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Keywords:

Alfalfa silage, oregano, cumin, cinnamon, essential oil.

Effect of Oregano, Cumin and Cinnamon Essential Oils on Fermentation Quality in Alfalfa Silages

Yonca Silajlarında Kekik, Kimyon ve Tarçın Uçucu Yağlarının Fermantasyon Kalitesine Etkisi

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ABSTRACT

Objective: The aim of this study was to determine the effects of the oregano (OEO), cumin (CEO) and cinnamon (CINEO) essential oils on fermentation quality, metabolic energy (ME) contents and relative feed value (RFV) of alfalfa silages.

Material and Methods: Alfalfa was harvested in the end of April, at the early bloom-stage, was wilted for 3 hours and chopped to approximately to a size of 2.0 cm. OEO, CEO and CINEO essential oils were added as additivies in an amount of 600 mg/kg wet chopped alfalfa, yet no additives were added to the control (CONT) group. The packages of silage were kept in a covered storage (18±4 °C) for 60 days for fermentation.

Results: The addition of cumin and cinnamon led to a significant increase in dry matter (DM) ($P<0.05$). The addition of essential oils at high level caused an increase in lactic acid (LA), while the amount of water soluble carbohydrate (WSC) decreased inversely correlated with LA ($P<0.05$). The treatment was increased lactic acid bacteria (LAB) numbers, and also caused a decrease in the number of yeast and mould at a significant level ($P <0.05$). All three essential oils led to a decrease in pH, the amount of weigth loss (WL) and ammonia nitrogen (NH_3-N). In the cumin and cinnamon essential oil treatments increased amount of enzimatic solubility organic matter (ESOM) ($P<0.05$).

Conclusion: It was concluded that OEO, CEO and CINEO promote to increase LAB population. In addition, ME_{ESOM} content calculated through ESOM was increased in OEO and CINEO treatments.

Anahtar Sözcükler:

Yonca silajı, kekik, kimyon, tarçın, uçucu yağ

ÖZ

Amaç: Bu araştırmanın amacı kekik, kimyon ve tarçın uçucu yağlarının yonca silajlarının fermantasyon kalitesi, *in vitro* metabolik enerji (ME) içerikleri ve nispi yem değeri (NYD) üzerine etkilerini belirlemektir.

Materyal ve Metot: Araştırmada kullanılan yonca, nisan sonu çiçeklenme başlangıç döneminde hasad edilerek 3 saat süreyle soldurulmuş ve yaklaşık 2.0 cm boyutunda parçalanmıştır. Denemede, kontrol grubuna hiçbir uçucu yağ ilave edilmemiş, diğerlerine her bir uçucu yağdan 600 mg/kg düzeyinde ilave edilmiştir. Paketler kapalı bir depoda (18±4 °C) 60 gün boyunca fermantasyona bırakılmıştır.

Bulgular: Kimyon ve tarçın ilavesi, kuru maddede (KM) önemli bir artışa yol açtı ($P <0.05$).Uçucu yağların yüksek düzeyde eklenmesi, laktik asitte bir artışa neden olurken ($P<0.05$), suda çözülebilir karbonhidrat miktarı, laktik asit ile ters orantılı olarak azalmıştır. Muameleler laktik asit bakteri sayısını artırırken maya ve küf sayısında önemli bir düşüşe neden olmuştur ($P <0.05$). Kimyon ve tarçın uçucu yağ muamelelerinde enzimde çözünebilen organik madde miktarı (EÇOM) artmıştır ($P <0.05$).

Sonuç: Yapılan çalışmada kekik, kimyon ve tarçın uçucu yağlarının ilavesi laktik asit bakterilerinin gelişimini teşvik ettiği sonucuna varılmıştır. Ayrıca, kekik ve tarçın muamelelerinde enzimde çözünen organik madde miktarından hesaplanan $ME_{EÇOM}$ içeriği artmıştır.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the first among the feed plants being planted, and is usually used in animal nutrition in forms of dried hay or fresh forage. In recent years, in regions of high level of precipitation, silage production became widespread particularly in April-May months from first-cutting or last-cutting alfalfa (Kurtoglu, 2011).

Alfalfa takes place in the category of difficultly ensilaged plants depending on the composition of its nutrients. However, very good results are obtained when various additives are used and ensilage is done in complete manner. Numerous chemical and biological based additives are used in silage production (Filya, 2000; Kurtoglu, 2011). Due to some silage additives being banned due to having carcinogenic effect, it has brought up the search for alternative silage additives that are harmless for the environment, human or animals (Slottner and Bertilsson, 2006). It has been stated that plant extracts contain essential oils and are used in many areas due to their antimicrobial effects for centuries (Davidson and Naidu, 2000), that they are believed to be safe for human consumption in the European Union, and that their antimicrobial characteristics stem from the terpenoids (carvacrol, carvone, thymol, terpinene-4-ol) and from phenylpropanoids (cinnamaldehyde, eugenol, anethol) included in their structure (Busquet et al. 2005). Kung et al. (2008) stated that the addition of 40 and 80 mg/kg essential oils mixture to corn silage did not affect the fermentation and aerobic stability of the silage. Chaves et al. (2012) added different amounts of essential oils of cinnamon leaf, oregano and sweet orange, and could not determine mould at 7 days of aerobic stability. Turan and Soycan Önenç (2018) stated that the addition of 300 mg/kg cumin essential oil into the last harvest (5th harvest) provided the fragmentation of the cell membrane through the stimulation of cell membrane fragmenting enzymes, increased the number and activity by promoting the development of LAB, and correspondingly increased the transformation of sugar into LA, and that LA, found in the medium at high amount, inhibited protein fragmenting enzymes by decreasing pH level, and also decreased the fragmentation of proteins into ammonia.

This study was conducted to determine the effects of the essential oils of oregano, cumin and cinnamon on the fermentation quality, *in vitro* ME contents, and RFV of alfalfa silages as antimicrobial additives.

MATERIAL and METHODS

Preparation of additives

OEO, CEO and CINEO essential oils, the chemical composition of which are given in Table 1, were added as additivies in an amount of 600 mg/kg wet chopped alfalfa, yet no additives were added to the CONT group.

Preparation of silages

Alfalfa was harvested in the end of April, at the early bloom-stage, was wilted for 3 hours and chopped to approximately to a size of 2.0 cm. Essential oils were sprayed homogeneously onto alfalfa. Approximately 2 kg of sample was placed into a plastic bag and air was vacuumed out. Plastic bags were covered 10-12 times and finally with one layer of adhesive tape. 16 packages of silage, being 4 pacakages for each group, were kept in a covered storage (18±4 °C) for 60 days for fermentation.

Chemical and microbiological analysis

After opening the silages, pH values were determined via a digital pH meter, buffer capacity (Bc) through the statements of Playne and McDonald (1966), and LA was detected through spectrophotometric method (Barker and Summerson, 1941). The NH_3-N and WSC contents of silages were determined according to the methods stated in Anonymous (1986). The aerobic stability test was carried out using the method developed by Ashbell et al. (1991). LAB as well as yeast and mould analysis were determined through the method developed by Seale et al. (1990), and total mesophilic aerobic bacteria (TMAB) was done according to Anonymous (2014). TMAB, LAB, enterobacter, yeast and mould counts were transformed into colony forming unit (cfu/g).

Table 1. The chemical composition of essential oils, %

Çizelge 1. *Uçucu yağların kimyasal bileşimi, %*

Oregano		Cumin		Cinnamon	
Compounds	Value	Compounds	Value	Compounds	Value
Carvacrol	69.10	Cuminaldehyde	44.47	Cinnamaldehyde propilene glycol acetal	46,69
Thymol	10.70	Carvacrol	12.12	Cinnamaldehyde	44,17
P-Cymene	4.00	Para cymen	8.82	Carbitol	9,14
Borneol+ α-Terpineol	3.00	Safranal	6.57		
γ-Terpinene	2.50	Gamma terpinen	5.64		
		Beta-pinen	4.93		
		(-) Alpha cedren	4.48		
		Carotol	2.49		
		Diğerleri	7.37		
Unknown	10.70	Unknown	3.11		
Total compounds	100	Total compounds	100	Total compounds	100

The DM was determined by drying the samples at 105 °C for 16 h. The crude protein (CP) content of feed samples were determined according to the methods of AOAC (1990). The organic matter (OM), crude fibre (CF) and ether extract (EE) contents of the feed was determined by Nauman and Bassler (1993). Nitrogen-free extract (NFE), hemicellulase and cellulase (Close and Menke 1986) was determined by calculation. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents, which are components of the cell membrane of the samples, were determined according to the method stated by Van Soest et al. (1991). The ESOM were determined via cellulase method (De Boever et al., 1986; Naumann and Bassler, 1993). In the technique, pre-treatment with pepsin-hydrochloric acid solution, followed treatment by cellulase (Onozuka R 10 from *Trichoderma viride,* Merck).

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

In vitro ME contents in silages were calculated using crude nutrition components (CNC), NDF, ADF, ADL and ESOM determined because of chemical analysis according to the equation given below:

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).

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.

Equations developed by Van Dyke and Anderson (2000) and given below were used in the determination of RFV. Firstly, digestible dry matter (DDM), secondly dry matter intake (DMI) was estimated and finally RFV was predicted.

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

RFV = %, DDM x % DMI x 0.775

Statistical Analyses

The statistical assessment of the data was calculated via SPSS v.18 (SPSS 2009) statistical package programme according to ANOVA procedures, and Duncan test was applied to the differences among the averages.

RESULTS

Wilting alfalfa (WA) was contained 28.07 % DM, 6.05 pH, 80 g/kg DM WSC and 728 MeqNaOH/kg DM Bc. CNCs of the alfalfa silages uncovered on the 60th day of ensilage are given in Table 2. While OM amount was found out to be high at CONT and CINEO groups, CA level was found low. In oregano and cumin groups; however, OM amount was low, and CA level was high. The highest CP levels of silages were determined in CEO and CINEO groups (P<0.05). EE contents of alfalfa silages in comparison to the CONT group revealed an increase of approximately 1% in OEO and CINEO groups. NFE contents of alflafa silages were found out to be significantly low in the experimental group in comparison to the CONT group (P<0.05). NDF and ADF contents of experimental groups increased according to CONT (P<0.05).

Table 2. Chemical composition of wilting alfalfa and silage ensiled at 60th days (DM %)

Çizelge 2. *Soldurulmuş yonca ve 60. gün silajların kimyasal bileşimi (% KM)*

Treatments	OM	CP	EE	CF	NFE	CA	NDF	ADF	ADL	Hemicellulose	Cellulose
WA	90.04	22.25	2.84	27.83	37.12	9.75	44.66	34.01	8.82	10.65	25.19
CONT	90.01±0.08^{a}	18.21±0.09^{c}	3.54±0.09^{c}	23.16±0.08^{d}	45.10±0.18^{a}	9.99±0.08^{b}	41.68±0.17^{d}	27.32±0.21^{d}	7.95±0.21^{a}	14.36±0.38^{b}	19.37±0.32^{c}
OEO	89.56±0.11^{b}	18.49±0.06^{b}	4.43±0.04^{a}	25.53±0.07^{c}	41.11±0.25^{b}	10.44±0.11^{a}	42.40±0.16^{c}	31.78±0.15^{a}	7.18±0.09^{b}	10.62±0.03^{d}	24.61±0.06^{a}
CEO	89.44±0.10^{b}	18.84±0.08^{a}	3.76±0.01^{b}	27.62±0.07^{a}	39.22±0.13^{d}	10.56±0.09^{a}	44.16±0.02^{a}	28.68±0.21^{c}	6.57±0.25^{c}	15.48±0.22^{a}	22.11±0.36^{b}
CINEO	89.92±0.08^{a}	18.77±0.08^{a}	4.44±0.04^{a}	26.87±0.14^{b}	39.83±0.15^{c}	10.08±0.08^{b}	43.32±0.06^{b}	30.91±0.04^{b}	8.01±0.04^{a}	12.41±0.09^{c}	22.89±0.08^{b}
P	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

WA: Wilting alfalfa, CONT: control, OEO: oregano essential oil, CEO: cumin essential oil, CINEO: cinnamon essential oil, 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. ± SEM, standart error of means, $^{abc:}$ Means with different letters in the same column are statistically significant (P<0.05).

Table 3. Fermentation quality of alfalfa silage ensiled at 60th days
Çizelge 3. *Yonca silajlarının 60.gün fermantasyon kalitesi*

Treatments	DM (%)	pH	WSC (g/kg DM)	LA (g/kg DM)	NH_3-N(g/kgTN)	WL (%)
CONT	25.42±0.08^{c}	5.50±0.12^{a}	95.33±0.88^{a}	26.45±0.29^{d}	105.60±8.07^{a}	1.91±0.03^{a}
OEO	25.40±0.16^{c}	5.20±0.12^{b}	90.00±0.58^{b}	55.99±0.08^{c}	99.29±3.49^{a}	1.59±0.03^{b}
CEO	25.92±0.04^{b}	5.27±0.03^{b}	73.33±0.33^{c}	70.10±0.11^{b}	95.40±0.49^{a}	1.45±0.17^{b}
CINEO	27.02±0.12^{a}	5.03±0.03^{b}	40.00±0.58^{d}	110.08±0.58^{a}	76.77±3.45^{b}	1.39±0.02^{b}
P	0.001	0.001	0.001	0.001	0.001	0.001

CONT: control, OEO: oregano essential oil, CEO: cumin essential oil, CINEO: cinnamon essential oil, DM: Dry matter, WSC: Water-soluble carbohydrates, LA: Lactic acid, NH_3-N: Ammonia nitrogen, TN: Total nitrogen, WL: Weight loss, ± SEM, standart error of means, $^{abc:}$ Means with different letters in the same column are statistically significant (P<0.05).

In the research, there was no difference found out between CONT and OEO groups in terms of DM (Table 3). On the other hand, the addition of cumin and cinnamon led to a significant increase in DM (P<0.05). The pH level of silages decreased significantly in comparison to the CONT group (P<0.05). In this research, the addition of oregano, cumin, and cinnamon essential oils at high level (high level, 600 mg/kg wet alfalfa; versus, 200 or 300 mg/kg wet alfalfa) caused an increase in LA, while the amount of WSC amount decreased inversely correlated with LA.

The NH_3-N concentrations of the silages decreased in the experimental groups and the differences between the control silage and the essential oil added silage were significant only in the cinnamon group (P <0.05). The highest WL was determined in the between CONT group (1.91%) and the lowest in the cinnamon added group (1.39%). The addition of oregano and cumin also influenced the prevention of WL when compared to the CONT group (P <0.05).

The microbiological analysis results of alfalfa silages are given in Table 4. The addition of oregano, cumin and cinnamon essential oils did not affect the TMAB numbers of alfalfa silages uncovered on the 60th day of ensilage, increased LAB numbers, and caused a decrease in the number of yeast and mould at a significant level (P <0.05). Enterobacter could not be identified in any group but it was found out that mould growth was high and that the addition of essential oil at high level decreased mould formation considerably but could not prevent it.

The addition of cumin and cinnamon essential oils increased the amount of ESOM (P<0.05). It is also seen that ME_{ESOM} values are highest in cinnamon (P<0.05). ME contents calculated using different regression equations revealed differences in groups according to the equation used.

Table 4. Effects of essential oils on microbial counts of alfalfa silages, log_{10} cfu/g
Çizelge 4. *Uçucu yağların yonca silajlarının mikrobiyal sayılara etkileri, log_{10} cfu/g*

Treatments	TMAB	LAB	Enterobacter	Yeast	Mould
CONT	7.03±0.01	4.87±0.03^{c}	ND	5.52±0.08^{a}	5.31±0.08^{a}
OEO	7.26±0.05	5.25±0.10^{b}	ND	3.26±0.11^{b}	4.49±0.06^{b}
CEO	7.03±0.11	5.36±0.06^{b}	ND	0.44±0.29^{c}	4.51±0.05^{b}
CINEO	7.12±0.14	6.27±0.07^{a}	ND	0.67±0.33^{c}	4.55±0.03^{b}
P	0.246	0.001	-	0.001	0.001

CONT: control, OEO: oregano essential oil, CEO: cumin essential oil, CINEO: cinnamon essential oil, TMAB: Total mesophilic aerobic bacteria, LAB: Lactic acid bacteria. ND:Not defined. ± SEM, standart error of means, $^{abc:}$ Means with different letters in the same column are statistically significant (P<0.05).

Table 5. ESOM (DM%) and ME contents of alfalfa silages, kcal/kg DM
Çizelge 5. *Yonca silajlarının EÇOM ve ME içerikleri, kcal/kg KM*

Treatments	ESOM	ME_{ESOM}	ME_{CNC}	ME_{NDF}	ME_{ADF}	ME_{ADL}
CONT	58.54±0.14^{b}	1506±3.93^{c}	1958±2.49^{a}	2549±3.21^{a}	2534±7.55^{a}	1948±21.40^{c}
OEO	58.45±0.16^{b}	1603±2.65^{b}	1817±6.73^{b}	2535±3.28^{b}	2374±5.51^{d}	2027±9.26^{b}
CEO	59.64±0.16^{a}	1513±6.49^{c}	1754±2.39^{d}	2500±0.33^{d}	2485±1.53^{b}	2089±25.24^{a}
CINEO	59.85±0.16^{a}	1623±7.09^{a}	1776±8.77^{c}	2516±1.33^{c}	2405±7.57^{c}	1941±4.16^{c}
P	0.001	0.001	0.001	0.001	0.001	0.001

CONT: control, OEO: oregano essential oil, CEO: cumin essential oil, CINEO: cinnamon essential oil, 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.

Table 6. Dry matter digestibility, dry matter intake and relative feed value of alfalfa silages.

Çizelge 6. *Yonca silajlarının kuru madde sindirilebilirliği, kuru madde tüketimi ve relatif yem değeri*

Treatments	DDM,%	DMI,%	RFV
WA	62.41	2.69	130.11
CONT	67.62±0.16 [a]	2.88±0.01 [a]	150.88±0.29 [a]
OEO	64.14±0.12 [d]	2.83±0.01 [b]	140.68±0.79 [b]
CEO	66.56±0.03 [b]	2.72±0.01 [d]	140.16±0.32 [c]
CINEO	64.82±0.16 [c]	2.77±0.01 [c]	139.16±0.14 [c]
P	0.001	0.001	0.001

WA: Wilting alfalfa, CONT: control, OEO: oregano essential oil, CEO: cumin essential oil, CINEO: cinnamon essential oil, 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).

The WA of alfalfa and DDM, DMI, and RFV of alfalfa silages are given in Table 6. The DDM values of silages were found out to be between 64.14% and 67.62%. DMI values were found out to be 2.88%, 2.83%, 2.72%, and 2.77% for CONT, OEO, CEO, and CINEO respectively. The RFV of silages were determined as 150.88 for the CONT group, 140.68 for OEO treatment, 140.16 for CEO treatment, and 139.16 for CINEO treatment.

DISCUSSION

In the study, due to the OEO and CEO groups being prepared last, it may be due to containing more soil etc. that therefore they had more CA contents when compared to the other groups. In this case, the difference among OM values may be due to the difference among CA contents of groups. CP contents of silages were highest in CEO and CINEO treatments statistically when compared to the CONT group. However, when NH3-N levels in this period were analyzed, it was found out that CINEO addition appeared to be more effective in protein fragmentation. Hence, Turan and Soycan Önenç (2018) stated that the addition of 300 mg/kg CEO was effective on the prevention of protein fragmentation. In another study, the addition of OEO and CINEO on field pea at the end of 60 day ensilage decreased the level of protein fragmentation at a significant level (Soycan-Önenç et al., 2015). The addition of cinnamon leaf oil to barley silage at different levels led to an increase in CP amount (Chaves et al., 2012). In the study conducted, the effect of high level use of essential oils in alfalfa silages on CP amount, was consistent with OEO and CINEO used by Soycan-Önenç et al. (2015), and the cinnamon extract used by Chaves et al. (2012).

Kung et al. (1991) determined that the addition of antibiotics caused a slight decrease in the amount of ADF. In the another study of Soycan-Önenç et al. (2015), the addition of 400 mg/kg of OEO, CINEO, and OEO+ CINEO increased the NDF and ADF contents of the silages. Chaves et al. (2012) found out that the ethanol extract of cinnamon leaves decreased the NDF and ADF, while the higher level increased it, and that oregano and orange extracts also caused an increase in NDF and ADF levels. The level of increase in NDF and ADF determined in this study is compatible with the results of Soycan-Önenç et al. (2015).

In the present study, the addition of OEO at high level was not effective on DM, was not compatible with the results of Soycan-Önenç et al. (2015), but CEO and CINEO revealed similarity. While pH level is expected to be 3.5-4.0 in a high quality silage, pH values of 4.0 and above are frequently in legume silages. The pH level of OEO and CINEO added field pea silages was found out as 4.4 and 4.47 (Soycan-Önenç et al. 2015). Turan and Soycan Önenç (2018) determined that CEO decreased the pH level of alfalfa silages. In this study, the effect of essential oils on the pH level was compatible with the results of Soycan-Önenç et al. (2015) and Turan and Soycan Önenç (2018).

In high-quality silages, the LA content should be above 2.0% (Alçiçek and Özkan, 1997). When the limit values reported for LA are taken into consideration, the LA amounts obtained in all groups in the study are compatible with the study of Alçiçek and Özkan, (1997). The use of OEO, CEO and CINEO at high level promoted LAB activities, and the increased LAB transformed WSC into LA. As a result, WSC decreased while LA increased. This result was similarly to Turan and Soycan Önenç (2018).

In legumes harvested very early, CP contents being high (>23-24%) (since more acid is required to decrease pH at fermentation process due to the buffer capacity being high) may function as a limiting effect on fermentation (Kung and Shaver, 2001). Cinnamon essential oil used in the study decreased the NH3-N amounts of alfalfa silages at the end of ensilage period. The reason of this is the prevention of proteolysis in silages with CINEO addition. Hence, Polan et al. (1998) reported that NH3-N amounts decreased when formic acid was added to alfalfa silages. In fact, essential oils have an antimicrobial effect similar to formic acid. Organic acids, particularly formic acid reveal antibacterial effect due to the hydrogen ion concentration in their structure and the selective bactericidal effect of the undissolved acid (Henderson, 1993). Phenols within the structure of the essential oils break down of the membrane proteins in cell membranes. They cause failure in the mainly function of the cell by altering the transmittance of cations such as H^+ and K^+ (Helander et al, 1998). It was suggested that the antimicrobial effect of cinnamaldehyde is related to interactions between proteins in the periplasm or in the inner part of the cell (Helander et al, 1998). In this study the selective antimicrobial effect of CINEO on microorganisms causing proteolysis may be due to the presence of 44.17% cinnamaldehyde. Henderson (1993) stated that a good silage

additive should be safe and reduce WL. When considered in this respect, the hypothesis that oregano, cumin and cinnamon essential oils may be an alternative silage additive is supported.

In this study, unlike the results of Chaves et al. (2012), it was found out that TMAB counts were not affected; however yeast counts similarly decreased with the use of essential oils. Unlike Chaves et al. (2012), LAB counts increased similarly to the results of Soycan-Önenç et al. (2015) and Turan and Soycan Önenç (2018). Similar to this study, Chaves et al. (2012) also found that *O. vulgare* could not prevent mould growth. However, Turan and Soycan Önenç (2018) stated that 300 and 500 mg/kg of CEO prevented mould growth in alfalfa silages. Unlike the results of the present study, Soycan-Önenç et al. (2015) stated that OEO, CINEO and OEO+CINEO prevented mould growth in field pea silages. The antimicrobial effect of phenolic compounds such as thymol and carvacrol is reported to be higher than other secondary plant components due to the presence of the hydroxyl group present in their phenolic structures (Helander et al, 1998; Ultee et al, 2002). In addition, while OEO used by Soycan-Önenç et al, (2015) contained 59.03% carvacrol and 12.04 % thymol as basic component, CINEO contained 41.50% cinnamaldehyde propilene glycol acetal and 35.28% cinnamaldehyde.

In the present study, OEO contained 69.10% carvacrol and 10.70% thymol as basic component, CINEO contained 46.69% cinnamaldehyde propilene glycol acetal and 44.17% cinnamaldehyde. The amounts of active substance and the level used being higher than the ones used by Soycan-Önenç et al. (2015) may have affected the results negatively. Although CEO contained components (44.47% cuminaldehyde, 12.12% carvacrol and 8.82% paracymen) at the same level, the results being different from Turan and Soycan Önenç (2018) may be correlated similarly with the increase in the level used. LAB counts necessary for silage fermentation increased despite the high amount of essential oils, and this revealed that the use of OEO, CEO and CINEO in high amount had an incentive effect on LAB.

Nadeau et al. (2000) determined that cynodon dactylon and alfalfa silages made by using formic acid led to an increase in the DM digestibility in ruminants. Soycan-Önenç et al. (2015) stated that while OEO and CINEO did not affect the ESOM contents of field peas in 60 day-ensilage, it led to an increase numerically in 120 days (Soycan-Önenç et al. 2017). CEO and CINEO used in the study revealed a formic acid like effect, and increased the ESOM significantly in comparison to the CONT group. However, ME_{ESOM} content calculated through ESOM amount increased in OEO and CINEO groups. The reason is that the levels of EE (Table 2) in the OEO and CINEO groups is higher than the others (see in ME_{ESOM} equality). This result reveals that CINEO also affects the ME content of silage positively. While, the highest ME_{CNC} , ME_{NDF} ve ME_{ADF} were determined in the CONT group, MEADL was determined in the cumin group. This was due to the CEO addition revealing a decreasing effect in ADL amount while it did not show the same effect in CF, NDF and ADF.

In this study, RVF of the CONT group was found out to be above 150, and it was determined that there was an increase based on the use of additives. In contrary to our results, Turan and Soycan Önenç (2018) stated that the use of 300 mg and 500 mg of CEO affected RFV positively. However, increased NDF in experimental groups caused to decreased in the RFV. One of the most commonly used measure of forage quality has NDF, which stimulated chewing, salivation, and thus enhances ruminal fermentation and milk fat production (Cao et al., 2013). Additionally, in the rainfall areas, the last cutting alfalfa cannot be dried. It was more advantageous to ensilage alfalfa when compared to the wilting alfalfa RFV value (130.11).

CONCLUSIONS

The high levels (high level,600 mg/kg wet alfalfa; versus, 200 or 300 mg/kg wet alfalfa) of essential oils used in the study, the increase in LAB counts in comparison to the CONT group, the decrease of pH, TMAB, yeast and mould counts emerged due to the selective antimicrobial effect of essential oils. In addition, ME_{ESOM} content calculated through ESOM amount increased in OEO and CINEO treatments. However, the RFV of silage decresed OEO, CEO and CINEO treatments by means of NDF and ADF increased. The NDF ratio affected rumen pH, which was especially important for rumen health.

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REFERENCES

Alçiçek A, Özkan K. 1997. Determination of silage quality in silo feeds by physical and chemical methods. First silage congress in Turkey, p. 241-246.

Anonymous, 1986. The analysis of agricultural material. Reference book, London, pp. 427-428.

Anonymous, 1991. Animal feeds-determination of metabolizable energy (chemical method). Turkish Standards Institute (TSE). Publ. no. 9610, pp.1-3.

Anonymous, 2014. Microbiology-food and animal feeds-horizontal method for the enumeration of microorganisms. Colony count at 30 °C by the spreading plate technique. TS EN ISO 4833.

AOAC, 1990. Official methods of analysis of the Association of Official Analytical Chemistry. 15th ed. Arlington. VA, USA.

Ashbell G, Weinberg ZG, Azrieli A, Henand Y, Horev B. A. 1991. A Simple system study the aerobic deterioration of silages. Canadian Agricultural Engineering 33:391-393.

Busquet M, Calsamiglia S, Ferret A, Kamel C. 2005. Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. Journal of Dairy Science 88:2508-2516.

Barker SB, Summerson WH. 1941.The colorimetric determination of lactic acid in biological material. Journal of Biological Chemistry 138:535-554.

Cao YC, Gao Y, Xu M, Liu NN, Zhao XH, Liu CJ, Liu Y, Yao JH. 2013. Effect of ADL to aNDF and ryegrass particle length on chewing, ruminal fermentation, and in sutu degradability in goats. Animal Feed Science and Technology 186:112-119.

Chaves AV, Baah J, Wang Y, McAllister TA, Benchaar C. 2012. Effects of cinnamon leaf, oregano and sweet orange essential oils on fermentation and aerobic stability of barley silage. Journal of Science Food Agriculture 92:906-915.

Close W, Menke KH.1986. Selected topics in animal nutrition university. p.170+85 Hohenheim.

Davidson PM, Naidu A S. 2000. Phyto-phenols. Natural food antimicrobial systems. ISBN:084932047, 265-294 p.

De Boever JL, Cottyn BG, Buysse FX, Wainman FV, Vanacker J M.1986. The use of an enzymatic technique to predict digestibility, metabolizable and net energy of compound feedstuffs for ruminants. Animal Feed Science Technology14:203-214.

Filya I. 2000. New developments in silage quality improvement. International Animal Nutrition Congress, 4-6 September Isparta p.243-250.

Helander IM, Alakomi H-L, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LGM, von Wright A. 1998. Characterization of the action of selected essential oil components on gram-negative bacteria. Journal of Agriculture Food Chemistry 46:3590-3595.

Henderson N. 1993. Silage additives. Animal Feed Science and Technology 45:35-56.

Jeroch H, Drochner W, Simon O. 1999. Nutrition on farm livestock. Verlag Eugen Ulmer Stuttgart, 525 p.

Kirchgessner M, Kellner RJ. 1981. Estimation of the energetic feed value of green and forage feed through the cellulas method. Landwirtschschaftliche Forschung 34:276-281.

Kirchgessner M, Kellner RJ, Roth FX, Ranfft K. 1977. For estimating the feed value using crude fiber and the cell wall fractions of the detergent analysis. Landwirtschaftliche Forschung 30:245-250.

Kung LJr, Tung RS, Maciorowski K. 1991. Effect of microbial inoculant (Ecosyl-TM) and/ or a glcopeptide antibiotic on fermantation and aerobic stability of wilted alfalfa silage. Animal Feed Science Technology 35:37-48.

Kung LJr, Shaver RD. 2001. Interpretation and use of silage fermentation analysis reports. Focus on forage 3(13):1-5. University of Wisconsin.

Kung Jr, Williams L, Schmidt P, Hu W. 2008. A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. Journal of Dairy Science 91:4793-4800.

Kurtoğlu V. 2011. Silage and silage additives. Aybil Press. Konya-Turkey p.356.

Nadeau EMG, Buxton DR, Russell JR, Allison MJ, Young JW. 2000. Enzyme, bacterial inoculant, and formic acid effects on silage composition of orchardgrass and alfalfa. Journal of Dairy Science 83:1487-1502.

Naumann C, Bassler R. 1993. Method book. B III. The chemical analysis of feeds. VDLUFA-Verlag, Darmstadt.

Playne MJ, McDonald P. 1966. The buffering constituent of herbage and silage. Journal of Science Food Agriculture17:264-268.

Polan CE, Stieve DE, Garrett JL. 1998. Protein preservation and ruminal degradation of ensiled forage treated with heat, formic acid, ammonia, or microbial inoculant. Journal of Dairy Science 81:765-776.

Seale DR, Pahlow G, Spoelstra SF, Lindgren S, Dellaglio F, Lowe JF. 1990. Methods for the microbiological analysis of silage. Proceeding of the Eurobac Conference Uppsala p.147.

Slottner D, Bertilsson J. 2006. Effect of ensiling technology on protein degradation during ensilage. Animal Feed Science Technology 127 (1–2):101–111.

Soycan-Önenç S, Fisun K, Coşkuntuna L, Özdüven ML, Gümüş T. 2015. The effect of oregano and cinnamon essential oils on fermentation quality and aerobic stability of field pea silages. Asian-Australas J Animal Science 28(9):1281-87.

Soycan-Önenç S, Coşkuntuna L, Koç F, Özdüven ML, Gümüş T. 2017. Effects of essential oils of oregano and cinnamon on fermentation quality and ın vitro metabolic energy of field pea silages. Animal Production 58(2):39-44.

SPSS. 2009. PASW Statistics for Windows, Version 18.0. Chicago, SPSS Inc.

Turan A, Soycan Önenç S. 2018. Effect of cumin essential oil usage on fermentation quality, aerobic stability and in vitro digestibility of alfalfa silage. Asian Australas. Asian-Australas J Animal Science 31(8):1252-1258.

Ultee A, Bennik HJ, Moezelaar R. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen. *Bacillus cereus.* Applied Environmental Microbiology 3:1561-1568.

Van Dyke NJ, Anderson PM. 2000. Interpreting a forage analysis. Alabama Cooperative Extension. Circular ANR-890.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Method for dietary fiber, neutral detergent fiber and nostarch polysaccharides in relation to animal nutrition. Journal of Dairy Scienc 74:3583-3597.