

Nutrient matter, fatty acids, *in vitro* gas production and digestion of herbage and silage quality of yellow sweet clover (*Melilotus officinalis* L.) at different phenological stages

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ABSTRACT. Yellow sweet clover (*Melilotus officinalis* L.) is a legume species, widely used as a forage crop for herbivores. This study aimed to research the nutritional compositions of herbage and silage *M. officinalis* at vegetative, early flowering and full flowering stages. The crude protein (CP) and ash contents of *M. officinalis* herbages and silages in vegetative and early flowering stages were higher than those in the full flowering stage ($P < 0.05$). The linoleic and alpha-linolenic acid percentages in total fatty acids of *M. officinalis* herbages in vegetative and early flowering stages were greater than those in full flowering stage ($P < 0.05$). The neutral and acid detergent fibres without ash contents of *M. officinalis* herbages and silages at the full flowering stage were higher than those of vegetative and early flowering ones ($P < 0.05$). The malondialdehyde (MDA) concentrations (1.12–1.67 g/kg DM) of silages were higher than those (0.38–0.48 g/kg DM) of herbages. The *in vitro* gas production, metabolizable energy, net energy for lactation and organic matter digestibility values and the molarities of volatile acids in fermentation fluid of *M. officinalis* herbages were similar for all phenological stages ($P > 0.05$). The lactic acid contents of silages at vegetative and early flowering stages were higher than that of the full flowering one ($P < 0.05$). The linoleic acid contents of forages were negatively correlated with MDA forage content ($P < 0.05$). Consequently, the values of CP, alpha-linolenic acid and *in vitro* digestion for herbage, and the nutrient matter compositions of silage, in especially vegetative and early flowering stages, were at non-objectionable levels for quality forage. So, it is suggested that *M. officinalis* can be used as a good quality silage.

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Introduction

Yellow sweet clover (*Melilotus officinalis* L.) is a legume species that can be widely used as a forage crop worldwide (Ehlert, 2020). Yellow sweet clover is one of the most common species in the *Melilotus* genus. These species have adapted to environmental constraints such as drought, cold and salinity (Turkington et al., 1978; Al Sherif, 2009).

Yellow sweet clover is used as a ground cover in depleted soils (Allen and Allen, 1981), especially in moderately saline areas where traditional forage legumes cannot be grown (Luo et al., 2014).

Yellow sweet clover develops a root system during the first season, and then produces flowers and seeds during the second season. Second-year yellow sweet clover may appear bushy and reach a height of 1.2–2 m (Klebesadel, 1992; Luo et al., 2014; Cornara et al., 2016). Yellow sweet clover

is a legume, similar to alfalfa and other clovers; it is rich in crude protein (CP) (about 16–25% in dry matter (DM)) (Canbolat and Karaman, 2009; Çaçan et al., 2015). Since this forage plant is digested well, it has a satisfactory level of metabolizable energy (ME) (about 10 MJ/kg DM for ruminant) as a forage (Canbolat and Karaman, 2009; Ehlert, 2020). It is a good forage source for mule, deer, elk, antelope, and a suitable nesting habitat for pheasants, grouse and other upland birds. Also, sweet clover is extremely attractive to pollinators such as honey bees (Ehlert, 2020). The plant is also mainly known for its anti-inflammatory, anti-oedematous, spasmolytic, diuretic and sedative properties (Cornara et al., 2016).

The primary concern with sweet clover is the possibility of sweet clover poisoning, which occurs when hay is inadequately allowed to dry or becomes mouldy. Sweet clover contains a substance called coumarin (0.14–1.14% in DM) (Luo et al., 2014), which is converted to dicoumarol by fungus in mouldy hay. Dicoumarol is an anti-clotting agent that causes severe haemorrhage in cattle. The conversion of coumarin to dicoumarol can occur even if there is only a little mould in the herbage, so mould level is not a good indicator of the dicoumarol level (Ogle et al., 2008; Ehlert, 2020). The best way to prevent sweet clover poisoning is strict control of silage making or hay curing (Ogle et al., 2008; Ehlert, 2020). *Melilotus* species, including sweet yellow clover, have not been widely used in forage production due to their high coumarin content. Studies have been carried out to reduce the plant's coumarin level and use it as common roughage (Luo et al., 2014). All *Melilotus* species, when flowering, have a peculiar sweet odour, which, through drying, becomes more aromatic and more agreeable. The characteristic sweet odour is derived from its coumarins (Jasicka-Misiak et al., 2017). The contamination of all forages and silages with mould fungi often occurs in the field and can also occur during harvesting, transport or storage. However, when they can find a suitable environment (such as moisture and heat in the material), these fungi release their metabolites to the environment. Moreover, poor postharvest management can lead to rapid spoilage. When the DM value drops off below 14% during the drying phase of forages, it will not be possible for the fungus to multiply and release its metabolites to the environment. Such roughages, prepared under appropriate conditions, will not cause ruminants' health problems (Lacey, 1991; Alonso et al., 2013).

Grazing sweet clover is not problematic because the conversion of coumarin to dicoumarol is impossible without the mould fungus in herbage. The conversion of coumarin to dicoumarol can be prevented by silage making rather than hay as long as the ensiling fermentation process is rapid and complete so that the silage is stable and does not allow mould growth (Ehlert, 2020). We hypothesize that yellow sweet clover can be silaged like other legumes, alfalfa silage, and good quality silage can be obtained (Améndola-Massiotti et al., 2018). Also, that the silage quality and the biochemical/nutritional compositions of yellow sweet clover may vary at different phenological stages (Ersahince and Kara, 2017; Kara et al., 2018). Little is known about the change of fatty acids and lipid oxidation in silage (Han and Zhou, 2013). Han and Zhou (2013) proved that saturated fatty acids (SFA; C16:0 and C18:0) percentages and malondialdehyde (MDA) concentration of maize silage tended to increase with the ensiling process, whereas unsaturated fatty acids (C18:1, C18:2 and C18:3) tended to decrease. At the same time, the MDA concentration in yellow sweet clover herbage content may increase during ensilaging due to the cell structure deteriorated by crushing at ensilage and during silage fermentation. Another hypothesis is that with increasing MDA in silage, there may be a possible change in the silage's fatty acid profile. This study aimed to explore the nutritional compositions of the herbage and silage of yellow sweet clover harvested at vegetative, early flowering and full flowering stages.

Material and methods

This study was approved (number: 21/114) by the Local Ethics Committee for Animal Experiments of Erciyes University, Kayseri, Turkey.

The area of yellow sweet clover samples collection

The yellow sweet clover samples were collected from Kayseri province, located (38°56' N, 34°24' E) in the centre (Cappadocia district) of Turkey.

Plant samples

A random sampling method was used from native grassland. The three phenological stages of yellow sweet clover were investigated. Plant samples were gathered in the vegetative (n = 8), early flowering (n = 8) and full flowering (n = 8) stages. The plant samples were gathered from eight different plants for each phenological stage.

The samples included all aerial parts (leaf, stem or bud-flower) of the plant. Eight samples of yellow sweet clover were randomly collected from four different areas in Kayseri. The sampling amount was about 2 kg for each replicate and about 16 kg for each phenological stage. Cutting was manually performed at 1 cm above the soil. Subsequently, about a 500-g representative sample for each phenological stage was dried in a thermostatically controlled cabinet (Lovibond®, Dortmund, Germany) for 48 h at 60 °C, and the DM content of herbage was calculated.

Silage of yellow sweet clover

For each phenological stage, fresh yellow sweet clover samples were manually (with a knife) cut into 2–3 cm pieces. The herbage (about 500 g) of yellow sweet clover was ensilaged in a polyethylene (25 × 35 cm sizes) silage bag (Caso-01201, Caso Design, Arnsberg, Germany) and mixed (n = 4). These polyethylene size bags were vacuumed using a vacuum machine (Bar Vacuum Sealer, Caso-VC100, Caso Design, Arnsberg, Germany). The silage bags were stored in laboratory conditions in a sun-free environment for 60 days. The silage materials in opened silage bags were dried in a thermostatically controlled cabinet (Lovibond®, Dortmund, Germany) for 48 h at 60 °C, and DM content was calculated.

Determination of the chemical composition of herbage and silages

Dried yellow sweet clover samples were milled in a grinder mill (IKA Werke, Staufen im Breisgau, Germany) to a maximum particle size of 1 mm. Dry matter, ash, CP (nitrogen × 6.25) (DK6 Kjeldahl Digestion Unit, Velp Scientifica, Usmate, Italy) and diethyl ether extract (EE) levels (SER 148/3, Velp Scientifica, Usmate, Italy) were determined according to the method reported by the AOAC International (1995). Neutral detergent fibre without ash (NDFom) and acid detergent fibre without ash (ADFom) contents were analysed using a fibre analyser (FIWE3 Fibre Analyzer, Velp Scientifica, Usmate, Italy) according to the methods reported by Van Soest et al. (1991). The NDFom analyses were carried out without amylase. The non-fibrous carbohydrate (NFC) values were calculated according to National Research Centre (NRC, 2001). All analyses were carried out in triplicate.

Determination of malondialdehyde concentration in herbage and silages

In the analysis, 73.2 mg of 1,1,3,3-tetraethoxypropane (TEP) in a solid form were taken into a tube and dissolved with 10 ml of HCl (0.1N).

Test tubes were incubated at 105 °C for 5 min and then cooled. This MDA/TEP stock solution (230 µg/ml) was completed to 100 ml volume of distilled water to obtain the stock standard solution (MDA for 23 mg/100 ml). The MDA/TEP standard (0.024–239 µg/ml MDA/TEP) curve ($f(x) = 0.00972x + 0.1674$, $R^2 = 0.9929$) was generated using TEP standard. The MDA concentration was given as mg/kg DM of forage.

Thiobarbituric acid-reactive substances (TBARS) of samples were determined by the spectrophotometric method reported by Botsoglou et al. (1994). About 2-g dried forage sample was mixed in a tube with 8 ml of trichloroacetic acid (TCA; 5% in distilled water) and 5 ml of butylated hydroxytoluene (BHT; 0.8% in hexane). The tubes were vortexed (at 2000 rpm for 30 sec.; Velp Scientifica™ Classic Vortex Mixer, Velp Scientifica, Usmate, Italy) and then centrifuged (at 5000 rpm for 5 min; NF 800R, Nüve, Ankara, Turkey). After centrifugation, the upper hexane layer was removed, and the second layer, the aqueous layer, was pipetted into capped tubes containing 10 ml of 5% TCA. Later, this 2.5-ml mixture was taken into capped tubes containing 1.5 ml of 0.8% BHA and incubated for 30 min at 70 °C. The MDA concentrations of the samples were determined at a wavelength of 535 nm in a spectrophotometer (UviLine 8100, Xylem Analytics Germany Sales GmbH & Co. KG, Mainz, Germany).

Determination of fatty acid composition

The EE residues for herbage and silages of yellow sweet clover were methylated with the three-stage modified procedure of Wang et al. (2015), which used KOH (10M), methanol, H₂SO₄ (10M). The methylated fatty acids in n-hexane were taken in a vial with PTE caps and analysed in a gas chromatograph (GC, Thermo1300, Thermo Scientific, Waltham, MA, USA) with flame-ionization detection (FID), connected with automatic sampling (Thermo AI 1310, Thermo Scientific, Waltham, MA, USA). The device was equipped with a fatty acid methyl esters (FAME) column (length: 60 m, ID: 0.25 mm, film thickness: 0.25 µm and maximum temperature: 250–260 °C) and the used parameters were: injection split temperature 255 °C, column temperature 140 °C, and flow rate 30 ml/min processing method for 42 min. FAME mix (37C) standard solution (CL.40.13093.0001, Chem-Lab, Zedelgem, Belgium) in dichloromethane was used for the identification of peak. Fatty acid identification was performed by comparing the chromatogram peaks with the standard's retention times (Kara, 2020). Polyunsaturated fatty acid (PUFA), monounsaturated

rated fatty acid (MUFA), medium-chain fatty acids (MCFA) (fatty acids with chains containing from 6 to 12 atoms of C), long-chain fatty acids (LCFA) (fatty acids with chains containing from 14 to 20 atoms of C) and very-long-chain fatty acids (VLCFA) (fatty acids with chains containing above 20 atoms of C) were detected.

Determination of ammonia concentration in silages

The ammonia concentration in silage fluid was determined using a commercial ammonia assay procedure (Megazyme, K-AMIAIR 02/20, Wicklow, Ireland). The 0.10 ml of silage fluid was mixed with 2.0 ml of distilled water, 0.3 ml of buffer plus 2-oxoglutarate and sodium aside (0.02% w/v) and 0.2 ml of NADPH. The absorbance value of the solutions was read at 340 nm on a UviLine 9100 spectrophotometer (Xylem Analytics Germany Sales GmbH & Co. KG, Mainz, Germany). Glutamate dehydrogenase suspension was added to the solution. The absorbance value of the solution was read at 340 nm on a spectrophotometer (Xylem Analytics Germany Sales GmbH & Co. KG, Mainz, Germany) again. The ammonia concentration (g/l) was calculated using the formula in the ammonia assay procedure of Megazyme (K-AMIAIR 02/20, Wicklow, Ireland).

Determination of acidity values in silages

The pH values of opened silages were immediately determined. First, 25 g of wet yellow sweet clover silage sample was shredded for 15 sec with a laboratory-type blender (Waring Commercial, Torrington, CT, USA) and then shredded for 10 sec with 100 ml of distilled water. The filtered mixture's pH value was measured with a digital pH meter (S220 pH/ion meter, Mettler Toledo, Greifensee, Switzerland) (Bernardes et al., 2019). The lactic acid (LA) content in silage fluid was determined according to a modified spectrophotometric method of Barnett (1951) (Tekin and Kara, 2020). The amount of LA in the sample fluid was calculated as lactate equivalent from the calibration curve ($R^2 = 0.95$) of standard lithium lactate (0.312–160 $\mu\text{g/ml}$). The % of LA content in silage DM was calculated using the equations as follows:

$$\text{LA, \% in DM} = \text{absorbance value} \times 10^{-2} \times (100 - \text{DM}) / \text{DM}.$$

The 1.5 ml of silage fluid mixed with 0.3 ml of meta-phosphoric acid (25%, w/v) in a microcentrifuge tube was centrifuged at 15000 rpm for 15 min using a microcentrifuge (Gyrozen 1524, Gyrozen Co. Ltd., Daejeon, South Korea). The

supernatant was taken into a gas chromatograph vial. The analysis of organic acids (or volatile fatty acids; VFAs) (acetic (AA), butyric (BA), propionic (PA), *iso*-butyric (IBA), valeric (VA) and *iso*-valeric (IVA) acids) in silage fluid was measured by using a gas chromatograph device (GC, Thermo Trace 1300, Thermo Scientific, Waltman, MA, USA) with an auto-sampler (Thermo AI-1310, Thermo Scientific, Waltman, MA, USA) (Ersahince and Kara, 2017). According to the retention time and peak area in chromatograms, the concentrations (mmol/l) of organic acids were identified using a Xcalibur software program (Thermo Scientific, Waltman, MA, USA). The % concentrations of organic acids in DM of silage were calculated.

Determination of *in vitro* ruminal digestion and estimated digestion values

The *in vitro* digestion potential of the yellow sweet clover herbage at different phenological stages was analysed using an *in vitro* gas production technique. Rumen fluid, which was used in the *in vitro* gas production technique (Hohenheim Gas Test), was taken *via* an oesophageal tube from two Brown Swiss-Simmental mixed breed cattle. The 500 ml was collected from each and mixed. The cattle have consumed the ration, which included maize silage (5 kg/day as fed basis), wheat straw (1.7 kg/day as fed basis), alfalfa herbage (1.5 kg/day as fed basis), sugar beet pulp (4.5 kg/day as fed basis) and concentrated mix feed (5 kg/day as fed basis). The cattle consumed 9.8 kg DM/day, which included CP of 1231 g/day (ruminal degradable protein of 833 g/day), ME of 24.3 Mcal/day and NDF of 3.95 kg/day (40.3% in ration DM) (National Academies of Sciences, Engineering, and Medicine, 2016). Approximately one litter of rumen fluid was collected in a thermos, which included water, at 39 °C using CO₂ gas, and filtered with six layers of cheesecloth in the laboratory. The samples were incubated in rumen fluid and buffer mix in 100 ml capacity calibrated anaerobic glass fermenter (Fortuna®, Poulten & Graf Ltd., Wertheim, Germany) following the procedures by Menke et al. (1979). One litter of buffer mixture included 474 ml of bi-distilled water, 237.33 ml of macro-mineral solution (5.7 g of Na₂HPO₄, 6.2 g of KH₂PO₄ and 0.6 g of MgSO₄ in one litter of bi-distilled water), 237.33 ml of buffer solution (35 g of NaHCO₃ and 4 g of NH₄HCO₃ in one litter of bi-distilled water), 0.12 ml of trace-mineral solution (13.2 g of CaCl₂ • 2H₂O, 10 g of MnCl₂ • 4H₂O, 1 g of CoCl₂ • 6H₂O and 0.8 g of FeCl₃ • 6H₂O in

100 ml of bi-distilled water), 1.22 ml of resazurin solution (0.1 g of resazurin in 100 ml of bi-distilled water) and 50 ml of reducing solution (285 mg of $\text{Na}_2\text{S} \cdot 7\text{H}_2\text{O}$ and 4 ml of 1 N NaOH in 96 ml of bi-distilled water). The reducing solution was used to provide the oxidation-reduction potential (ORP) and the anoxic conditions. The pH and ORP of the *in vitro* digestion fluids were measured using Mettler Toledo InLab® Expert Pro-ISM sensor probes in pH-Ion meter (Seven Compact™ pH/ Ion S220, Mettler-Toledo, Schwerzenbach, Switzerland). There was no statistical difference between the *in vitro* incubation fluids of pH (6.7–6.85) and ORP values.

The 0.200 ± 0.010 g of dried herbage sample was incubated with 20 ml of buffer mixture and 10 ml of filtered rumen fluid. This mixture was incubated in a glass fermenter (Model Fortuna, Wertheim, Germany) at 39 ± 0.5 °C in an incubator for 24 h. The initial volumes of the *in vitro* glass fermenter were recorded. Each sample was studied in triplicate. Also, three blank syringes (without sample, rumen fluid + buffer mixture) were used to calculate the total gas production. After 24 h of incubation, the total gas volume was recorded from the calibrated scale in the *in vitro* glass fermenter.

The ME and organic matter digestibility (OMd) values of the herbage were calculated using equations by Menke and Steingass (1988) as follows:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136 \times \text{Gas24h} + 0.057 \times \text{CP},$$

$$\text{OMd (g/kg DM)} = 14.88 + 0.889 \times \text{Gas24h} + 0.45 \times \text{CP} + 0.0651 \times \text{ash}.$$

Net energy for lactation (NE_L) was calculated using the equation:

$$\text{NE}_L \text{ (MJ/kg DM)} = 0.115 \times \text{GP} + 0.0054 \times \text{CP} + 0.014 \times \text{EE} - 0.0054 \times \text{ash} - 0.36;$$

where: Gas24h – 24h net gas production (ml/200 mg), CP – crude protein (g/kg DM), CA – crude ash content (g/kg DM) and EE – ether extract (g/kg DM).

Determination of organic acids in the *in vitro* digestion fluid

The total gas volume at 24 h of *in vitro* incubation was recorded, and 10 ml of digestion fluid in the glass fermenter was collected in Falcon tubes. The fluids were frozen at -20 °C until analyses when the tubes were thawed at room temperature. Then, 2 ml of digestion fluid were placed into micro-centrifuge tubes and centrifuged at 15000 rpm for 15 min in a micro-centrifuge (Gyrozen 1524, Gyrozen Co. Ltd., Daejeon, Korea). After that, 1.25 ml of the supernatant acidified with 0.25 ml of meta-phosphoric acid solution (25%, w/v) for deproteinization, was mixed

in a vial for gas chromatography (GC). Analysis of VFAs in the *in vitro* digestion fluid was determined by using a GC device (Thermo Trace 1300, Thermo Scientific, Waltham, MA, USA) with an auto-sampler (Thermo AI-1310, Thermo Scientific, Waltham, MA, USA). The GC device was equipped with a FID and a polyethylene glycol column (length: 60 m, ID: 0.25 mm, film thickness: 0.25 μm) (TG-WAXMS, Thermo Scientific, Waltham, MA, USA). The operation procedure of the device was according to the study of Ersahince and Kara (2017). A standard organic acid mixture was used to determine retention time and calibration curve ($R^2 = 0.99$). The concentrations (mmol/l) of AA, PA, BA, IBA, VA and IVA were identified using the Xcalibur software program (Thermo Scientific, Waltham, MA, USA) according to retention time and peak area in the chromatograms.

Statistical analysis

The data were first subjected to Levene's test to detect the variance homogeneity. One-way variance analyses (ANOVA) were implemented for homogeneous variances by General Linear Model procedures to test treatment differences.

The data were analysed by the following statistical model:

$$Y_i = \mu + S_i + e_i;$$

where: Y_i – investigated parameter (e.g., gas production), μ – general mean, S_i – i^{th} effect of phenological stage and e_i – random residual effect.

The means were separated by Tukey's multiple range test at $P < 0.05$. Eight samples of yellow sweet clover herbage for each phenological stage and four samples of yellow sweet clover silage for each phenological stage were analysed by the aforementioned statistical model (SPSS 17.0 software, IBM Corp., Armonk, NY, USA).

Linear relations among the CP, NDFom, ADFom, NFC and MDA of yellow sweet clover herbage and the pH, DM, MDA, BA, PA and AA of yellow sweet clover silage were determined using Pearson's correlation through SPSS 17.0 software (IBM Corp., Armonk, NY, USA).

Results

Chemical composition of yellow sweet clover herbage

The CP and ash contents of yellow sweet clover herbage at vegetative and early flowering stages were higher than those of the full flowering stage ($P < 0.05$). The ash content of yellow sweet clover herbage linearly decreased with plant phenological

Table 1. Nutrient matter, MDA and fatty acid content of *Melilotus officinalis* herbage at different phenological stages

Indices	CP	NDFom	ADFom	NFC	EE	Ash	MDA, mg/kg DM	Palmitic acid	Oleic acid	Linoleic acid	Alpha-linolenic acid
	% in DM							% in total fatty acids of DM			
Phenological stages											
vegetative stage	19.15 ^a	38.98 ^b	27.18 ^b	29.49 ^a	2.66	9.69 ^a	434.07	17.07 ^b	5.79 ^b	16.04 ^a	43.57 ^a
early flowering stage	21.44 ^a	40.87 ^b	28.51 ^b	25.40 ^b	2.67	9.60 ^a	379.49	16.91 ^b	4.48 ^b	16.33 ^a	45.07 ^a
full flowering stage	16.95 ^b	44.64 ^a	31.11 ^a	26.63 ^b	3.07	8.68 ^b	481.26	17.95 ^a	6.73 ^a	14.57 ^b	40.69 ^b
SD	2.15	2.70	2.72	1.95	0.32	0.50	84.71	0.64	1.37	0.86	2.39
SEM	0.87	1.10	1.11	0.79	0.13	0.20	34.58	0.21	0.45	0.28	0.79
P-value											
L	0.114	0.013	0.037	0.029	0.284	0.003	0.262	0.720	0.929	0.002	0.077
Q	0.029	0.379	0.802	0.024	0.526	0.022	0.958	0.007	0.010	0.005	0.045

CP – crude protein, NDFom – neutral detergent fibre without ash, ADFom – acid detergent fibre without ash, NFC – non-fibre carbohydrate, EE – diethyl ether extract, DM – dry matter, MDA – malondialdehyde, SD – standard deviation, SEM – standard error of means, L – linear term, Q – quadratic term; ^{ab} – average values with different superscripts in the same column are significantly different at $P < 0.05$

stages ($P < 0.05$). The NDFom and ADFom contents of yellow sweet clover herbage linearly increased with plant phenological stages ($P < 0.05$). For all phenological stages of yellow sweet clover herbage, the highest NFC content was in the vegetative stage. The NFC content linearly decreased with increasing phenological stages ($P < 0.05$). The EE and MDA contents of yellow sweet clover herbage were similar for all phenological stages ($P > 0.05$) (Table 1).

The fatty acids at the highest ratio in total fatty acids were palmitic, oleic, linoleic and alpha-linolenic ones. The palmitic and oleic acid contents

($P < 0.05$) (Table 1). The MUFA and n-9 content in vegetative stage herbage did not differ from full flowering stage but was higher than in early stage ($P < 0.05$). The PUFA content was the highest in early stage ($P < 0.05$). The n-3 content in early stage herbage did not differ from full flowering stage but was higher than in vegetative stage ($P < 0.05$). MCFA was the lowest in vegetative stage and the highest in full flowering stage ($P < 0.05$). LCFA was the lowest in full flowering stage. There was no difference in n-6 and VLCFA ($P > 0.05$) (Table 2).

Table 2. The fatty acid content of *Melilotus officinalis* herbage at different phenological stages, as % in total fatty acids of dry matter

Indices	MUFA	PUFA	n-3	n-6	n-9	MCFA	LCFA	VLCFA
Phenological stage								
vegetation stage	9.60 ^a	65.06 ^b	44.72 ^b	20.33	6.91 ^a	0.51 ^c	98.47 ^a	0.99
early flowering stage	7.13 ^b	69.66 ^a	48.24 ^a	21.42	4.68 ^b	0.71 ^b	98.13 ^a	1.14
full flowering stage	8.77 ^{ab}	65.03 ^b	46.06 ^{ab}	18.97	6.78 ^{ab}	1.52 ^a	97.34 ^b	1.11
SD	1.28	2.39	2.06	1.62	1.32	0.46	0.51	0.12
SEM	0.42	0.79	0.68	0.54	0.44	0.15	0.17	0.04
P-value								
L	0.243	0.967	0.342	0.284	0.864	0.001	0.001	0.259
Q	0.010	0.001	0.044	0.129	0.012	0.001	0.083	0.306

MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, MCFA – medium-chain fatty acids, LCFA – long-chain fatty acids, VLCFA – very long-chain fatty acids, SD – standard deviation, SEM – standard error of means, L – linear term, Q – quadratic term; ^{abc} – average values with different superscripts in the same column are significantly different at $P < 0.05$

of yellow sweet clover herbage at full flowering stage were the highest than those of other phenological stages ($P < 0.05$). The palmitic and oleic acid contents of herbage linearly increased with the plant growing stage ($P < 0.05$). The linoleic and alpha-linolenic acid contents of yellow sweet clover herbage at vegetative and early flowering stages were higher than those of the full flowering stage ($P < 0.05$). The linoleic and alpha-linolenic acid contents of herbage decreased with the plant growing stage

***In vitro* digestion values of yellow sweet clover herbage**

The *in vitro* total gas production, ME, NE_L and OMD values for all phenological stages of yellow sweet clover herbage were similar ($P > 0.05$) (Table 3). The molarities and percentages of AA, PA, BA, IVA, VA, IBA, other volatile acids and TVFA in the *in vitro* fermentation fluid of yellow sweet clover herbage at different phenological stages were similar ($P > 0.05$) (Table 4).

Table 3. The *in vitro* total gas production and estimated ruminal fermentation values of *Melilotus officinalis* herbage at different phenological stages

Indices	Total gas production, ml/0.2 g DM	ME _i , MJ/kg DM	NE _L , MJ/kg DM	OMd, % DM
Phenological stage				
vegetation stage	43.14	9.16	5.61	62.48
early flowering stage	43.17	9.29	5.75	63.54
full flowering stage	42.95	9.01	5.49	61.09
SD	2.70	0.38	1.32	2.62
SEM	0.78	0.11	0.09	0.75
P-value				
L	0.930	0.615	0.612	0.479
Q	0.943	0.416	0.374	0.309

DM – dry matter, ME – metabolizable energy, NE_L – net energy for lactation, OMd – organic matter digestibility, SD – standard deviation, SEM – standard error of means, L – linear term, Q – quadratic term,

flowering stage ($P < 0.05$). The CP and ash content of silage linearly decreased with the plant phenological stages ($P < 0.05$). The MDA, oleic acid, linoleic acid, MUFA, n-3, n-6, n-9 and VLCFA contents of yellow sweet clover silages did not differ between phenological stages ($P > 0.05$) (Table 5).

The alpha-linolenic acid percentage in total fatty acids of yellow sweet clover silages at the vegetative stage were higher than that of the full flowering stage but similar to that of the early flowering stage ($P < 0.05$) (Table 5). The PUFA and LCFA percentages in total fatty acids of silages in vegetative and early flowering stages were the same, but higher than those of full flowering stage ($P < 0.05$) (Table 6). The MCFA percentage in total fatty acids of yellow sweet clover silages at the full flowering stage was higher than those of other phenological stages ($P < 0.05$) (Table 6).

Table 4. The organic acids in the *in vitro* digestion of *Melilotus officinalis* herbage at different phenological stages

Indices	Phenological stage			SD	SEM	P-value	
	vegetative stage	early flowering stage	full flowering stage			L	Q
Organic acids as mM/l for <i>in vitro</i> fermentation fluid							
acetic acid (AA)	75.03	79.14	76.92	3.82	1.27	0.575	0.295
propionic acid (PA)	18.79	20.12	19.11	1.05	0.35	0.708	0.149
butyric acid (BA)	13.56	14.40	13.82	0.70	0.24	0.659	0.202
iso-valeric acid (IVA)	2.52	2.70	2.68	0.12	0.04	0.142	0.266
valeric acid (VA)	1.90	2.07	1.97	0.10	0.03	0.285	0.060
iso-butyric acid (IBA)	1.35	1.42	1.42	0.06	0.02	0.263	0.461
other volatile acids (OVA)	0.76	0.80	0.77	0.02	0.01	0.629	0.116
TVFA	113.93	120.67	116.71	5.85	1.95	0.582	0.244
Organic acids as % in TVFA of <i>in vitro</i> fermentation fluid							
acetic acid	65.84	65.58	65.90	0.15	0.05	0.323	0.071
propionic acid	16.49	16.67	16.37	0.15	0.05	0.165	0.112
butyric acid	11.90	11.94	11.85	0.05	0.02	0.144	0.086
iso-valeric acid	2.22	2.24	2.30	0.05	0.02	0.070	0.628
valeric acid	1.67	1.72	1.70	0.03	0.01	0.121	0.129
iso-butyric acid	1.19	1.18	1.22	0.02	0.01	0.175	0.165
other volatile acids	0.66	0.66	0.66	0.01	0.01	0.513	0.960

SD – standard deviation, SEM – standard error of means, L – linear term, Q – quadratic term; TVFA (total volatile fatty acids; mmol/l) = AA (mmol/l) + PA (mmol/l) + BA (mmol/l) + IBA (mmol/l) + VA (mmol/l) + IVA (mmol/l) + OVA (mmol/l); other volatile acids = n-heptanoic acid + hexanoic acid

Chemical composition of yellow sweet clover silages

The DM, NDFom and ADFom contents, and palmitic acid percentage in total fatty acids of yellow sweet clover silages at the full flowering stage were higher than those of other stages ($P < 0.05$). The DM, NDFom, ADFom and palmitic acid of silage linearly increased with the plant phenological stages ($P < 0.05$). The CP and ash contents of yellow sweet clover silages at vegetative and early flowering stages were higher than those of the full

The acidity values and ammonia contents of yellow sweet clover silages

Melilotus officinalis silages pH values were 3.93 for the vegetative stage, 3.88 for the early flowering stage and 4.25 for the full flowering stage ($P < 0.05$). The pH value in silage fluid and PA in the silage DM of the full flowering stage was higher than those of other stages ($P < 0.05$). The LA contents in the DM of yellow sweet clover silages at vegetative and early flowering stages were higher than that of the full flowering stage ($P < 0.05$). The silage acidity

Table 5. Nutrient matter, MDA and fatty acids of *Melilotus officinalis* silages at different phenological stages

Indices	DM	CP	NDFom	ADFom	NFC	EE	Ash	MDA, mg/kg DM	Palmitic acid	Oleic acid	Linoleic acid	Alpha-linolenic acid
	% in DM								% in total fatty acids of silage DM			
Phenological stage												
vegetative stage	23.65 ^b	21.24 ^a	35.01 ^c	23.26 ^c	30.55	3.53	9.67 ^a	1518.13	17.35 ^b	4.58	18.85	47.46 ^a
early flowering stage	23.00 ^b	22.78 ^a	37.60 ^b	25.95 ^b	27.02	2.80	9.79 ^a	1164.92	17.41 ^b	5.14	19.25	46.37 ^{ab}
full flowering stage	25.00 ^a	16.48 ^b	42.16 ^a	29.36 ^a	29.69	2.90	8.75 ^b	1672.45	20.24 ^a	6.46	16.60	39.76 ^b
SD	0.88	2.96	3.25	2.77	1.81	0.57	0.51	267.59	1.51	1.36	1.51	3.98
SEM	0.29	1.21	1.32	1.13	0.74	0.23	0.20	109.24	0.61	0.55	0.61	1.62
P-value												
L	0.010	0.003	<0.001	0.002	0.453	0.368	0.001	0.432	0.007	0.261	0.119	0.024
Q	0.014	0.003	0.045	0.526	0.037	0.467	0.003	0.062	0.036	0.765	0.188	0.179

DM – dry matter, CP – crude protein, NDFom – neutral detergent fibre without ash, ADFom – acid detergent fibre without ash, NFC – non-fibre carbohydrate, EE – diethyl ether extract, MDA – malondialdehyde, SD – standard deviation, SEM – standard error of means, L – linear term, Q – quadratic term; ^{abc} – average values with different superscripts in the same column are significantly different at $P < 0.05$

Table 6. Fatty acids of *Melilotus officinalis* silages at different phenological stages, as % in total fatty acids of dry matter

Indices	MUFA	PUFA	n-3	n-6	n-9	MCFA	LCFA	VLCFA
Phenological stage								
vegetative stage	6.65	70.01 ^a	50.46	19.54	4.68	0.62 ^b	98.70 ^a	0.68
early flowering stage	6.87	69.94 ^a	50.24	19.70	5.24	0.67 ^b	98.63 ^a	0.68
full flowering stage	8.09	62.39 ^b	45.50	16.88	6.57	1.56 ^a	97.53 ^b	0.91
SD	1.18	4.43	3.08	1.68	1.37	0.47	0.58	0.13
SEM	0.48	1.80	1.25	0.68	0.56	0.19	0.23	0.05
P-value								
L	0.330	0.045	0.121	0.107	0.263	<0.001	<0.001	0.059
Q	0.676	0.204	0.342	0.237	0.770	0.002	<0.001	0.159

MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, MCFA – medium-chain fatty acids, LCFA – long-chain fatty acids, VLCFA – very long-chain fatty acids, SD – standard deviation, SEM – standard error of means, L – linear term, Q – quadratic term; ^{ab} – average values with different superscripts in the same column are significantly different at $P < 0.05$

Table 7. Acidity values of *Melilotus officinalis* silages at different phenological stages

Indices	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Hexanoic acid	Ammonia, g/l silage fluid
		% in DM of silage					
Phenological stage							
vegetative stage	3.93 ^b	6.30 ^a	0.658	0.010 ^b	0.002	0.012	0.27
early flowering stage	3.88 ^b	5.76 ^a	0.712	0.009 ^b	0.003	0.011	0.33
full flowering stage	4.25 ^a	3.68 ^b	0.727	0.013 ^a	0.002	0.007	0.26
SD	0.18	0.740	0.114	0.002	0.004	0.004	0.089
SEM	0.06	0.112	0.038	0.001	0.001	0.001	0.029
P-value							
L	0.001	0.038	0.527	0.005	0.805	0.174	0.981
Q	0.005	0.165	0.836	0.006	0.226	0.574	0.458

DM – dry matter, SD – standard deviation, SEM – standard error of means, L – linear term, Q – quadratic term; ^{ab} – average values with different superscripts in the same column are significantly different at $P < 0.05$

and LA concentration linearly decreased with the plant phenological stage ($P < 0.05$). The AA, BA and HA contents in the DM of yellow sweet clover silages were similar for all phenological stages ($P > 0.05$). The ammonia content (g/l) in silage fluid did not differ between examined phenological stages of yellow sweet clover ($P > 0.05$) (Table 7).

Pearson correlations among chemical compositions in *Melilotus officinalis* silages and herbage

Yellow sweet clover herbage's EE content was positively correlated with herbage's ADF content ($P < 0.05$). The CP content of yellow sweet clover herbage was negatively correlated with NDFom,

Table 8. Pearson correlations among chemical compositions in silages and herbage of *Melilotus officinalis*

Indices	Herbage contents					Silage values						
	CP	NDFom	ADFom	NFC	MDA	pH	DM	MDA	BA	PA	AA	
Herbage contents	EE	-0.477	0.502	0.840**	-0.207	0.009	0.608	0.513	-0.137	-0.153	0.471	-0.268
	CP	1	-0.731*	-0.613*	-0.316	-0.541*	-0.809**	-0.937**	-0.785*	0.685*	-0.828**	0.162
	NDFom		1	0.637*	-0.397	0.434	0.808**	0.804**	0.584	-0.200	0.770*	0.227
	ADFom			1	-0.110	0.054	0.693*	0.621	0.152	-0.323	0.590	-0.322
	NFC				1	0.134	-0.037	0.148	0.365	-0.643	0.044	-0.464
	MDA					1	0.499	0.523	0.837*	-0.625	0.730	0.265
Silage values	pH						1	0.925**	0.674	-0.375	0.854**	0.132
	DM							1	0.795	-0.577	0.920**	0.110
	MDA								1	-0.787	0.825*	0.231
	BA									1	-0.564	0.111
	PA										1	0.253

EE – diethyl ether extract, CP – crude protein, NDFom – neutral detergent fibre without ash, ADFom – acid detergent fibre without ash, NFC – non-fibre carbohydrate, MDA – malondialdehyde, DM – dry matter, BA – butyric acid, PA – propionic acid, AA – acetic acid; * – $P < 0.05$, ** – $P < 0.01$

Table 9. Pearson correlations among fatty acids and other chemical contents in herbage and silage for *Melilotus officinalis*

Indices	Silage		Herbage MDA	Silage				
	DM	MDA		LA	BA	PA	AA	
Silage								
	palmitic acid (C16:0)	0.926**	0.716	0.611	-0.449	-0.461	0.947**	0.239
	oleic acid (C18:1)	0.697*	0.506	0.482	-0.429	-0.517	0.844**	0.198
	linoleic acid (C18:2)	-0.852**	-0.816*	-0.855*	0.295	0.542	-0.924**	-0.253
	alpha-linoleic acid (C18:3)	-0.885**	-0.654*	-0.549*	0.483	0.444	-0.941**	-0.280
Herbage								
	palmitic acid (C16:0)	0.586	0.451	0.006	-0.475	-0.569	0.345	-0.469
	oleic acid (C18:1)	0.612	0.875*	0.594	-0.531	-0.582	0.620	0.440
	linoleic acid (C18:2)	-0.929**	-0.777*	-0.796*	0.543	0.432	-0.935**	-0.450
	alpha-linoleic acid (C18:3)	-0.803**	-0.842*	-0.738*	0.430	0.448	-0.842**	-0.568

DM – dry matter, MDA – malondialdehyde, LA – lactic acid, BA – butyric acid, PA – propionic acid, AA – acetic acid; * – correlation is significant at the 0.05 level (2-tailed), ** – correlation is significant at the 0.01 level (2-tailed)

ADFom and MDA contents of herbage and pH value, DM, MDA and PA contents of silage; and positively correlated with the BA contents of silage ($P < 0.05$). The NDFom content of herbage was positively correlated with herbage ADF content, silage pH value, silage DM and silage PA content ($P < 0.05$). The ADFom content of herbage was positively correlated with silage pH ($P < 0.05$).

The MDA content of herbage was positively correlated with silage MDA content ($P < 0.05$).

The PA content of silage was correlated with pH value, DM and MDA contents of silage ($P < 0.05$) (Table 8).

The silage palmitic acid content was positively correlated with silage DM and silage PA content ($P < 0.05$). Silage oleic acid content was positively correlated with silage DM and silage PA content ($P < 0.05$). The linoleic acid content of herbage and silage were negatively correlated with silage DM, silage MDA, herbage MDA and silage PA contents

($P < 0.05$). The alpha-linoleic acid contents of herbage and silage were negatively correlated with silage DM, silage MDA, herbage MDA and silage PA contents ($P < 0.05$).

Discussion

Yellow sweet clover (*Melilotus officinalis* L.), which is a common legume species, has the potential to be used as a forage crop for herbivores. The species is native to Eurasia and naturalized in North America, Africa and Australia (Cornara et al., 2016). The yellow sweet clover did not culture like other legume forages, such as *Medicago sativa*, *Onobrychis sativa*, *Trifolium pratense* and *Trifolium repens* (Dumlu and Tan, 2009; Luo et al., 2014). There are few studies about the nutritional content and digestibility capacity of yellow sweet clover. In the present study, yellow sweet clover herbage's CP contents at different phenological stages ranged

from about 17 to about 21% in DM. Yisehak (2008) stated that yellow sweet clover herbage's CP content at the middle of the flowering stage was 22% of DM. Canbolat and Karaman (2009) found that the CP of yellow sweet clover herbage at the flowering stage included at a similar level with our study results. The CP content (24.7%) of yellow sweet clover herbage at the flowering stage in the study of Çağan et al. (2015) was higher than the results of the present study. The CP and ash contents of yellow sweet clover herbage at vegetative and early flowering stages were higher than those of the full flowering stage. The NDFom and ADFom contents of yellow sweet clover herbage at the full flowering stage were higher than those of vegetative and early flowering stages; such results were similar to the results of previous researchers (Ersahince and Kara, 2017; Kara et al., 2018). Yisehak (2008) stated that the NDF and ADF contents of yellow sweet clover herbage at 50% flowering stage were 37 and 33% in DM, respectively. For all phenological stages of yellow sweet clover herbage, the highest non-structural carbohydrates (NSC) content was in the vegetative stage, similar to the results of other forage plants by previous researchers (Kara et al., 2018). Previous researchers determined that the yellow sweet clover herbage at the flowering stage included about 7% ash, 38–43% NDF and 22–33% ADF in DM (Canbolat and Karaman 2009; Çağan et al., 2015). In another study, Ateş and Seren (2020) demonstrated that the CP content of *Melilotus caeruleus* herbage was decreased by the growth stage, and ADF and NDF contents were increased by the growth stage. The CP content of yellow sweet clover was correlated with ADFom and NDFom contents. The nutritional difference between the present and previous studies on yellow sweet clover herbage can be related to soil type, salinity, water restriction, and temperature and climate variables.

The DM contents of yellow sweet clover silage at different growing stages in the present study were ranged from 23 to 25%. The highest silage DM content was in the full flowering stage. According to the results, silage DM increases as the plant growth period progresses. In line with the study results, the researchers determined that the DM value of yellow sweet clover silage harvested at the end of the flowering stage was 30.6% (Dumlu and Tan, 2009). In the present study, the CP value of yellow sweet clover silage varied from approximately 23 to 16.5% in DM. The CP of yellow sweet clover silage for all phenological stages in the present study was higher than the CP content (about 12% in DM) in the silage

of yellow sweet clover at the end of the flowering stage of Dumlu and Tan (2009).

The total gas production volume of *in vitro* fermentation depends on the composition of nutrient content, the ratio of structural and NSC, the presence of inhibitor for gas production, the diet of the donor animal, and the quality of fermentation provided by the microflora in the rumen fluid (Szumacher-Strabel and Cieślak, 2012; Kara et al., 2015). The *in vitro* gas production and *in vitro* estimated digestion values of forage decrease with the increase in NDF and ADF value; and increase with the increase in NFC (Ersahince and Kara, 2017; Kara et al., 2018). In the present study, although NDFom and ADFom values increased linearly as the plant phenological period progressed, this effect was not observed on *in vitro* digestion values. Although the NFC value of yellow sweet clover herbage ranged from about 25 to 29%, there was no significant difference in the *in vitro* gas production and estimated digestion values. This result was interesting. Similar *in vitro* digestion levels in different phenological stages of the plant may also be due to the level of hemicellulose, which is an easily digested fibre source. Because it was seen that the hemicellulose levels (NDFom-ADFom) according to the plant growth stage of yellow sweet clover herbage were approximately 12, 12 and 13%, respectively. In general, the *in vitro* gas production and *in vitro* estimated digestion values for the silage and herbage samples of yellow sweet clover in the presented study were similar to the values of the lucerne herbage and maize silage (Canbolat and Karaman, 2009; Kara, 2015; Kara et al., 2016). This showed that the *in vitro* total gas production, OMD and ME values of herbage of yellow sweet clover at the flowering stage were 51.2 ml/0.2 g, 72% and 10 MJ/kg DM at 24 h of incubation. Yisehak (2008) showed that yellow sweet clover herbage's ME value at 50% flowering stage was 9.2 MJ/kg DM. *In vitro* rumen fermentation, TVFA molarities (114–120 mM/l) in digestive fluids for yellow sweet clover herbage at different phenological stages were at reference values (Ersahince and Kara, 2017; Kara et al., 2018). The molarities of VFAs in rumen fluid change by plant cell wall carbohydrates, easily digestible carbohydrates, and other nutrient compositions in substrates (Kara, 2015). The AA molarity in rumen fluid was also in values that could be achieved by fermentation of a plant cell wall carbohydrates-weighted substrate.

Yellow sweet clover silage's pH values for vegetative and early flowering stages were at ideal

fermentation acidity range (pH 3.88 and 3.93, respectively). The LA contents of yellow sweet clover silages for vegetative and early flowering stages were higher than that of the full flowering stage. However, yellow sweet clover silage's pH value for full flowering stages was high for ideal fermentation acidity (pH 4.25). The pH value of yellow sweet clover silage for all phenological stages in the present study was lower than the pH content (pH 4.6) in the silage of yellow sweet clover at the end of the flowering stage of Dumlu and Tan (2009). For yellow sweet clover silage, it is understood that silage acidity decreases (pH value increases) as the plant growing period progresses. Yellow sweet clover silage's pH value was positively correlated with silage DM content and silage PA content. The PA acidity value (pKa 4.88) was lower than the acidity values of LA (pKa 3.86) and AA (pKa 4.75). Yellow sweet clover silage's pH value being positively correlated with silage PA content did not reduce silage acidity. The reason for this may be the acidity value (pKa 4.88) of PA. In the present study, the NDFom contents of yellow sweet clover silage ranged from 35 to 42% in DM. The NDFom contents of yellow sweet clover silage were lower than that of yellow sweet clover silage at the end of the flowering stage of Dumlu and Tan (2009).

Forages include the changing of fatty acids at different ratios according to plant species, plant part, plant phenological stage, etc. In the present study, the palmitic acid (C16:0) has among all SFA the highest concentration in yellow sweet clover herbage and silages at different phenological stages. In a previous study, it was demonstrated that palmitic acid concentrations (in total fatty acids) of common forages used in dairy cattle diet were 15–30% for alfalfa (herbage, silage or hay), 16–20% for perennial ryegrass (herbage), 14–20% for red clover (fresh, silage or hay), 16% for white clover (herbage, silage) and 16% for maize silage (Vanhatalo et al., 2007; Van Ranst et al., 2009; Dierking et al., 2010; Glasser et al., 2013). The approximately 17–20% palmitic acid concentrations in total fatty acids of yellow sweet clover (herbage and silage) for different phenological stages were similar to other legume forage such as red clover (14–20% for fresh, silage or hay), white clover (16% for herbage and silage) and alfalfa (15–30% for herbage, silage or hay) (Vanhatalo et al., 2007; Van Ranst et al., 2009; Dierking et al., 2010; Glasser et al., 2013). In the present study, palmitic acid in yellow sweet clover herbage and silages increased with a plant growing similarly to that in

Mir et al. (2006) study. The palmitic acid contents of yellow sweet clover herbage and silages in the full flowering stage were very close (about 18 and 20% in total fatty acids, respectively) which demonstrates that fermentation does not remarkably affect this fatty acid. The oleic acid percentages, which were the highest MUFA in forages in the present study, were about 4–6% in total fatty acids of yellow sweet clover silages and herbage. The oleic acid and MUFA concentrations in total fatty acid of herbage increasing up to full flowering stage may be related to plant height, leaf:stem ratio, flower ratio and other environmental conditions. The highest percentage of unsaturated fatty acid among total fatty acids in yellow sweet clover had alpha-linolenic acid. The alpha-linolenic acid (C18:3n-3) percentage in total fatty acids of yellow sweet clover silage (about 40–48%) and herbage (about 41–45%) lowered with increasing plant growth stage. The alpha-linolenic acid percentage in total fatty acids of herbage (42%), silage (32%) and hay (22%) of alfalfa, and the herbage (58%) and silage (59%) of white clover (Glasser et al., 2013) were very variable.

Lipid oxidation of plants depends on the activity of lipoxygenases. Lipoxygenase activity is found in a plant at all phenological stages, and this activity increases when a plant enters the mature and senescence stages or after tissue injury (Han and Zhou, 2013). Lipid peroxidation refers to a series of free radical's reactions conducted in unsaturated fatty acids and has been widely used to indicate cell oxidative damage (DaCosta and Huang, 2007; Talbi et al., 2015). The MDA reflects lipid peroxidation in plant cells and external stress responses (Prasad, 1996; Thompson et al., 1998). Han and Zhou (2013) proved that SFA (C16:0 and C18:0) and MDA contents of maize silage tended to increase with the ensiling process. In contrast, unsaturated fatty acids (C18:1, C18:2 and C18:3) tended to decrease with the ensiling process. In the present study, the MDA value of yellow sweet clover increased up to 3.5–4 times with silage making according to herbage. An increase in MDA concentration with the ensiling of yellow sweet clover in the present study can result from the decomposition of the plant into fresh material during silage making, crushed-compression and acidic activity in anaerobic fermentation. The linoleic acid (C18:2) and alpha-linolenic acid (C18:3) of yellow sweet clover silage were negatively correlated with MDA of silage and herbage which was parallel with the results of Han and Zhou (2013). Elgersma et al.

(2003) demonstrated that virtually all fatty acids in fresh grass are in the form of esterified fatty acids, whereas in silage, a large proportion is in the form of free fatty acids. Palmitic acid (C16:0) content of yellow sweet clover silage at the full flowering stage was greater than of herbage, which was similar to maize silage results of Han and Zhou (2013). The decrease in unsaturated fatty acids can be related to the increasing degradation of unsaturated fatty acids by lipid peroxidation in the plant. The increasing MCFA ratio in % in total fatty acids of yellow sweet clover herbage and silage with plant growing can be related to flower ratio, leaf:stem ratio or plant metabolism changes.

Conclusions

The fact that the yellow sweet clover herbages at different phenological stages have similar *in vitro* digestibility for each period can be valuable for producers. While this plant is used as forage in ruminant rations, it should not be overlooked during the growth period of the plant. This legume forage plant is a quality forage rich in crude protein, energy, organic matter digestibility and alpha-linolenic acid. The ensiling of yellow sweet clover can be recommended to be found at a certain level of functional fatty acids, but there is also a disadvantage that enhances lipid peroxidation. For ideal silage acidity, it can be recommended to make silage at the vegetative and early flowering stages.

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Conflict of interest

The authors declare that there is no conflict of interest.

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