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Chemical Composition, *In Vitro* Digestibility and Fatty Acid Profile of *Amaranthus caudatus* Herbage During its Growth Cycle

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ABSTRACT

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The aim of this study was to investigate the effects of plant ageing on the chemical composition, gross energy, *in vitro* true digestibility, neutral detergent fibre digestibility and fatty acid (FA) profile of amaranth. The herbage was harvested at seven maturity stages, namely early-, mid- and late-vegetative, shooting, budding, early flowering and grain fill. The effects of maturity were analysed by polynomial contrasts. The quality of crop decreased with increasing morphological stages. The chemical composition of amaranth was found closely connected to plant development; while the OM and CP contents decreased ($P<0.05$), the contents of NDF, ADF, and lignin(sa) increased from the first to the last stage ($P<0.05$). Consequently, nutritive parameters decreased with increasing growth stage. Even though the lipid content did not differ significantly during plant development, the pattern of FA changed during plant growth. The α-linolenic acid content decreased (linear $P<0.01$; quadratic $P<0.05$; cubic $P<0.01$), while linoleic acid content increased (linear $P<0.01$; quadratic $P<0.05$) with increasing maturity. As far as other FAs are concerned, palmitic, stearic and oleic acid increased ($P<0.01$), while stearidonic acid decreased ($P<0.05$) in later growth stage. A minor FA such as α-linolenic acid did not differ significantly during the growth cycle. It is concluded that since its nutritional quality deteriorates and polyunsaturated FA content decreases when cutting is delayed, the first cut of amaranth should be before or at the shooting stage.

Keywords: Amaranth, Forage, Lipids, Morphological stage, Nutritive value.

INTRODUCTION

High nutritional costs, human-animal competition and increasing interest in the nutraceutical properties of crops have made it necessary to search for alternative feed

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sources. Recently *Amaranthus* spp., an ancient, unconventional and multipurpose Andean crop has been rediscovered, attracting researchers' attention on several levels (Venskutonis and Kraujalis, 2013). Due to its high adaptability to diverse environmental conditions including poor soils and shortage of water (Barba de la Rosa *et al.*, 2009; Rezaei *et al.*, 2014), it is currently cultivated in all temperate and tropical areas of the world (Svirskis, 2003).

It has been shown by different authors that *Amaranthus* has potential as a source of animal feed (Pospišil *et al.*, 2009; Seguin *et al.*, 2013; Alegbejo, 2013; Rezaei *et al.*, 2013, 2014), even though it is commonly used in human nutrition for its beneficial health properties (anti-hypercholesterolemic, antitumor, and antioxidant, etc.) and for the high nutritional value of its grain (Rodríguez *et al.*, 2011; Caselato-Sousa and Amaya-Farfan, 2012).

In livestock nutrition, amaranth can be used in different ways, as a grain and as fresh, dried or ensiled forage (Rezaei *et al.*, 2009; Rodríguez *et al.*, 2011). Studies have demonstrated that this plant could be considered as a nutrient substitute for conventional feed for rabbits (Molina *et al.*, 2015), pigs (Zraly *et al.*, 2004; Kambashi *et al.*, 2014), chickens (Písaøíková *et al.*, 2006) and ruminants (Sleugh *et al.*, 2001; Abbasi *et al.*, 2012), especially in tropical and sub-tropical regions.

The advantage of amaranth grain lies in its high content of CP of high quality, rich in essential amino acids, especially lysine, which is a limiting amino acid in other cereal crops (Svirskis, 2003), and acceptable levels of tryptophan and methionine. Amaranth grain also has a higher concentration of soluble fibre than many cereals, such as wheat, corn or oat (Plate and Arêas, 2002) and minerals (Shukla *et al.*, 2006). Moreover, Plate and Arêas (2002) demonstrated the cholesterol-lowering effect of *Amaranthus caudatus* that, when included in rabbit diet (either as oil or extruded) reduces low-density lipoprotein and total cholesterol levels in the blood. *Amaranthus caudatus* can produce leafy edible vegetables and grains and has several features that make it attractive as a potential nutraceutical crop due to its antioxidant activity and fatty acids (FAs), tocopherol, squalene and phenolic contents (Bruni *et al.*, 2001; Conforti *et al.*, 2005).

The interest in amaranth as a feedstuff has been aroused due to its high nutritive value. This depends on the development stage at which cutting was carried out (Pospišil *et al.*, 2009), but generally the plants are capable of producing high yields (up to 86.4 t fresh forage/ha) of very nutritious herbage (Kadoshnikov *et al.*, 2008; Abbasi *et al.*, 2012). With regard to digestibility, the data from Seguin *et al.* (2013) indicated that fresh and ensiled amaranth are both highly degradable in the rumen.

In contrast to its grain, *Amaranthus caudatus* forage has received significantly less research attention. The objective of this study was to describe the nutritive characteristics of *Amaranthus caudatus* plant and to evaluate how development stage affects its digestibility, chemical composition and FA profile, in order to determine its potential for use as dietary supplement for livestock.

MATERIALS AND METHODS

Plant material

Amaranthus caudatus seeds were kindly furnished by Pedon S.p.A. (Molvena, Italy). The study was carried out at the Department of Agriculture, Forestry, and Food Sciences of the University of Turin. Field trials were carried out in Grugliasco, Piedmont, NW Italy (45°03'57.9"N 7°35'36.9"E, 293m above sea level), in a sandy soil, low in organic matter with moderately alkaline pH (Subedi *et al.*, 2016) and taxonomically classified as Entisol according to the USDA soil classification system (USDA, 1999). The climate is temperate sub-continental, characterized by two main rainy periods in spring and autumn. Total precipitation falling during the soybean growing varies from 76.2 mm per month (in May) to 139.0 mm per month (in July), while relative humidity and mean temperature during the growing period are 68.6% and 20.3°C, respectively.

The amaranth stands were seeded in the spring (April 2014), and no fertilizers or irrigation were applied after sowing. The herbage samples were collected with edging shears (10 cm cutting width) at seven progressive morphological stages from early vegetative to grain fill stage from May to July 2014. Plants were cut to a 1–2 cm stubble height from 2 m^2 subplots randomly located in 2×7 m^2 plots with two replicates. Sampling was done in the morning after evaporation of dew and was never carried out on rainy days.

Chemical analysis

The herbage samples were immediately dried in a forced-draft air oven to a constant weight at 65°C. Samples were then brought to air temperature, weighed, ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass through a 1-mm screen and stored for qualitative analyses. Dried herbage samples were analyzed by methods of AOAC (1990) for DM (#925.40), N (#984.13), and ash (#923.03). Neutral detergent fibre (NDFom), acid detergent fibre (ADFom) and lignin(sa) were determined with the Ankom200 Fibre Analyzer (Ankom Technology Corp., Macedon, NY, USA), following the Ankom Technology Method and corrected for residual ash. The gross energy (GE) was determined using an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany). Fresh samples (200g) of the herbage were refrigerated, freeze-dried and ground to pass through a 1-mm screen. Lipid content was quantified on freeze-dried samples according to Hara and Radin (1978). The FAs were analyzed as their methyl esters according to Peiretti *et al.* (2013).

In vitro digestibility

In vitro digestibility at 48h was performed using the DaisyII Incubator (Ankom Technology Corp.) as per Robinson *et al.* (1999). Duplicate samples of the freeze-dried herbage were inserted into an Ankom F57 filter bag, sealed and incubated in a jar containing pre-warmed (39°C) buffer solution. Sample incubation was performed in one fermentative run with 400 ml of rumen liquor collected at a slaughterhouse, from three cattle of the same farm fed a fibre-rich diet, as described by Spanghero *et al.* (2010).

After incubation, samples were analyzed for NDFom content with the Ankom200 Fibre Analyzer and incinerated to correct the residual neutral detergent fibre for the residual ash. *In vitro* true digestibility (IVTD) and NDF digestibility (NDFD) were calculated as reported by Tassone *et al*. (2014).

Statistical analysis

The variability in the FA and herbage quality characteristics harvested at seven different stages of maturity were analyzed for their statistical significance by analysis of variance using the Statistical Package for Social Science (SPSS, 2002) to test the effect of the growth stage. In addition, single-degree-of-freedom polynomial contrasts were used to test for linear, quadratic and cubic effects due to morphological stage (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical composition and nutritional quality

The DM content of the plant during growth (Table 1) was lowest during the three vegetative stages and then increased with advancing morphological stage from the shooting to grain-fill stages (linear $P<0.01$ and cubic $P<0.01$), while ash and CP content decreased from early vegetative stage to early flowering (linear $P<0.01$ and quadratic $P<0.01$). Lipid content did not differ significantly during plant development. The NDFom, ADFom and lignin(sa) content increased with maturity (all fibers: linear $P<0.01$; lignin(sa): quadratic $P<0.05$; ADFom and lignin(sa): cubic $P<0.05$ and $P<0.01$, respectively) likely due to progressive translocation of the soluble cell contents from the stems and leaves to the seed. The GE content was at its lowest at the late vegetative stage and highest at the early flowering stage. The IVTD at 48h of incubation decreased (linear $P<0.01$ and quadratic $P<0.05$), while NDFD decreased (linear and cubic $P<0.01$) with increasing stages of plant maturity.

The results of the trial indicate that the development stage influenced the chemical composition and digestibility of *Amaranthus caudatus*, in accord with other authors (Sleugh *et al*., 2001; Pospišil *et al*., 2009). Except for lipids, a decline in the quality of the forage was observed with plant development.

The increasing DM content with plant maturity was consistent, as described by Sleugh *et al*. (2001) and Abbasi *et al*. (2012). Abbasi *et al*. (2012) assessed the effects of harvest date and N fertilization rate on the nutritive value of amaranth (*Amaranthus hypochondriacus*) forage. They found that the later harvest date (60d after planting) increased ($P<0.05$) DM, ether extract, NDFom, ADFom, and lignin(sa); while CP, *in vitro* gas production, OM disappearance and NDFom disappearance decreased ($P<0.05$) in comparison with the first harvest date (40 d after planting). With increasing N fertilization rate yield, CP concentration and nutrient digestibility increased ($P<0.05$). Generally, in our study, CP content was higher (243.8–105.8 g/kg during maturity) than those of other vegetable types in other studies (Seguin *et al*., 2013; Molina *et al*., 2015) and similar to *Amaranthus hybridus* spp. in the work of Kambashi *et al*. (2014).

Table 1. Chemical composition (% DM basis), gross energy (GE), *in vitro* dry matter digestibility (IVDMD) and *in vitro* neutral detergent fibre digestibility (IVNDFD) of *Amaranthus caudatus* plant at seven morphological stages

Attributes	Stage of growth[†]							SEM	Contrasts[‡]		
	Early vegetative (d43)	Mid vegetative (d50)	Late vegetative (d57)	Shooting (d64)	Budding (d71)	Early flowering (d79)	Grain fill (d90)		L	Q	C
DM	11.45	12.83	11.48	13.69	14.64	17.86	16.00	0.62	**	NS	**
Ash	24.29	20.51	18.11	16.78	15.76	14.84	15.31	0.88	**	**	NS
Crude protein	24.38	19.16	12.57	11.00	9.36	7.33	10.58	1.57	**	**	NS
Lipid	1.46	1.58	1.36	1.44	1.07	1.39	1.42	0.07	NS	NS	NS
NDFom	32.44	36.61	39.02	43.03	45.82	42.68	51.50	1.65	**	NS	NS
ADFom	22.77	24.26	26.66	29.29	34.86	32.62	34.91	1.29	**	NS	*
Lignin(sa)	7.53	8.48	8.60	8.73	9.22	9.88	10.93	0.28	**	*	**
GE (MJ/kg DM)	15.59	16.56	15.54	16.56	16.62	16.95	16.42	0.14	**	*	*
IVTD (% DM)	97.62	96.51	96.12	90.88	82.94	84.60	78.10	2.00	**	*	NS
NDFD (% NDF)	92.63	90.65	90.28	77.78	61.56	60.65	57.48	4.06	**	NS	**

[†]Data within parentheses indicate period after sowing in days.

[‡]Contrasts: L, linear; Q, quadratic; C, cubic. Significance: *$P<0.05$; **$P<0.01$; NS, not significant.

The NDFom, ADFom and lignin(sa) concentration tended to increase with advancing stage, due to the decline of the leaf:stem ratio (Freer and Dove, 2002), even though at the early flowering stage the NDFom and ADFom values were lower than at the budding stage. *Amaranthus caudatus* fiber values were similar to those for other vegetable-type amaranths during maturation (Sleugh *et al.*, 2001; Pospišil *et al.*, 2009). In agreement with the increase in ADF and lignin content, IVTD decreased significantly from early vegetative to grain fill (0.98–0.78 g/g). The trend was similar for NDFD (0.93–0.57 g/g). Sleugh *et al.* (2001) evaluated the forage quality of various amaranth accessions at different harvest dates. Averaged over accessions, they showed that *in vitro* DM digestibility decreased ($P<0.05$) from 780 g/kg at 42d after planting to 680 g/kg at 112d after planting and CP content decreased from 270 g/kg to 100 g/kg at the later harvest. NDFom increased from 310 g/kg to 430 g/kg. They concluded that forage quality of the amaranth accessions at most harvest dates was consistent with what would be expected for relatively good-quality forage.

Fatty acid composition

The FA profile of the plant during growth (Table 2) was characterized by a high proportion of á-linolenic acid (ALA, C18:3 n-3) and of linoleic acid (LA, C18:2 n-6), which represent from 683 to 516 g/kg of total FA during plant growth. ALA content decreased (linear $P<0.01$; quadratic $P<0.05$; cubic $P<0.01$), while LA content increased (linear $P<0.01$; quadratic $P<0.05$) with increasing growth stages. As far as other FAs are concerned, palmitic (C16:0) increased (linear $P<0.01$) and stearidonic acid (C18:4 n-3) decreased (linear $P<0.05$), while stearic (C18:0) and oleic acid (C18:1 n-9) increased (linear $P<0.01$; quadratic $P<0.01$; cubic $P<0.01$ and $P<0.05$, respectively) with increasing growth stages. Minor FAs such as ã-linolenic acid (C18:3 n-6) did not differ significantly during the growth cycle.

The oil content of amaranth grain ranged from 58 to 71 g/kg for *Amaranthus cruentus* (Berganza *et al.*, 2003), while Hlinková *et al.* (2013) showed that oil content of *Amaranthus cruentus* ranged from 64 to 82 g/kg and from 63 to 87 g/kg for *Amaranthus hypochondriacus*.

The FA profile of amaranth grain is characterized by three dominant FAs (LA, palmitic, and oleic acids) and other minor FAs (ALA, stearic, and arachidic acids) (Hlinková *et al.*, 2013). Berganza *et al.* (2003) found in decreasing order: LA (335–440 g/kg total FAs), oleic (203–320 g/kg total FAs), palmitic (171–213 g/kg total FAs), and stearic acid (30–38 g/kg total FAs). The major FAs reported by Jahaniaval *et al.* (2000) in the grain of five *Amaranthus* accessions (*Amaranthus cruentus*, *Amaranthus hypochondriacus* and *Amaranthus hypochondriacus* × *Amaranthus hybridus*) were LA (394–491 g/kg total FAs), oleic (228–315 g/kg total FAs) and palmitic acid (214–238 g/kg total FAs) with a low content of ALA (6.5–9.3 g/kg total FAs). As far as the FA profile of *Amaranthus caudatus* seeds is concerned, Bruni *et al.* (2001) showed that major FAs were: LA (516–530 g/kg total FAs), oleic (248–268 g/kg total FAs), palmitic (166–199 g/kg total FAs) and stearic acid (26–32 g/kg total FAs). He and Corke (2003)

Table 2. Fatty acid composition (% of total FA) of *Amaranthus caudatus* plant at seven morphological stages

Attributes	Stage of growth[†]							SEM	Contrasts[‡]		
	Early vegetative (d43)	Mid vegetative (d50)	Late vegetative (d57)	Shooting (d64)	Budding (d71)	Early flowering (d79)	Grain fill (d90)		L	Q	C
C16:0	9.73	8.02	9.33	12.36	14.32	14.50	15.20	0.81	**	NS	NS
C18:0	2.38	2.70	3.09	3.59	5.23	4.66	3.62	0.27	**	**	**
C18:1n-9	3.75	3.13	3.46	4.81	5.13	6.18	13.30	0.92	**	**	*
C18:2n-6	14.15	11.08	12.92	16.60	17.44	18.76	27.13	1.45	**	*	NS
C18:3n-6	1.03	0.52	0.52	1.23	1.37	1.37	0.42	0.20	NS	NS	NS
C18:3n-3	54.18	44.19	46.25	47.38	38.74	38.16	24.48	2.47	**	*	**
C18:4n-3	6.47	7.11	5.93	7.90	5.92	5.11	3.67	0.43	*	NS	NS
Others	8.28	23.23	18.48	6.12	11.82	11.23	12.15	1.79	NS	NS	*

[†]Data within parentheses indicate period after sowing in days.

[‡]Contrasts: L, linear; Q, quadratic; C, cubic. Significance: *$P < 0.05$; **$P < 0.01$; NS, not significant.

found that the average contents of three major FAs in amaranth grain were 446, 291, and 222 g/kg total FAs for LA, oleic and palmitic acid, respectively.

The major FAs of four genotypes of *Amaranthus* (*Amaranthus crassipes*, *Amaranthus cruentus*, *Amaranthus dubius*, and *Amaranthus hypochondriacus*) leaf lipids were ALA (from 565 to 620 g/kg total FAs), LA (from 155 to 247 g/kg total FAs), and palmitic (from 135 to 155 g/kg total FAs). As for the FA compositions at different growth stages (from seedling to mature plant), these authors found that FA content in leaf lipid was higher in young leaves than in mature leaves and concluded that *Amaranthus* leaves are a source of nutritionally important FAs, such as ALA, and make them potentially a better nutritional source of feeds and vegetables.

Amaranth is a widely adapted plant, tolerant of adverse conditions. This work showed that the high content of CP and high level of digestibility, in the period between the early- and late-vegetative stages, suggests that the plant could be used as a nutrient substitute for conventional forage, as demonstrated by Molina *et al.* (2015) and Rezai *et al.* (2013). The oil fraction of the plant is considerably higher in ALA compared to many other cereal or pseudocereal sources. However, animal trials must be performed in order to assess the nutritional effects of *Amaranthus caudatus* on livestock production.

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