



Effect of harvesting frequency and maturity stage of Jerusalem artichoke forage on yield, chemical composition and *in vitro* fermentation of the tubers and forage

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Abstract

Aim of study: To evaluate the effect of maturity stage and harvesting frequency of Jerusalem artichoke (JA) forage on the nutritional quality of the tubers and forages.

Area of study: The plant cultivation and laboratory experiments were carried out in Karaj (Alborz, Iran) and Tehran (Tehran, Iran), respectively.

Material and methods: Forages were harvested every 60, 90 and 120 days during the growing season (four, three and two harvests per year, respectively). Tubers were harvested just once, at the end of the growing season, from plots with four, three and two forage cuts per year. Biomass production, chemical composition and *in vitro* ruminal fermentation of the forages and tubers were assessed.

Main results: Compared to 90 and 120 days, the forages harvested every 60 days contained the highest ($p < 0.05$) yearly dry matter (DM) biomass (27.16 t/ha), crude protein (98.6 to 145 g/kg DM), organic matter digestibility (0.607 to 0.691) and microbial biomass production (350 to 369 g/kg DM). Compared to 60 and 90 days, harvesting JA forage every 120 days caused the tubers with the higher ($p < 0.05$) water-soluble carbohydrates (WSC), *in vitro* digestibility and DM yield (7.63 t/ha). Jerusalem artichoke forages and tubers contained the low phenolics (4.93 to 13.2 g/kg DM) and nitrate (1.12 to 3.19 g/kg DM). Overall, the best harvesting interval of JA forage to achieve tubers with the highest yearly yield, WSC and digestibility was every 120 days, while the highest nutritive value and yield of the forages were observed with harvesting JA every 60 days.

Research highlights: The best harvesting interval of JA forage to obtain the highest yearly DM, protein and energy biomass from both tubers and forage was every 60 days.

Additional keywords: aerial biomass; cutting interval; digestibility; *Helianthus tuberosus*; nutritive value; plant maturity.

Abbreviations used: ADF (acid detergent fibre); ADL (acid detergent lignin); B (asymptotic value of gas production); c (first order fractional rate constant of produced gas); CP (crude protein); DM (dry matter); EE (ether extract); GP (gas production); JA (Jerusalem artichoke); MBP (microbial biomass production); ME (metabolisable energy); NDF (neutral detergent fibre); NFC (non-fibre carbohydrates); OMD (organic matter disappearance); PF (partitioning factor); SEM (standard error of means); TDS (truly degraded substrate); TEP (total extractable phenolics); TET (total extractable tannins); THn (tubers from plots with n forage cuts per year); VFA (volatile fatty acids); WSC (water-soluble carbohydrates).

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Introduction

Helianthus tuberosus L. (Jerusalem artichoke; JA), is an economically valuable plant having a hopeful future for feeding animals, especially in low-water areas of the world (Ma *et al.*, 2011; Razmkhah *et al.*, 2017). It can grow well, with the least to zero fertiliser requisites, in low-nutritious and sandy soils. Jerusalem artichoke is resistant to frostiness (further resistant than maize), drought, saline-alkali lands and pests (Ma *et al.*, 2011; Yang *et al.*, 2015). Cultivation of JA could be beneficial in areas where traditional crops (*e.g.*, lucerne and maize) are challenging to grow (Yang *et al.*, 2015; Razmkhah *et al.*, 2017). Moreover, it could be an alternative for soil erosion control (Ma *et al.*, 2011).

It is well established that JA is characterised by its high potential yield, producing between 75 (fresh tubers) and 120 t/ha (fresh forage) (Diederichsen, 2010). From a nutritional point of view, the tubers are rich in soluble-carbohydrates (750 g/kg dry matter; DM) and metabolisable energy (ME; 11.8 to 12.5 MJ/kg DM) (Kosaric *et al.*, 1985; Kays & Nottingham, 2008; Heuzé *et al.*, 2015). However, forage is considered a source of crude protein (CP) containing between 95 and 173 g CP/kg DM (Kays & Nottingham, 2008); digestibility varies between 0.459 and 0.769 (Hay & Offer, 1992; Kays & Nottingham, 2008). Therefore, both parts are considered valuable livestock feed resources without any undesirable effects on ruminant performances and health (Kays & Nottingham, 2008; Papi *et al.*, 2017; Razmkhah *et al.*, 2017). Moreover, studies reported by Kays & Nottingham (2008) and Ma *et al.* (2011) recorded the presence of high contents of health-promoting components such as inulin and fructo-oligosaccharides in this plant.

Conventionally, JA tubers are harvested in late autumn when the carbohydrates transferred from the plant aerial parts to underground tubers (Ma *et al.*, 2011). Moreover, due to the high regrowth capability of shoot, it is possible to harvest JA forage three to four times per year (Izsáki & Kádi, 2013). Variation in yield and chemical composition of JA tubers and forage would be occurred due to different growth stages at harvesting (Hay & Offer, 1992; Izsáki & Kádi, 2013). However, at our best knowledge, there are no studies on the effect of harvesting intervals of JA forage on yield and nutritive value of the tubers. Thus, our main objective in the present study was to evaluate how the harvesting frequency (two, three or four times per year) of JA forage affects yield, chemical composition and *in vitro* ruminal fermentability of the tubers and forage.

Material and methods

Trial site, design, planting and harvesting of JA

The plant cultivation was carried out at the Animal Science Research Institute situated in Karaj city (Iran) at a latitude of 35°56' N, longitude of 50°58' E and altitude of 1312.5 m. The mean annual rainfall and temperature are 250.7 mm and 15.8°C, respectively. The soil is loamy containing 420 g/kg silt, 200 g/kg clay and 380 g/kg sand.

This trial was performed as a randomised complete block design with three replicates. JA tubers of local origin (from Tiran city, Isfahan, Iran) were planted manually (24 February 2016) on the rows (every 20 to 25 cm) at 10 cm depth. The plants were furrow-irrigated to maintain soil under unlimited water supply for maximisation of the plant growth. According to the soil test results of the field, fertiliser was not applied. The soil contained the requested minerals (75 mg NO₃, 68.4 mg NH₄, 15.6 mg available P and 463.6 mg available K per kilogram) for plant growth. Weeds were removed manually during the first weeks after the plant emerging and no pesticides were applied.

After emerging the plants on the soil surface (33 days after planting; 27 March), JA forage was harvested every 60, 90 or 120 days during the growing season (*i.e.*, four, three and two harvests/year, respectively). Thus, there were nine (4+3+2) treatments including the combination of harvesting interval and number. The forages were: 1) forage harvested after 60 days on 25 May (F60H1) at stem elongation stage (BBCH-39); 2) forage harvested after 60 days on 24 July (F60H2) at BBCH-39; 3) forage harvested after 60 days on 22 September (F60H3) at inflorescence emergence (BBCH-51); 4) forage harvested after 60 days on 20 November (F60H4) at BBCH-39; 5) forage harvested after 90 days on 25 June (F90H1) at inflorescence emergence (BBCH-53); 6) forage harvested after 90 days on 23 September (F90H2) at flowering (BBCH-63); 7) forage harvested after 90 days on 21 December (F90H3) at BBCH-39; 8) forage harvested after 120 days on 24 July (F120H1) at flowering (BBCH-69); and 9) forage harvested after 120 days on 20 November (F120H2) at BBCH-63, according to the phenological development stages of *Helianthus* described by Lancashire *et al.* (1991). The forages were cut with a knife about five cm above the ground level.

The tubers were harvested just once, at the end of the growing season (late autumn), from nine plots; *i.e.*, three

replicates by three treatments consist of four (TH4), three (TH3) and two (TH2) forage harvests per year.

Biomass production and chemical analysis

After each harvesting, the forage issued from the different experimental units was transported to the laboratory where it was weighed and oven-dried at 60°C (48 h) to determine the DM content (%) and biomass production per hectare. Moreover, DM percentage and biomass production were determined for the tubers. After recording the fresh yield, DM yields of the forages and tubers were calculated as the forage or tuber DM percentage \times the fresh biomass yield/ha. Similarly, the CP (t/ha) and ME (MJ/ha) yields were calculated.

Samples of the forages and tubers were ground in a hammer mill (Swedesboro, USA) using a 1-mm sieve. Ash, ether extract (EE) and CP were measured following the methods of the AOAC (2012), whereas neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991). An anthrone method was used to measure concentration of water-soluble carbohydrates (WSC) in the samples (AFIA, 2014). Non-fibre carbohydrates (NFC) content was obtained by subtracting the sum of four compounds (CP, NDF, EE and ash, as g/kg DM) from 1000 (NRC, 2001).

The method proposed by Singh (1988) was followed to determine nitrate concentration in tubers and forages of JA: 100 mg of DM was gently mixed with 50 mL of acetic acid (2%) for 20 min and filtered through filter paper; nitrate concentration was measured colourimetrically at 540 nm. Phenolic compounds of the forages and tubers were extracted with acetone solution. Total extractable phenolics (TEP) concentration was measured by the

Folin-Ciocalteu reagent and tannic acid as the standard. Non-tannin phenolics (NTP) level was measured after reaction of the extract with insoluble polyvinylpyrrolidone and Folin-Ciocalteu. Finally, the content of total extractable tannins (TET) was calculated as $TET = TEP - NTP$ (Makkar, 2003).

In vitro fermentation of forages and tubers

Animals and rumen fluid extraction

Three mature Shal sheep (BW of 59.3 ± 2.68) fitted with a permanent ruminal cannula were used for the extraction of rumen fluid to carry out the *in vitro* incubations of JA forages and tubers. Animals were fed with 873 g DM of the balanced diet (Table 1) twice per day and had free access to freshwater. The FASS (2010) guidelines were followed, and the Animal Science Committee of Tarbiat Modares University confirmed (No. 9330381006; 1394-10-15) all the procedures. A sample of rumen contents was withdrawn before the morning feeding, transferred to the thermos flasks and taken immediately to the laboratory. Rumen fluid from the sheep was mixed, strained through various layers of cheesecloth and kept at 39°C under a CO₂ atmosphere.

In vitro 24-h gas production (GP) experiment

Gas production profiles were obtained using an adaptation of the technique described by Menke *et al.* (1979). Ground samples (200 mg DM) of the forages and tubers were placed in the 100-mL Hohenheim glass syringes. Silicone tube was put on the capillary mouthpiece, and the tube was fitted with a clip to close the syringe. The syringe piston was greased with some vaseline to ease

Table 1. Ingredients and chemical composition (g/kg DM or as stated) of the diet.

Ingredients		Chemical composition	
Lucerne	450	Crude protein	141
Wheat straw	50	Neutral detergent fibre	361
Jerusalem Artichoke forage	50	Acid detergent fibre	224
Jerusalem Artichoke tubers	50	Ether extract	24.3
Soybean meal	60	Ash	8.25
Barley grain	180	Ca	7.76
Wheat bran	150	P	3.91
Premix ^[1]	10	Metabolisable energy (MJ/kg DM)	9.90

^[1]Containing (/kg) 60 g Ca, 30 g P, 20 g Mg, 3 mg Se, 2.5 mg Co, 80 mg Mn, 25 mg I, 40 mg Cu, 900 mg Zn, 400,000 IU vitamin A, 100,000 IU vitamin D3 and 8,000 IU vitamin E.

movement and prevent escape of the gas. The samples were incubated in 30 mL of diluted rumen fluid (10 mL of the mixed rumen fluid + 20 mL of the buffered medium) in the syringes under a CO₂ atmosphere. Three syringes containing only diluted rumen fluid inoculum were used as blanks to correct for GP. After pumping the diluted rumen fluid into each syringe, the plastic clip on the silicon tube was closed, and the syringe was shaken. The piston position at the beginning of incubation was read, and the syringe was placed in the incubator at 39°C. The gas volume was recorded by reading the piston position after 24-h incubation.

Regarding forages, incubation of 222 syringes was done in a split-plot, including nine treatments (main plot), three replicates, two samples per replicate, two syringes per sample, two separate runs (subplot), conducted in different weeks, and three blanks in each run. For tubers, incubation of 78 syringes was performed, including three blanks, three treatments, three replicates, two samples, two syringes per sample and two runs conducted in different weeks.

Gas produced and estimated variables

Gas production at the 24-h incubation (GP₂₄) was defined as the total gas in the syringe with substrate minus gas produced in blank syringes. Then, organic matter disappearance (OMD) and ME were estimated using the equations proposed by Menke *et al.* (1979):

$$\begin{aligned} \text{OMD (\%)} &= 14.88 + (0.8893 \times \text{GP}_{24}) + \\ &\quad (0.448 \times \text{CP}) + (0.651 \times \text{XA}) \\ \text{ME} &= 2.20 + (0.1357 \times \text{GP}_{24}) + \\ &\quad (0.057 \times \text{CP}) + (0.002859 \times \text{EE}^2) \end{aligned}$$

In these equations, GP₂₄ is 24-h net gas production (mL/200 mg DM), CP is crude protein (%), XA is ash (%), EE is ether extract (%), and ME is metabolisable energy (MJ/kg DM).

Twelve syringes per forage or tubers (three replicates × two samples × one syringe per sample × two runs) were used for evaluating truly degraded substrate (TDS). The entire contents of each syringe were centrifuged (at 20,000 ×g for 30 min), and the supernatant was removed. The pellet remained after centrifugation was boiled (for 1 h) with neutral detergent solution, and the contents were filtered. The residues were washed, dried at 60°C and weighed. The amount of TDS (g/kg DM) was estimated as [(the initial mass incubated – the residues weight) / the initial mass × 1000] (Blümmel *et al.*, 1997b).

Microbial biomass production (MBP; mg/g DM) was calculated as mg TDS – (mL GP₂₄ × 2.2) (Blümmel *et al.*, 1997a). Partitioning factor after 24-h incubation (PF₂₄; an indicator of fermentation effectiveness) of each sample

was estimated as mg TDS / mL GP₂₄ (Blümmel *et al.*, 1997b).

In vitro ruminal pH, ammonia-N, volatile fatty acids (VFA) and protozoa

These variables were measured in the contents of 12 syringes per forage or tubers (three replicates × two samples × one syringe per sample × two runs). After ending the 24-h incubation, pH was measured using the Sartorius PT-10 pH meter (Sartorius AG, Germany). A portion (5 mL) of the syringe contents was preserved (–20°C) in combination with 0.1 mL of H₂SO₄ (50%) for VFA analysis. For ammonia-N assay, 2.5 mL of the syringe contents was mixed with 0.5 mL of HCl (0.2 N) and kept frozen at –20°C. Later, the contents were analysed for VFA using the UNICAM 4600 gas chromatograph (SB Analytical, UK) equipped with a capillary column (10 m × 0.535 mm × 1.00 μm, 19095F-121; Agilent Technologies, CA), with 2-ethyl butyric acid as the internal standard (Galyean, 2010). The phenol-hypochlorite method (Galyean, 2010) was followed to determine ammonia-N concentration.

For protozoa enumeration, a 5-mL sample of each syringe contents was mixed with 5 mL of formalin solution (50%). Then, total ciliate protozoa were counted using a Haemocytometer (Marienfeld, Germany) and a light microscope (Dehority, 2003).

In vitro 96 and 120-h gas production experiments

For evaluating GP kinetics of the forages and tubers, the *in vitro* 96 and 120-h GP experiments were conducted by the method proposed by Menke *et al.* (1979), as described above. Gas production was recorded at incubation times of 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h for the forages, and 2, 4, 6, 8, 12, 24, 48 and 72 h for the tubers. The kinetic variables, including *B* and *c*, were predicted using the model $y = B(1 - e^{-ct})$, as described by Blümmel *et al.* (2003). In this model, *y*, *B* and *c* are gas volume (mL) at time *t*, asymptotic value of GP (mL/200 mg sample DM) and first-order fractional rate constant of GP (/h), respectively. Estimation of *B* and *c* was done using the Proc NLIN of SAS (vers. 9.1; SAS Inst Inc, Cary, NC, USA).

Statistical analyses

Data were analysed using the PROC GLM of SAS (vers. 9.1; SAS Inst., Cary, NC, USA). Data of chemical composition of the samples were analysed in a randomised complete block design (nine and three treatments for the forage and tubers, respectively, × three replicates ×

two samples). The model was $Y_{ijk} = \mu + H_i + B_j + e_{ij} + e_{ijk}$, in which Y_{ijk} is general observation, μ is overall mean, H_i is treatment effect, B_j is block effect, e_{ij} is experimental error, and e_{ijk} is sampling error.

A split-plot in a randomised complete block design (nine and three treatments for forage and tubers, respectively, \times three replicates \times two samples \times two runs) was used to analyse the *in vitro* GP data. Treatment was considered as the main plot and run as the subplot. The model was $Y_{ijkl} = \mu + T_i + B_j + e_{ij} + R_k + (TR)_{ik} + e_{ijk} + e_{ijkl}$, in which Y_{ijkl} , μ , T_i , B_j , e_{ij} , R_k , $(TR)_{ik}$, e_{ijk} and e_{ijkl} are general observation, overall mean, treatment effect, block effect, main plot error (treatment \times block), run effect, treatment \times run, split-plot error (treatment \times block \times run) and sampling error (treatment \times block \times run \times sample), respectively.

The presence or absence of differences among the treatments were examined using LSMEANS with PDIF option adjusted by Bonferroni. The statistical significance of the means was defined by $p \leq 0.05$.

Results

Forage and tuber biomass

Data on the yields of JA forages and tubers are presented in Table 2. The highest dry forage yield/ha was recorded in the forages harvested at F90H1, F90H2 and F120H1 (10.68, 11.57 and 12.72 t/ha, respectively), and the lowest in those collected at F60H4 and F90H3 (2.89 and 2.92 t/ha, respectively) ($p=0.011$). The forages harvested at F90H2 and F120H1 contained the highest CP and ME yields, while the lowest yields of CP and ME were observed in those collected at F60H4 and F90H3 ($p<0.05$). Regarding tuber yield/ha, decreasing the harvest frequency of JA forage had a positive influence on the fresh material, DM, CP and ME yields of the tubers ($p<0.05$). The highest tuber yields of DM, CP and ME (7.36 t, 0.839 t and 92.74×10^3 MJ/ha, respectively) were recorded in those harvested at TH2, and the lowest

Table 2. Effect of harvesting interval and maturity stage of Jerusalem artichoke forage on yields of fresh material, dry matter, crude protein (t/ha) and metabolisable energy ($\times 10^3$ MJ/ha) of the forage and tubers.

Harvesting interval	60 days				90 days			120 days		SEM ^[1]	p-value
	F60H1	F60H2	F60H3	F60H4	F90H1	F90H2	F90H3	F120H1	F120H2		
Forage^[2]											
FW ^[3]	45.84 ^a	45.97 ^a	43.87 ^a	17.96 ^b	45.86 ^a	46.10 ^a	18.35 ^b	47.45 ^a	45.36 ^a	2.03	0.009
DM	6.69 ^c	8.50 ^{de}	9.08 ^{cd}	2.89 ^f	10.68 ^{abc}	11.57 ^{ab}	2.92 ^f	12.72 ^a	7.80 ^{de}	0.637	0.011
CP	0.970 ^b	1.16 ^{ab}	1.11 ^{ab}	0.285 ^d	1.10 ^{ab}	1.33 ^a	0.270 ^d	1.25 ^a	0.743 ^c	0.076	0.009
ME	62.69 ^{de}	78.29 ^c	76.54 ^{cd}	22.60 ^f	88.54 ^{abc}	97.19 ^{ab}	22.45 ^f	101.6 ^a	60.68 ^e	4.31	0.008
Tuber^[4]		TH4			TH3		TH2				
FW		22.97 ^b			24.07 ^b		28.99 ^a			0.864	0.037
DM		4.87 ^c			5.70 ^b		7.36 ^a			0.273	0.012
CP		0.516 ^b			0.581 ^b		0.839 ^a			0.060	0.023
ME		59.41 ^b			68.97 ^b		92.74 ^a			4.58	0.018
Total (Forage + Tubers)											
FW		176.6 ^a			134.4 ^b		121.8 ^b			6.76	0.016
DM		32.03 ^a			30.87 ^a		26.88 ^b			0.901	0.025
CP		4.08 ^a			3.30 ^b		2.81 ^b			0.183	0.019
ME		399.9 ^a			277.0 ^b		254.9 ^b			16.02	0.009

^[1]SEM, standard error of the means. ^[2]F60H1, forage harvested after 60 days on June (stem elongation); F60H2, forage harvested after 60 days on August (elongation); F60H3, forage harvested after 60 days on October (inflorescence emergence); F60H4, forage harvested after 60 days on November (elongation); F90H1, forage harvested after 90 days on July (inflorescence); F90H2, forage harvested after 90 days on October (flowering). F90H3, forage harvested after 90 days on December (elongation); F120H1, forage harvested after 120 days on July (flowering); F120H2, forage harvested after 120 days on November (flowering). ^[3]FW, fresh weight; DM, dry matter; CP, crude protein; ME, metabolisable energy. ^[4]TH4, tubers from plots with four forage cuts per year; TH3, tubers from plots with three forage cuts per year; TH2, tubers from plots with two forage cuts per year. Means in the same row with different superscripts differ ($p \leq 0.05$).

Table 3. Impact of harvesting interval and maturity stage of Jerusalem artichoke forage on chemical composition (g/kg DM or as stated) of the forage and tubers.

Harvesting interval	60 days				90 days			120 days		SEM ^[1]	<i>p</i> -value
	Forage ^[2]	F60H1	F60H2	F60H3	F60H4	F90H1	F90H2	F90H3	F120H1		
DM ^[3] (g/kg FW)	146 ^c	185 ^C	207 ^b	161 ^{de}	233 ^a	251 ^a	159 ^{de}	247 ^a	172 ^{cd}	6.34	0.010
CP	145 ^a	137 ^a	122 ^b	98.6 ^d	103 ^{cd}	115 ^{bc}	92.5 ^d	98.6 ^d	95.3 ^d	4.08	0.013
NDF	340 ^d	350 ^d	401 ^c	427 ^b	385 ^c	444 ^{ab}	458 ^a	449 ^{ab}	467 ^a	7.16	0.008
ADF	255 ^e	268 ^{de}	297 ^c	319 ^b	276 ^d	325 ^b	333 ^{ab}	330 ^{ab}	347 ^a	5.22	0.008
ADL	59.6 ^c	73.0 ^c	91.6 ^b	99.2 ^{ab}	89.7 ^b	97.2 ^{ab}	105 ^a	99.9 ^{ab}	110 ^a	4.12	0.012
NFC	345 ^{ab}	330 ^{bc}	333 ^{bc}	302 ^{de}	358 ^a	308 ^{de}	308 ^{de}	317 ^{cd}	299 ^e	5.20	0.011
WSC	107 ^{ab}	102 ^{bc}	121 ^a	68.4 ^{de}	102 ^{bc}	88.5 ^c	48.7 ^f	80.4 ^{cd}	56.5 ^{ef}	5.11	0.010
EE	14.3	18.7	17.6	17.5	16. ⁶	15.5	16.4	17.1	16.7	2.53	0.33
Ash	156 ^a	164 ^a	126 ^{bc}	155 ^a	137 ^b	118 ^c	125 ^{bc}	118 ^c	122 ^{bc}	5.12	0.015
TEP	7.91 ^e	7.68 ^e	8.01 ^{de}	7.56 ^e	10.1 ^{cd}	10.9 ^{bc}	9.57 ^{cde}	12.8 ^{ab}	13.2 ^a	0.679	0.017
TET	3.86 ^f	4.91 ^{de}	5.80 ^d	4.24 ^{ef}	6.69 ^c	7.34 ^{bc}	6.60 ^c	8.19 ^a	7.99 ^{ab}	0.235	0.008
Nitrate	2.52 ^{bc}	3.19 ^a	1.43 ^{ef}	2.76 ^{ab}	1.83 ^{de}	2.09 ^{cd}	2.54 ^{bc}	1.12 ^f	1.35 ^f	0.174	0.012

Tuber ^[4]	TH4	TH3	TH2	SEM	<i>p</i> -value
	DM ^[3] (g/kg FW)	212 ^b	237 ^a		
CP	106	102	114	7.93	0.25
NDF	93.2	94.2	81.2	7.40	0.44
ADF	63.3	64.0	53.2	9.30	0.61
ADL	18.8	16.2	15.3	1.59	0.27
NFC	729	740	741	11.8	0.76
WSC	550 ^b	544 ^b	591 ^a	10.2	0.035
EE	13.2	13.4	11.8	0.864	0.21
Ash	58.9	50.1	51.8	3.72	0.21
TEP	5.39	5.23	4.93	0.492	0.61
TET	4.07	3.90	3.61	0.353	0.49
Nitrate	1.74	1.97	1.79	0.167	0.46

^[1]SEM, standard error of the means. ^[2]F60H1, forage harvested after 60 days on June (stem elongation); F60H2, forage harvested after 60 days on August (elongation); F60H3, forage harvested after 60 days on October (inflorescence emergence); F60H4, forage harvested after 60 days on November (elongation); F90H1, forage harvested after 90 days on July (inflorescence); F90H2, forage harvested after 90 days on October (flowering). F90H3, forage harvested after 90 days on December (elongation); F120H1, forage harvested after 120 days on July (flowering); F120H2, forage harvested after 120 days on November (flowering). ^[3]DM, dry matter; FW, fresh weight; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent fibre; NFC, non-fibre carbohydrates; WSC, water soluble carbohydrates; EE, ether extract; TEP, total extractable phenolics; TET, total extractable tannins. ^[4]TH4, tubers from plots with four forage cuts per year; TH3, tubers from plots with three forage cuts per year; TH2, tubers from plots with two forage cuts per year. Means in the same row with different superscripts differ ($p \leq 0.05$).

(4.87 t, 0.516 t and 59.41×10^3 MJ/ha, respectively) in the tubers collected at TH4. Total yearly yields (forage + tuber) of DM, CP and ME reached their highest values (32.03 t, 4.08 t and 399.9×10^3 MJ/ha, respectively) with harvesting JA forage every 60 days ($p < 0.05$).

Chemical composition

Chemical compositions of the forages and tubers are presented in Table 3. The forage DM ranged between

146 and 251 g/kg fresh weight for the forages harvested at F60H1 and F90H2, respectively ($p = 0.010$). The highest tuber DM content was recorded in the tubers harvested at TH2, and the lowest in those collected at TH4 ($p = 0.030$).

The forage maturity had adverse effect on its CP concentration so that the forages harvested after 90 and 120 days and F60H4 had the lower ($p = 0.013$) CP concentrations (92.5 to 98.6 g/kg DM) compared to those collected at F60H1, F60H2 and F60H3 (122 to 145 g/kg DM). Advancing the maturity stage increased ($p < 0.05$)

Table 4. Impact of harvesting interval and maturity stage of Jerusalem artichoke forage on *in vitro* gas production and estimated variables of the forage and tubers.

Harvesting interval	60 days				90 days			120 days		SEM ^[1]	p-value
	F60H1	F60H2	F60H3	F60H4	F90H1	F90H2	F90H3	F120H1	F120H2		
Forage^[2]											
GP ₂₄ ^[3] (mL/200 mg DM)	42.3 ^a	42.0 ^a	37.7 ^b	35.3 ^b	38.3 ^{ab}	38.0 ^{ab}	34.8 ^b	36.5 ^b	35.2 ^b	1.39	0.027
OMD	0.691 ^a	0.691 ^a	0.620 ^b	0.607 ^{bc}	0.625 ^b	0.616 ^b	0.581 ^c	0.594 ^{bc}	0.584 ^c	0.009	0.012
ME (MJ/kg DM)	9.37 ^a	9.21 ^a	8.43 ^b	7.82 ^{bcd}	8.29 ^{bcd}	8.40 ^{bc}	7.69 ^d	7.99 ^{bcd}	7.78 ^{cd}	0.195	0.018
TDS (g/kg DM)	835 ^a	813 ^a	775 ^b	737 ^{cd}	759 ^{bc}	750 ^{bc}	717 ^d	725 ^{cd}	710 ^d	9.23	0.011
MBP (g/kg DM)	369 ^a	351 ^{ab}	361 ^a	350 ^{ab}	337 ^{bc}	332 ^{bc}	334 ^{bc}	324 ^c	323 ^c	5.84	0.016
PF ₂₄ (mg TDS/mL GP ₂₄)	3.96	3.88	4.12	4.19	3.96	3.95	4.12	3.97	4.03	0.156	0.25
B (mL/200 mg DM)	51.8 ^a	50.1 ^{ab}	45.6 ^c	44.5 ^c	47.2 ^{bc}	46.0 ^c	44.1 ^c	45.5 ^c	43.9 ^c	1.22	0.026
c (h)	0.077	0.081	0.071	0.072	0.077	0.077	0.070	0.074	0.069	0.005	0.24
Tuber^[4]		TH4			TH3		TH2				
GP ₂₄ ^[3] (mL/200 mg DM)		66.7 ^b			66.7 ^b		69.3 ^a			0.727	0.043
OMD		0.828 ^b			0.820 ^b		0.850 ^a			0.005	0.029
ME (MJ/kg DM)		12.2 ^b			12.1 ^b		12.6 ^a			0.108	0.028
TDS (g/kg DM)		858 ^b			867 ^b		887 ^a			6.22	0.032
MBP (g/kg DM)		125			133		124			5.17	0.38
PF ₂₄ (mg TDS/mL GP ₂₄)		2.58			2.60		2.56			0.053	0.75
B (mL/200 mg DM)		73.5			72.9		75.0			0.832	0.21
c (h)		0.136			0.144		0.149			0.004	0.23

^[1]SEM, standard error of the means. ^[2]F60H1, forage harvested after 60 days on June (stem elongation); F60H2, forage harvested after 60 days on August (elongation); F60H3, forage harvested after 60 days on October (inflorescence emergence); F60H4, forage harvested after 60 days on November (elongation); F90H1, forage harvested after 90 days on July (inflorescence); F90H2, forage harvested after 90 days on October (flowering). F90H3, forage harvested after 90 days on December (elongation); F120H1, forage harvested after 120 days on July (flowering); F120H2, forage harvested after 120 days on November (flowering). ^[3]GP₂₄, *in vitro* gas production at 24 h; OMD, organic matter disappearance; ME, metabolizable energy; TDS, truly degraded substrate; MBP, microbial biomass production; PF₂₄, partitioning factor at 24 h of incubation; B, the asymptotic value of gas production; c, the first order fractional rate constant of gas production. ^[4]TH4, tubers from plots with four forage cuts per year; TH3, tubers from plots with three forage cuts per year; TH2, tubers from plots with two forage cuts per year. Means in the same row with different superscripts differ ($p \leq 0.05$).

the forage NDF, ADF and ADL concentrations by 37.4, 36.1 and 84.6%, respectively. However, the CP, NDF, ADF and ADL concentrations (102 to 114, 81.2 to 94.2, 53.2 to 64.0 and 15.3 to 18.8 g/kg DM, respectively) in the tubers were not changed ($p > 0.05$) by harvesting frequency of JA forage.

Jerusalem artichoke forages contained 48.7 to 121 g WSC/kg DM, with the lowest value in the forage harvested at F90H3 and the highest in that harvested at F60H3. The maturity stage of JA had a decreasing effect on the forage WSC. The WSC concentrations in the forages collected at F60H1, F60H2, F60H3 and F90H1 (102 to 120 g/kg DM) were higher ($p = 0.010$) than those harvested at F60H4, F90H3 and F120H2 (48.7 to 68.4 g/kg DM). Decreasing the harvest frequency of JA forage increased ($p = 0.035$) the WSC concentration in the tubers so that the highest WSC observed in the tubers harvested at TH2 (591 g/kg DM).

Advancing JA maturity decreased ($p = 0.015$) the forage ash concentration. The forages harvested after 60 days had the highest ash (126 to 164 g/kg DM), those collected after 90 days were intermediate (118 to 137 g/kg DM), and those harvested after 120 days had the lowest concentrations (118 to 122 g/kg DM). The ash content of JA tubers (50.1 to 58.9 g/kg DM) was lower than the forages, and it was not affected ($p = 0.21$) by harvesting frequency of the forage.

The concentrations of TEP and TET in JA forages were 7.56 to 13.2 and 3.86 to 8.19 g/kg DM, respectively. The highest TEP and TET were recorded in the more mature forages harvested at F120H1 and F120H2 (TEP of 12.8 to 13.2 and TET of 7.99 to 8.19 g/kg DM, respectively), and the lowest in those harvested after 60 days (TEP of 7.56 to 8.01 and TET of 3.86 to 5.80 g/kg DM, respectively) ($p < 0.05$). The nitrate concentration in JA forages (1.12 to 3.19 g/kg DM) decreased ($p = 0.012$)

Table 5. Impact of harvesting interval and maturity stage of Jerusalem artichoke forage on *in vitro* ruminal fermentation variables of the forage and tubers.

Harvesting interval	60 days				90 days			120 days		SEM ^[1]	p-value
	F60H1	F60H2	F60H3	F60H4	F90H1	F90H2	F90H3	F120H1	F120H2		
Forage ^[2]											
pH	6.91	6.84	7.04	6.85	6.86	6.78	6.91	6.77	6.80	0.096	0.28
Ammonia-N (mg/dL)	8.81	7.91	8.45	7.85	7.82	8.71	7.90	7.57	7.42	0.612	0.29
Total VFA ^[3] (mmol/L)	53.8 ^{ab}	54.2 ^a	48.5 ^{abc}	47.7 ^c	50.7 ^{abc}	48.9 ^{abc}	48.0 ^{bc}	48.1 ^{bc}	47.6 ^c	1.80	0.039
Acetate (%)	71.96	73.69	72.92	73.33	72.59	71.67	72.2	73.61	73.31	1.68	0.52
Propionate (%)	18.07	16.82	17.36	16.81	17.43	17.48	17.34	16.88	16.60	0.696	0.31
Butyrate (%)	7.68	7.27	7.60	7.59	7.80	8.87	7.93	7.41	8.01	0.647	0.16
Isobutyrate (%)	0.660	0.775	0.788	0.735	0.711	0.601	0.791	0.681	0.702	0.083	0.15
Valerate (%)	0.418	0.309	0.341	0.325	0.374	0.410	0.418	0.426	0.397	0.054	0.19
Isovalerate (%)	1.21	1.14	0.992	1.21	1.10	0.978	1.32	0.996	0.977	0.206	0.35
Total protozoa ($\times 10^5$ /mL)	7.06 ^a	7.24 ^a	5.18 ^b	4.84 ^b	4.88 ^b	4.91 ^b	4.91 ^b	5.10 ^b	4.86 ^b	0.252	0.014
Tuber ^[4]		TH4			TH3			TH2			
pH		6.66			6.72			6.70		0.062	0.67
Ammonia-N (mg/dL)		8.25			8.07			7.77		1.07	0.95
Total VFA ^[3] (mmol/L)		70.6 ^b			73.1 ^{ab}			77.5 ^a		2.09	0.038
Acetate (%)		58.07			56.66			57.75		1.32	0.58
Propionate (%)		19.99			21.70			21.78		0.580	0.12
Butyrate (%)		18.90			18.93			17.97		1.01	0.66
Isobutyrate (%)		0.892			0.874			0.840		0.086	0.78
Valerate (%)		0.698			0.696			0.650		0.090	0.80
Isovalerate (%)		1.45			1.14			1.01		0.373	0.51
Total protozoa ($\times 10^5$ /mL)		7.08			6.72			6.81		0.498	0.77

^[1]SEM, standard error of the means. ^[2]F60H1, forage harvested after 60 days on June (stem elongation); F60H2, forage harvested after 60 days on August (elongation); F60H3, forage harvested after 60 days on October (inflorescence emergence); F60H4, forage harvested after 60 days on November (elongation); F90H1, forage harvested after 90 days on July (inflorescence); F90H2, forage harvested after 90 days on October (flowering). F90H3, forage harvested after 90 days on December (elongation); F120H1, forage harvested after 120 days on July (flowering); F120H2, forage harvested after 120 days on November (flowering). ^[3]VFA, volatile fatty acid. ^[4]TH4, tubers from plots with four forage cuts per year; TH3, tubers from plots with three forage cuts per year; TH2, tubers from plots with two forage cuts per year. Means in the same row with different superscripts differ ($p \leq 0.05$).

with plant maturity so that the lowest nitrate was observed in the forage harvested at F120H1. The tubers TEP, TET and nitrate were not affected ($p > 0.05$) by harvesting frequency of the forage.

In vitro GP and fermentation variables

Increasing JA maturity decreased ($p < 0.05$) the *in vitro* GP₂₄, OMD, ME, TDS, MBP and 'B' in the forages (Table 4). The highest GP₂₄ (42.0 to 42.3 mL/200 mg DM), OMD (0.691), ME (9.21 to 9.37 MJ/kg DM), TDS (813 to 835 g/kg DM) and 'B' (50.1 to 51.8 mL/200 mg DM) were recorded in the forages harvested at F60H1 and F60H2, while the lowest in those collected at F90H3 and F120H2 (34.8 to 35.2 mL/200 mg DM, 0.581 to

0.584, 7.69 to 7.78 MJ/kg DM, 710 to 717 g/kg DM and 43.9 to 44.1 mL/200 mg DM, respectively). The PF₂₄ and 'c' remained unchanged ($p > 0.05$) with harvesting JA forages in different maturity stages. Compared to the forages, the higher GP₂₄ (66.7 to 69.3 mL/200 mg DM), OMD (0.820 to 0.850), ME (12.1 to 12.6 MJ/kg DM) and TDS (858 to 887 g/kg DM) were recorded in JA tubers. The GP₂₄, OMD, ME and TDS of the tubers increased ($p < 0.05$) with decreasing the harvest frequency of the forage. However, MBP, PF₂₄, 'B' and 'c' were not different among the tubers ($p > 0.05$).

The *in vitro* ruminal pH (6.77 to 7.04) and ammonia-N (7.42 to 8.81 mg/dL) were not changed ($p > 0.05$) by the maturity stage of JA forage (Table 5). The highest *in vitro* total VFA concentrations were recorded in the forages harvested at F60H1 and F60H2 (53.8 to 54.2

mmol/L), while the lowest in those collected at F60H4 and F120H2 (47.6 to 47.7 mmol/L) ($p=0.039$). The higher ($p=0.014$) *in vitro* ruminal protozoa count was observed in the forages collected at F60H1 and F60H2 (7.06 to $7.24 \times 10^5/\text{mL}$), compared to those harvested at F60H3, F60H4, F90H1, F90H2, F90H3, F120H1 and F120H2 (4.84 to 5.18 mmol/L). Decreasing the harvest frequency of JA forage improved ($p=0.038$) the *in vitro* total ruminal VFA concentration of the tubers. However, the pH, ammonia-N, individual VFA proportions and protozoa were not affected ($p>0.05$).

Discussion

Chemical composition

The increased forage DM concentration as JA advanced in maturity could be related to the better photosynthetic capability caused by the higher Rubisco (ribulose-1, 5-bisphosphate carboxylase/oxygenase) activity, which increases carbon dioxide assimilation and thus DM storage in the plant aerial parts (Sarmadi *et al.*, 2016). Among the forages, the lowest DM concentration was observed in those harvested in early spring (F60H1) and late autumn (F60H4, F90H3 and F120H2) because adequate heat and sunlight for best maturity and DM storage are not available (McDonald *et al.*, 2011). On the other hand, the increased DM concentration in the tubers with decreasing the harvest frequency of JA forage may be due to the further transferring of nutrients from the plant aerial parts to the tubers and the lower nutrient utilisation to support forage regrowth after harvest. In confirmation, Kays & Nottingham (2008) reported that longer persistence of optimum leaf area enables a superior assimilation of DM in the tubers.

The negative effect of JA maturity on the forage CP concentration could be attributed to the increased stem to leaf ratio because the plant stems contain lower CP compared to the leaves. Another reason is the decrease in the leaves CP with maturity (Kays & Nottingham, 2008). Among the forages, the lowest CP was observed in those harvested in late autumn because unsuitable climatic conditions, such as insufficient heat and sunlight, decrease nitrogen accumulation in plants (Hopkins & Hüner, 2008). Others also reported different CP concentrations (41.3 to 167 g/kg DM) in JA forages (Karsli & Bingöl, 2009; Ma *et al.*, 2011; Razmkhah *et al.*, 2017) due to the maturity stage, climate, season, soil and management (Hopkins & Hüner, 2008; McDonald *et al.*, 2011). On the other hand, the tuber CP concentrations in the present study were similar to those reported by Kays & Nottingham (2008). It is worth noting that the CP concentration in JA forages

and tubers was more than 70 g/kg DM, which provides sufficient ruminal ammonia to optimum growth of the rumen microorganism (Sampaio *et al.*, 2010).

The increases of NDF, ADF and ADL concentrations in the forage as JA advanced in maturity could be attributed to the decrease in the leaf to stem ratio and a higher requirement for lignified tissues for providing structural strength to the plant during growth (McDonald *et al.*, 2011). Furthermore, variable ecological factors (*e.g.*, temperature, light and moisture) during the growing season, affecting uptake and distribution of nutrients in plants, could change the cell wall concentration in JA forages harvested at the different seasons (Hopkins & Hüner, 2008; Jouanin & Lapierre, 2012). Other researchers reported different concentrations of NDF (233 to 392 g/kg DM), ADF (216 to 299 g/kg DM) and ADL (48 to 72 g/kg DM) in JA forages harvested at different developmental stages (Hay & Offer, 1992; Karsli & Bingöl, 2009; Papi *et al.*, 2017). Compared to the forages, JA tubers contained the lower NDF, ADF and ADL concentrations, confirming the results (NDF, ADF and ADL of 85 to 95, 53 to 60 and 9 to 12 g/kg tuber DM, respectively) reported by Heuzé *et al.* (2015).

The lower WSC storage in the more mature JA forages could be due to the higher utilisation of the plant sugars for synthesis cellulose and starch (McDonald *et al.*, 2011; Smith *et al.*, 2012). Another reason may be related to the transfer of nutrients, *e.g.*, sugars, from the aerial biomass to the tubers with increasing the age of JA forage (Denoroy, 1996; Hopkins & Hüner, 2008). Moreover, the leaves of JA harvested at F60H4, F90H3, F120H1 and F120H2 were partially lost or corrupted, which resulted in the lower forage WSC because JA leaves contained higher WSC than the stems (data not shown). Climatic conditions at the harvesting season also affect the forage WSC content (Hopkins & Hüner, 2008; McDonald *et al.*, 2011). On the other hand, the increased tuber WSC concentration with decreasing the harvest frequency of JA forage was probably due to more photosynthesis of carbohydrates and their translocation from the aerial parts to the tubers (Kays & Nottingham, 2008). This result was consistent with the decreased forage WSC as JA advanced in maturity.

The decrease in the forage ash concentration with JA maturity was probably due to natural dilution process as the plant biomass enhanced and higher OM production than minerals uptake as the photosynthetic areas increased (Denoroy, 1996; Sarmadi *et al.*, 2016). Another reason could be attributed to the increased proportion of the plant stems, including lower ash (data not shown) compared to the leaves (Kays & Nottingham, 2008). However, the ash content in JA forages (118 to 164 g/kg DM) was higher than common forages such as lucerne and maize (NRC, 2001).

High tannin concentrations in diets may negatively affect performance and health of animals (Aboagye & Beauchemin, 2019). The increased TEP and TET in JA forage with increasing plant maturity were consistent with those results reported for other plants (Sarmadi *et al.*, 2016). On the other hand, the tuber TEP in the present work was higher than that reported (1.4 to 1.7 g/kg DM) by Kapusta *et al.* (2013). However, the levels of TET in both forage and tubers of JA were below the toxic level (> 50 g/kg DM) for animals (Aboagye & Beauchemin, 2019).

The nitrate concentrations in JA forages and tubers were lower than the dangerous dosage (> 5 g/kg DM) reported for ruminants (Norberg & Llewellyn, 2014). Decreasing effect of maturity on the nitrate concentration in JA forages (inflorescence emergence and flowering stages vs. stem elongation stage) could be related to the more nitrate reduction to ammonia-N by the nitrate-reductase and its incorporation into the plant proteins (Hopkins & Hüner, 2008). In addition, unsuitable environmental conditions increase the nitrate accumulation in plants due to lower production of photosynthetic proteins (Hopkins & Hüner, 2008; Sarmadi *et al.*, 2016). Accordingly, the nitrate concentrations in JA forages harvested at F60H4 and F90H3 were high, although they contained the lower CP because their growth and harvesting occurred in autumn when adequate heat and sunlight for the best assimilation of nitrate into plant proteins were not available (Hopkins & Hüner, 2008).

***In vitro* GP and fermentation variables**

The negative effect of JA maturity on GP₂₄, OMD, ME, TDS and 'B' of the forages (*i.e.*, F60H1, F60H2 > F60H3, F90H1, F90H2 > F60H4, F90H3, F120H1, F120H2) could be attributed to the higher stem proportion and ADL, and the lower WSC concentration in the more mature forages (McDonald *et al.*, 2011; Wu, 2018). The OMD of all JA tubers (0.828 to 0.850) was higher than the forages due to their higher WSC and lower cell wall concentrations. The positive effect of decreasing the frequency of JA forage harvest on the OMD, ME and TDS of the obtained tubers was owing to the increased WSC concentration, because of the positive correlation between digestibility and WSC (McDonald *et al.*, 2011). Scarce information is available on the effect of harvesting frequency of JA forage on *in vitro* GP variables; however, the OMD and ME of different JA forages at various climates and areas were reported from 0.591 to 0.713 and 8.2 to 9.55 MJ/kg DM, respectively (Kosaric *et al.*, 1985; Karsli & Bingöl, 2009).

The effect of maturity on the MBP of JA forages and tubers has not been investigated in other studies. In this work, the negative impact of maturity on the MBP of JA

forages was somewhat related to the higher concentrations of ADL and TEP, which prevent the colonization and activity of the ruminal cellulolytic bacteria and hence decline the ammonia incorporation into bacterial proteins (Sarmadi *et al.*, 2016). Another reason may be due to the lower TDS and CP in more mature JA forages, compared to the younger forages (Makkar, 2010). On the other hand, no effect of the harvesting frequency of JA forage on the MBP of the tubers could be attributed to the same CP, ADL and TEP concentrations in the tubers.

The PF₂₄ values of all JA forages and tubers were within the theoretical range (2.75 to 4.41 mg/mL) defined by Makkar (2010). No effect of the plant maturity and harvesting frequency on the PF₂₄ of JA forages and tubers could be attributed to the parallel changes occurred in the amounts of TDS and GP.

The *in vitro* ruminal pH of all JA forages (6.77 to 7.04) and tubers (6.66 to 6.72) was within the normal physiological range (5.5 to 7), defined by Dehority (2003). The *in vitro* ruminal ammonia-N (7.42 to 8.81 mg/dL) of all JA forages and tubers was above the minimum level (5 mg/dL) needed to optimal grow of bacteria in the rumen (Wu, 2018). Ruminal ammonia accumulation results from ammonia release and its assimilation into microbial proteins, which are affected by CP degradability and energy availability (Wu, 2018). In the present study, reduction of the forage CP with JA maturity was accompanied by decreases in the TDS and MBP; hence, the *in vitro* ruminal ammonia-N concentration remained unchanged. Moreover, the same *in vitro* ammonia-N concentration among the tubers indicated that ammonia production activity and ammonia incorporation into the ruminal microbial biomass were not affected by different harvesting frequencies of JA forage (McDonald *et al.*, 2011; Wu, 2018).

The negative effect of JA maturity on the *in vitro* ruminal total VFA concentration of the obtained forages could be owing to the decrease in TDS, because of the positive correlation between VFA production in the rumen and TDS (Wu, 2018). The improving effect of the lower harvesting frequency of JA forage on the *in vitro* total ruminal VFA of the tubers was in parallel with the increased TDS (Wu, 2018).

The lower *in vitro* ruminal protozoa population of the more mature JA forages was probably due to the increased TEP (disrupting protozoa metabolism; De Paula *et al.*, 2016) and the decreased NFC (involving starch as a primary substrate for protozoa; Dehority, 2003) as the plant advanced in maturity. On the other hand, no effect of the forage harvesting frequency on *in vitro* protozoa population of the obtained tubers was maybe related to the similar TEP, TET and NFC concentrations in the tubers, as factors influencing the protozoa population (Dehority, 2003; De Paula *et al.*, 2016).

In summary, the nutritive value of JA forage decreased with maturity. The best harvesting interval of JA forage to achieve the tubers with the highest yearly yield/ha, WSC and digestibility was every 120 days, while the highest nutritive value and yield of the forages were observed with harvesting JA every 60 days. The best harvesting interval of JA forage to obtain the highest yearly DM, protein and energy biomasses from both tubers and forage was every 60 days. Thus, the best strategy of JA harvesting depends on the interest in above- and/or below-ground biomass.

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