/kg, CMN3: Cumin essential oil 300 mg/kg. CMN5: Cumin essential oil 500 mg/kg, LA: Lactic acid, NH_3-N:Ammonia nitrogen, WL: Weight loss, DM: Dry matter, WSC: Water-soluble carbohydrates.
$^{abc:}$ Means with different letters in the same column are statistically significant ($P<0.05$).

Table 5. Effects of cumin essential oil on microbiology of vetch-oat silages, log_{10} cfu/g

Çizelge 5. *Kimyon uçucu yağının fiğ-yulaf silajlarının mikrobiyolojisine etkisi,* log_{10} *cfu/g*

Treatments	TMAB	Lactobacilli	Enterobacter	Yeast	Mould
FM	6.35	2.11	0.89	2.26	1.80
CON	5.35 ± 0.01^{c}	3.46 ± 0.03^{d}	0.85 ± 0.05^{a}	1.87 ± 0.05^{a}	1.87 ± 0.05^{a}
CMN2	6.97 ± 0.06^{a}	6.30 ± 0.01^{a}	0.06 ± 0.01^{b}	0.51 ± 0.11^{b}	0.35 ± 0.15^{b}
CMN3	6.14 ± 0.02^{b}	5.30 ± 0.01^{b}	0.06 ± 0.01^{b}	0.30 ± 0.01^{c}	0.1 ± 0.01^{c}
CMN5	6.11 ± 0.01^{b}	5.15 ± 0.03^{c}	0.06 ± 0.01^{b}	0.30 ± 0.01^{c}	0.1 ± 0.01^{c}
P	<0.001	<0.001	<0.001	<0.001	<0.001

FM: Fresh material, CON: Control, CMN2: Cumin essential oil 200 mg/kg, CMN3: Cumin essential oil 300 mg/kg. CMN5: Cumin essential oil 500 mg/kg: TMAB: Total mesophilic aerobic bacteria, LAB: Lactic acid bacteria. ± SEM, standart error of means, $^{abc:}$ Means with different letters in the same column are statistically significant (P<0.05).

Table 6. Effects of cumin essential oil on aerobic exposure of vetch-oat silages

Çizelge 6. *Kimyon uçucu yağının fiğ-yulaf silajlarının aerobik stabilite test sonuçları*

Treatments	DM, %	pH	TMAB log_{10} cfu/g	Yeast, log_{10} cfu/g	Mould, log_{10} cfu/g
CON	27.24 ± 0.09^{c}	8.07 ± 0.07^{a}	7.97 ± 0.06^{a}	4.76 ± 0.02^{a}	4.28 ± 0.02^{a}
CMN2	29.02 ± 0.07^{a}	7.5 ± 0.06^{c}	6.35 ± 0.03^{d}	3.30 ± 0.03^{d}	3.60 ± 0.10^{b}
CMN3	28.49 ± 0.31^{ab}	7.5 ± 0.06^{c}	6.81 ± 0.17^{c}	3.94 ± 0.02^{c}	3.24 ± 0.06^{c}
CMN5	28.21 ± 0.27^{b}	7.87 ± 0.03^{b}	7.11 ± 0.01^{b}	4.21 ± 0.2^{b}	3.34 ± 0.05^{c}
P	0.002	0.001	0.001	0.001	0.001

CON: Control, CMN2: Cumin essential oil 200 mg/kg, CMN3: Cumin essential oil 300 mg/kg. CMN5: Cumin essential oil 500 mg/kg: TMAB: Total mesophilic aerobic bacteria± SEM, standart error of means, $^{abc:}$ Means with different letters in the same column are statistically significant (P<0.05).

The addition of cumin essential oil to vetch-oat silages decreased the yeast and mould formation significantly on the 5th day in comparison to the CON group (P<0.05). While the lowest yeast count was determined as 3.30 log_{10} cfu/g in CMN2, the lowest mould count was determined as 3.24 log_{10} cfu/g in CMN3. In this period, it was found out that there was no change in the dry matter, and that pH values increased between 3.07 and 3.67 in all groups. The yeast and mould counts at the aerobic period were suppressed with the addition of cumin essential oil in comparison to the CON group (P<0.05).

While the addition of cumin essential oil at low levels did not affect ESOM (Table 7), it had a decreasing effect when added at high levels (P<0.05). When ME contents of silages were analysed, it was found out that CMN3 level, which was calculated via different regression equations, revealed an increasing effect on ME contents (P<0.05).

Table 7. ESOM (% DM) and ME contents of vetch-oat silages, kcal/kg DM

Çizelge 7. Fiğ-yulaf silajlarının EÇOM ve ME içerikleri, kcal/kg KM

Treatments	ESOM	ME_{ESOM}	ME_{CNC}	ME_{NDF}	ME_{ADF}	ME_{ADL}
CONT	37.27 ± 0.43^{a}	1152 ± 8.96^{b}	1259 ± 6.45^{d}	2098 ± 1.70^{c}	1966 ± 6.47^{c}	2080±20.17
CMN2	37.68 ± 0.13^{a}	1177 ± 9.54^{ab}	1382 ± 3.83^{b}	2085 ± 1.00^{b}	2053 ± 6.72^{a}	2078±1.64
CMN3	37.88 ± 0.01^{a}	1193 ± 7.76^{a}	1439 ± 8.55^{a}	2115 ± 0.38^{a}	2043 ± 3.30^{a}	2086±5.53
CMN5	36.04 ± 0.10^{b}	1166 ± 3.20^{b}	1350 ± 9.54^{c}	2108 ± 0.66^{d}	2020 ± 6.85^{b}	2082±17.30
P	0.002	0.032	0.001	0.001	0.001	0.978

CON: Control, CMN2: Cumin essential oil 200 mg/kg, CMN3: Cumin essential oil 300 mg/kg. CMN5: Cumin essential oil 500 mg/kg. ESOM: Enzyme soluble organic matter, ± SEM, standart error of means, $^{abc:}$ Means with different letters in the same column are statistically significant (P<0.05). ***** ME contents were translated into kilocalories.

The beginning material of vetch-oat and the DDM, DMC and RFV of the vetch-oat silages are given in Table 8. It was found out that the addition of cumin essential oil improved the DDM and RFV of the silages.

Table 8. Dry matter digestibility, dry matter intake and relative feed value of vetch-oat silages.

Çizelge 8. *Fiğ-yulaf silajlarının sindirilebilir kuru madde, kuru madde tüketimi ve nispi yem değerleri*

Treatments	DDM,%	DMI,%	RFV
FM	57.05	1.97	86.89
CON	55.28±0.14^{c}	1.87±0.002^{c}	80.02±0.12^{c}
CMN2	57.17±0.15^{a}	1.85±0.001^{d}	81.94±0.19^{b}
CMN3	56.97±0.07^{a}	1.89±0.001^{a}	83.58±0.08^{a}
CMN5	56.46±0.15^{b}	1.88±0.001^{b}	82.40±0.20^{b}
P	<0.001	<0.001	<0.001

FM: Fresh material, CON: Control, CMN2: Cumin essential oil 200 mg/kg, CMN3: Cumin essential oil 300 mg/kg. CMN5: Cumin essential oil 500 mg/kg.DDM: Digestible dry matter, DMI: Dry matter intake, RFV: Relative feed value, ± SEM, standart error of means, $^{abc:}$ Means with different letters in the same column are statistically significant (P<0.05).

DISCUSSION

The cumin essential oil addition into vetch-oat silages enabled the quality to be at very high level. Konca et al. (2005) stated in their study that the flieg score of corn silages varied between 29 and 97; whereas it was 57 in vetch-oat silages. In the study conducted by Turan and Soycan-Önenç (2018), it was revealed that the addition of cumin essential oil at 300 mg/kg and 500 mg/kg levels to alfalfa increased the flieg score. In this study it was found out that the flieg score was higher than the ones in the results of Konca et al. (2005), and similar to the results of the study of Turan and Soycan-Önenç (2018) on alfalfa silages. The addition of cumin essential oil to the vetch-oat silages affected positively the flieg score.

In a study, carried out by Chaves et al. (2012), it was stated that the addition of cinnamon leaf extract to barley silage led to an increase in CP amount. In this study, it was found out that the addition of cumin essential oil prevented the degradation of protein, and that CP level was determined to be high similar to the studies conducted by Chaves et al. (2012) and Turan and Soycan-Önenç (2018). The decrease in the ammonia nitrogen level through the addition of cumin essential oil also supports the results of CP.

The ADF and ADL, which are components of the cell walls of the feeds are expected to be as little as possible in the ration due to their digestion level being very slow as well as low (Van Soest 1994). Kung et al. (1991) stated that the antibiotic addition into alfalfa silages led to a slight decrease in the ADF amount. Soycan-Önenç et al. (2015, 2017) stated in their studies that oregano, cinnamon, and oregano+cinnamon essential oil addition to field peas, which were silage for 60 and 120 days, had an increasing effect on NDF and ADF contents. Chaves et al. (2012); however, stated that the use of ethanol extract of cinnamon leaves at low levels decreased the NDF and ADF levels, yet had an increasing effect when used at high levels. It was found out in this study that the addition of cumin essential oil to vetch-oat silages decreased the CF, cellulose and ADF, while it did not affect ADL, and that the increasing effect on hemicellulose at CMN2 level was compatible with the results of Soycan-Önenç et al. (2015, 2017) and Chaves et al. (2012).

The DM amount in CMN3 group was determined to be higher than the amounts in the CON group and the other two experimental groups, this difference was also reflected in the loss of dry matter, and the DM in CMN3 group decreased compared to CON. However, the addition of cumin essential oil was not effective on the decrease in WL (P>0.05). Konca et al. (2005) stated that the DM amount in vetch-oat silages from Bayındır-İzmir region was 23.89%. In this study it was found out that the DM amount of vetch-oat silages was higher that the results found by Konca et al. (2005). Since the silage materials are protected by LA, it is essential that there is sufficient LAB in the silaging environment and also WSC in order to enable the production of LA to prevent the decay of the silage. If there is sufficient WSC in the LAB environment, the necessary lactic acid for the fermentation can be produced (Filya, 2000).

In this study, it was determined that the LAB counts of vetch-oat silages depending of the addition of cumin essential oil increased significantly ($P<0.05$) in comparison to the CON group. Concordantly, there was a decrease in WSC, and an increase in LA amount. The LAB count being low in the control group limited the transformation of WSC into LA. On the other hand, the high level of LAB in CMN2 group increased the transformation of WSC into LA. Therefore, the highest LA was found in CMN2 group. In this research it was concluded that the addition of 200 mg/kg of cumin essential oil into vetch-oat silage revealed a promoting effect on LAB progress, and that it improved the silage fermentation.

The cumin essential oil used in this study decreased the NH_3-N concentration of the vetch-oat silages at the end of the ensilaging period similar to the results of the studies conducted by Soycan-Önenç et al. (2015) and Turan and Soycan-Önenç (2018). Cumin essential oil includes 44.47% cinnamaldehyde, and is one of phenylpropanoids, which have an antimicrobial effect. Therefore, cumin essential oil decreased the NH_3-N concentration in silages, and was effective in the prevention of proteolysis.

The progress of *Enterobacteria*, *Clostridia*, *Listeria*, and mould in bunkers, and their metabolic activities create a great danger for the hygienic structure of the silages (Filya, 2000). It was found out that, on the day of the silages being unwrapped, the *Enterobacteria*, yeast, and mould counts in the CON group was close to the counts at the beginning material, whereas with the addition of cumin essential oil into the experimental groups, there was a decrease in the growing of these microorganisms. When TMAB counts were analysed, it was found out that LAB count was low in the beginning material, and the undesired microorganism count was high, whereas in ensilage the TMAB count was similar, yet in cumin groups the majority of TMAB consisted of LAB.

In this study, the increase of LAB counts, and the decrease in *Enterobacteria*, yeast, and mould, which are considered to be detrimental for silages, in groups with cumin essential oil addition, could be explained by the selective antimicrobial activity of essential oils. The addition of cumin essential oil into vetch-oat silages decreased the yeast counts; however, did not have any preventive effect on their growing. Since it was impossible to have any exposure to air during fermentation, it was believed that the yeast count of the silage may be the ones in the fresh material (FM: 2.26 $\log_{10}$ cfu/g). Turan and Soycan-Önenç (2018) stated that the cumin essential oil addition led to an increase in the LAB counts, and a decrease in *Enterobacteria*, yeast, and mould counts similarly to the results in this study.

Antibiotic addition into silages is not able to prevent the aerobic degradation completely in silages. It was stated that the most effective antibiotics against silage microorganisms at pH4 and pH5 was primaricin. In the treatment with antibiotics, it was found out that the total microorganism, LAB, and yeast counts were affected significantly, while mould was affected quite slightly (Kurtoğlu 2011). Essential oil treatment, which was shown as an alternative to antibiotics, revealed a similar effect (Soycan-Önenç et al. 2015, 2017; Turan and Soycan-Önenç 2018; Soycan-Önenç and Korkmaz-Turgud 2019). The addition of 200 and 300 mg/kg of cumin essential oil into vetch-oat silages improved the aerobic stability. However, the WSC in the environment formed a source for the growing of yeast and mould.

Therefore, cumin essential oil could not suppress the development of yeast and mould sufficiently, yet the yeast counts were found out to be below the hygienic value. The count of yeasts exceeding 5 log cfu / g (naturally) in silage is considered as an indicator that the silage is disrupted (Wilkinson and Davies, 2013). The yeast count in experimental groups being below the critical level (5 log cfu/g) on the 5th day of both the aerobic and anaerobic period can be explained in that cumin prevents the disrupted of the hygienic structure of the silage; yet is not able to prevent the increase in pH level. Moreover, it was also stated in a study that bacteria such as yeast and mould lead to aerobic degradation, and that the bacteria contribute the aerobic degradation through the use of lactic and acetic acid (Woolford and Cook, 1978). The pH increase and the TMAB count in the anaerobic period being high in the CON group as well as in CMN5; can be explained with the fact that bacteria used the LA to increase the pH level.

In a study carried out by Soycan-Öncenç et al. (2015), it was stated that origanum and cinnamon essential oils did not affect ESOM contents of the field peas in a 60-day-storage, yet, increased them numerically in 120 days (Soycan-Önenç et al. 2017). In this study it was determined that cumin essential oil at high level (500 mg/kg) decreased the ESOM amount, and that 300 mg/kg addition of the essential oil affected the energy content (ME_{ESOM}) of the silage positively.

In the study conducted, RFV was found out to be below 100 in all groups, and increased as a little with the use of the additive. This increase resulted from the decrease of cell wall components (NDF and ADF). In studies conducted by Van Soest (1994) and Yavuz (2005), it was stated that the increase in NDF and ADF levels in feeds decelerated digestion, led to the animals feeling full, and that this resulted in the limitation of feed consumption. In this study, the addition of 300 and 500 mg of cumin essential oil into vetch-oat silages decreased NDF and ADF. Therfore, the DMI and RFV in CMN3 and CMN5 groups have improved.

CONCLUSION

In this study, it was concluded that the addition of cumin essential oil had a positive effect on the chemical and microbiological qualities of the vetch-oat silages. Particularly the addition of 200 mg/kg of cumin essential oil provided the cell wall to break down through the stimulation of the enzyme activities. Concordantly, the increase in the water soluble carbohydrate amount and LAB counts inhibited the enzymes, which degrade proteins, by decreasing the pH level, thus decreased the degradation of proteins to ammonia. It also improved the aerobic stability.

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