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Improving Ruminant Fermentation Characteristics with Addition of Apple Pulp and Essential Oil to Silage

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Abstract

One of the main categories of environmental challenges are process discards including apple pulp (AP). This by-product contains nutrients making it an ideal candidate as feed additive. In this study, the potential of AP as animal feed was examined. Alfalfa silage was supplemented with fresh AP and essential oil (EO) and the in vitro effects were tested on gas production (GP), dry matter (DM), organic matter and crude protein degradability. Ensiled for 90 days, the treatments were the following: T1) alfalfa silage alone (control), T2) EO processed alfalfa silage (AE), T3) 75% alfalfa + 25% AP silage (AA1), T4) 75% alfalfa + 25% AP EO processed silage (AA1E), T5) 50% alfalfa + 50% AP silage (AA2), T6) 50% alfalfa + 50% AP and EO processed silage (AA2E), T7) 25% alfalfa + 75% AP silage (AA3) and T8) 25% alfalfa + 75% AP and EO processed silage (AA3E). It was observed that the highest BP volume for 25% AP and EO (189.64 mL/g DM) supplemented silage and the lowest for 50% AP and EO (141.07 mL/g DM) supplemented silage after 72-h incubation. The results showed that the supplementation of silage with AP at 50 and 75% levels increased BP parameters ($p < 0.01$). Effective DM degradability increased by adding EO and AP at 75% level ($p < 0.01$). It can be concluded that AP can be used in the preparation of alfalfa silage and has the potential to affect ruminal fermentation efficiency.

***Keywords**:* alfalfa silage, apple pulp, degradability, essential oil, in vitro gas production

1. Introduction

Preservation of the forage in form of silage is a common method of providing feed resources for ruminants, especially when fresh forage is not available (Collins and Moore, 2017; Besharati *et al*., 2020a; Santos *et al*., 2020). Many producers are currently interested in alfalfa silage but its production is difficult due to high protein level, high buffering capacity and low water-soluble carbohydrate content. To overcome the issue, additives should be used during the process of alfalfa silage production.

Numerous studies have tested carbohydrate and bacterial additives for the improvement of alfalfa silage quality (Rambau *et al*., 2017; Tao *et al*., 2017; Zhao *et al*., 2019; Besharati *et al*., 2020b). Various agricultural processing by-products (e.g., apple pomace and its silage, tomato pomace, citrus pulp, sugar beet pulp and pistachio peel) have demonstrated considerable potential as animal feed (Ayaşan *et al*., 2012; Rahbarpour *et al*., 2012; Palangi *et al*., 2013; Ulger *et al*., 2018, 2020; Fayed, 2019; Marcos *et al*., 2019; Naderi *et al*., 2019; Selçuk *et al*., 2019; Yurtseven *et al*., 2019; Zhou *et al*., 2019; Çayıroğlu *et al*., 2020; Davies *et al*., 2020; Gharehbagh *et al*., 2020). These low-value products, generally considered waste, can be fermented and incorporated into silages as a source of carbohydrates. Moreover, plant-extracted essential oils (EO) are widely used in animal nutrition for improving performance (Hundal *et al*., 2019; Zhou *et al*., 2020). A handful of studies have tested their antimicrobial activities (Besharati *et al*., 2020b). In general, the most important active ingredients of herbal EOs are divided into two groups: terpenoids and phenylpropanoids (Amini *et al*., 2020). The most important role of these compounds is their antimicrobial and antibacterial activities (Gomes *et al*., 2019; Ravikumar *et al*., 2019).

Herbal EOs may be used in ruminant feeds to alter fermentation because fermentation in silage and rumen depends on microbial activity that can be affected by EOs. Antimicrobial activity of plant EOs against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, has been shown to inhibit the synthesis of phenolic and terpenoid compounds (Chaves *et al*., 2008). Chemical components and structural groups in EOs, or interactions between them, probably lead to these effects (Costa *et al*., 2020). In general, EO is composed of hydrophobic compounds that are structurally comparable to the lipids of bacterial cell membranes. Hydrophilic feature favors antibacterial and antifungal properties of EO. This mechanism is highly related to the lipophilic properties of the constituents of EO and the ability of their functional group (Lian *et al*., 2019). Ruminal protozoa provide suitable environment for methanogens that are abundant in rumen; however, EO could prevent the proliferation of protozoa reducing the methane production (Kamra and Singh, 2019; Stanton *et al.*, 2020). Part of the methane production is due to the presence of ciliated prostheses (Newbold and Ramos-Morales, 2020). The aim of this experiment was to investigate the effects of adding EOs (Essential®) and apple pulp (AP) in alfalfa silage production on gas production (GP), dry matter (DM), organic matter (OM) and crude protein (CP) degradability using in vitro method.

2. Methodology

2.1 Ensiling Method and Treatment Composition

Alfalfa (300 kg) was harvested in the fourth cut before the flowering stage, then chopped and wilted at room temperature (25-28 °C) for 24 h. Fresh AP (50 kg) was provided by fruit juice factory and wilted for 24 h. The mixture of AP and silage were supplemented with 500 mg of EO (30% ricinoleic acid, 30% cardol and 20% cardanol) and ensiled for 90 days. EO, used in this study, was a commercial preparation (Essential®, Oligo Basics Agro Ind. Ltd., Cascavel, Brazil) containing castor oil as the carrier and the active ingredients (ricinoleic acid, cardol and cardanol). EO was dissolved in 1-mL ethanol (Merck) and sprayed on forage. Ethanol, used to dissolve the EO additive, was also added to the control. The laboratory silos (three per treatment) were used with a height of 90 cm and a diameter of 10 cm and were manually compacted after filling the silos (Besharati *et al.*, 2017). Experimental treatments were designed according to the factorial model and included the following: treatment 1 (T1) alfalfa silage alone (control); T2) EO processed alfalfa silage (AE); T3) 75% alfalfa + 25% AP silage (AA1); T4) 75% alfalfa + 25% AP EO processed silage (AA1E); T5) 50% alfalfa + 50% AP silage (AA2); T6) 50% alfalfa + 50% AP and EO processed silage (AA2E); T7) 25% alfalfa + 75% AP silage (AA3); and T8) 25% alfalfa + 75% AP and EO processed silage (AA3E).

2.2 In Vitro Gas Production and Digestibility

The study used the previously described method of Fedorak and Hrudey (1983) for the measurement of gas production. A sample of 300 mg of each treatment was weighed (A&D Company Ltd., N-92, Tokyo, Japan) and transferred into 50-mL sterile serum bottles (five replications for each treatment). The rumen liquid was collected from three slaughtered sheep in a livestock slaughterhouse in Ahar, Iran by a four-layered cheese cloth and immediately transported to the laboratory inside a 39 °C water flask. Rumen liquid was mixed with a buffer prepared by McDougall method (1948) in a ratio of 1:2 (one-part rumen liquid and two parts buffer) and then transferred to serum bottles. Mixture of 20-mL rumen and buffer liquid was added to the experimental treatment containing bottles and tightly closed with a rubber cap and a metal press. All the bottles were transferred to the shaking incubator (IKA, K 4000i, Germany) with a speed of 120 rpm and a temperature of 39 °C to measure the produced gas. Produced gas was recorded at 2, 4, 6, 8, 12, 16, 24, 36, 48 and 72 h after incubation.

Metabolizable energy (ME), net energy lactation (NEL), and organic matter digestibility (OMD) were calculated using Equations 1, 2 and 3, respectively, following the methods of Menke and Steingass (1988).

$$ME\ (MJ/kg\ DM) = 2.2 + 0.136GP + 0.0057CP + 0.000286CF^2 \tag{1}$$

$$NEL\ (MJ/kg\ DM) = 0.54 + 0.096GP + 0.0038CP + 0.000173CF^2 \tag{2}$$

$$OMD\ (\%) = 16.49 + 0.9042GP + 0.0492CP + 0.0387CA \tag{3}$$

where *GP* is 24-h net gas production (mL/200 mg DM)*; CP* is crude protein; *CF* is crude fat; and *CA* is crude ash.

Short-chain fatty acid (SCFA) were calculated using Equation 4 (Menke *et al.*, 1979).

$$SCFA\ (mmol/200\ mg\ DM) = 0.0222GP - 0.00425 \tag{4}$$

In this regard, GP, CP, CF, and CA are the produced gas in 24 h (mL/200 mg DM), crude protein, crude fat, and crude ash in dry matter, respectively. The amount of microbial protein was calculated on the basis of 19.3 g of microbial nitrogen per kilogram of digestible organic matter.

In vitro degradability test, the procedure as gas production measurement test, was performed. However, in this experiment, 15 repetitions for each treatment were designed. Three repetitions of each treatment were collected from the incubator at 2, 4, 8, 12 and 24 h and recorded the pH of each treatment. In order to completely remove all microbial germs, each laboratory tube was centrifuged twice at 4,000 rpm (Hettich, Universal 320R, GmbH & Co., Tuttlingen, Germany) for 4 min at 25 °C, and each time after centrifugation, the liquid was slowly drained and washed with phosphate buffer 70%. The laboratory tubes were then oven dried (UNB10, Memmert, Schwabach, Germany) at 65 °C for 48 h (Besharati *et al.*, 2017). The data obtained from the in vitro degradability test was analyzed using NEWAY software based on Orskov and McDonald's (1979) equation (Equation 5) and the values of *a*, *b* and *c* were obtained.

$$Y = a + b\,(1 - e^{-ct}) \tag{5}$$

where *y* degraded part at time t (h); *a* is soluble part; *b* is the potentially degradable part; and *c* is the rate of degradable *b* fraction.

2.3 Statistical Analysis

The data was analyzed with a completely randomized 2 x 4 (EO x AP) factorial treatment design. Analysis of variance was performed using SAS software (version 9.4) (2018). Significant differences ($p < .05$) between the mean values were compared using Duncan's test.

3. Results and Discussion

The additive effect of EO and AP on the estimated parameters of gas production are given in Tables 1 and 2. Treatment AA3E had the highest gas production from 2 to 72 h of incubation time compared with other treatments ($p < 0.05$). The gas production of supplemented alfalfa silage with EO treatment was lower than control at 24-h incubation. At the end of 72 h, the highest gas production was related to the treatment of AA3E, and the lowest was related to AA1 and AA2E treatments (Figure 1).

The results showed that EO had a significant effect on GP rate, and AP caused more GP by 75% due to the presence of high pectin (Yuan and Wan, 2019). The present results agree with those of Nanon *et al.* (2014), Hodjatpanah-Montazeri *et al.* (2016) and Abdelrahman *et al.* (2019). Gorbani *et al.* (2013) reported the gas production inhibitory effects of peppermint and fennel EO (150 mg) in the fermentable part of corn silage.

Table 1. Effects of adding essential oil and apple pulp on gas production parameters

Parameters	Adding essential oil (mg/kg)			Apple pulp (%)					Pr > F		
	0	500	SEM	0	25	50	75	SEM	Apple pulp	Essential oil	Interactions
ME	4.61^{b}	4.83^{a*}	0.022	4.54^{c}	4.54^{c}	4.65^{b}	5.17^{a}	0.031	< 0.0001	< 0.0001	< 0.0001
NEL	2.24^{b}	2.40^{a}	0.015	2.19^{c}	2.19^{c}	2.27^{b}	2.64^{a}	0.022	< 0.0001	< 0.0001	< 0.0001
OMD	32.54^{b}	34.03^{a}	0.146	32.06^{c}	32.06^{c}	32.8^{b}	36.28^{a}	0.207	< 0.0001	< 0.0001	< 0.0001
GP	18.78^{b}	19.39^{a}	0.162	17.21^{c}	17.21^{c}	18.03^{b}	21.88^{a}	0.229	< 0.0001	< 0.0001	< 0.0001
MP	6.28^{b}	6.48^{a}	0.006	5.89^{d}	6.28^{c}	6.33^{b}	7.02^{a}	0.009	< 0.0001	< 0.0001	< 0.0001
SCFA	0.38^{b}	0.42^{a}	0.003	0.37^{c}	0.37^{c}	0.40^{b}	0.48^{a}	0.004	< 0.0001	< 0.0001	< 0.0001

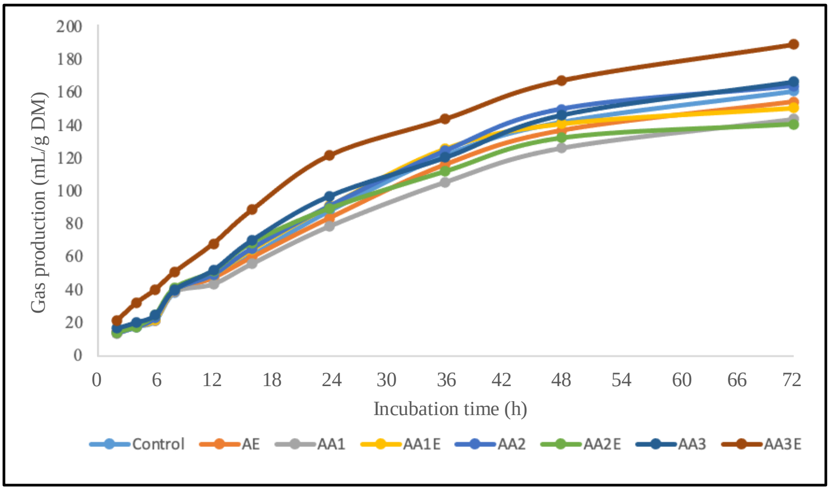
*Means within same column with different superscripts differ; ME – metabolizable energy (MJ/kg DM); SCFA – short chain fatty acid (mmoL/0.2 g DM); OMD – digestible organic matter in dry matter (%); NEL – net energy lactation (MJ/kg DM); MP – microbial protein

AP contains high amounts of pectin and easily digestible carbohydrates that are used as a substrate by bacteria. Amount of substrate, the fermentation and gas production were positively correlated. Thyme EO when used as additive to alfalfa silage to feed ruminants had similar effect to peppermint and fennel EO (Newbold *et al*., 2004; Jahani-Azizabadi *et al*., 2014; Davoodi *et al*., 2019). According to the findings of Kurniawati *et al.* (2019) and Molho-Ortiz *et al.* (2019), cinnamon EO reduced the amount of 24-h incubation gas production. In another study, garlic EO reduced 17-h incubation in vitro gas production dose dependently (Castillejos, 2005).

In an experiment, adding 80% AP to 20% alfalfa in 72 h of incubation significantly increased gas production (Losa, 2001). Razzaghi *et al.* (2015) investigated the nutritional value of AP, date and date kernel by gas production method and found that the highest volume of GP was related to AP. Due to the fact that alfalfa silage has a large amount of ammonia nitrogen by synchronizing it with a soluble carbohydrate source, the amount of microbial protein production and fermentation products resulting from the metabolic activities of microbes can be optimized. AP had increased in vitro digestibility because of its soluble carbohydrates. Also, the addition of EO reduced the production of in vitro gas production probably linked to the inhibition of deamination.

The effects of using thyme EO as an additive to alter the fermentation of alfalfa silage in ruminants by in vitro methods showed that thyme EO reduced the amount of gas produced compared with the treatment without additives (Pour *et al*., 2017; El-Essawy *et al*., 2019). Effect of EO and AP on GP is shown in Figure 1.

Gas production is mainly affected by chemical compounds and physical properties of feed as well as rumen microbial activity (Besharati *et al*., 2017). In this study, increasing the EO had a significant effect on GP parameters compared with the control, which is consistent with the results of other researchers (Garcia *et al*., 2019; Jahani-Azizabadi *et al*., 2019; Jafari et *al*., 2019). Talebzadeh *et al.* (2012) reported the inhibitory effects of thyme EO (0, 150, 300 and 600 μg/m) on in vitro gas production in different incubation times. Findings of Gorbani *et al.* (2013) for the similar parameter did not fully agree with those of Talebzadeh *et al.* (2012) as peppermint, lavender, basil, peppermint, and thyme EO did not significantly change the rumen fermentation pattern, but reduced in vitro methane production.



alfalfa silage alone (control), EO processed alfalfa silage (AE), 75% alfalfa + 25% AP silage (AA1), 75% alfalfa + 25% AP EO processed silage (AA1E), 50% alfalfa + 50% AP silage (AA2), 50% alfalfa + 50% AP and EO processed silage (AA2E), 25% alfalfa + 75% AP silage (AA3), 25% alfalfa + 75% AP and EO processed silage (AA3E).

Figure 1. Effect of EO and AP on gas production

AP (80%) in alfalfa silage increased gas production in 72 h of incubation (Khatooni *et al.*, 2014). In another study, AP resulted in more gas production in comparison to date and date kernel (Alizadeh *et al.*, 2010). Alfalfa silage provides high ammonia nitrogen, and supplementing it with a carbohydrate source allows ruminal bacteria to use nitrogen and energy simultaneously producing more microbial protein. The present results were consistent with the findings of Afshar Hamidi *et al.* (2014) that the amount of gas produced by the treatment containing AP in 72 h was 177.4 versus 189.4 mL/g. AP with considerable amounts of soluble carbohydrates increased in vitro digestibility of DM. EO additives also reduced in vitro gas production possibly due to inhibition of deamination.

The additive effect of EO and AP on the estimated parameters of GP are given in Table 2. All the tested parameters were stimulated with EO addition ($p < 0.01$). Supplementation of alfalfa silage with AP (50% and 75%) increased the estimated parameters compared with control, and the highest values was observed at 75% AP ($p < 0.01$). The results showed that the interactions between EO and AP were significant. As noted in Table 2, AA3E had the highest estimation parameters while the lowest values were related to AA1 ($p < 0.05$). Similarly, low doses of thymol (50 mg/L) had no effect on in vitro microbial fermentation, but at higher doses (500 mg/L), total volatile fatty acid concentration and ammonia nitrogen decreased and the acetate to propionate ratio was increased (Castillejos *et al.*, 2005).

Table 2. Effects of adding essential oil and apple pulp on gas production parameters

Item	Estimated parameters					
	GP	ME	NEL	OMD	SCFA	MP
Control	17.5^{cd}	4.58^{cd}	2.22^{cd}	32.32^{cd}	0.387^{c}	6.23^{f}
AE	16.92^{de}	4.50^{de}	2.16^{de}	31.80^{de}	0.367^{d}	6.31^{d}
AA1	16.17^{e}	4.40^{e}	2.09^{e}	31.11^{e}	0.345^{e}	6.00^{g}
AA1E	18.25^{c}	4.68^{c}	2.29^{c}	33.00^{c}	0.400^{c}	5.78^{h}
AA2	17.90^{cd}	4.63^{cd}	2.25^{cd}	32.68^{cd}	0.400^{c}	6.30^{e}
AA2E	18.17^{c}	4.67^{c}	2.28^{c}	32.93^{c}	0.393^{c}	6.35^{c}
AA3	19.56^{b}	4.86^{b}	2.41^{b}	34.18^{b}	0.427^{b}	6.59^{b}
AA3E	24.20^{a}	5.49^{a}	2.86^{a}	38.38^{a}	0.538^{a}	7.40^{a}
SEM	0.324	0.044	0.031	0.293	0.00507	0.0017

Means within same column with different superscripts differ; ME – metabolizable energy (MJ/kg DM); SCFA – short chain fatty acid (mmoL/0.2 g DM); OMD – digestible organic matter in dry matter (%); NEL – net energy lactation (MJ/kg DM); MP – microbial protein; SEM – standard error or means; alfalfa silage alone (control), EO processed alfalfa silage (AE), 75% alfalfa + 25% AP silage (AA1), 75% alfalfa + 25% AP EO processed silage (AA1E), 50% alfalfa + 50% AP silage (AA2), 50% alfalfa + 50% AP and EO processed silage (AA2E), 25% alfalfa + 75% AP silage (AA3), 25% alfalfa + 75% AP and EO processed silage (AA3E).

Banchaar *et al.* (2007) did not observe a significant difference in the apparent digestibility of dry matter and organic matter between treatments receiving EO (2, 3 and 4 g/day) in cattle. Ethanol-extract or methanol-extract of fennel and clove reduced gas production in in vitro condition. In general, the addition of oregano EO to corn silage significantly increased gas production (Patra *et al*., 2008). The same material increased GP, ME, volatile fatty acid, OMD and higher digestibility of OM compared with the control. It seems that oregano EO has been able to protect nutrients well during the ensiling process (Patra *et al*., 2008). Busquet *et al.* (2005) reported that the amount of gas production can be affected by the total amount and pattern of volatile fatty acids. However, the chemical composition of corn silage was slightly different, and it is possible that the residual substrate in each treatment used by the microbial population could influence the volatile fatty acid pattern. This is due to the fact that microorganisms use different pathways to produce volatile fatty acids depending on the available substrate. The rate of gas production can indicate the rate of digestion in the rumen followed by the rate of passage and consumption of dry matter.

The average of in vitro disappearance of DM is shown in Table 3. According to the present observations, DM disappearance at 2-h incubation significantly differed from one to another treatment ($p < 0.05$).

Table 3. Effect of apple pulp and essential oil additive on the fermentation characteristics of dry matter

Item	Incubation time (h)				
	2	4	8	12	24
Control	22.10^{c}	23.80^{d}	26.60^{c}	27.80^{f}	38.10^{b}
AE	13.86^{fe}	19.93^{e}	22.96^{d}	27.13^{f}	37.43^{b}
AA1	14.46^{ef}	25.26^{c}	23.13^{d}	28.60^{e}	36.53^{b}
AA1E	13.11^{f}	17.87^{f}	22.13^{d}	31.00^{cd}	36.40^{c}
AA2	14.96^{e}	19.46^{e}	27.86^{bc}	30.33^{d}	53.73^{a}
AA2E	19.76^{d}	25.93^{c}	28.53^{b}	32.20^{c}	40.67^{b}
AA3	25.10^{b}	27.13^{b}	29.33^{b}	36.20^{b}	40.76^{b}
AA3E	27.5^{a}	30.40^{a}	37.76^{a}	38.33^{a}	57.40^{a}
SEM	0.438	0.318	0.520	0.441	1.59

Means within same column with different superscripts differed, SEM – standard error or means, alfalfa silage alone (control), EO processed alfalfa silage (AE), 75% alfalfa + 25% AP silage (AA1), 75% alfalfa + 25% AP EO processed silage (AA1E), 50% alfalfa + 50% AP silage (AA2), 50% alfalfa + 50% AP and EO processed silage (AA2E), 25% alfalfa + 75% AP silage (AA3), 25% alfalfa + 75% AP and EO processed silage (AA3E).

Adding AP increased in vitro degradability ($p < 0.05$). This can be explained by the higher soluble carbohydrate and insoluble fiber content of AP. Losa (2001) examined the impact of substitution AP (20, 40, 60 and 80% to alfalfa silage) on in vitro gas production and reported its stimulatory effect on dry matter degradability and effective degradability compared with control.

Rodrigues *et al.* (2008), testing the effect of AP addition to straw and forage (50, 70 and 85%), reported significant OMD increase in treatments containing higher concentrations of AP. Interestingly, cinnamon leaves, oregano and orange EO had no effect on digestibility of barley silage (Hodjatpanah-Montazeri *et al.*, 2016).

Effect of AP and EO additive on the fermentation characteristics of organic matter are shown in Table 4. According to results, AA3E treatment had the highest OM degradability while AE had the lowest value. The mean degradability parameters (a, b and c) of DM and OM are shown in Tables 5 and 6. The a and b fractions were significantly higher in 75% AP compared with control ($p < 0.05$). EO additives did not have a significant effect on a and b sections but EO + AP containing treatments considerably influenced a and b fractions. The addition of EO and AP (75%) had significantly increased the effective degradability of alfalfa silage ($p < 0.05$).

Table 4. Effect of apple pulp and essential oil additive on the fermentation characteristics of organic matter

Item	Incubation time (h)				
	2	4	8	12	24
Control	26.39^{e}	33.67^{d}	36.91^{b}	39.23^{cd}	43.26^{f}
AE	25.53^{e}	28.63^{f}	32.92^{c}	37.27^{e}	40.29^{g}
AA1	30.61^{c}	35.85^{c}	38.06^{b}	40.00^{c}	45.43^{de}
AA1E	26.72^{e}	28.67^{f}	34.57^{bc}	40.20^{c}	44.47^{ef}
AA2	28.60^{d}	31.02^{e}	35.11^{bc}	39.62^{c}	52.01^{c}
AA2E	35.17^{b}	38.35^{b}	43.14^{a}	42.36^{b}	53.88^{b}
AA3	29.35^{d}	32.53^{d}	35.45^{bc}	37.72^{de}	46.16^{d}
AA3E	42.25^{a}	44.41^{a}	46.62^{a}	47.72^{a}	59.77^{a}
SEM	0.3852	0.4672	1.184	0.5615	0.4517

Means within same column with different superscripts differed, SEM – standard error or means, alfalfa silage alone (control), EO processed alfalfa silage (AE), 75% alfalfa + 25% AP silage (AA1), 75% alfalfa + 25% AP EO processed silage (AA1E), 50% alfalfa + 50% AP silage (AA2), 50% alfalfa + 50% AP and EO processed silage (AA2E), 25% alfalfa + 75% AP silage (AA3), 25% alfalfa + 75% AP and EO processed silage (AA3E).

Table 5. The effect of experimental treatments on *a*, *b* and *c* of dry matter degradability

Parameters	Adding essential oil (mg/kg)			Apple pulp					Pr > F		
	0	500	SEM	0	25	50	75	SEM	Apple pulp	Essential oil	Interactions
a	15.47	15.06	0.276	14.24^{b}	10.12^{c}	14.03^{b}	22.69^{a}	0.405	< 0.0001	0.326	< 0.0001
b	30.07	32.87	1.004	33.15^{a}	33.13^{a}	33.93^{a}	25.36^{b}	1.42	0.001	0.068	0.0005
c	0.068	0.071	0.003	0.068^{a}	0.073^{a}	0.064^{a}	0.073^{a}	0.005	0.647	0.648	< 0.0001
ED	38.19^{b}	39.54^{a}	0.434	38.33^{b}	35.57^{c}	39.25^{b}	42.30^{a}	0.614	< 0.0001	0.044	0.082

Means within same column with different superscripts differed, SEM – standard error or means, a – gas production from the immediately soluble fraction (mL), b – gas production from the insoluble fraction (mL), c – gas production rate constant for the insoluble fraction (mL/h)

Table 6. The effect of experimental treatments on *a*, *b* and *c* of organic matter degradability

Parameters	Adding essential oil (mg / kg)			Apple pulp					Pr > F		
	0	500	SEM	0	25	50	75	SEM	Apple pulp	Essential oil	Interactions
a	26.22	25.47	1.10	20.44^{c}	25.05^{bc}	27.32^{ab}	30.57^{a}	1.56	0.0025	0.639	0.083
b	29.25	23.97	2.04	21.97^{b}	22.89^{b}	23.55^{b}	38.01^{a}	2.88	0.0032	0.85	0.0153
c	0.127	0.088	0.34	0.154	0.090	0.157	0.029	0.048	0.230	0.429	0.233
ED	45.96	44.01	1.01	39.69^{c}	53.69^{bc}	45.46^{b}	50.61^{a}	0.58	0.0006	0.256	0.994

Means within same column with different superscripts differed, SEM – standard error or means, a – gas production from the immediately soluble fraction (mL), b – gas production from the insoluble fraction (mL), c – gas production rate constant for the insoluble fraction (mL/h)

According to the results (Table 6), addition of EO had no significant effect on the degradability parameters (*a*, *b* and *c*) of the organic material ($p > 0.05$). Section *a* was significantly higher in 50 and 75% AP while section *b* was only affected by 25% AP ($p < 0.05$). The degradability of part *c* was not affected by the addition of AP. Effective degradability was increased significantly with the addition of EO and AP (75%) compared to control ($p < 0.05$). The effect of EO and AP on the degradability of CP by in vitro method is given in Table 7. At 2 h of incubation, the highest rate of CP disappearance was achieved in AE and AA1E treatments, and the other treatments showed the lowest rate of disappearance. At 2 h of incubation, AE showed the highest, and AA2 showed the lowest degradability.

Table 7. Effect of apple pulp and essential oil additive on the fermentation characteristics of crude protein

Item	Incubation time (h)				
	2	4	8	12	24
Control	29.11^{a}	29.59^{d}	39.14^{c}	40.41^{cd}	41.82^{b}
AE	31.68^{a}	38.24^{a}	39.76^{b}	46.21^{a}	46.91^{a}
AA1	19.46^{b}	33.74^{b}	39.70^{bc}	41.02^{c}	46.15^{a}
AA1E	29.63^{a}	33.79^{b}	40.67^{a}	43.23^{b}	38.96^{c}
AA2	19.86^{b}	28.64^{d}	36.15^{d}	39.68^{d}	41.93^{b}
AA2E	27.95^{b}	31.17^{c}	33.21^{e}	36.78^{e}	38.87^{bc}
AA3	21.77^{b}	31.66^{c}	32.52^{f}	36.33^{e}	37.03^{c}
AA3E	21.89^{b}	29.34^{d}	31.98^{g}	32.10^{f}	37.92^{c}
SEM	2.43	0.879	0.350	0.735	1.84

Control – alfalfa silage without additives, SEM – standard error or means, alfalfa silage alone (control), EO processed alfalfa silage (AE), 75% alfalfa + 25% AP silage (AA1), 75% alfalfa + 25% AP EO processed silage (AA1E), 50% alfalfa + 50% AP silage (AA2), 50% alfalfa + 50% AP and EO processed silage (AA2E), 25% alfalfa + 75% AP silage (AA3), 25% alfalfa + 75% AP and EO processed silage (AA3E)

The mean pH of the cultivation environment during incubation times is given in Table 8. The results demonstrated that the experimental treatments did not affect the pH in 2 h after incubation. At the end of 8-h incubation, AA3E showed the lowest pH among treatments. From 4 h to the end of incubation, a decrease in pH was observed, which can be justified by the increase in feed degradability by microorganisms and increasing digestive products in the environment. McIntosh *et al.* (2003) reported that the addition of EOs prevented the growth of hyper ammonia producing bacteria (HAP) (e.g., *Clostridium stecyland* and *Peptostreptococcus N-aerobiosis*), yet other bacteria of the same group were less sensitive (e.g., *Clostridium amylofilus*).

Castellijos *et al.* (2006) investigated the effect of eugenol, guaiacol, limonene, thymol and vanillin on in vitro ruminal microbial fermentation and feed utilization. Effects of these compounds on ruminal microbial activity were concentration dependant. Monensin and thymol reduced the digestion of dry matter and organic matter (Castellijos *et al.*, 2006). Many years ago, Roy and Wright (1974) reported the positive effects of higher concentrations of peppermint EO on the ratio of extinct dry matter to gas production in extracorporeal conditions. In summary, plant extracted EO differ in chemical structure, source, and bioactivity, thus having different effects on rumen fermentation and animal function.

Table 8. Effect of experimental treatments on pH of fermentation characteristics

Item	Incubation time (h)				
	2	4	8	12	24
Control	7.3	6.96^{a}	6.90^{a}	6.6^{a}	6.5^{a}
AE	7.3	6.95^{ab}	6.90^{a}	6.6^{a}	6.5^{a}
AA1	7.3	6.93^{ab}	6.78^{ab}	6.5^{b}	6.2^{b}
AA1E	7.3	6.86^{d}	6.66^{ab}	6.4^{c}	6.2^{b}
AA2	7.3	6.88^{d}	6.65^{ab}	6.4^{cd}	6.03^{c}
AA2E	7.3	6.83^{cd}	6.53^{c}	6.4^{cd}	6.06^{c}
AA3	7.3	6.8^{cd}	6.56^{c}	6.36^{d}	6.1^{c}
AA3E	7.3	6.9^{abc}	6.5^{c}	6.4^{cd}	6.1^{c}
SEM	0.026	0.026	0.026	0.026	0.020

Control – alfalfa silage without additives, SEM – standard error or means, alfalfa silage alone (control), EO processed alfalfa silage (AE), 75% alfalfa + 25% AP silage (AA1), 75% alfalfa + 25% AP EO processed silage (AA1E), 50% alfalfa + 50% AP silage (AA2), 50% alfalfa + 50% AP and EO processed silage (AA2E), 25% alfalfa + 75% AP silage (AA3), 25% alfalfa + 75% AP and EO processed silage (AA3E)

4. Conclusion

The addition of EO increased all predictive parameters of gas production. Alfalfa silage supplemented with AP (50 and 75% levels) increased the gas production parameters compared with control, and the highest values were observed for 75% AP. AP at a level of 75% significantly increased *a* and *b* sections of dry matter degradability. The effective degradability of the dry matter was significantly increased by addition of EO and 75% AP compared than the control. In general, it can be concluded that EOs have the ability to alter fermentation processes.

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6. References

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