The effect of lactic acid bacteria inoculation, molasses, or wilting on the fermentation quality and nutritive value of amaranth (*Amaranthus hypochondriacus*) silage¹

Mehrangiz Abbasi,[†] Yousef Rouzbehan,^{†,2} Javad Rezaei,[†] and Sven-Erik Jacobsen[‡]

[†]Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, PO Box 14115-336, Tehran, Iran [‡]Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Højbakkegård Allé 13, 2630 Taastrup, Denmark

ABSTRACT: This study assessed the influence of wilting, lactobacillus (LAB), and/or molasses on the chemical composition, phenolic compounds, in situ degradability, and in vitro ruminal fermentation parameters of amaranth (var. Maria) silage using a randomized complete block design with 6 replicates. Treatments were fresh amaranth forage (FAF), ensiled amaranth without additive (EA), EA inoculated with LAB (EAB), EA + 5% of molasses (EAM), EA inoculated with LAB + 5% of molasses (EABM), and 24-h wilted EA (WEA). The ensiled materials were stored anaerobically for a period of 45 d. Chemical composition, oxalic acid and nitrate levels, silage fermentation characteristics, DM disappearance (DMD), OM disappearance (OMD), in vitro ruminal ammonia-N, volatile fatty acids, cellulolytic bacteria and protozoa counts, and in situ DM and CP

degradability were determined. Compared with FAF, EA had lesser values of water-soluble carbohydrates ($P = 0.023$), nitrate ($P = 0.001$), total phenolics ($P = 0.04$), total tannins ($P = 0.01$), DMD ($P = 0.01$), ruminal cellulolytic bacteria ($P = 0.02$), soluble and very rapidly degradable fraction ($P = 0.014$), and effective degradability ($P = 0.01$). The EA had lesser OMD and CP degradability compared with FAF ($P < 0.05$). Adding molasses to EA resulted in increased ash and lactate concentrations and decreased ammonia ($P < 0.05$), but had no effect on OMD. The WEA had lesser ammonia and greater lactate compared with EA ($P < 0.05$). Overall, fresh amaranth var. Maria can be preserved as a valuable silage to feed ruminants. Ensiling amaranth var. Maria decreased the antiquality compounds, and molasses addition improved the fermentation quality of the silage.

Key words: additive, amaranth silage, degradability, in vitro fermentation, silage quality

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INTRODUCTION

Agricultural crop production can be limited in parts of the world due to an insufficiency of water (Jacobsen *et al.*, 2013; Jacobsen, 2014; Rezaei *et al.*, 2015). Crops adapted to poor soils,

water scarcity, and high temperatures are useful as feedstuffs for ruminants under these harsh conditions. Amaranth (*Amaranthus hypochondriacus*) is an example of such a crop. Amaranth is a C₄ dicotyledonous crop (Seguin *et al.*, 2013; Alemayehu *et al.*, 2015; Jacobsen *et al.*, 2015) with a yield of up to 85 t fresh weight/ha and 16.7 t DM/ha (Abbasi *et al.*, 2012). Compared with corn silage, amaranth has a greater CP concentration (up to 28% of DM), a low lignin(sa) content (4% of DM) (Rezaei *et al.*, 2009), and a low concentration of antiquality compounds (e.g., oxalates and nitrates) (Karimi Rahjerdi *et al.*, 2015).

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²Corresponding author: rouzbeh_y@modares.ac.ir

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Amaranth has a moderate to great OM disappearance (**OMD**; 618 to 734 g/kg; Abbasi et al., 2012). The main stem axis and high moisture concentration make quick drying of a large biomass of grain-type amaranth difficult (Abbasi et al., 2012; Rezaei et al., 2014), and this limits its use as a dried forage. Therefore, ensiling is recommended to conserve amaranth (Rezaei et al., 2014). Replacing corn silage with ensiled amaranth (**EA**) has been found practical in fattening lambs at up to 30% of dietary DM (Rezaei et al., 2014), and in lactating cows at up to 21% of dietary DM (Rezaei et al., 2015).

A new variety of amaranth, *A. hypochondriacus* var. Maria, has been described as having a great grain yield and also as a dual purpose crop (grain and forage) compared with other varieties (S.-E. Jacobsen, personal communication). Adding molasses, lactobacillus bacteria (**LAB**), and wilting have all improved the fermentation characteristics of ensiled crops (McDonald et al., 1991), but there is little information on the effects of wilting, LAB inoculant, and/or molasses on the nutritive value of amaranth (*A. hypochondriacus* var. Maria). This study was done to assess the effects of wilting, LAB inoculant and/or molasses on the chemical composition, silage fermentation parameters, in vitro DM disappearance (**DMD**) and **OMD**, and in situ degradability of amaranth silage var. Maria.

MATERIALS AND METHODS

The Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) was followed for housing, feeding, transport, proper and humane care and use of animals, veterinary care, occupational health and safety, program management, and procedures. The Committee of Animal Science of Tarbiat Modares University approved the experimental protocols.

Forage and Silage Preparation

The study considered one accession of amaranth (var. Maria) provided by S.-E. Jacobsen (University of Copenhagen, Denmark). The amaranth was hand-sown in an experimental field (covers 600 m²) located at Tarbiat Modares University (Tehran, Iran) at an altitude of 1215 m above sea level with a mean annual rainfall and temperature of 306 mm and 15 °C, respectively. Weather data during the growing season and ensiling period are presented in Table 1. The soil at the experimental field was soft loam-sandy (90 g/kg of clay, 100 g/kg of silt, and 810 g/kg of sand) Calcic Xerosol in the

Table 1. Average monthly temperature (°C) and precipitation (mm) during the growing season of the plant and ensiling period

Month	Average temperature			Precipitation
	Minimum	Maximum	Mean	
July	18.5	40.8	30.7	0.2
August	20.7	38.1	29.7	0
September	12.1	37.1	26.4	0
October	9.3	29.2	18.9	0.2
November	-8.9	25.2	9.2	2.4
December	-3.3	17.4	5.9	34.4

Food and Agriculture Organization of the United Nations taxonomy. Fertilizer formula 46–0–0 of N was applied at a rate of 360 kg of urea/ha (166 kg of N/ha). Seeding rate was about 0.5 kg/ha. Amaranths were planted in a randomized complete block design with 6 replicates. The forage was sown manually on the 24th of July (2016), harvested, after 85 d, by cutting the whole-plants with knife to a 5-cm stubble height, and chopped into fragments of 2 to 4 cm in lengths before ensilage. Six treatments were as follows: 1) fresh amaranth forage (**FAF**), 2) ensiled amaranth without any additive (**EA**), 3) EA inoculated with LAB (**EAB**), 4) EA + 5% of molasses on a fresh basis (**EAM**), 5) EA with LAB + 5% of molasses (**EABM**), and 6) 24-h wilted EA (**WEA**). Plant materials in the ensiled treatments containing lactobacillus were inoculated using 0.313 g of LAB diluted in 240 mL of distilled water and then kept for 4 h before being sprayed onto 100 kg of fresh material. The homogeneous mixtures for silage were packed tightly into 10-kg double-layer plastic bags (total 36 bags) and vacuumed to remove excess air before sealing. The mixtures were ensiled for 45 d, after which representative 3-kg samples were taken from each bag for later analyses.

Chemical Composition

The fresh samples were weighed, oven-dried at 60 °C to a constant weight for DM determination, then ground to pass through a 1-mm sieve (Wiley mill; Thomas Scientific, Gloucester, NJ), and analyzed for OM (method 924.05), CP (method 984.13), ether extract (**EE**; method 954.02), ash-free ADF (**ADFom**), and ADL (method 973.18) (AOAC, 1998). Ash-free NDF (**NDFom**) was determined according to the method described by Mertens (2002), and water-soluble carbohydrates (**WSC**) using the anthrone method (MAFF, 1982).

Nitrate was assayed by a colorimetric method (Singh, 1988). Oxalic acid concentration was determined spectrophotometrically by extraction of total oxalate from 1 g of sample using 50 mL of HCl (2 M) in a water bath (80 °C for 15 min) (Savage *et al.*, 2000). Total phenolics (TP) and nontannin phenolics (NTP) were measured by the Folin-Ciocalteu methods using tannic acid (Merck GmbH, Germany) as the standard (Makkar, 2000). Total tannins (TT) were calculated as the difference between TP and NTP.

To measure pH, 50 g of the fresh silage was mixed thoroughly with 125 mL of distilled water and kept for 1 h at 25 °C with occasional stirring. The pH value was recorded (Faithfull, 2002) using a portable digital pH meter (Sartorius PT-10; Sartorius AG, Göttingen, Germany). Two milliliters of the silage juice were pipetted into a micro-centrifuge tube with 0.5 mL of an acid solution (containing 20% orthophosphoric acid and 20 mM 2-ethyl butyric acid, as the internal standard) and then centrifuged at $15,000 \times g$ for 15 min at a temperature of 4 °C. The supernatant was used to quantify the volatile fatty acids (VFA) using a gas chromatograph (UNICAM 4600; SB Analytical, Cambridge, UK) equipped with a capillary column (Agilent J&W HP-FFAP, 10 m by 0.535 mm by 1.00 μ m, 19095F-121; Agilent, Santa Clara, CA).

To determine ammonia-N, 9 mL of the silage extract was mixed with 1 mL of 7.2 N H₂SO₄ and stored at -20 °C. After thawing, the ammonia-N of the extract was measured using the phenol-hypochlorite method (Galyean, 1997).

In Vitro OM and DM Disappearances

The two-stage procedure of Tilley and Terry (1963) was used to determine disappearance by ruminal microorganisms plus HCl/pepsin. Prior to morning feeding, ruminal fluid was collected from 3 fistulated Shal breed sheep, filtered through 4 layers of cheesecloth, and gassed with CO₂. The filtered rumen fluid was mixed with McDougall buffer at a ratio of 1:4 to obtain the inoculum. Fifty milliliters of the inoculum were added to each bottle containing 500 mg of the sample. After gasifying with CO₂, bottles were incubated at 39 °C for 48 h. At the end of the 48-h incubation, the tubes were removed from the incubator, centrifuged for 15 min at $2,000 \times g$, and the liquid was suctioned off by vacuum. Then, pepsin digestion of the tube contents was done for 48 h. After the completion of the digestion, samples were filtered using a Buchner funnel and ashless filter paper. The filter papers

containing the samples were oven-dried at 100 °C, weighed, then burned in a furnace at 500 °C, and reweighed. Finally, DMD, OMD, and ME were calculated. The DMD (g/kg of DM) was estimated as [(initial sample DM weight – DM weight after 24 h incubation)/initial sample DM weight] $\times$ 1000. The OMD (g/kg of OM) was calculated as [(initial sample OM weight – OM weight after incubation)/initial sample OM weight] $\times$ 1000. Digestible OM in the DM (DOMD; g/kg of DM) was calculated as OMD $\times$ % OM. Finally, the ME concentration (MJ/kg of DM) of the forage and silage was estimated as $0.0157 \times \text{DOMD}$ and $0.0160 \times \text{DOMD}$, respectively (Alderman *et al.*, 1993).

In Vitro Ruminal Fermentation Parameters

The effect of the treatments on in vitro ruminal protozoa, cellulolytic bacteria, VFA, ammonia-N, and pH was assessed using a 24-h in vitro gas production technique (Menke and Steingass, 1988). Rumen fluid was collected via rumen fistula from 3 adult sheep of the Shall breed just before the morning feeding. The experimental animals were fed alfalfa hay, amaranth silage, barley grain, soybean meal, and mineral-vitamin premix (at a ratio of 20:40:30:9:1 on a DM basis) twice daily at 0800 and 1700 h and had free access to water. The ingredients of the diet were mixed thoroughly by hand and offered as total mixed ration. Two hundred milligrams of the ensiled amaranth samples were incubated with 30 mL of buffered rumen fluid (mixed strained rumen fluid and anaerobic mineral buffer at a ratio of 1:2 [vol/vol]; Menke *et al.*, 1979) in 100-mL glass syringes (Fortuna, Häberle Labortechnik, Lonsee-Ettlenschieb, Germany) at 39 °C. A total of 168 syringes (5 treatments $\times$ 4 replicates $\times$ 3 individual samples $\times$ 2 runs in different weeks), with 3 syringes as blanks (rumen fluid + buffer) at each run, were incubated for 24 h. At the end of incubation, the volume of gas production was recorded. After 24 h of in vitro incubation, the pH of syringe contents was determined with a portable digital pH meter. A strained sample of 2.5 mL was preserved immediately, with 0.5 mL of 0.2 N HCl, at -20 °C until analysis of ammonia-N which was determined using the phenol-hypochlorite procedure described by Galyean (1997). For VFA analysis, a strained sample of 2 mL was preserved, at -20 °C, with 0.5 mL of an acid solution (containing 20% orthophosphoric acid and 20 mM 2-ethyl butyric acid, as the internal standard). After thawing, the mixture was centrifuged at $15,000 \times g$ at 4 °C for 15 min. The supernatant was

used to quantify VFA by gas chromatography as described above.

For enumeration of cellulolytic bacteria, the anaerobic technique of Bryant (1972) was used to prepare anaerobic culture media. Hungate tubes containing media and filter paper (Whatman no. 1), as the sole carbohydrate source for cellulolytic bacteria, were prepared. The syringe contents were diluted and inoculated into Hungate tubes. Cultures were grown at 39 °C for 21 d. The cellulolytic bacteria were counted in the broth medium using the most probable number procedure of Dehority (2003).

The ruminal protozoa population was enumerated by pipetting 2 mL of syringe contents into a screw-capped test tube containing 5 mL of formalized physiological saline (20 mL of formaldehyde in 100 mL of saline containing 0.85 g of NaCl in 100 mL of distilled water). Total counts and sub-family numbers of protozoa were determined using a light microscope and a haemocytometer (Neubauer-improved, Marienfeld, Germany), according to the method of Dehority (2003).

In Vivo DM and CP Degradability

The AFRC (1992) method was used to determine the degradability coefficients of the samples by suspending nylon bags in the rumens of rumen-fistulated male Shal breed sheep. The bag size was 21 × 10 cm with a pore size of 45 µm (Bucksburn, Aberdeen, UK). The sheep were fed with a diet containing alfalfa, amaranth, wheat bran, barley grain, soybean meal, and mineral-vitamin premix, at 0700 and 1900 h, and had free access to water. The samples were milled through a 4-mm sieve. Five grams of each sample were put into each nylon bag and incubated in the rumen for 4, 8, 12, 24, 48, 72, and 96 h. After each incubation time, the bags were washed in a washing machine (Hoover OPHS 612; Hoover, London, UK) for 1 h and oven-dried at 55 °C to a constant weight. The degradability value at time 0 was obtained by washing 3 bags per sample in a washing machine for 1 h. Bags and contents were weighed to estimate degraded DM. The bag residues were analyzed for CP (method no. 988.05) according to AOAC (1998). The percentage of ruminal DM and CP degradability (Y) at time (t) was estimated from an exponential curve as $Y = A + B(1 - e^{-Ct})$ which was fitted to the data by iterative regression analysis (Ørskov and McDonald, 1979). Then, the effective degradability (ED) of DM and CP was estimated as $ED(\%) = (A + B \times C)/(C + k)$, according to Ørskov

and McDonald (1979). In these equations, “e” is the base of natural logarithms, “A” is the soluble and very rapidly degradable fraction, and “B” is the insoluble but potentially degradable fraction which degrades at a constant fractional rate (C) per unit time (t), ED is the effective degradability, and “k” refers to the fractional outflow rate from the rumen. An assumed value for “k” was 0.05 fraction/h. Finally, the amounts of the effective rumen degradable protein, digestible undegradable protein, and metabolisable protein were estimated according to AFRC (1992) equations.

Statistical Analysis

Data on the quality of the amaranth silages were subjected to ANOVA using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC) in a randomized complete block design. Prior to ANOVA, residual normality for data was tested using Proc UNIVARIATE. Multiple comparisons among the means of the silages were performed with the Duncan's multiple range test. Differences between FAF and EA were examined using a t-test. Statistical significance was defined by *P* values of <0.05, and trends were declared if *P* > 0.05 and *P* < 0.10.

RESULTS AND DISCUSSION

Chemical Composition

The DM concentration of the silages was low (Table 2), because in Iran, forage corn and amaranth are planted as part of a double cropping system in summer, and harvested in the fall when lesser temperatures and light levels are not adequate for plant maturity. These crops are ensiled with a DM concentration of less than 250 g/kg of fresh weight (Rezaei et al., 2014). The DM concentrations of *A. hypochondriacus* in the studies by Abbasi et al. (2012) and Rezaei et al. (2014) were less (i.e., 159 to 212 g/kg of fresh weight) than those in the present study (250 g/kg of fresh weight). This difference in DM content is probably due to the variety and harvest time of these forages and weather conditions. Addition of molasses resulted in increased (*P* = 0.01) DM concentrations in EAM and EABM when compared with EA and EAB, due to the greater DM concentration of molasses than amaranth (i.e., 870 g/kg of fresh wt), which was in accordance with the results by Rezaei et al. (2009).

The CP content of FAF was clearly above 80 g/kg of DM which, according to Norton (1998), should

Table 2. Chemical composition and antiquality compounds levels (g/kg of DM or as stated) of pre-ensiled amaranth and ensiled amaranth without or with molasses, *Lactobacillus plantarum* or their combination, or wilted

Item	FAF ¹	Silages					SEM	P-value	
		EA	EAB	EAM	EABM	WEA		FAF vs. EA	Among silages
DM, g/kg of fresh wt.	233	250 ^c	258 ^c	303 ^b	304 ^b	364 ^a	4.45	0.90	0.01
CP	187	171	168	170	168	173	3.54	0.80	0.88
NDFom ²	420	400	402	398	395	398	6.14	0.03	0.77
ADFom	275	267	271	268	265	263	2.17	0.80	0.90
ADL	44.6	43.0	45.0	41.4	41.5	44.6	0.26	0.70	0.60
Ether extract	57.0	103	99.0	100	101	94.0	4.50	0.01	0.06
WSC	62.5	19.7	19.6	21.0	21.0	19.5	1.06	0.02	0.70
Ash	145	170 ^b	171 ^b	188 ^a	189 ^a	171 ^b	2.35	0.01	0.01
Ca	11.1	10.8	10.4	10.9	11.2	11.3	0.41	0.06	0.20
P	6.6	5.8	6.8	6.3	6.1	5.7	0.11	0.50	0.90
Mg	3.6	4.0	3.6	4.1	4.2	3.6	0.33	0.30	0.70
Fe, mg/kg of DM	718	713	740	715	731	721	49.6	0.10	0.90
Cu, mg/kg of DM	42.6	40.6	42.7	40.6	41.4	41.8	0.18	0.06	0.90
Zn, mg/kg of DM	37.8	40.4	39.5	36.9	38.1	40.0	0.19	0.10	0.80
ME, MJ/kg of DM	8.4	7.5	7.9	7.5	7.6	7.6	0.19	0.01	0.52
Anti-quality compounds									
Nitrate	3.30	0.21	0.24	0.20	0.20	0.20	0.02	0.01	0.40
Oxalic acid	7.61	7.50	7.94	7.31	7.47	7.30	0.63	0.50	0.90
Total phenolic	11.1	8.06	8.76	8.23	8.03	8.22	0.33	0.04	0.42
Total tannins	7.82	5.31	5.77	5.24	5.08	5.12	0.24	0.01	0.08

^{a-c}Among the silages, means in the same row with different superscripts differ ($P < 0.05$).

¹FAF = fresh amaranth forage; EA = ensiled amaranth forage without additives; EAB = ensiled amaranth with *Lactobacillus plantarum*; EAM = ensiled amaranth with molasses; EABM = ensiled amaranth with molasses + *Lactobacillus plantarum*; WEA = wilted-ensiled amaranth.

²NDFom = ash-free neutral detergent fiber; ADFom = ash-free acid detergent fiber; WSC = water-soluble carbohydrates; ME = metabolizable energy of the forage and silages was estimated as $0.0157 \times \text{DOMD}$ and $0.0160 \times \text{DOMD}$.

provide an adequate ruminal ammonia level to support optimum growth of rumen microorganisms. The CP content was greater than those reported by Sleugh *et al.* (2001) for *A. hypochondriacus* (120 g/kg of DM). The CP concentration was not affected by ensiling ($P = 0.80$). Moreover, adding molasses and LAB and wilting had no effect ($P = 0.88$) on CP concentration of the plant silage.

The EA had less ($P = 0.03$) concentration of NDFom than FAF. Compared with FAF, the lesser NDFom in EA was probably due to cell wall degradation by plant enzymes, cellulolytic clostridia, or acid hydrolysis during silage fermentation (McDonald *et al.*, 1991). The cell wall components of the silage were not affected ($P > 0.59$) by treating with molasses, LAB, or wilting. This disagrees with the results by Rezaei *et al.* (2009) who added molasses onto amaranth silage and found a decrease in the NDFom content, possibly due to greater lactic acid concentration (121 to 127 g/kg DM) than those observed in the current experiment (59.0 to 70.0 g/kg DM). Hence, according to Baytok *et al.* (2005), higher lactic acid concentration (i.e., greater acidity) had led to enhance more cell wall

degradation in the work of Rezaei *et al.* (2009) than those observed in the current experiment.

The EA had a greater ($P = 0.01$) EE concentration than the FAF, which is probably due to the reduction of WSC, CP, and NDF, during ensiling, that led to a proportional increase in EE in the silage. No effect ($P = 0.06$) of molasses, LAB, and wilting on silage EE was observed in our study.

In comparison with FAF, the lower ($P = 0.02$) concentration of residual WSC in EA indicated that WSC was utilized by bacteria resulting in lactic acid production (Table 3). WSC are the basic food source for microorganisms during silage fermentation (McDonald *et al.*, 1991). Similar to Babaeinasab *et al.* (2015), adding molasses and molasses plus LAB, in present study, had no effects ($P = 0.70$) on residual WSC content of EA. Rezaei *et al.* (2009), however, reported more residual WSC content when molasses was mixed with amaranth silage.

Amaranth had a great ash content because of its C_4 metabolism and great carbon uptake per unit area of the plant (Edwards and Walker, 1983). The different ash concentrations of amaranths

Table 3. The values of pH, ammonia-N (g/kg of total N), lactic acid, and individual volatile fatty acids (g/kg of DM) of ensiled amaranth without or with molasses, *Lactobacillus plantarum* or their combination, or wilted

Item	EA ¹	EAB	EAM	EAMB	WEA	SEM	P-value
pH	4.49 ^b	4.63 ^b	3.90 ^c	3.70 ^c	5.01 ^a	0.03	0.01
Ammonia-N	54.3 ^a	53.2 ^a	51.0 ^b	51.8 ^b	45.9 ^c	0.50	0.01
Lactic acid	57.0 ^c	55.0 ^c	70.0 ^a	69.2 ^a	59.0 ^b	0.41	0.01
VFA							
Acetate	17.1	17.0	17.3	17.9	16.8	0.32	0.90
Propionate	0.30	0.31	0.30	0.28	0.35	0.02	0.05
Butyrate	0.30	0.40	0.34	0.36	0.29	0.01	0.60

^{a-c}Among the silages, means in the same row with different superscripts differ ($P < 0.05$).

¹EA = ensiled amaranth forage without additives; EAB = ensiled amaranth with *Lactobacillus plantarum*; EAM = ensiled amaranth with molasses; EAMB = ensiled amaranth with molasses + *Lactobacillus plantarum*; WEA = wilted-ensiled amaranth.

considered in various experiments (92 to 220 g/kg of DM; Rezaei et al., 2014) are related to factors such as plant variety, harvest stage, climate, and soil type (Rezaei et al., 2014). The EA had the greater ash concentration than FAF ($P = 0.01$). The increase in the ash content was due to the reduction of OM materials during the ensiling process. The ensiling process had limited effect on the mineral composition of amaranth. Calcium is the most abundant mineral in amaranth forage, followed by P and Mg. In our study, the great level of Ca was consistent with the results of Rezaei et al. (2009). The Ca to P ratio in fresh and ensiled amaranths was comparable to the recommended ratio of 1.75:1 by NRC tables (NRC, 2001), indicating a balanced source of Ca and P. Overall work on the mineral composition of amaranth forage is limited. Our results are similar to the values previously reported for amaranth species in terms of Ca, Mg, and Zn contents (Seguin et al., 2013), but P, Fe, and Cu contents were greater in our study. Differences between various studies might be in part attributable to differences in biotic and/or abiotic factors to which plants were exposed during their growth.

Antiquality Compounds

Ensiling amaranth resulted in the reduction of nitrate ($P = 0.01$) when compared with FAF. Molasses addition, LAB, and wilting had no effect ($P = 0.40$) on nitrate concentration of amaranth silages. However, the nitrate level in FAF, of 3.30 g/kg of DM, was less than the toxic level (i.e., > 6 g/kg of DM) for animals (Radostits et al., 2007). Nitrate content in the silages decreased due to the capability of some microorganisms to reduce nitrate to ammonia-N via nitrite in the ensiling process (McDonald et al., 1991).

Ensiling amaranth resulted in the reduction of TP ($P = 0.04$) and TT ($P = 0.01$) concentrations. In all silages, the concentrations of TP and TT were less than 1% which may not have an inhibitory effect on the ruminal fermentation of ruminants (Benchaa et al., 2008).

Ensiling of amaranth had no effect ($P = 0.50$) on oxalic acid concentration. Also, the silage concentration of this antiquality compound was not affected ($P = 0.90$) by wilting, LAB, and molasses addition. In the present study, oxalic acid concentrations of the pre-ensiled and ensiled amaranths, 7.30 to 7.94 g/kg of DM, were less than the toxic levels for ruminants (i.e., 20 g/kg of DM) proposed by Rahman et al. (2013). Researchers (Teutonico and Knorr, 1985; Rezaei et al., 2015) reported a wide range of oxalic acid concentrations (i.e., 2 to 114 g/kg of DM) for amaranth. The range in oxalic acid seems huge, which is probably related to the effects of plant maturity, species, climate, and field management on secondary metabolites (Bressani, 1993; McDonald et al., 2011). Abbasi et al. (2012) noted that with increasing plant maturity from 40 to 60 d after planting, concentration of total oxalic acid decreased (from 20.2 to 11.1 g/kg of DM) due to the rise in stem proportion as it contains lesser levels of oxalates than leaves (Abbasi et al., 2012). In the present study, amaranth was harvested 85 d after planting which had lower oxalic acid concentration (i.e., 0.73 to 0.76 g/kg of DM) than those observed by Abbasi et al. (2012) study.

Silage Fermentation Characteristics

Among the silages, there were differences ($P = 0.01$) in pH values (Table 3). The decrease in pH in the silage made with molasses was consistent with the results of Rezaei et al. (2009), but Dönmez et al. (2003) reported that pH was not affected or increased

in silage treated with molasses. Lactic acid production was greater in the silages treated with molasses (EAM and EABM) compared with the other silages ($P = 0.01$) because of an initial increase in the number of LAB with the addition of a WSC source (McDonald *et al.*, 1991). Among the silages, there were differences ($P = 0.01$) in ammonia-N contents, in which EA and EAB were the greatest, and WEA was the lowest. Molasses-treated silages produced less ammonia-N because the greater sugar levels led to a rapid pH decline inhibiting deamination and decarboxylation of amino acids (McDonald *et al.*, 1991). The molasses addition, LAB, and wilting had no considerable influences on the silage concentrations of acetate, propionate, and butyrate ($P > 0.05$); however, the concentrations of these acids indicated good fermentation quality of silages (Rezaei *et al.*, 2009). Results of this study indicate that LAB inoculation did not improve the fermentation parameters of amaranth silages. In other literature, responses in fermentation parameters to LAB inoculation are variable (McDonald *et al.*, 1991).

In Vitro Fermentation Parameters

In vitro ruminal pH of the treatments varied between 6.73 and 6.75, which were within a normal range from 5.6 to 7.0 (Dehority, 2003) (Table 4).

The EA had a greater ruminal ammonia concentration than the FAF ($P = 0.03$), probably due to the relatively greater in vitro ruminal protozoa population in EA in comparison to those in FAF (6.38 vs. 6.62, respectively) (Dehority, 2003). Wilted ensiled amaranth resulted in lesser ($P = 0.01$) ruminal ammonia-N concentration compared with other silages, which is probably related to the comparatively lower in vitro ruminal protozoa population in WEA in comparison to those in other silages (Dehority, 2003). Also, the lower soluble-rapid degradable protein (fraction "A") in WEA compared with other silages may have led to decline in the ruminal ammonia-N concentration. However, the in vitro ruminal ammonia-N concentrations for all the experimental silages were adequate to support optimal growth of rumen microorganisms (>5 mg/dL; Sinclair *et al.*, 1993). Total VFA concentration, the molar proportions of individual fermentative fatty acids, and acetate-to-propionate ratio did not differ among treatments, which suggests that the additives (molasses and/or bacteria) did not modify silage fermentability and energy availability. There was no difference ($P > 0.62$) in in vitro ruminal protozoa populations among the treatments and this was probably due to the normal physiological range of pH which has an important role in ciliated protozoa activity (Dehority, 2003).

Table 4. In vitro ruminal pH, ammonia-N (mg/dL), and VFA concentrations of the fermentation characteristics, ruminal protozoa ($\times 10^5$ /mL digesta), and cellulolytic bacteria enumeration ($\log_{10}$ /g digesta) of pre-ensiled and ensiled amaranth without or with molasses, *Lactobacillus plantarum* or their combination, or wilted

Item	FAF ¹	Silages					SEM	<i>P</i> -value	
		EA	EAB	EAM	EAMB	WEA		DF vs. EA	Among silages
pH	6.75	6.73	6.75	6.76	6.76	6.74	0.01	0.10	0.30
Ammonia-N	11.1	14.8 ^a	13.3 ^a	13.3 ^a	13.4 ^a	9.73 ^b	0.28	0.03	0.01
Total VFA, mmol/L	51.3	53.1	55.7	54.6	54.1	53.2	0.70	0.81	0.60
VFA, mol/100 mol									
Acetate	75.1	75.7	76.0	76.1	74.0	76.3	0.70	0.50	0.43
Propionate	15.8	13.9	13.8	14.1	14.2	15.4	0.40	0.42	0.70
Butyrate	7.6	9.0	7.9	8.8	9.7	7.14	0.27	0.50	0.08
Isovalerate	1.0	0.9	1.0	1.0	1.0	0.9	0.07	0.11	0.79
Valerate	0.54	0.40	0.45	0.40	0.46	0.39	0.03	0.40	0.21
Acetate: propionate	4.6	5.5	5.5	5.4	5.2	5.0	0.32	0.12	0.48
Total protozoa	6.38	6.62	6.12	6.22	6.20	6.14	0.17	0.30	0.63
<i>Isotrichidae</i>	1.32	1.18	1.24	1.24	1.18	1.40	0.11	0.21	0.88
<i>Entodiniinae</i>	3.24	3.62	3.10	3.08	2.96	2.88	0.12	0.30	0.92
<i>Diplodiniinae</i>	1.30	1.24	1.26	1.28	1.32	1.28	0.10	0.13	0.90
<i>Ophrioscolecina</i>	0.52	0.58	0.52	0.62	0.68	0.58	0.07	0.30	0.91
Cellulolytic bacteria	8.41	7.57 ^a	7.31 ^{ab}	6.71 ^{bc}	6.71 ^{bc}	6.47 ^c	0.14	0.02	0.02

^{a-c}Among the silages, means in the same row with different superscripts differ ($P < 0.05$).

¹FAF = fresh amaranth forage; EA = ensiled amaranth forage without additives; EAB = ensiled amaranth with *Lactobacillus plantarum*; EAM = ensiled amaranth with molasses; EAMB = ensiled amaranth with molasses + *Lactobacillus plantarum*; WEA = wilted-ensiled amaranth.

Table 4 shows that the decline ($P = 0.02$) of the in vitro ruminal cellulolytic bacteria population for incubated EA in comparison with FAF corresponds to the decrease in the NDF substrate concentration (Van Soest, 1994). Similarly, a decrease ($P = 0.02$) in the ruminal cellulolytic bacteria population by in vitro incubation of EAM, EAMB, and WEA is concurrent with the decline of their substrate NDF concentration.

In Vitro OM and DM Disappearances

Disappearance of OM and DM in EA was less ($P = 0.001$) than those in FAF due to depletion of fermentable substrates during ensilage (i.e., when degradable OM is lost during silage fermentation, less is available for rumen digestion). Silage

digestibility is affected by its quality, including the pH value which influences the stability of the silage during fermentation (McDonald *et al.*, 1991). Forage with levels of 50 to 80 g of WSC per kg of DM should reach a low pH that is sufficient to produce a stable silage (McDonald *et al.*, 1991). In the current study, all forages had WSC concentrations above 50 g/kg of DM, and good stable silages were achieved. All silages have similar DMD or OMD (650 to 690 g/kg), which were comparable with those of the amaranth silage (650 to 719 g/kg) observed by Rezaei *et al.* (2009). Molasses addition, LAB, and wilting had no effects ($P > 0.23$) on the silages digestibility of the experimental silages, because of their similar ADL, CP, and residual WSC contents (McDonald *et al.*, 2011) (Table 5).

Table 5. In vitro disappearance of pre-ensiled amaranth and ensiled amaranth without or with molasses, *Lactobacillus plantarum* or their combination, or wilted

Item	FAF ¹	Silages					SEM	P-value	
		EA	EAB	EAM	EAMB	WEA		FAF vs. EA	Among silages
DMD ² , g/kg of DM	703	668	673	657	657	683	15.5	0.001	0.48
OMD, g/kg of OM	662	617	615	603	602	610	5.69	0.001	0.24

¹FAF = fresh amaranth forage; EA = ensiled amaranth forage without additives; EAB = ensiled amaranth with *Lactobacillus plantarum*; EAM = ensiled amaranth with molasses; EAMB = ensiled amaranth with molasses + *Lactobacillus plantarum*; WEA = wilted-ensiled amaranth.

²DMD = DM disappearance was estimated as [(initial sample DM weight – DM weight after 24 h incubation)/initial sample DM weight] × 1,000; OMD = OM disappearance was calculated as [(initial sample OM weight – OM weight after incubation)/initial sample OM weight] × 1000. Digestible OM in the DM (DOMD; g/kg of DM) was calculated as OMD × % OM.

Table 6. In situ DM and CP degradability of pre-ensiled amaranth and ensiled amaranth without or with molasses, *Lactobacillus plantarum* or their combination, or wilted

		Silages						P-value	
Item	FAF ¹	EA	EAB	EAM	EABM	WEA	SEM	FAF vs. EA	Among silages
DM degradation									
A ² , g/kg of DM	208	258 ^c	259 ^c	284 ^a	285 ^a	241 ^b	7.39	0.01	0.01
B, g/kg of DM	370	356	356	347	361	347	5.85	0.70	0.39
C, /h	0.058	0.062	0.061	0.063	0.063	0.066	0.007	0.63	0.90
ED, g/kg of DM	471	459 ^b	461 ^b	503 ^a	501 ^a	421 ^c	3.10	0.01	0.01
CP degradation									
A, g/kg of CP	474	443	440	443	428	383	9.96	0.02	0.10
B, g/kg of CP	387	448	444	444	442	433	6.09	0.04	0.41
C, /h	0.066	0.068	0.069	0.064	0.059	0.061	0.005	0.30	0.73
ED, g/kg of CP	750	590 ^c	629 ^a	612 ^b	540 ^d	535 ^e	3.50	0.90	0.01
ERDP, g/kg of DM	109	97.0	101	100	95.0	96.0	2.10	0.04	0.31
DUP, g/kg of DM	28.0	28.5	28.2	29.1	30.0	29.0	1.16	0.26	0.92
MP, g/kg of DM	97.5	90.5	92.7	93.0	90.7	90.3	1.48	0.01	0.50

^{a-c}Among the silages, means in the same row with different superscripts differ ($P < 0.05$).

¹FAF = fresh amaranth forage; EA = ensiled amaranth forage without additives; EAB = ensiled amaranth with *Lactobacillus plantarum*; EAM = ensiled amaranth with molasses; EAMB = ensiled amaranth with molasses + *Lactobacillus plantarum*; WEA = wilted-ensiled amaranth.

²A = soluble and very rapidly degradable fraction; B = insoluble but potentially fermentable fraction; C = fractional degradation rate of B; ED = effective degradability calculated for an outflow rate of 0.05/h; ERDP = effective rumen degradable protein; DUP = digestible undegradable protein; MP = metabolizable protein.

In Situ Degradability of DM and CP

In comparison with FAF, ensilage increased ($P = 0.01$) the in situ soluble DM fraction, probably due to the OM degradation during silage fermentation (McDonald *et al.*, 1991). In the current study, the DM soluble fractions were less than those values reported by Seguin *et al.* (2013) and Karimi Rahjerdi *et al.* (2015) for silages from other amaranth varieties (i.e., Plainsman, D136, Kharkovskiy, and Sem). The concentration of fraction “A” and ED was greater ($P = 0.01$) in EAM and EABM in comparison with the other silages (Table 6) probably due to the availability of more fermentable substrates from the molasses. The CP degradation parameters, fraction “A,” fraction “B,” ED, and effective rumen degradable protein of FAF were greater than those in ensiled treatments probably due to availability of more CP in the FAF in comparison to those in silages. However, soluble and degradable CP fractions and effective CP degradability of the amaranth variety were not influenced by either wilting or adding any additive to the silage.

The chemical composition, particularly CP, ash, and ADL, antiquality compounds, and in vitro disappearance suggest that ensiled amaranth (var. Maria) made with or without the use of additives has potential as a ruminant feedstuff. However, an in vivo trial, especially on animal performance, is needed to confirm the nutritional characteristics reported in this study.

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