Yield, chemical composition, fermentation characteristics, in vitro ruminal variables, and degradability of ensiled amaranth (*Amaranthus hypochondriacus*) cultivars compared to corn (*Zea mays*) silage

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ABSTRACT

Silages from four amaranth varieties (A5, A12, A14 and A28) were compared to corn silage (CS) in terms of their yield, chemical composition, phenolic compounds, oxalic acid and nitrate levels, silage fermentation characteristics, in vitro methane production, organic matter disappearance (OMD), microbial crude protein (MCP), ruminal ammonia (NH₃-N), pH, volatile fatty acids, cellulolytic bacteria numbers, protozoa counts and in situ dry matter (DM) and crude protein (CP) degradability were determined. Forages were harvested 93 days after planting, chopped and ensiled in plastic buckets for 60 days. The study was based on a randomized complete block design, and data were analyzed using SAS, GLM procedure. Compared to CS, amaranth silages (AMS) had lower ash-free neutral detergent fiber nitrate, OMD (P<0.001), phosphorus (P=0.003), and metabolizable energy (ME) (P=0.043) but higher (P<0.001) CP, calcium, non-fiber carbohydrates (NFC), ADL, ether extract, ash, total phenolics, pH, NH₃-N concentration, MCP, digestible undegradable protein (DUP) and metabolizable protein (MP). Fresh, OM, OMD, ME (P<0.001) and DM (P=0.032) yields of AMS from different varieties were higher than CS, with the exception of A5. Overall, amaranth made good quality silage, with some variation, and A28 had the highest yield and nutritional value (CP, NFC, MCP, DUP, and MP). The yield, CP concentration and nutritional value of A28 silage was higher than CS. Although these in vitro results are promising, they also need to be validated with future in vivo research.

Key words: amaranth silage, chemical composition, degradability, in vitro gas production, yield.
List of Abbreviations: A, soluble and very rapidly degradable fraction; ADFom, ash-free acid detergent fiber; ADL, lignin measured by solubilizing cellulose with sulphuric acid; AMS, amaranth silages; B, insoluble but potentially fermentable fraction; C, fractional degradation rate of B; CP, crude protein; CS, corn silage; DM, dry matter; DUP, digestible undegradable protein; ED, effective degradability; EE, ether extract; ERDP, effective rumen degradable protein; GP, gas production; MCP, microbial crude protein; ME, metabolizable energy; MP, metabolizable protein; N, nitrogen; NDF, ash-free neutral detergent fiber; NFC, non-fiber carbohydrates, OM, organic matter; OMD, organic matter disappearance; PF, partitioning factor; TDS, truly degraded substrate; TP, total phenolics; TT, total tannins; VFA, volatile fatty acids; WSC, water soluble carbohydrate.
INTRODUCTION

Many amaranth varieties have been introduced into the Middle Eastern region, but their acclimation and the evaluation of their agronomic and nutritional values in the peculiar agro-ecologies of the region are unknown (Akin-Idowu et al., 2016). Amaranth has a lower water requirement than corn (Ofitserov, 2001) and can yield up to 85 t fresh weight/ha (Abbasi et al., 2012) with a higher crude protein (CP) (122 vs. 77 g/kg DM) and a lower acid detergent lignin (ADL) (35 vs. 45 g/kg DM) (Rezaei et al., 2015). The CP in most forages is predominantly rumen degradable protein (RDP). However, Lotfi et al. (2018) showed that amaranth silage (AMS) had higher CP levels than corn silage (CS), and particularly more digestible undegradable protein (DUP). The DUP is available in the lower gut and is more efficiently used in post-ruminal digestion (Van Soest, 1994). Increasing DUP in heifer diets improved feed efficiency and live weight gain (Tomlinson et al., 1997) and dairy cow milk yield (Vagnoni and Broderick, 1997). Low protein solubility feeds with high levels of RUP were reported to result in lower methane production compared to high protein solubility feeds, i.e., fermentation of rumen protein resulting in methane production (Preston et al., 2012; Ho Quang Do et al., 2013).

Genotypes of Amaranthus are characterized by fast growth after germination (Pisarikova et al., 2006), early maturity, and water requirement (Kauffman and Weber, 1990), and high nutritive value of AMS as a ruminant feed (Rezaei et al., 2009). This has led to its use as a substitute for corn, especially in arid regions (Rezaei et al., 2015). Replacing CS with AMS had similar effect on Holstein dairy cow performance (Rezaei et al., 2015), and improved the growth rate of lambs (Rezaei et al., 2014). Hence, testing different varieties AMS to select the best of them is desirable. Therefore, in this study, four new varieties of amaranth considered suitable for silage production in semi-arid zones were chosen and their silage characteristics including yield, chemical composition, anti-nutrient levels, silage fermentation characteristics, in vitro digestibility and in situ DM and CP degradability were compared to those of CS.
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 (Iran) approved the experimental protocols.

Forage and silage preparation and sampling method
Corn (Z. mays, var. hybrid SC 704) (Seed and Plant Research Improvement Institute, Karaj, Iran) and four amaranth varieties (A5, A12, A14, and A28) provided by S.-E Jacobsen (Quinoa Quality ApS, Teglværksvej 10, DK-4420 Regstrup, CVR 40610588). According to the agronomic performance of these varieties, they were selected by S.-E. Jacobsen, who is a member of the European project entitled PROTIEN2FOOD, and working on amaranth and Quinoa plants which ended in February 2020. The cultivated species of amaranth (Amaranthus sp.) originated from Latin America, and belong to the species Amaranthus hypochondriacus, A. caudatus and A. cruentus. The corn and four amaranth varieties were grown near the city of Nishapur (Khorasan-e Razavi Province, Iran); at 1250 m above sea level, with an average yearly (2017) rainfall of 250 mm and average temperature of 20 °C in a soil characterized as soft loam. A randomized complete block design with four replicates (four plots of 25 m² each per treatment) was used in the field study. The forages were sown on April 30 (2017). Amaranth cultivation was initially conducted in special culture trays (industry standard) with a coconut coir bed then planted out when the plants reached 10 cm height (industry standard). The corn was sown with a four-row precision drill (Tarashkadeh Co., Karaj, Iran). All plants were spaced in 50 cm rows. There was daily irrigation of amaranth plants growing in coco coir beds and every two days during the vegetative period in the field plots in the form of droplets with special drip irrigation strips (20 cm hole spacing, bar type). Mean total water volumes applied to the
amaranths and corn were 250 and 350 mm (i.e., 2500 and 3500 m³/ha), respectively. Ninety-three days after planting, the forages were harvested leaving a 10-cm stubble, and the corn was harvested at the milk stage of its kernels (growth stage BBCH-75; Lancashire et al., 1991). The forages were chopped into approximately 2 cm length cut. Homogeneous mixtures of each of the five treatments were packed tightly into 20-L plastic barrels, and excess air removed before the barrels were vacuumed to remove excess air before sealing to maintain the anaerobic environment. The barrels were ensiled for 60 days (one for each plot), and individual samples weighing 3-kg were taken before (i.e., fresh samples were taken per silo) and after ensiling for later analyses.

For measuring silage pH, 50 g of fresh silage was blended with 125 mL of distilled water and allowed to stand at room temperature for 1 h (Faithfull, 2002). After decanting the silage extract into a small beaker, the pH was measured using a digital pH meter (Sartorius PT-10; Germany). Two milliliters of juice from the silages were pipetted into centrifuge tubes containing 0.2 mL of acid (25% meta-phosphoric acid and 2-ethyl butyric acid 2 g/L as the internal standard), then centrifuged at 10,000 × g for 10 min at 4 °C (Galyean, 1997). Volatile fatty acids in the supernatant were quantified using gas chromatography (UNICAM 4600; SB Analytical, Cambridge, UK) with a flame ionization detector (FID; 250 °C), split-injection port (1.0 μL injection), capillary column (Agilent J & W HP-FFAP, 10 m by 0.535 mm by 1.00 μm, 19095F-121; Agilent, CA) and helium as the carrier gas (column head pressure of 10 psi). To determine NH₃-N, an extract was obtained by squeezing the silage material, filtered using Whatman 54 filter paper, then a 9 mL of aliquot was taken, mixed with 1 mL of 7.2 N H₂SO₄, and stored at −20 °C. After thawing, the silage extracts were analyzed for NH₃-N using a phenol-hypochlorite assay (Galyean, 1997).
Chemical analyses

Weighed samples were dried at 60 °C for 48 h period to determine DM concentration, ground to 1-mm sieve (Wiley mill; Thomas Scientific, Gloucester, NJ), and analyzed for organic matter (OM) (method 924.05), CP (method 984.13), ether extract (EE; method 954.02), ash-free acid detergent fiber (ADFom) and ADL (method 973.18) by AOAC (1998) procedures. Ash-free neutral detergent fiber (NDFom) was determined by Mertens (2002) method. The determination of water-soluble carbohydrates (WSC) concentration was carried out using the anthrone reaction assay and absorbance of the extract was measured by a spectrophotometer (MAFF, 1986). The colorimetry was used to measure nitrate (Singh, 1988). The levels of oxalic acid were measured using a spectrophotometer after extracting the total oxalate from 1 g of ground sample with 50 mL 2 M HCl at 80 °C for 15 min (Savage et al., 2000). The Folin-Ciocalteau method (Makkar, 2000) was used to measure total (TP) and non-tannin phenolics (NTP). The difference between TP and NTP gives the amount of total tannins (TT) with tannic acid (Merck GmbH, Darmstadt, Germany) used as the standard.

In vitro gas production and related variables

In vitro gas production (GP) was carried out for 24 h (Menke et al., 1979) to assess treatment gas production and fermentation variables (pH, NH₃-N, lactate, volatile fatty acids, cellulolytic bacteria and protozoa numbers, and methane). A probe used for ruminal fluid collection from rumen of three fistulated adult Shall sheep before the morning feeding. The sheep’s diet contained 10, 15, 40, 14, 15, 5, and 1% of AMS, CS, alfalfa hay, wheat bran, rolled barley, soybean meal, and a vitamin-mineral mix, respectively (on a DM basis). Sheep feeding times were 0700 and 1900 h, and they had free access to water. After filtering through cheesecloth (4 layers), the rumen fluid was mixed with an anaerobic mineral buffer (1:2, v/v) in CO₂ flushed thermos flasks warmed to 39 °C and stirred under CO₂ until use (Menke et al., 1979). Each silage sample of 200 mg was incubated for 24 hours at 39 °C in a pre-warmed 100 mL glass syringe containing 30 mL of rumen fluid and buffer (1:2, v/v). One hundred and sixty-
six syringes (five treatments × four blocks [replicates] × two individual samples per block [replicate] × two syringes per sample × two runs, with three blanks in each run) were used in total. After 24 h, the volume of gas (GP) was measured, and the disappearance of OM (OMD) and metabolizable energy (ME) were estimated as follows (Menke et al., 1979).

\[
\text{OMD (g/kg)} = 148.8 + (8.893 \times \text{GP}_{24}) + (0.448 \times \text{CP}) + (0.651 \times \text{XA})
\]

\[
\text{ME (MJ/kg DM)} = 2.20 + (0.1357 \times \text{GP}_{24}) + (0.0057 \times \text{CP}) + (0.00002859 \times \text{CP}^2)
\]

In the above equations, OMD is the disappearance of OM, GP_{24} is net gas produced over 24 h (mL/200 mg DM), CP is crude protein in g/kg DM, XA is ash in g/kg DM, and ME is the metabolizable energy.

Truly degraded substrate (TDS), partitioning factor after 24 h (PF_{24}) and microbial CP (MCP) of the treatments were measured by taking the contents of eight syringes per treatment from each run (four blocks × two individual samples × one syringe per sample), removing soluble products using a neutral detergent solution (Van Soest et al., 1991) then weighing the un-dissolved feedstuffs in crucibles, after washing and drying at 60 °C for 48 h. Loss in weight after drying was the measure of TDS (mg/g DM) (Blümmel et al., 1997). The PF_{24} (to estimate the efficiency of fermentation) was derived from the equation: PF_{24} (mg/mL) = TDS (mg)/GP_{24} (mL), where PF_{24} is the partitioning factor after 24 h (mg substrate truly degraded in vitro/mL gas) and GP_{24} is gas produced after 24 h (Blümmel et al., 1997). The MCP (mg/g DM) was derived from the equation: TDS (mg) - (mL GP_{24} × 2.2 mg/mL), the 2.2 mg/mL being a stoichiometric factor expressing mg of carbon, hydrogen and oxygen required for the short chain fatty acids–gas complex production needed for 1 mL of gas production (Blümmel et al., 1997).

Eight syringes per treatment per run (four blocks × two samples × one syringe per sample) were used to determine in vitro pH, VFA, NH₃-N, cellulolytic bacteria and protozoa numbers. After 24 h, the pH of the syringe contents was measured with a digital pH meter (Sartorius PT-10, Germany). A strained sample of 2.5 mL was mixed with 0.5 mL of 0.2 N HCl, and analyzed for NH₃-N using the phenol-hypochlorite assay (Galyean, 1997). For analysis of VFAs, 2 mL of
supernatants was preserved, at −20 °C, with 0.5 mL of an acid solution containing 20% orthophosphoric acid and 20 mM 2-ethylbutyric acid. Total VFAs were measured by gas liquid chromatography using ethyl-butyric acid as the internal standard. Counting total and sub-family numbers of protozoa from syringe contents was done using the method of Dehority (2003). Five mL of syringe contents, which was strained through 3 layers of cheesecloth into a CO₂-filled sterilized bottle (39°C), was used to enumerate the cellulolytic bacteria population. The anaerobic techniques of Hungate (1966) as modified by Bryant (1972) were used to prepare anaerobic culture media. Hungate tubes with anaerobic media and Whatman number 1 filter paper, as the carbohydrate source, were made. Strained rumen fluid was serially diluted and added to the tubes which were incubated at 39 °C for 21 days.

Methane produced in the syringes was determined using the method of Anele et al. (2011). The total GP24 from each syringe was measured then 4 mL of 10 M NaOH was added to each syringe to absorb CO₂, then the remaining gas volume was recorded as CH₄.

The GP kinetics of the treatments were evaluated using a 96-h in vitro gas production. Gas volumes were measured at 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h. Kinetic parameters were estimated using the exponential model described by Blümmel et al. (2003), \( y = B (1 - e^{-ct}) \), where \( y \) is volume of gas at time \( t \), \( B \) is the asymptotic value of GP (mL/200 mg DM) and \( c \) is the first order fractional constant rate of GP (/h).

**In situ degradability of DM and CP**

The degradability of DM and CP of the samples were calculated according to the nylon bag method, pre-weighed bags were placed in the rumens of four fistulated male Shal sheep of 62 ± 2.1 kg live weight (AFRC, 1992). The sheep’s diet contained 10, 15, 40, 14, 15, 5 and 1% of AMS, CS, alfalfa hay, wheat bran, rolled barley, soybean meal, and a vitamin-mineral mix, respectively (on a DM basis), fed twice daily at 0700 and 1900 h as a total mixed ration, and with 24 h access to water. The bag size was 21 cm × 10 cm with a pore size of 45 µm (Bucksburn, Aberdeen, UK). Dried silage samples were ground to 4 mm sieve in a Cyclotec TM
Sample Mill (Foss Companies, Hillerød, Denmark). Samples of 5g DM were placed into bags, and incubated for 2, 4, 8, 12, 24, 48, 72 and 96 h. One bag per sample per sheep for each time was used. Following incubation time, bags were removed by hand and rinsed under tap water, washed in a washing machine (Hoover OPHS 612; London, UK) using cold water for 1 h then dried in a 55 °C oven for 48 h. Time zero degradability was measured by cold water machine washing three extra bags per sample for 1 h. Bags and contents were weighed to estimate degraded DM. The percentage of ruminal degradability (Y) at time (t) was obtained from an exponential curve of the type \( Y = a + b (1 - e^{-ct}) \), which was fitted to the experimental data by iterative regression analysis (Ørskov and McDonald, 1979). In this equation, e is the base of natural logarithms, the constant a represents the soluble and very rapidly degradable fraction, and b represents the insoluble but potentially degradable fraction, which degrades at a constant fractional rate (c) per unit time. Effective DM degradability (ED) in the silages was then estimated (Ørskov and McDonald, 1979) as:

\[
ED = \frac{(a + b \times c)}{(c + k)},
\]

where ED is the effective DM degradability; constant a is the soluble and very rapidly degradable fraction; b is the insoluble but potentially degradable fraction, which degrades at a constant fractional rate (c) per unit time; and k is the fractional outflow rate of small particles from the rumen. An assumed value of \( k = 2\%/h \) was used, which is an average value for sheep given a mixed diet at a very low level of feeding, equivalent to approximately maintenance (Alderman et al., 1993). CP from bag residues was measured using the method No. 988.05 of AOAC (1998). The ruminal degradability percentage (Y) of DM and CP at time (t) was taken from an exponential curve where \( Y = A + B (1 - e^{-Ct}) \) which fitted the experimental data using iterative regression analysis (Ørskov and McDonald, 1979). The effective degradability (ED) of DM and CP was then estimated (Ørskov and McDonald, 1979) as \( ED(\%) = \frac{(A + B \times C)}{(C + k)} \). In these equations, ‘e’ is the base of natural logarithms, ‘A’ is the soluble and very rapidly degradable fraction, and ‘B’ represents the insoluble but potentially degradable CP 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 accepted value for ‘k’ was 0.02 fraction/h (Alderman et al., 1993). Effective RDP (ERDP), DUP and metabolizable protein (MP) levels were calculated from equations recommended by AFRC (1992).

Statistical analysis

Data on the yield, chemical composition and silage fermentation characteristics were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) in a randomized complete block design with the fixed effect of treatment. The model was $Y_{ijk} = \mu + T_i + B_j + e_{ij} + e_{ijk}$, where $Y_{ijk}$ is observation, $\mu$ is general mean, $T_i$ is treatment effect, $B_j$ is block effect, $e_{ij}$ is experimental error and $e_{ijk}$ is sampling error.

A split-plot in a randomized complete block design (including the effects of treatment, block [replicate], sample per block and run) was used for analyzing the in vitro GP data, where treatment was considered as the main plot and run as the subplot. The analysis was performed based on the model $Y_{ijkl} = \mu + T_i + B_j + e_{ij} + R_k + (TR)_{ik} + e_{ijk} + e_{ijkl}$. In this model, $Y_{ijkl}$, $\mu$, $T_i$, $B_j$, $e_{ij}$, $R_k$, $(TR)_{ik}$, $e_{ijk}$ and $e_{ijkl}$ are observation, general mean, treatment effect, block effect, treatment × block (main plot error), run effect, treatment × run, treatment × block × run (split-plot error) and treatment × block × run × sample (sampling error), respectively.

Prior to ANOVA, residual normality for data was tested using Proc UNIVARIATE. Multiple comparisons among the means were performed with the Tukey’s multiple range test. Statistical significance was defined by $P$-values ≤ 0.05.

RESULTS

Yield and chemical Composition

Yield of the forages before and after ensiling are shown in Table 1. When comparing yields per hectare of pre-ensiled corn and amaranth varieties, it was found that A28 had the highest fresh weight ($P<0.001$), DM ($P=0.032$), OMD, CP and MP ($P<0.001$).
Freshly harvested corn had higher \( (P<0.001) \) NDFom, ADFom, WSC, and phosphorus concentrations compared to the amaranth forages (Table 2). Corn silage had higher NDFom, ADFom, non-fiber carbohydrates (NFC) \( (P<0.001) \), and phosphorus \( (P=0.003) \) levels compared to AMS. The cultivar A28 had the highest CP level \( (P<0.001) \) compared to all other forages and silages. Among the amaranth varieties, A12 had the highest \( (P<0.001) \) NDFom level and A5 had the lowest \( (P<0.001) \) ADFom, ADL, and ash concentrations but higher concentration \( (P<0.001) \) of EE compared to the other amaranth forages and silages. Moreover, WSC concentration of the fresh forage \( (P<0.001) \) and silage \( (P=0.028) \) from variety A5 was higher than the other amaranth varieties.

Amaranth forage varieties had higher \( (P<0.001) \) nitrate, TP and TT concentrations than corn (Table 3). Among the silages, CS had the highest nitrate concentration, A12 had the highest oxalate and TP concentration, and A28 had the highest TT concentration \( (P<0.001) \).

**Silage fermentation characteristics**

Ammonia-N concentration and pH were higher \( (P<0.001) \) in AMS, compared to CS, their values ranging from 54.5 to 60 g/kg of total N and 4 to 4.6, respectively (Table 4). Among AMS, A5 had the lowest \( (P<0.001) \) pH and \( \text{NH}_3-N \) concentration.

**In vitro GP and fermentation variables**

The *in vitro* ruminal pH did not differ \( (P=0.93) \) among ensiled forages, but the level of \( \text{NH}_3-N \) was lower in CS \( (P<0.001) \), compared to AMS (Table 5). The incubated CS had higher *in vitro* total VFA concentration \( (P=0.005) \) and cellulolytic bacteria numbers \( (P=0.003) \) compared to the other silages, with no difference in total protozoa. Amaranth silages had higher \( (P=0.008) \) Entodiniinae protozoa count than CS, but CS and A5 had higher \( (P=0.009) \) Isotrichidae protozoa count than other silages. Corn silage had higher \( \text{GP}_{24} \), OMD, TDS \( (P<0.001) \), ME \( (P=0.043) \), and *in vitro* methane production \( (P<0.001) \) than AMS (Table 6). Amaranth silages had higher \( (P<0.001) \) *in vitro* ruminal MCP compared to CS. However, there was no difference
(P=0.060) among AMS in PF24. The \textit{in vitro} incubation of A12 and A28 resulted in lower methane production (P<0.001) than the other AMS.

\textbf{In situ degradability of DM and CP}

There were differences in CP and DM degradation in CS and amaranth varieties (Table 7). Among all silages, A5 had the highest (P<0.001) ED of DM. The ED of CP for A28 was the highest among AMS, but they were all lower (P<0.001) than that of CS. The cultivar A28 had higher (P<0.001) ERDP and MP in comparison with the other silages, and the DUP values of A12 and A28 were higher (P<0.001) than the other AMS.

\section*{DISCUSSION}

\textbf{Yield and chemical composition}

Among all the forages, the yields of A12, A18 and A28 were relatively good (11.8 to 14.1 t/ha on a DM basis) (Table 1). Yields of up to 86.4 t/ha as fed and 13.2 t/ha of DM have been recorded for amaranth forage, respectively (Mehrani et al., 2012) with differences in yield being attributed to differences in variety, soil, climate, season and N fertilizer usage (Klemencic and Kramberger, 2006).

Differences in the nutritional values of the amaranth varieties compared to corn are reflected in their chemical composition. The amaranth forage DM (> 25% of fresh weight) in this study was more than that observed by Rezaei et al. (2014), but was within the range reported by Abbasi et al. (2018). The lowest level (8% of DM) of CP needed to provide the necessary level of \( \text{NH}_3 \)-N to support optimum rumen microbiota growth (Norton, 1998) was found in all AMS suggesting that they have potential as a CP source in ruminant rations.

Compared to CS, AMS had lower levels of NDFom and ADFom showing their potential for ruminant fodder (Rezaei et al., 2014), as these compounds (plant cell wall derivatives) in high concentrations limit feed intake and energy availability (Jung and Allen, 1995). Seguin et al. (2013) showed that the cell wall contents of AMS fairly similar to our findings (361 to 405 g/kg}
DM), whereas Olorunnisomo and Ayodele (2009) and Rezaei et al. (2015) reported higher NDFom levels. The ADL concentrations in these AMS varieties (46 to 65 g/kg DM) were higher than those reported for AMS from other varieties (Seguin et al., 2013; Rezaei et al., 2015). Differences in ADL depend on the growth stage at harvest and the cultivar (Abbasi et al., 2012). Fermentation requires an adequate WSC concentration for conversion into lactic acid to lower pH for good quality silage (Kaiser et al., 2004). Forages with levels of 50 to 80 g WSC/kg DM should develop low pH sufficient for a stable silage (McDonald et al., 1991). In the current study, all pre-ensiled forages had WSC concentrations above 50 g/kg DM, and good stable silages were achieved. Low levels of residual WSC in the silages indicate increased bacterial utilization leading to higher lactic acid production. After ensiling, reductions in WSC, protein and NDF led to a proportional increase in silage EE levels. Similar to the present study, Abbasi et al. (2012) and Seguin et al. (2013) reported high ash concentration in amaranth. The ash content in AMS (182 to 215 g/kg DM) was genetically higher than that of CS, which was comparable to the results reported by the others (Olorunnisomo and Ayodele, 2009; Karimi-Rahjerdi et al., 2015). Compared to CS, AMS had higher Ca and lower phosphate concentrations. These Ca levels were lower than in other studies (Rezaei et al., 2014), and the Ca:P ratio in AMS, which ranged from 2.5:1 to 3.3:1, was higher than the recommended 2:1 ratio. However a Ca:P ratio of 2:1 or greater is recommended to prevent urinary calculi in sheep (NRC, 1985). The amaranth varieties had higher Mg concentrations compared to Plainsman and D136 varieties, but these differences may be caused by other changes in factors that influence plant growth (Seguin et al., 2013).

The Amaranth forage had higher levels of nitrate than corn but nitrate concentration decreased over time following a decline in CP (i.e., from 253 to 160 g/kg DM at 40 and 60 days after planting, respectively), similar to that reported by Sleugh et al. (2001). Ensiled amaranth had lower nitrate levels than the fresh forage, because of the action of microorganisms reducing nitrate to NH$_3$-N via nitrite in the ensiling process (McDonald et al., 1991). The nitrate content
of AMS, at 0.3 to 0.69 g/kg DM, was lower than the toxic level for ruminants \((i.e., > 6 \text{ g/kg DM})\) (Radostits et al., 2007).

High levels of oxalate ingestion can cause hypocalcaemia and kidney failure, and high doses can prove fatal (Knight and Walter, 2001). In this study, the ensiling of amaranth had no effect on oxalic acid concentration with oxalate in the silages ranging from 4.5 to 12.0 g/kg DM, which is lower than toxic level for ruminants \((i.e., 20 \text{ g/kg DM})\) proposed by Rahman et al. (2013). Teutonico and Knorr (1985) found that amaranth varieties showed a large variation in total oxalate concentration \((i.e., 2.0 \text{ to } 114 \text{ g/kg DM})\) and this may be related to cultivar, plant maturity, climate and field management (Bressani, 1993).

Tannin supplementation up to 40 g/kg DM in lambs’ diets showed no negative effects on ruminal fermentation or animal productivity in a long-term feeding trial (Salami et al., 2018). In the current study, the levels of TT in AMS were low, between 1.2 to 4.9 g/kg DM, and therefore had no adverse effect on the \textit{in vitro} fermentation. Tannins may bind to protein in a ration and reduce its digestibility, but their effect is variable and may depend less on their concentration than their structure and molecular weight (Rochfort et al., 2008).

\textit{Silage fermentation characteristics}

Among the silages, the pH of CS was lower than that of the AMS because its higher WSC content led to higher lactic acid production (Kaiser et al., 2004). The AMS pH ranged from 4.0 to 4.6 which is considered low enough for an acceptable preservation (Faithfull, 2002). The pH of amaranth silages from other cultivars were within the range of AMS obtained in current study (Rezaei et al., 2014, 2015) and (Seguin et al., 2013).

Protein degradation during fermentation is minimized in good quality silage (McDonald et al., 1991) and the lower level of NH₃-N in CS (39.5 g/kg total N) compared to the (54 to 60 g/kg total N) in AMS probably resulted from its lower pH, as this reduces protein breakdown (Kaiser et al., 2004). The NH₃-N concentration in AMS was relatively low for a low DM (< 300 g/kg).
silage (Demarquilly, 1990) indicating good preservation but Rezaei et al. (2009) reported lower NH$_3$-N concentrations in low DM AMS.

Silage lactic acid levels (59 to 65 g/kg DM) in the present study were within the range of good quality silage (30 to 140 g/kg DM) characterized by ZoBell et al. (2004). In other work, higher lactic acid levels have been recorded for AMS (Seguin et al., 2013; Rezaei et al., 2014). Low levels of butyric acids (Table 4) in the AMS silages indicated that there had been good fermentation (Kaiser et al., 2004).

**In vitro GP and fermentation variables**

The *in vitro* ruminal pH values of the present treatments varied within the normal range of 5.9 to 7.0 reported by Dehority (2003) and the NH$_3$-N levels were within the range for optimal growth of the rumen microbiota (> 50 mg/L) (Sinclair et al., 1993). The *in vitro* incubation of CS led to a lower NH$_3$-N concentration compared to AMS, related to its lower CP level. Rumen VFA production depends on the levels of cellulose, hemicellulose, CP and WSC in the feed (Bureenok et al., 2011). The *in vitro* ruminal total VFA levels differed among the treatments suggesting that the ruminal fermentability of OM differed among the silages. The greater degradation (TDS) of CS compared to AMS explains the higher total VFA concentrations from CS during *in vitro* fermentation (Van Soest, 1994).

The higher *in vitro* ruminal cellulolytic bacteria population of CS in comparison with AMS corresponds to its higher NDF substrate concentration (Van Soest, 1994).

The lower *in vitro* GP$_{24}$, OMD, TDS and ME in AMS compared to CS was possibly due to the lower NFC and cellulolytic bacteria numbers, and higher ADL concentration (McDonald et al., 2011). Amaranth varieties used in the current study had lower GP$_{24}$, OMD, ME and TDS than those observed by Sarmadi et al. (2016), and this may be due to their higher ash and ADL levels.
The PF$_{24}$ values of AMS were between 2.75 and 4.41 mg/mL, within a theoretical range defined by Makkar (2010), but the value for CS was below this range. Feedstuffs that have a higher PF$_{24}$ (mg degraded substrate divided by mg gas produced in vitro) have more degraded substrate incorporated into microbial mass (i.e., improved efficiency of MCP production; Makkar, 2010). Consequently, the MCP of AMS was higher than that of CS incubated in vitro. The lower in vitro methane production of AMS compared to CS relates to their lower NDF$_{om}$ concentration, as methane production positively correlates with levels of NDF (Pinares-Patino et al., 2007). The higher DUP in AMS compared to CS corresponded to the decline in in vitro methane, i.e., lower rumen protein fermentation results in lower methane production (Preston et al., 2012; Ho Quang Do et al., 2013).

**In situ degradability of DM and CP**

For DM degradability, the increased fraction B in AMS compared to CS could be related to their higher CP content (i.e., more CP for ruminal bacteria to ferment fraction B) (McDonald et al., 2011). Van Soest (1994) observed that there was a correlation between ED and NFC, however, in current study such correlation was not obtained in the AMS silages. Compared to reports by Karimi-Rahjerdi et al. (2015) and Sarmadi et al. (2016), the AMS in this study had a lower ED of DM which is probably due to its higher ADL content, as high ADL concentrations can limit ruminal degradability (Jung and Allen, 1995).

Data on ruminal CP degradability (i.e., protein quality) of AMS are limited. In this study, AMS contained less fraction A and more fraction B and DUP compared to CS and this may be due to more CP being bound to cell wall components (Abbasi et al., 2012). The higher DUP in AMS may be due to the presence of tannins reducing proteolytic bacteria activity in the in vitro fermentation (Molan et al., 2001).

Since yield was measured using one year and one location, further research is warranted.
CONCLUSION

In comparison to CS, the ensiled amaranth from the cultivar A28 could be a potential forage resource for ruminants based on its high DM yield, CP and DUP content, with low anti-nutrient compounds and in vitro methane production (i.e., A28 could be used instead of CS). However, more in vivo work is needed to assess the amaranth forage quality particularly in terms of intake.
Conflict of interest statement

The authors declare that there were no conflicts of interest.

ACKNOWLEDGMENTS

The authors would like to thank the Iran National Science Foundation for the financial support given to carry out this trial, and gratefully acknowledge Mr. Gary Easton for his English language correction of the manuscript.
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Table 1. Yields of the fresh forages and silages of four amaranth varieties (A5, A12, A14, A28) and corn.

| Item                  | Corn | A5    | A12   | A14   | A28   | SEM² | P-value |
|-----------------------|------|-------|-------|-------|-------|------|---------|
| Fresh forage          | 35.0d| 19.0e | 44.2c | 51.0b | 56.0a | 1.20 | <0.001  |
| DM                    | 9.10c| 4.91d | 11.8ab| 12.9ab| 14.1a | 0.47 | 0.032   |
| OM                    | 8.40b| 4.01c | 9.58ab| 10.3a | 11.0a | 0.52 | <0.001  |
| OMD                   | 5.68a| 2.23b | 5.22a | 6.02a | 6.13a | 0.50 | <0.001  |
| CP²                   | 0.683d|0.351e | 1.92b | 1.52c | 2.57a | 0.03 | <0.001  |
| MP                    | 0.410|       |       |       |       |      |         |
|                       | 0.225d|1.23b | 0.984c| 1.59a |       | 0.008| <0.001  |

¹DM = dry matter; OM = organic matter; OMD = organic matter disappearance; ²CP = crude protein estimated from total nitrogen content x 6.25; MP = metabolizable protein. ²SEM = standard error of the means.
Table 2. Chemical composition (g/kg DM) of the fresh forages and silages of four amaranth varieties (A5, A12, A14, A28) and corn.

| Item | Fresh forages | Ensilied |
|------|--------------|----------|
|      | Corn | A5 | A12 | A14 | A28 | SEM | P-value | Corn | A5 | A12 | A14 | A28 | SEM | P-value |
| DM   | 252  | 251 | 258 | 246 | 247 | 4.8 | 0.12    | 260  | 258 | 267 | 253 | 251 | 7.0 | 0.13    |
| CP   | 80d  | 83d | 176b | 129c | 192a | 1.9 | <0.001  | 75.0d | 81.4d | 163b | 118c | 182a | 1.8 | <0.001  |
| NDFom| 515a | 402b | 413b | 373c | 376c | 4.0 | <0.001  | 490a | 391b | 405b | 361c | 367c | 4.2 | <0.001  |
| ADFom| 318a | 246d | 273c | 249d | 289b | 3.3 | <0.001  | 292a | 235d | 265c | 239d | 281b | 2.3 | <0.001  |
| ADL  | 51.0c | 47.3d | 61.3b | 59.3b | 64.6a | 1.0 | <0.001  | 48.7c | 53.0b | 59.3ab | 56.0b | 62.0a | 1.3 | <0.001  |
| WSC  | 111a | 75.5b | 59.6d | 63.3b | 53.8a | 0.73 | <0.001  | 15.2ab | 15.1b | 11.0c | 15.0b | 14.5b | 0.87 | 0.028  |
| EE   | 32.5c | 44b | 40b | 41.5b | 42b | 0.93 | <0.001  | 42.5c | 90.3a | 81.6b | 77b | 77b | 1.1 | <0.001  |
| NFC  | 310a | 321a | 209b | 269b | 200c | 2.89 | <0.001  | 317a | 265b | 163d | 241c | 159d | 1.8 | <0.001  |
| Ash     | 63<sup>c</sup> | 150<sup>c</sup> | 163<sup>b</sup> | 188<sup>a</sup> | 190<sup>a</sup> | 1.1 | <0.001 | 76.0<sup>c</sup> | 182<sup>d</sup> | 188<sup>c</sup> | 203<sup>b</sup> | 215<sup>a</sup> | 0.90 | <0.001 |
|---------|----------------|-----------------|-----------------|-----------------|-----------------|-----|---------|----------------|----------------|----------------|----------------|----------------|------|---------|
| Calcium | 4.5<sup>b</sup> | 12.1<sup>a</sup> | 11.6<sup>a</sup> | 12.4<sup>a</sup> | 12.0<sup>a</sup> | 0.49 | <0.001 | 4.6<sup>b</sup> | 12.1<sup>a</sup> | 11.8<sup>a</sup> | 12.7<sup>a</sup> | 12.0<sup>a</sup> | 0.48 | <0.001 |
| Phosphorus | 7.1<sup>a</sup> | 4.0<sup>b</sup> | 3.8<sup>b</sup> | 3.7<sup>b</sup> | 3.6<sup>b</sup> | 0.42 | <0.001 | 7.1<sup>a</sup> | 4.0<sup>b</sup> | 3.8<sup>b</sup> | 3.8<sup>b</sup> | 3.6<sup>b</sup> | 0.38 | 0.003 |
| Magnesium | 3.5            | 3.5             | 3.7             | 3.9             | 3.6             | 0.28 | 0.11   | 3.7             | 3.6             | 3.8             | 3.8             | 3.8             | 0.19 | 0.19   |

<sup>1</sup>DM = dry matter (g/kg as fed); <sup>2</sup>CP = crude protein estimated from total nitrogen content x 6.25; NDFom = ash-free neutral detergent fiber; ADFom = ash-free acid detergent fiber; ADL = lignin measured after solubilization of cellulose with 72% sulphuric acid; EE = ether extract; WSC = water-soluble carbohydrates; NFC = non-fiber carbohydrates. <sup>2</sup>SEM = standard error of the means.
Table 3. Antinutritional factors levels (g/kg DM) of the fresh forages and silages of four amaranth varieties (A5, A12, A14, A28) and corn.

| Item     | Fresh forages |          |          |          |          |          |          |          |          |          |          |          | Ensilaged |          |          |          |          |          |          |          |          |          |          |
|----------|---------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
|          |               | Corn A5  | A12 A14  | A14 A28  | SEM³     | P-value  | Corn A5  | A12 A14  | A14 A28  | SEM³     | P-value  |
| Nitrate  |               |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| Oxalate  |               |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| TP       |               |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |
| TT       |               |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |          |

¹TP = total phenolics; ²TT = total tannins. ³SEM = standard error of the means.
Table 4. Fermentative characteristics of the corn and amaranth silages varieties (A5, A12, A14, A28).

| Item                      | Corn | A5  | A12 | A14 | A28 | SEM\(^1\) | P-value |
|---------------------------|------|-----|-----|-----|-----|-----------|---------|
| pH                        | 3.8\(^c\) | 4.0\(^d\) | 4.2\(^c\) | 4.4\(^b\) | 4.6\(^a\) | 0.057    | <0.001  |
| NH\(_3\)-N, g/kg total N  | 39.5\(^c\) | 54.5\(^b\) | 58.0\(^a\) | 59.5\(^a\) | 60.0\(^a\) | 0.8      | <0.001  |
| Lactic (g/kg DM)          | 65.2 | 63.0 | 61.0 | 59.1 | 58.5 | 4.7      | 0.64    |
| Acetic (g/kg DM)          | 19.0 | 17.8 | 16.7 | 16.4 | 16.0 | 1.01     | 0.91    |
| Propionic (g/kg DM)       | 3.2  | 3.2  | 3.9  | 4.2  | 4.4  | 0.6      | 0.70    |
| Butyric (g/kg DM)         | 0.21 | 0.25 | 0.40 | 0.46 | 0.52 | 0.04     | 0.73    |

\(^1\)SEM = standard error of the means.
Table 5. *In vitro* ruminal fermentation characteristics, ruminal protozoa numbers ($\times 10^5$/mL digesta) and cellulolytic bacteria numbers (log$_{10}$/g digesta) of the amaranth silages varieties (A5, A12, A14, A28) and corn.

| Item                        | Corn  | A5    | A12   | A14   | A28   | SEM$^2$ | P-value |
|-----------------------------|-------|-------|-------|-------|-------|---------|---------|
| pH                          | 6.72  | 6.72  | 6.76  | 6.79  | 6.80  | 0.58    | 0.93    |
| NH$_3$-N (mg/dL)            | 11.7$^d$ | 14.8$^c$ | 15.5$^c$ | 17.3$^a$ | 16.3$^b$ | 0.23    | <0.001  |
| Total VFA$^1$ (mmol/L)      | 63.0$^a$ | 57.5$^b$ | 53.9$^b$ | 57.0$^b$ | 53.5$^b$ | 1.4     | 0.005   |
| VFA (mol/100 mol)           |       |       |       |       |       |         |         |
| Acetate                     | 72.5  | 68.6  | 70.0  | 70.6  | 69.6  | 5.7     | 0.59    |
| Propionate                  | 20.2  | 21.5  | 20.8  | 20.5  | 21.3  | 2.5     | 0.88    |
| Butyrate                    | 5.6   | 7.7   | 7.1   | 6.1   | 6.8   | 0.9     | 0.38    |
| Isovalerate                 | 1.7   | 2.2   | 2.5   | 2.8   | 2.3   | 0.44    | 0.97    |
| Total protozoa              | 6.68  | 6.78  | 6.66  | 6.78  | 6.60  | 0.08    | 0.59    |
| *Isotrichidae*              | 1.93$^a$ | 1.92$^a$ | 1.60$^{bc}$ | 1.80$^{ab}$ | 1.51$^c$ | 0.07    | 0.009   |
| *Entodiniinae*              | 2.81$^e$ | 3.03$^d$ | 3.11$^{bc}$ | 3.08$^{cd}$ | 3.25$^a$ | 0.029   | 0.008   |
| *Diplodiniinae*             | 1.12  | 1.01  | 1.15  | 1.08  | 1.02  | 0.06    | 0.57    |
| *Ophrioscolecinae*          | 0.823 | 0.823 | 0.798 | 0.822 | 0.823 | 0.024   | 0.97    |
| Cellulolytic bacteria        | 8.8$^a$ | 8.2$^b$ | 8.3$^b$ | 8.4$^b$ | 8.2$^b$ | 0.086   | 0.003   |

$^1$VFA = volatile fatty acids. $^2$SEM = standard error of the means.
Table 6. *In vitro* ruminal gas production and estimated parameters, truly degraded substrate (TDS) and microbial protein (MCP) of the amaranth silages varieties (A5, A12, A14, A28) and corn.

| Item                          | Corn | A5  | A12 | A14 | A28 | SEM² | p- value |
|-------------------------------|------|-----|-----|-----|-----|------|----------|
| 24-h incubation               |      |     |     |     |     |      |          |
| GP₂₄ (mL/200 mg DM)¹          | 50ª  | 29ª | 22.5ª| 28ª | 21ª | 0.88 | <0.001   |
| OMD (g/kg)                    | 676ª | 557ª| 545ª| 584ª| 557ª| 5.7  | <0.001   |
| ME (MJ/kg DM)                 | 9.57ª| 6.69ª| 6.95ª| 7.08ª| 6.93ª| 1.18 | 0.043    |
| TDS (g/kg DM)                 | 658ª | 625ª| 635ª| 632ª| 614ª| 2.6  | <0.001   |
| PF₂₄ (mg TDS/mL GP₂₄)²        | 2.63ª| 4.31ª| 5.64ª| 4.80ª| 5.84ª| 0.88 | 0.060    |
| MCP (g/kg DM)                 | 108ª | 306ª| 388ª| 364ª| 383ª| 1.95 | <0.001   |
| *In vitro* methane (mL/g DM)  | 29.5ª| 25.6ª| 21.5ª| 25.0ª| 20.0ª| 0.68 | <0.001   |
| 96-h incubation               |      |     |     |     |     |      |          |
| B (mL/200 mg DM)              | 57.3ª| 50.0ª| 40.0ª| 42.1ª| 50.0ª| 0.51 | <0.001   |
| C (/h)                        | 0.030ª| 0.034ª| 0.034ª| 0.070ª| 0.035ª| 0.002| <0.001   |

¹GP₂₄ = *in vitro* ruminal gas production at 24 h; OMD = estimated organic matter disappearance; ME = metabolizable energy; TDS = truly degraded substrate; ²F₂₄ = partitioning factor at 24 h of incubation; MCP = microbial crude protein; B = the asymptotic value of gas production; C = constant rate of gas production. ²SEM = standard error of the means.
Table 7. *In situ* degradability of dry matter and crude protein of the silages (4 amaranth varieties and corn).

| Item       | Corn | A5  | A12 | A14 | A28 | SEM² | P-value |
|------------|------|-----|-----|-----|-----|------|---------|
| DM degradation¹ |      |     |     |     |     |      |         |
| A (g/kg DM) | 420a | 425a | 373b | 436a | 378b | 8.6  | <0.001  |
| B (g/kg DM) | 340c | 408b | 354b | 353b | 535a | 8.5  | <0.001  |
| C (/h)     | 0.038c | 0.062a | 0.380c | 0.040b | 0.014d | 0.008 | <0.001  |
| ED (g/kg DM) | 602b | 632a | 573c | 583bc | 509d | 6.53 | <0.001  |
| CP degradation |      |     |     |     |     |      |         |
| A (g/kg CP) | 460a | 361d | 334e | 387c | 426b | 6.9  | <0.001  |
| B (g/kg CP) | 324d | 347c | 372b | 330cd | 454a | 6.9  | <0.001  |
| C (/h)     | 0.041a | 0.030b | 0.029c | 0.017c | 0.019c | 0.006 | <0.001  |
| ADIN       | 1.10c | 1.16e | 2.42a | 1.75b | 2.66a | 0.1  | <0.001  |
| ED (g/kg CP) | 605a | 492c | 480c | 471c | 550b | 6.8  | <0.001  |
| ERDP (g/kg DM) | 38.5d | 30.2e | 67.1b | 46.3c | 85.1a | 1.01 | <0.001  |
| DUP (g/kg DM) | 20.0d | 26.5c | 62.7a | 46.2b | 59a  | 1.21 | <0.001  |
| MP (g/kg DM) | 45.1d | 46.0d | 105b | 76.3c | 113a | 1.27 | <0.001  |

¹A = 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 0.02/h; ERDP = effective rumen degradable protein; DUP = digestible undegradable protein; MP = metabolizable protein. ²SEM = standard error of the means.