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The investigation of fatty acids compositions of Jerusalem artichoke (*Helianthus tuberosus*) herbage harvested at different phenological stages

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Abstract: This study was aimed to determine fatty acid compositions of Jerusalem artichoke herbages (*Helianthus tuberosus*) at five different phenological stages. Jerusalem artichoke was harvested at early vegetative, vegetative, early flowering, full flowering, and early seed stages and its herbage samples were obtained. In the herbages, the saturated fatty acid (∑SFA), unsaturated fatty acid (∑UFA), polyunsaturated fatty acid (∑PUFA), monounsaturated fatty acid (MUFA), medium chain fatty acids (∑MCFA), long chain fatty acids (∑LCFA) and very long chain fatty acids (∑VLCFA) were analyzed. The linoleic acid (C18:2n 6c) concentrations of herbages were changed from about 21 to 23% at different growing stages (P<0.05). The oleic acid (C18:2n 6t) concentrations of this forage increased with plant growing (20 to 34% in total fatty acids) (P<0.001). The ∑PUFA, w-3, w-6, ∑MCFA and ∑VLCFA concentrations of Jerusalem artichoke herbage were positively correlated with plant growing stage (from vegetation to after flowering) (P<0.05). The ∑MUFA, w-9 and ∑LCFA concentrations of this herbage were negatively correlated with plant growing stage of plant (P<0.05). As a result of the study, the ∑UFA, ∑MUFA and w-9 fatty acids compositions of Jerusalem artichoke herbage, harvested at early flowering stage, were high than those of other plant growing stages. Besides, the Jerusalem artichoke herbage, harvested at flowering stage, was rich from ∑PUFA, w-3 and ∑VLCFA fatty acids. Therefore, Jerusalem artichoke herbage, harvested at early flowering and full flowering stages, has high functional properties for ruminants and other herbivorous.
Keywords: Forage, plant growing, polyunsaturated fatty acid, ruminant.

Farklı fenolojik dönemlerde hasat edilen yerelması hasılının (*Helianthus tuberosus*) yağ asiti kompozisyonun araştırılması

Özet: Bu çalışmada farklı fenolojik dönemlerde hasat edilen yerelması (*Helianthus tuberosus*) hasılının yağ asiti kompozisyonunun saptanması amaçlandı. Yerelması vejetasyonun başlangıcı, vejetasyon dönemi, çiçeklenme başlangıcı, tam çiçeklenme ve tohum başlama başlangıcı olmak üzere beş farklı fenolojik dönemde hasat edildi ve örnekleri alındı. Bitki örneklerinde doymuş yağ asitleri (∑SFA), doymamış yağ asitleri (∑UFA), çoklu doymamış yağ asitleri (∑PUFA), tekli doymamış yağ asitleri (∑MUFA), orta zincirli yağ asitleri (∑MCFA), uzun zincirli yağ asitleri (∑LCFA) ve çok uzun zincirli yağ asitleri (∑VLCFA) analiz edildi. Hasılın linoleik asit (C18:2n 6c) konsantrasyonu büyüme dönemine göre %21 ile 23 arasında değişmekteydi (P<0,05). Bu kaba yemin oleik asit (C18:2n 6t) konsantrasyonu bitki büyümesi ile arttı (%20-34, toplam yağ asitleri içinde) (P<0,001). Yerelması hasılının ∑PUFA, w-3, w-6, ∑MCFA ve ∑VLCFA konsantrasyonu bitkinin (vejetasyondan çiçeklenmeye kadar) büyüme dönemiyle pozitif korelasyon içindeydi (P<0,05). Bu hasılın ∑MUFA, w9 ve ∑LCFA konsantrasyonu bitki büyüme dönemiyle negatif korelasyonluydu (P<0,05). Çalışmanın sonucu olarak, çiçeklenme başlangıcında hasat edilen yerelması hasılı ∑UFA, ∑MUFA ve w-9 yağ asitleri kompozisyonu diğer bitki büyüme dönemlerinkinden yüksekti. Bunun yanında, çiçeklenme döneminden hasat edilen yerelması hasılı ∑PUFA, w3 ve ∑VLCFA yağ asitleri açısından zengindi. Bunların göstergesi olarak, çiçeklenme başlangıcı ve tam çiçeklenme döneminde hasat edilen yerelması hasılı ruminantlar ve diğer herbivorlar için fonksiyonel özelliklere sahiptir.
Anahtar sözcükler: Bitki gelişimi, doymamış yağ asitleri, kaba yem, ruminant.

Introduction

Forage-feedstuff resources have major significant effects on diet of dairy cattle. They are important sources for fiber, as well as protein, energy, mineral, vitamin and unsaturated fatty acid (UFA) (22). The amount of lipids in forage feedstuffs reach up to 8% in dry matter (DM).

Lipids in the leaf are often localized in chloroplasts, which contain 22 to 25% lipids on a DM basis. Complex lipids constitute most leaf tissues, mainly as glycolipids and phospholipids (6). The esterified fatty acids (FA) in forages represent two-thirds of the total lipids (5% of DM). Their composition includes simple lipids, i.e. diglycerides, free FA, waxes, and sterol esters (33%), galactolipids, i.e. mono- and digalactosyl diglycerides (50%), and phospholipids (17%). The FA composition of forage lipids is dominated by high proportions of linolenic (C18:3) and linoleic acids (C18:2) from polyunsaturated fatty acids (PUFA), but also small amounts of oleic acid (C18:1) (12, 15). Moreover, forage lipids sometimes contain significant amounts of polyunsaturated fatty acids (FA). Nutritional quality of milk and meat in dairy cattle and beef cattle, which fed with diets based on pasture and grass silage, can be improve by shifting their FA composition toward less saturated FA and more PUFA, particularly toward omega-3 fatty acids (9).

Animal products obtained in such systems contain high levels of fatty acids (FA) which is beneficial to human health, such as conjugated linoleic acid (CLA) and polyunsaturated FA (PUFA) from the omega-3 fatty acids (9). Collomb et al. (7) reported that the concentration of these beneficial FA in milk fat increased with altitude and suggested that they could be related to a higher percentage of herbs in the dairy cow ration. Vanhatalo et al. (26) stated that changes in the rates of grass and legume forages in the dairy cattle ration resulted in differentials in milk FA composition. In conclusion, it is possible to alter the milk fat composition on high-forage-based diets through the selection of forage species and manipulation of the herbage harvesting stage (26). Similarly, the present results suggest that it is possible to manipulate the milk PUFA content by increasing the intake of forage PUFA (26).

Jerusalem artichoke (*Helianthus tuberosus* L.), which is a tuberous plant and belongs to *Asteraceae* family, can grow naturally or be cultivated in various areas of the world and originates from North America (13). Jerusalem artichoke is a perennial plant and it can reach up to 3-4 meters in length. The length of this plant, color of its tubers, the numbers of branches and stems, and the leaf ratio in the plant change according to variety, soil type and climatic conditions (18, 23). It is suitable for various soil types (pH 4.5-8.2 and salinity) and different climatic conditions (about 6-27°C) and also includes natural taste substances in leaves of this plant (14, 24, 30). In addition, Jerusalem artichoke has a number of advantages, such as low input cultivation, high crop yield and strong resistance to pests and plant diseases (30).

The FA concentration in forages depends on many factors, including species and senescence, growth stage, conservation method, as well as wilting, shading, and silage additives (6, 12, 15). Jerusalem artichoke herbage has the potential to be used as quality forage, especially at the vegetative stage, in terms of high/moderate nutrient composition (crude protein, ether extract, ash, non-fibrous carbohydrate, neutral detergent fiber, and lutein, zeaxanthin, lycopene, and α-, β-, and γ-carotenes) and satisfactory digestion values (metabolic energy, true dry matter disappearance, true organic matter disappearance) for both horses and ruminants (11). The present study hypothesizes that the fatty acid profile of the Jerusalem artichoke plants in different growth periods will change and these values can be used to adjust the plant harvesting time for animal nutrition. The aim of this study was to determine fatty acid compositions for different phenological stages (/growing stages) of Jerusalem artichoke herbage, which has the potential of quality forage.

Materials and Methods

The samples of Jerusalem artichoke plant were collected from Karaman province, Turkey. Karaman is located (36°33’50” N, 32°56’52” E) in Turkey’s Central Anatolia region. Arid conditions and desert-like steppe vegetation are dominant in the Karaman province due to temperature and rainfall amounts (2). Plant samples were gathered at five different stages: early vegetative (May 2015) (n=6), vegetative (Jun 2015) (n=6), early flowering (July 2015) (n=6), full flowering (August 2015) (n=6), and early seed (September 2015) (n=6) (Table 1; 11). For each phenological stage, six different plants were gathered, which included all the aerial parts (leaf, stem, and flower). Plants were harvested at above 5 cm from the soil in the morning. The amount of sample was about 3 kg for each phenological stage. Three samples for each different plant were used.

Table 1. Phenological stages of Jerusalem artichoke herbage used in the present study.

Phenological stages	Stage definition
Early vegetative	Stem length <50 cm; no bud or flowers; green leaves
Vegetative	Stem length <100 cm; no bud or flowers; green leaves
Early flowering	Stem length >100 cm; starting of flowering (yellow color); green leaves
Full flowering	Open flowers (yellow color); green leaves
Early seeding	Brown and dried flowers; first green pods; green leaves Leaves near the ground starting to dry

The samples of Jerusalem artichoke herbage were dried (48 hours, 55°C) using a thermostatically controlled cabinet (Lovidond, Dortmund, Germany). The dried samples were milled through a 1 mm sieve (IKA Werke, Staufen im Breisgau, Germany) for analysis. The ether extract (EE) levels were determined according to the method (method 920.39) reported by the AOAC (4) (Velp, Italy). For fatty acid analyses, the EE of dried Jerusalem artichoke herbage were methylated with the modified (17) three stage procedure of Wang et al. (29). According to this procedure, 40 µL of fats in falcon tubes with 15 mL volumes were mixed with 0.7 ml of KOH (10 M) and 5.3 mL of methanol and was vortexed. The mixture was incubated for 45 min at 55°C in an incubator (Nüve, Turkey) and cooled to 21°C. The mixture was combined with 0.58 mL of H_2SO_4 (10 M) and was vortexed. After this mixture was incubated for 45 min at 55°C, 3 mL of n-hexane was added. The tubes were centrifuged for 5 min at 4000 rpm. The 1.5 mL of supernatants was analyzed in a gas chromatograph (Thermo Scientific, USA) with autosampler (Thermo AI 1310, USA). Analyses were conducted by FAME column (Leigh 60 m, I.D: 0.25 mm, film: 0.25 µm and maximum temperature 250-260°C) at an injection split temperature of 255°C and a colon temperature of140 °C with a flow rate of 30 ml/min for 40 min. Fatty acid identification was performed by comparing the peaks in the chromatogram with the retention times at the standard (19). Total saturated fatty acid (∑SFA), unsaturated fatty acid (∑UFA), polyunsaturated fatty acid (∑PUFA), monounsaturated fatty acid (∑MUFA), medium chain fatty acids (∑MCFA), long chain fatty acids (∑LCFA) and very long chain fatty acids (∑VLCFA) were detected.

The experiment 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 analyzed based on the statistical model: $Yij = \mu ij + Si + ei$. Where, Yij = the general mean common for each parameter under investigation. S_i = the ith effect of phenological stages of Jerusalem artichoke herbage on the observed parameters, and ei = the standard error term. Polynomial contrast (linear, quadratic and cubic) of fatty acid values in different plant growing stages were analyzed to reveal the change in fatty acid profile according to the growth period of the plant. Linear relations between the maturity stage and fatty acid compositions of Jerusalem artichoke herbage were determined using Pearson's correlation coefficients (r). Analyses were performed using SPSS 17.0 software (IBM Corp., Armonk, NY, USA). All data were expressed as means ± standard error of means (SEM).

Results

Caprylic, tridecanoic, myristoleic, *cis*-10-pentadecenoic, palmitoleic, heptadecanoic, elaidic, oleic, arachidic and cis-13,16-docosadienoic acid concentrations of Jerusalem artichoke herbage linearly increased to early flowering stage ($P<0.05$) and decreased after early/full flowering stages ($P<0.05$). The palmitic acid (C16:0) concentrations of Jerusalem artichoke herbage was ranged from about 22% to 26% according to the growing stage ($P<0.001$). The linoleic acid (C18:2 n6 cis) concentration of Jerusalem artichoke herbage was changed from about 21% to 23% at different growing stages ($P<0.05$). The concentration of oleic acid (C18:2 n6 trans) of Jerusalem artichoke herbage was changed from about 20% to 34% with plant growing ($P<0.001$). The highest stearic acid concentration (9.66%) of Jerusalem artichoke herbage (C18:0) was determined at the vegetation stage ($P<0.001$). The alpha linolenic acid (C18:3n3) concentration of herbage changed from 0.44% to 5.99%; and the highest alpha linolenic acid concentration of herbage was in full flowering stage ($P<0.001$). The highest fatty acid concentrations in early vegetation, vegetation and early flowering stages were in oleic acid (C18:1n9); but the highest fatty acid concentrations in full flowering and after flowering stages were in palmitic acid (C16:0) (Tables 2 and 3).

The highest eicosapentaenoic acid (C20:5n3), docosahexaenoic acid (C22:6n3), tricosanoic acid (C23:0), lignoceric acid (C24:0) and nervonic acid (C24:1) concentrations of Jerusalem artichoke herbage at full flowering stage were different from other stages ($P<0.001$) (Tables 4 and 5).

The ∑SFA concentration of Jerusalem artichoke herbage was ranged from about 33 to 42% for different phenological stages of plant (linearly; $P<0.001$ and quadratic; $P=0.001$). The highest ∑UFA (67.43%), ∑MUFA (39.42%), w-9 (34.92%) and w-6/w-3 ratio (25.19) of Jerusalem artichoke herbage was at the early flowering stage. The highest ∑LCFA concentration (95.63%) of Jerusalem artichoke was at vegetation stage. The highest ∑PUFA (33.97%), w-3 (9.22%) and ∑VLCFA (10.97%) concentrations of Jerusalem artichoke herbage were at full flowering stage (Tables 6 and 7).

For phenological stages of Jerusalem artichoke herbage from vegetation to after flowering stage, ∑PUFA (r = 0.829), w-3 (r = 0.571), w-6 (r = 0.649), ∑MCFA (r = 0.891) and ∑VLCFA (r = 0.758) concentrations of plant were positively correlated with plant growing ($P<0.05$). The ∑MUFA (r = -0.711), w-9 (r = -0751) and ∑LCFA (r = -0.775) concentrations of Jerusalem artichoke herbage were negatively correlated with plant growing ($P<0.05$) (Table 8).

Table 2. The composition of C8-C15 fatty acids (as % in total fatty acids) of Jerusalem artichoke (*Helianthus tuberosus*) herbage at different growing stages.

	Caprylic acid	Capric acid	Undecanoic acid	Lauric acid	Tridecanoic acid	Myristic acid	Myristoleic acid	Pentadecanoic acid	*ci*- 10 pentadecenoic acid
	C8:0	C10:0	C11:0	C12:0	C13:0	C14:0	C14:1	C15:0	C15:1
Early vegetation	0.06±0.00^{d}	0.05±0.01^{c}	0.01±0.00	0.11±0.00^{d}	0.03±0.01^{a}	1.04±0.01^{e}	0.17±0.01^{a}	0.21±0.01^{c}	0.06±0.01^{c}
Vegetation	0.05±0.00^{e}	0.08±0.01^{a}	0.01±0.00	0.33±0.00^{c}	0.01±0.00^{c}	1.40±0.01^{c}	0.17±0.01^{a}	0.21±0.01^{c}	0.05±0.00^{d}
Early flowering	0.15±0.01^{a}	0.07±0.00^{ab}	0.003±0.00	0.09±0.01^{e}	0.03±0.00^{a}	1.27±0.01^{d}	0.18±0.01^{a}	0.20±0.01^{c}	0.13±0.01^{a}
Full flowering	0.10±0.00^{b}	0.07±0.01^{ab}	0.01±0.00	0.37±0.01^{b}	0.02±0.00^{b}	2.63±0.01^{a}	0.09±0.01^{c}	0.40±0.01^{a}	0.02±0.01^{e}
After Flowering	0.07±0.01^{c}	0.06±0.00^{bc}	0.01±0.00	0.46±0.00^{a}	0.02±0.00^{b}	1.94±0.01^{b}	0.13±0.01^{b}	0.30±0.01^{b}	0.08±0.01^{b}
SD	0.03	0.01	0.004	0.15	0.01	0.58	0.03	0.08	0.04
SEM	0.01	0.003	0.001	0.03	0.002	0.15	0.01	0.02	0.01
Linear	<0.001	0.496	0.628	<0.001	0.005	<0.001	<0.001	<0.001	<0.001
Quadratic	<0.001	<0.001	0.061	<0.001	0.014	<0.001	<0.001	0.285	<0.001
Cubic	<0.001	0.060	0.242	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

SD: Standard deviation of means, SEM: Standard error of means

[a,e:] Values within a column with different superscripts differ significantly at $P < 0.05$.

Table 3. The composition of C16-C18 fatty acids (as % in total fatty acids) of Jerusalem artichoke (*Helianthus tuberosus*) herbage at different growing stages.

	Palmitic acid	Palmitoleic acid	Heptadecanoic acid	*cis*-10 heptadecanoic acid	Stearic acid	Elaidic acid	Oleic acid	Linolelaidic acid	Linoleic acid	α-Linolenic acid
	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1 n9*t*	C18:1 n9*c*	C18:2 n6*t*	C18:2 n6*c*	C18:3 n3
Early vegetation	23.90±0.03^{c}	3.47±0.01^{b}	0.08±0.01^{b}	0.26±0.001^{d}	7.68±0.01^{b}	0.10±0.01^{b}	31.32±0.01^{b}	0.00±0.00^{c}	23.05±0.02^{b}	0.86±0.01^{c}
Vegetation	26.00±0.04^{ab}	2.96±0.01^{d}	0.06±0.01^{c}	0.32±0.001^{c}	9.66±0.01^{a}	0.10±0.01^{b}	30.13±0.01^{c}	0.01±0.01^{b}	22.16±0.01^{c}	1.02±0.01^{c}
Early flowering	22.21±0.01^{d}	4.25±0.01^{a}	0.31±0.01^{a}	0.05±0.001^{e}	7.37±0.01^{c}	0.16±0.01^{a}	33.73±0.01^{a}	0.02±0.01^{a}	23.01±0.01^{b}	0.44±0.01^{d}
Full flowering	26.22±0.19^{a}	1.88±0.01^{e}	0.02±0.01^{c}	0.45±0.01^{a}	6.13±0.01^{d}	0.15±0.01^{a}	20.45±0.19^{e}	0.01±0.01^{b}	21.43±0.21^{d}	5.99±0.08^{a}
After Flowering	25.62±0.10^{b}	3.33±0.01^{c}	0.03±0.01^{c}	0.41±0.001^{b}	7.71±0.02^{b}	0.14±0.01^{a}	22.29±0.05^{d}	0.01±0.01^{b}	23.64±0.05^{a}	2.12±0.01^{b}
SD	1.58	0.79	0.12	0.16	1.17	0.02	5.42	0.007	0.81	2.10
SEM	0.40	0.20	0.03	0.04	0.30	0.007	1.40	0.001	0.21	0.54
Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.031	0.196	<0.001
Quadratic	<0.001	<0.001	<0.001	<0.001	0.078	0.001	<0.001	<0.001	<0.001	<0.001
Cubic	<0.001	<0.001	0.010	<0.001	<0.001	0.003	<0.001	0.850	<0.001	<0.001

SD: Standard deviation of means, SEM: Standard error of means.

[a,e:] Values within a column with different superscripts differ significantly at $P < 0.05$.

Table 4. The composition of C20-C21 fatty acids (as % in total fatty acids) of Jerusalem artichoke (*Helianthus tuberosus*) herbage at different growing stages.

	Arachidic acid	*cis* 11 eicosenoic acid	*cis*11,14-eicosadienoic acid	*cis* 11,14,17 eicosatrienoic acid	Arachidonic acid	*cis* 5,8,11,14,17 eicosapenta enoic	Heneicosanoic acid
	C20:0	C20:1	C20:2	C20:3 n3	C20:4 n6	C20:5 n3	C21:0
Early vegetation	0.06±0.01	0.75±0.01^{b}	0.04±0.001^{a}	0.65±0.01^{c}	0.30±0.01^{b}	0.20±0.01^{b}	0.19±0.01^{c}
Vegetation	0.01±0.01	0.44±0.01^{c}	0.03±0.001^{a}	0.71±0.02^{b}	0.03±0.01^{d}	0.18±0.01^{b}	0.16±0.01^{d}
Early flowering	0.08±0.03	0.42±0.01^{c}	0.03±0.001^{a}	0.38±0.001^{d}	0.18±0.01^{c}	0.10±0.01^{b}	0.22±0.01^{b}
Full flowering	0.01±0.01	0.92±0.01^{a}	0.01±0.001^{b}	0.15±0.003^{e}	0.42±0.01^{a}	1.03±0.01^{a}	0.31±0.01^{a}
After Flowering	0.02±0.01	0.19±0.01^{d}	0.03±0.01^{a}	1.43±0.001^{a}	0.17±0.01^{c}	0.36±0.09^{b}	0.14±0.01^{e}
SD	0.03	0.27	0.02	0.45	0.14	0.36	0.06
SEM	0.01	0.07	0.003	0.12	0.04	0.09	0.02
Linear	0.077	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	0.709	<0.001	<0.001	<0.001	<0.001	0.220	<0.001
Cubic	0.348	<0.001	0.001	<0.001	<0.001	<0.001	<0.001

SD: Standard deviation of means, SEM: Standard error of means
$^{a,e:}$ Values within a column with different superscripts differ significantly at $P < 0.05$.

Table 5. The composition of C22-C24 fatty acids (as % in total fatty acids) of Jerusalem artichoke (*Helianthus tuberosus*) herbage at different growing stages.

	Erucic acid	Behenic acid	*cis* 13,16 docosadienoic acid	*cis* 4,7,10,13,16,19 docosahexaenoic	Tricosanoic acid	Lignoceric acid	Nervoric acid
	C22:1 n9	C22:0	C22:2	C22:6 n3	C23:0	C24:0	C24:1
Early vegetation	0.69±0.01^{b}	1.66±0.01^{a}	1.17±0.01^{d}	0.42±0.01^{c}	0.30±0.01^{d}	0.93±0.02	0.12±0.01^{b}
Vegetation	0.71±0.01^{b}	0.73±0.01^{d}	0.28±0.01^{e}	0.14±0.01^{d}	0.34±0.01^{c}	1.47±0.02	0.04±0.01^{cd}
Early flowering	0.43±0.01^{c}	0.16±0.003^{e}	3.79±0.02^{b}	0.15±0.01^{d}	0.14±0.01^{e}	0.12±0.01	0.02±0.01^{d}
Full flowering	0.22±0.01^{d}	1.50±0.03^{b}	2.89±0.04^{c}	2.04±0.02^{a}	1.22±0.01^{a}	2.57±0.95	0.22±0.01^{a}
After Flowering	1.52±0.01^{a}	0.96±0.01^{c}	3.95±0.01^{a}	0.58±0.01^{b}	0.69±0.01^{b}	1.52±0.10	0.05±0.01^{c}
SD	0.46	0.56	1.50	0.73	0.40	1.03	0.07
SEM	0.12	0.15	0.38	0.18	0.10	0.26	0.01
Linear	<0.001	<0.001	<0.001	<0.001	<0.001	0.122	<0.001
Quadratic	<0.001	<0.001	<0.001	<0.001	<0.001	0.711	0.030
Cubic	<0.001	<0.001	<0.001	<0.001	<0.001	0.264	<0.001

SD: Standard deviation of means, SEM: Standard error of means
$^{a,e:}$ Values within a column with different superscripts differ significantly at $P < 0.05$.

Table 6. The compositions of total saturated and unsaturated fatty acids (as % in total fatty acids) of Jerusalem artichoke (*Helianthus tuberosus*) at phenological stages.

Phenological stages	∑SFA	∑UFA	∑MUFA	∑PUFA	∑MCFA	∑LCFA	∑VLCFA
Early vegetation	36.33±0.40^{b}	63.66±0.46^{b}	36.95±0.45^{b}	26.71±0.80^{c}	0.23±0.01^{c}	94.23±0.99^{b}	5.49±0.20^{c}
Vegetation	40.48±0.38^{a}	59.51±0.20^{b}	34.93±0.83^{b}	24.58±0.42^{c}	0.46±0.01^{ab}	95.63±0.64^{a}	3.89±0.31^{c}
Early flowering	32.57±0.27^{b}	67.43±0.97^{a}	39.32±0.90^{a}	28.10±0.84^{b}	0.38±0.04^{b}	94.49±0.78^{b}	5.03±0.08^{c}
Full flowering	41.59±0.15^{a}	58.40±1.02^{b}	24.42±0.10^{b}	33.97±1.05^{a}	0.56±0.01^{a}	88.44±0.84^{c}	10.97±0.07^{a}
After Flowering	39.56±0.64^{ab}	60.45±0.81^{b}	28.16±0.23^{b}	32.28±1.10^{b}	0.60±0.02^{a}	89.96±0.49^{c}	9.42±0.49^{b}
SD	3.41	3.41	5.78	3.62	0.13	2.97	2.89
SEM	0.88	0.88	1.49	0.93	0.03	0.76	0.74
Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	0.001	0.001	<0.001	0.001	<0.001	0.009	0.007
Cubic	0.256	0.270	<0.001	<0.001	<0.001	<0.001	<0.001

SD: Standard deviation of means, SEM: Standard error of means, ∑LCFA: Total long chain fatty acids, ∑MCFA: Total medium chain fatty acids, ∑MUFA: Total monounsaturated fatty acids, ∑PUFA: Total polyunsaturated fatty acids, ∑SFA: Total saturated fatty acids, ∑UFA: Total unsaturated fatty acids, ∑VLCFA: Total very long chain fatty acids.
[a,c:] Values within a column with different superscripts differ significantly at $P < 0.05$.

Table 7. The compositions of total omega fatty acids (as % in total fatty acids) of Jerusalem artichoke (*Helianthus tuberosus*) herbage at phenological stages.

Phenological stages	w3	w6	w9	w6/w3
Early vegetation	2.13±0.04^{c}	24.57±0.72^{b}	33.03±0.95^{b}	11.50±0.78^{b}
Vegetation	2.06±0.48^{c}	22.52±0.52^{c}	31.56±0.87^{b}	10.93±1.14^{b}
Early flowering	1.07±0.07^{c}	27.03±0.09^{a}	34.92±1.23^{a}	25.19±0.97^{a}
Full flowering	9.22±0.44^{a}	24.75±0.21^{b}	21.84±0.40^{c}	2.68±0.46^{d}
After Flowering	4.48±0.80^{b}	27.79±0.43^{a}	24.28±0.51^{c}	6.20±0.88^{c}
SD	3.04	1.95	5.30	7.93
SEM	0.78	0.50	1.37	2.04
Linear	<0.001	<0.001	<0.001	<0.001
Quadratic	0.574	<0.001	<0.001	<0.001
Cubic	<0.001	0.007	<0.001	<0.001

SD: Standard deviation of means, SEM: Standard error of means
[a,d:] Values within a column with different superscripts differ significantly at $P < 0.05$.

Table 8. Pearson correlations (*r*) in among fatty acid compositions and phenological stage of Jerusalem artichoke (*Helianthus tuberosus*) herbage.

		∑SFA	∑UFA	∑MUFA	∑PUFA	w3	w6	w9	∑MCFA	∑LCFA	∑VLCFA
Phenological stage	*r*	0.325	-0.323	-0.711	0.829	0.571	0.649	-0.751	0.891	-0.775	0.758
	P value	0.238	0.240	0.003	0.000	0.026	0.009	0.001	0.000	0.001	0.001

∑LCFA: Total long chain fatty acids, ∑MCFA: Total medium chain fatty acids, ∑MUFA: Total monounsaturated fatty acids, ∑PUFA: Total polyunsaturated fatty acids, ∑SFA: Total saturated fatty acids, ∑UFA: Total unsaturated fatty acids, ∑VLCFA: Total very long chain fatty acids.

Discussion and Conclusion

The fatty acids have important biological functions for animals. Fibrous feedstuffs, which must be included in the rations of ruminants (such as cattle, goat, sheep) raised milk production or meat production, are ration components that continuously provide fatty acids for ruminants. Average total fatty acid content in wheat and legume forages used in feeding dairy cattle is in the range of 20-50 g per kg of dry matter. The fatty acid content in forages, which are the cheapest and safest source of fatty acids in ruminant ration, are affected by various different factors such as plant species, plant growing stage, climate, rainfall, soil and fertilization (15). The chemical compositions of in common forage plants change with plant growing stage (16). Ersahince and Kara (11) stated that nutrient composition of Jerusalem artichoke herbage changed by plant maturating. Nutritional quality and quantities of forage in ruminant diet are also not same for different phenological stage of plant. The amount of oil and fatty acid concentration in forage can change depending on plant species, plant growing stage and environmental conditions (9, 10, 21). According to results of the present study, individual fatty acid composition of Jerusalem artichoke herbage, which consist of leaf, stem or bloom/flower, changed depending on the plant growth stage.

The saturated fatty acid in the highest concentration (22-26% in total FA) of Jerusalem artichoke herbage was palmitic acid (C16:0) for all phenological stages in the present study. In a previous study, it was demonstrated that C16:0 concentrations (in total FA) of common forages used in dairy cattle diet were 15-30% for alfalfa (fresh, silage or hay), 16-20% perennial ryegrass (fresh), 14-20% for red clover (fresh, silage or hay), % 16 for white clover (fresh, silage) and 16% for corn silage (10, 12, 25, 26). Palmitic acid concentration of Jerusalem artichoke herbage reached to about 26% in vegetation and full flowering stages can relation with leaf: stem ratio and flower amount of plant. Other high saturated fatty acid of Jerusalem artichoke herbage in the present study was stearic acid (C18:0). The stearic acid concentration of herbage reached to about 9.7% in all fatty acids may connect more leaf amount in vegetation stage in the present study. However, stearic acid concentrations of common legume forages and grasses used in dairy cattle diet were range from about 2 to 4.6% in total fatty acids showed by previous researchers (10, 12, 25, 26). The C18:0 concentration of Jerusalem artichoke herbage in the present study was higher than those of common forages; C16:0 concentration of it was similar to legume forage and higher than that of grass forage (12). Mir et al. (21) stated that the concentrations of saturated C16:0, as weight percentage of the fat, increased in the orchardgrass (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perennne* L.) and tall fescue (*Festuca arundinacea* Schreb.), which used in dairy cattle ration, by increasing plant growth stage. In the present study, ∑SFA, 18:0 and C16:0 fatty acid concentrations of Jerusalem artichoke herbage increased with plant growing stage, expect for early flowering stage. The C14:0, C16:0 and C18:0 concentrations in total FA of Jerusalem artichoke herbage in present study decreased up to full flowering stage of plant were parallel with results of (6).

The oleic acid (C18:1n9c) concentration of Jerusalem artichoke herbage, which was the highest MUFA in plant of the present study, was in about 30-33% in all fatty acids of Jerusalem artichoke herbage up to early flowering stage. The oleic acid and MUFA concentrations in total fatty acid of herbage decreased after lowering stages may relation with plant height, leaf: stem ratio and other environmental conditions. The oleic acid concentrations in fat of orchardgrass (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perennne* L.) and tall fescue (*Festuca arundinacea* Schreb.) were found to be in a minimal amount (about 1.5-5%) and increased with plant growth stage as demonstrated by Mir et al. (21).

The UFA concentration in fatty acids of Jerusalem artichoke herbage increased to about 67% at early flowering stage, which was the highest levels for this plant in the present study,. The concentrations of PUFA, VLCFA and w-3, which are alpha linolenic acid (C18:3n3), EPA (C20:5n3), DHA (C22:6n3), fatty acids of Jerusalem artichoke herbage were found to be in the highest levels in full flowering stage can parallel with flower amount of plant. The alpha linolenic acid (C18:3n3) of Jerusalem artichoke herbage was higher than the value reported for corn silage by Glasser et al. (12). In addition, w6/w3 ratio of Jerusalem artichoke herbage at the full flowering stage decreased to 2.68 value which can be caused by high w-3 concentration in full flowering stage. The MUFA, w-9 and LCFA concentrations in total fatty acids of Jerusalem artichoke herbage lowed with plant growing stage demonstrate rich forage in terms of MUFA (oleic acid) and w-9 fatty acids, which have a positive effect on fertility and embryo implantation in uterus of the ruminant, up to early flowering stage.

Findings of this study shows that the C18:3 fatty acid concentrations of timothy decreased up to early flowering stage stated as reported by a previous study (6). The alpha linolenic acid (C18:3 n3) ratio in total fatty acids of Jerusalem artichoke herbage was increased up to full flowering stage by plant maturating, and then decreased after full flowering stage. In contrast, C18:3 concentrations of some pasture plants decreased with plant maturating by the results of Mir et al. (21). As similar with the results at full flowering and after flowering stages of

the present study, Vanhatalo et al. (26) showed that C18:3 n3 and PUFA concentrations in green herbage harvested early were higher than those of it harvested late.

The dairy cattle diet added with alpha-linolenic acid and rations which are rich in alpha-linolenic acid increases blood progesterone concentration, which is the hormone necessary for the healthy continuation of pregnancy in dairy cattle. Due to this increase, follicular and luteal cells are stimulated, and progesterone synthesis increased (20, 23). Ovarian follicles contain insulin receptors (5) and delay the resumption of postpartum ovarian activity and maintenance of regular estrus cycles in cows with low peripheral insulin levels immediately after calving (27, 28). The high alpha linolenic acid (C18:3, n3) concentration of herbage at full flowering and after flowering stages in the present study show that Jerusalem artichoke herbage at these stages will be a positive effect for the healthy continuation pregnancy in dairy cattle.

In the current study, the high oleic acid concentration of Jerusalem artichoke herbage up to early flowering stage shows that this forage at these stages will be on oocyte quality and fertility in ruminant (1). Aardema et al. (1) investigated the effect of three fatty acids (saturated palmitic and stearic acid and unsaturated oleic acid) on lipid storage and development of oocytes in vitro and showed that palmitic and stearic acid had inhibitory effects on oocyte development, but oleic acid (MUFA) eliminated this adverse effect and showed a positive effect.

As a result of the study, the Jerusalem artichoke herbage, harvested at early flowering stage, was rich from ∑UFA, ∑MUFA and w-9 fatty acids. Besides, the Jerusalem artichoke herbage, harvested at flowering stage, was rich from ∑PUFA, w-3 and ∑VLCFA fatty acids. The Jerusalem artichoke herbage, harvested at these phenological (early flowering and full flowering) stages, demonstrate that it has high functional and nutritional properties for ruminants.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declare that they have no conflict of interest.

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