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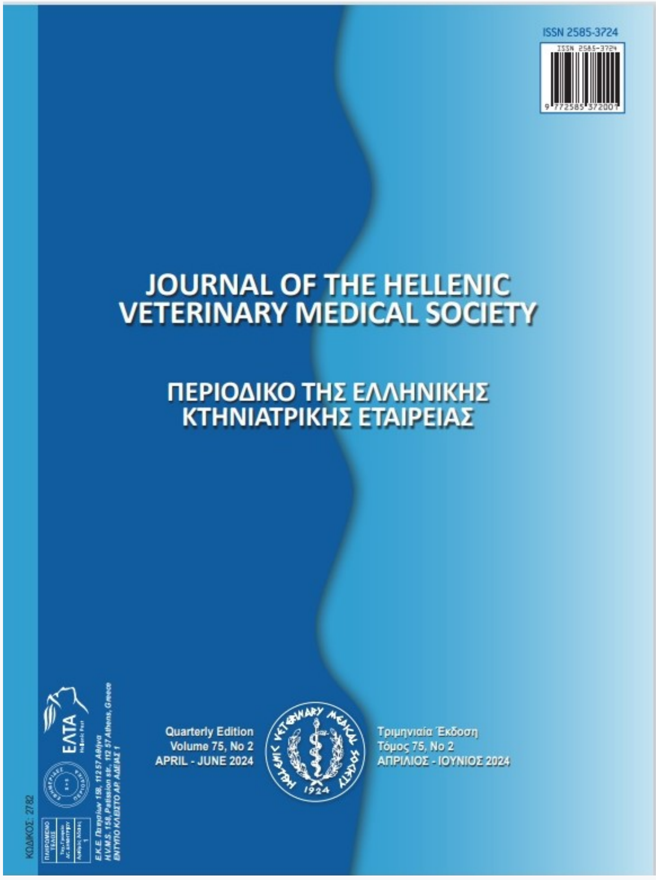

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Research article
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Determination of the effect of *Hypericum perforatum* oil supplementation on silage quality in ruminant feeding

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ABSTRACT: This study evaluated the effect of *Hypericum perforatum* oil (HPO) on chemical composition, fermentation traits and metabolic energy, *in vitro* organic matter digestibility, and net energy lactation of Hungarian vetch-wheat silages. The treatments were: (i) control (No HPO), (ii) 20 mg/kg HPO, (iii) 40 mg/kg HPO, (iv) 80 mg/kg HPO and (v) 160 mg/kg HPO in Hungarian vetch-wheat silages. After 75 days of ensiling, dry matter, neutral detergent fiber, and acid detergent fiber were increased by adding 20 mg/kg and 40 mg/kg *Hypericum perforatum* oil to Hungarian vetch-wheat silage ($p<0.01$). However, there was no significant in the content of silage in lactic acid, acetic acid, propionic acid, metabolic energy, *in vitro* organic matter digestibility, and net energy lactation ($p>0.05$). Butyric acid and ammonia nitrogen were not found in the experiment silages. Finally, it was concluded that the addition of 20 mg/kg *Hypericum perforatum* oil may have a positive effect on the quality of Hungarian vetch-wheat silages.

***Keywords*:** Hungarian vetch; *Hypericum perforatum* oil; *in vitro* digestibility; quality; silage

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INTRODUCTION

Hypericum perforatum L. which grows in Europe, Asia, North Africa, New Zealand, and America, is located in Clusiacea a family, that grows naturally in moist areas up to 2.500 meters above sea level in Turkiye (Davis, 1972; Hegi, 1975). A comprehensive account of the chemical constituents including flavonoids, tannins, and volatile oils is also included (Saddiqe et al., 2010). Antiseptic, anthelmintic, antibacterial, wound healing, anti-inflammatory, antidepressant, antifungal, and viral origin is used to prevent symptoms (Mascalo et al., 1987; Mervelo et al., 1988; Rao et al., 1991; Richer and Davies, 1995; Çakır et al., 1997; Danilov et al., 1999; Butterweck, et al., 2000; Kumar et al., 2000; Kumar et al., 2001; Cervo et al., 2002; Meral and Karabay, 2002; Rancic et al., 2005; Milosevic et al., 2007). In addition to these properties, it has the potential to be used as a therapeutic and apoptogenic herbal remedy (Saddiqe et *al*., 2010).

Essential oil treatment in silage reduced the pH of the silage in a very short time. This reduces the amount of fermentation products. In addition, it prevents the growth of harmful microorganisms in grass silages that are difficult to ensilage. Since the protein content is high and the carbohydrate content is low in vetch silage, additives should be used for quality silage and good fermentation. *Hypericum perforatum L* oil has antifungal and antibacterial effects. It is thought that by making use of this feature of the oil, it can provide good fermentation in Hungarian vetch-wheat silages and can be stored for a long time without spoiling.

It is important to determine the characteristics which are thought to have an important role in ruminant feeding and to be able to be evaluated as a contribution to silages. This study, it was aimed to determine the effects of *Hypericum perforatum* L. oil added to the Hungarian vetch-wheat on chemical composition, fermentation characteristics, and *in vitro* gas production parameters of silages.

MATERIAL AND METHODS

Silages and Ensiling Period

This study was carried out in the laboratories of the Department of Feeds and Animal Nutrition, Uşak University. *Hypericum perforatum* oil (HPO) is obtained from a cold-pressing commercial enterprise. Hungarian vetch-wheat (*Vicia pannonica Cronte*) was supplied from growers operating in the region (95% Hungarian vetch + 5% wheat) and was mown at the pod-filling stage. The chemical composition of fresh Hungarian vetch-wheat is summarized in Table 1.

Hungarian vetch-wheat was chopped into lengths of 1.5-2 cm. The treatments were: (i) control (No HPO), (ii) 20 mg/kg HPO, (iii) 40 mg/kg HPO, (iv) 80 mg/kg HPO and (v) 160 mg/kg HPO in Hungarian vetch-wheat silages. The experiment silages were ensiled 1-liter plastic anaerobic jars and kept at room temperature for 75 days.

Chemical Analyses

The pH values of the samples were determined by the digital pH meter (Polan *et al*., 1998). At the same time, 40 g of experiment silages were taken and shaken for at least 5 minutes by adding 360 ml of distilled water and filtered through Whatman No. 1 paper. NH3-N was determined by Kjeldahl distillation method by taking 100 ml of filtrate (Broderick and Kang, 1980). However, 10 ml of the same filtrate was taken, centrifuged at 14.000 rpm for 30 minutes and the volatile fatty acids (propionic acid, acetic acid, and butyric) and lactic acid contents were analyzed by HPLC (Leventini et al, 1990). Dry matter, crude ash, ether extract, and crude protein were assessed by the procedures of AOAC (1999). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the method of Van Soest (1982).

In vitro gas production Analyses: *In vitro* organic matter digestibility (OMD) and Metabolisable energy (ME) were determined by the *in vitro* gas production technique reported by Menke and Steingass (1988). Net energy lactation (NEL) was calculated by the equations reported by Blümmel and Ørskov (1993).

$$\text{ME (MJ/kg DM)} = 2,20 + 0,136\ \text{GP} + 0,0057\ \text{CP} + 0,00029\ \text{EE}^2$$

$$\text{NEL (MJ/kg DM)} = 0,1149\ \text{GP} + 0,0054\ \text{CP} + 0,0139\ \text{EE} - 0,0054\ \text{CA} - 0,36$$

Table 1. Chemical composition of fresh Hungarian vetch-wheat % DM

	DM*	CP**	NDF**	ADF**	CA**
Hungarian vetch-wheat	36.92	13.72	38.40	25.14	11.20

DM: Dry matter; CA: Crude ash; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; CP: Crude protein.
* %; ** % DM.

OMD (%) = 14,88 + 0,8893 GP + 0.0448 CP + 0.0651 CA

GP: Amount of gas production after 24 hours of fermentation (ml)

CP: Crude protein contents (g/kg DM)

EE: Ether extract contents of silages (g/kg DM)

CA: Crude ash contents of silages (g/kg DM)

Statistical Analyses: The data were analyzed with SPSS version 16.0 (SPSS, Inc. Chicago. Illinios, USA). The significance of treatment means was determined by one-way analysis of variance and mean separation was done with Duncan's Multiple Range Procedure (SPSS, 2007).

RESULTS

The dry matter, crude ash, NDF, ADF, crude protein, and ether extract of Hungarian vetch-wheat silages at the end of the experiment were summarized in Table 2.

After ensiling, dry matter contents of Hungarian vetch-wheat silages increased ($p<0.01$) 20 and 40 mg/kg with HPO supplement, but no differences were observed at 80 mg/kg and 160 mg/kg compared to the control group ($p>0.05$). NDF and ADF contents remarkably increased with HPO at 80 mg/kg and 160 mg/kg levels ($p<0.01$). However, HPO had no effect on crude ash, ether extract, and crude protein contents ($p>0.05$).

The effects of HPO on fermentation traits of Hungarian vetch-wheat silages were given in Table 3.

Accordingly, pH values of the silages with *Hypericum perforatum* oil treatment significantly decreased ($p<0.01$), but no significant change was detected in terms of lactic acid, acetic acid, and propionic acid contents ($p>0.05$). In addition, butyric acid and ammonia nitrogen contents could not be detected in the experiment silages.

The effects of HPO on metabolic energy, *in vitro* organic matter digestibility (IVOMD), and net energy lactation contents of Hungarian vetch-wheat silages were given in Table 4.

Table 2. Chemical composition of Hungarian vetch-wheat silages, % DM

HPO treatments, mg/kg	DM	CA	NDF	ADF	CP	EE
Control	38.46±0.80^{c}	12.28±0.54	35.68±1.31^{b}	24.20±0.77^{b}	13.74±0.43	1.42±0.06
20	40.36±0.22^{a}	12.44±0.27	36.25±0.79^{b}	24.28±1.20^{b}	13.53±0.75	1.32±0.26
40	39.56±0.68^{b}	12.07±0.31	37.23±1.37^{b}	24.74±1.01^{b}	12.79±0.38	1.52±0.49
80	38.12±0.38^{c}	12.28±0.44	39.60±1.52^{a}	26.83±1.04^{a}	13.13±0.70	1.61±0. 55
160	37.80±0.44^{c}	11.73±0.57	39.00±1.19^{a}	26.30±0.47^{a}	13.07±0.78	1.67±0. 44
$\bar{X}$ ±SD	38.68±1.02	12.18±0.47	37.54±1.99	25.15±1.40	13.27±0.67	1.50±0.36

$^{a-c}$: The different letters on the same coloumn stand for statistical differences ($P<0.01$).
HPO: *Hypericum perforatum* oil; DM: Dry matter; CA: Crude ash; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; CP: Crude protein; EE: Ether extract.

Table 3. Fermentation characteristics of Hungarian vetch-wheat silages

HPO treatments, mg/kg	pH	LA, %	AA, %	PA, %	BA, %	NH3-N, g/kg DM
Control	4.71±0.12^{a}	0.81±0.45	0.27±0.10	0.32±0.02	ND	ND
20	4.62±0.04^{b}	0.85±0.75	0.29±0.06	0.25±0.07	ND	ND
40	4.59±0.04^{b}	0.85±0.54	0.34±0.03	0.23±0.05	ND	ND
80	4.57±0.08^{b}	0.89±0.41	0.36±0.05	0.26±0.03	ND	ND
160	4.57±0.04^{b}	0.86±0.40	0.33±0.03	0.22±0.03	ND	ND
$\bar{X}$ ±SD	4.62±0.09	0.85±0.53	0.32±0.06	0.25±0.05		

$^{a-b}$: The different letters on the same coloumn stand for statistical differences ($P<0.01$).
HPO: *Hypericum perforatum* oil, LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid; NH3-N: Ammonia nitrogen; ND: Not determined.

Table 4. Net energy lactation, *in vitro* organic matter digestibility, and metabolic energy of Hungarian vetch-wheat silages

HPO treatments, mg/kg	ME, MJ/kg DM	NEL, MJ/kg DM	IVOMD, %
Control	9.03±0.63	5.55±0.44	70.77±4.59
20	9.97±1.02	5.86±0.09	73.78±0.97
40	8.66±0.61	5.29±0.40	69.12±1.69
80	9.08±1.09	5.60±0.83	70. 25±7.12
160	9.25±0.39	6.03±0.13	72.24±4.85
$\bar{X}$ ±SD	9.28±0.89	5.66±0.51	71.24±4.45

HPO: *Hypericum perforatum* oil; ME: Metabolic Energy, NEL: Net Energy Lactation. IVOMD: *in vitro* organic matter digestibility.

Supplement of HPO in the Hungarian vetch-wheat silages had no significant effect on metabolic energy, IVOMD, and net energy lactation ($p>0.05$). Compared to the control group, HPO treatment increased metabolic energy, net energy lactation, and IVOMD values numerically.

DISCUSSION

An additional and crucial method for long-term fodder preservation is ensiling (Soundharrajan et al., 2021). Antibacterial additives are utilized as fermenters in the ensiling period of hard ensiled forages (Haigh et al., 1987; Martinsson, 1991). Preserving the forages digestible fiber, protein content, and energy so that ruminants can use them effectively are generally the main goals. Çakir et al. (1997) stated that HPO produced in Turkey contains alpha-pinene (% 61.7), which is a strong antibacterial. Dry matter (DM) contents of fresh Hungarian vetch-wheat were found 36.92 (Table 1), which increased after the ensiling period. In the present work, DM contents remained stable or even significantly increased with the HPO supplement by the ensiling processes. Compared to the control group, at the end of the ensiling period, significant differences were observed between the group of silages having 20 mg/kg and 40 mg/kg HPO while no significant differences were found among other treatments. Although DM contents of good quality silage are expected to be 25-35 % and its pH values are expected to be 3.8-4.2 (Bolsen et al., 1996). It is reported that a pH value between 4.5-4.6 in especially legumes whose DM content is between 40-45 % is very satisfying (Weissbach, 1996). Also, pH values were reduced with HPO supplement in Hungarian vetch-wheat silages. pH values of experiment silages significantly decreased with HPO with an antibacterial property as compared to the control group. According to Keles and Demirci (2011), the treatment of Hungarian vetch-wheat silages had no effect on DM and Kung *et al*. (2008) indicated that it did not change with essential oil supplements. Similarly, Pursianien and Tuori (2008) identified that pH value decreased with formic acid and Weinberg et al. (1988) indicated that pH value decreased with the amount of added lactic acid bacteria to vetch silages.

If convenient fermentation condition is not provided in silages, undesired microorganisms such as *Saccarolytic clostridia* and *Proteolytic clostridia* can use water-soluble carbohydrates and essential oil acids as substrates. As a result of this, carbon dioxide and H2O come out. Besides this phenomenon, proteolysis is also observed in silages. Therefore, protease enzymes in plants first digest the proteins in the plant into amino acids and ammonia and then into peptides and amides. Finally, the crude ash content in the silage increases (Basmacıoğlu and Ergül, 2002). It was identified that there was no significant change in crude ash content of the experiment silages with HPO supplement and there was no negative effect during the fermentation. Soycan Önenç et al. (2017) reported that thyme essential oil had no effect on the crude ash concentrations of the silages, but they decreased with cinnamon, cinnamon + thyme essential oils. Aksu Elmalı and Duru (2012) stated that crude ash content decreased with a malic acid additive to vetch silages.

NDF and ADF, the components of cell walls, are required to be in fewer amounts in ration since their digestibility rate is low. NDF and ADF increased with 80 mg/kg and 160 mg/kg of *Hypericum perforatum* oil additive to experiment silages. Although there was no NDF and ADF content of HPO, the fact that it reduced the digestibility of cell wall components in silages as a result of decelerating the activity of lactic acids could be effective. Soycan-Önenç et al. (2015) stated that thyme, cinnamon, and thyme + cinnamon essential oil additives to the silages, and Chaves et al. (2012) indicated that ethanol extract of the high amount of cinnamon and NDF and ADF of thyme and orange essential oils had an increasing effect. Besharati et al. (2023) reported that 120 ml/kg flaxseed oil supplementation reduced NDF concentration in alfalfa silages.

In the first periods when the fresh material is ensiling, plant respiration goes on. Then, the protease enzyme in the plant first breaks down the proteins into amino acids and ammonia (Filya, 2001). In order to obtain good silage, first of all, lactic acid bacteria have to produce lactic acid by using water-soluble carbohydrates and then pH has to decrease (Bolsen *et al*., 1996). In the current work, pH values of Hungarian vetch-wheat silages (Table 3) had effectively reduced with HPO additive, additionally provided the amount of lactic acid and water-soluble carbohydrates proliferation which is sufficient in silages and prevented the ammonia nitrogen generation in all of the silages by protecting the proteins and preventing the proteolysis to go on longer. Using cinnamon, thyme, and cumin essential oil at various concentrations had no effect on the crude protein contents of the silages, according to Hodjatpanah-Montazeri et al. (2016) however; it increased with oregano and mint essential oils.

Ammonia concentration in silages is a crucial indicator of the degree of protein fragmentation caused by butyric acid bacteria during the ensiling process. Ammonia nitrogen levels in qualified silage reportedly need to be less than 80g/kg total N (Petterson, 1988). According to McDonald et al. (1991), the deamination of amino acids took place due to the high amounts of acetic acid in silages, which led to an increase in ammonia levels. The decrease in the pH values of silages mainly affects the production of organic acids. At the end of the ensiling period, acetic acid and butyric acid contents may have managed to keep the proteins by suppressing the activities of the microorganisms and plant proteases fermenting the amino acids and preventing the generation of ammonia nitrogen concentration in the experiment silages. Turan and Önenç (2018) stated that the ammonia nitrogen concentration of the silages decreased with the essential oil additives; however, Kung et al. (2008) indicated that the silages have not changed.

Lactic acid bacteria that consume water-soluble carbohydrates produce lactic acid, a fermentation product that should be in silages and, therefore, suppress acetic acid proliferation. As a result, protein transformation to ammonia is prevented. The fact that ammonia nitrogen contents were not observed in the experiment silages stated that acetic acid contents were sufficiently not produced to prevent the silages from deteriorating throughout the ensiling period. Foskolos et al. (2018) reported that lactic acid did not change with cinnamaldehyde and eugenol, but it decreased with thymol and carvacrol. In addition, with inoculant treatment to vetch silages Kung et al. (1990) and with lactic acid bacteria and propionic acid additives, Zhang et al. (2015) found increased lactic acid content in silages.

The main opponent of acetic acid bacteria during the ensiling process is butyric acid bacteria. Because a significant amount of nutrient loss occurs with the production of butyric acid in silages. These bacteria either reduce or completely consume the nutrients they need by using the carbohydrates used by acetic acid bacteria. For that reason, butyric acid is undesirable in silages (Alçiçek and Özkan, 1997). In order to prevent butyric acid proliferation, there must be sufficient lactic acid and the pH values must decrease (Kılıç, 1986). In the present study, it can be said that butyric acid proliferation was not observed due to obtaining sufficient lactic acid contents and pH values. The butyric acid level of Hungarian vetch-wheat silages was 0.3%, according to Demirci et al. (2011).

The harvesting period of the plants used in ensiling affects *in vitro* organic matter digestibility contents of the silages. Because the dry matter content of the fresh material increases at an unsuitable level for ensiling if the harvesting period of the plant is delayed, therefore, water-soluble carbohydrates contents and organic matter digestibility of silages decrease. When Hungarian vetch-wheat is ensiled after both early and late harvesting, in both cases its fermentation efficiency decreases. According to the data obtained, it indicated that Hungarian vetch-wheat was harvested in the most convenient period for ensiling. Arican and Arslan Duru (2020) stated that metabolic energy, *in vitro* organic matter digestibility, and net energy lactation contents did not change significantly with the supplement of black seed oil to Hungarian vetch-wheat silages. However, Turan and Önenç (2018) reported that enzyme-soluble organic matter and metabolic energy levels of the silages increased with the essential oil additives. The difference between the researchers may be due to the different essential oils and forages used in ensiling processes.

CONCLUSION

In the present study, we can say that good fermentation quality was obtained in experiment silages in general. The sufficient proliferation of lactic acid, no butyric acid, were and ammonia nitrogen which emerges as a result of poor fermentation quality in silages supports this phenomenon. It is understood that it has a positive effect on some important parameters such as a decrease in pH, the amount of lactic acid and *in vitro* organic matter digestibility, metabolic and net energy lactation, and crude ash with HPO. Therefore, it can be said that the use of *Hypericum perforatum* oil in Hungarian vetch-wheat silages especially at 20 mg/kg level will improve the fermentation quality more and can be kept intact for a long time.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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