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Influences of growth stage and nitrogen fertilizer on chemical composition, phenolics, *in situ* degradability and *in vitro* ruminal variables in amaranth forage

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ABSTRACT

This study was carried out to assess the influences of growth stage (early flowering or milk stage of kernels) and nitrogen (N) fertilizer level (120, 180 or 240 kg of N/ha) on the chemical composition, phenolic compounds, *in situ* degradability and *in vitro* ruminal fermentation variables in amaranth forage (*Amaranthus hypochondriacus*). The study was conducted as split plot arrangement based on randomized complete block design with four replicates of two samples each. Chemical composition, *in situ* degradability of dry matter (DM) and crude protein (CP), gas production (GP) and *in vitro* ruminal fermentation variables were determined. Data were analyzed using GLM procedure of SAS. The concentrations of DM, CP, ash-free neutral detergent fiber (NDFom), total phenolics (TP), and effective degradability (ED) of DM and CP were respectively 159 g/kg fresh weight, 224, 346 and 13.4 g/kg DM, and 767 g/kg DM and 810 g/kg CP for the amaranth harvested at early flowering and fertilized with 120 kg N/ha (treatment 1). *In vitro* organic matter (OM) disappearance (OMD), 24-h partitioning factor (PF_{24}), microbial CP (MCP), methane production, pH, ammonia-N, volatile fatty acids (VFA), protozoa and cellulolytic bacteria were 686 g/kg OM, 4.36 mg/mL, 201 mg/g DM, 35.5 mL/g degraded DM, 10.2 mg/dL, 68.3 mmol/L, 6.82×10^5/mL and 8.09 $\log_{10}$/g digesta, respectively, for treatment 1. With advancing the amaranth growth stage, the concentrations of DM, OM, NDFom, EE and TP increased, but CP, soluble and very rapidly degradable fraction (fraction 'A') and ED reduced ($P < 0.05$). Rising N fertilization caused a linear increase of the DM, CP, fraction 'A' and ED ($P < 0.05$). With increasing the plant age, the *in vitro* ruminal GP, OMD, PF_{24}, MCP, ammonia-N, VFA and cellulolytic bacteria decreased ($P < 0.05$), but methane production, per g degraded DM, increased ($P < 0.05$). The values of *in vitro* GP, OMD, VFA and cellulolytic bacteria increased linearly as N fertilization enhanced ($P < 0.05$). *In vitro* ruminal pH, ammonia-N and protozoa were not affected by fertilization.

Abbreviations: A, soluble and very rapidly degradable fraction; ADFom, ash-free acid detergent fiber; B, insoluble but potentially fermentable fraction; C, fractional degradation rate of B; CP, crude protein; DM, dry matter; DUP, digestible undegradable protein; ED, effective degradability; EE, ether extract; ERDP, effective rumen degradable protein; GP, gas production; Lignin(sa), lignin measured by solubilizing cellulose with sulphuric acid; MCP, microbial crude protein; ME, metabolisable energy; MP, metabolisable protein; N, nitrogen; NDFom, 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.

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Overall, increasing amaranth age decreased CP, degradability, *in vitro* ruminal VFA and MCP, but increased lignin(sa), phenolics and methane production per g of degraded substrate. Increasing N fertilization enhanced the forage quality at both growth stages. Thus, the decrease of amaranth quality in second growth stage can be reduced by increasing N fertilization. Though, the effect of growth stage on the forage quality was more pronounced than the effect of N fertilization.

1. Introduction

Due to economic and environmental causes, ruminants should be fed on non-conventional feed resources and fibrous roughages which are not in competition with the human food (Ørskov, 1998; Ben Salem et al., 2004). Moreover, available lands, water resources, soil type, climatic condition and economic factors affect cultivation of forages to feed livestock (Sotomayor-Ríos and Pitman, 2001). Unconventional crops adapted to poor soils, water scarcity and high temperature can be useful as feedstuff for ruminants under certain conditions (Rezaei et al., 2015). One of these crops is amaranth, *Amaranthus* spp., which has not been extensively studied (Seguin et al., 2013). This plant has been rediscovered as a promising food crop due to its resistance to diseases, pests, drought and heat, environmental plasticity and the great nutritive value of grain and leaf (Trucco and Tranel, 2011; Achigan-Dako et al., 2014). Amaranth can be used in developing and underdeveloped countries to improve nutrition, food security and income generation, and to reduce poverty, hunger and illness (Onyango, 2010). Forage amaranth, a C_4 plant, is a high-performance crop (up to 16.6 t dry matter [DM]/ha) with appropriate nutritional value for sheep and lactating cows (Abbasi et al., 2012; Rezaei et al., 2014, 2015). Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, and DM digestibility concentrations of *Amaranthus hypochondriacus,* between 42 and 112 days after planting, range from 130–285, 330–400, 191–281, 22–52 and 670–790 g/kg DM, respectively (Sleugh et al., 2001). Partial substitution of amaranth silage (*A. hypochondriacus*) for maize silage in diets of fattening lambs (up to 300 g/kg diet DM; Rezaei et al., 2014) and lactating Holstein cows (up to 210 g/kg diet DM; Rezaei et al., 2015) does not change animal performance and health.

Forages should be harvested at the optimum stage for yield and quality (Havilah, 2011) and growth stage at harvest is the most important factor influencing plant quality (Nordheim-Viken and Volden, 2009). With increasing plant age NDF and lignin increase, whereas CP and water-soluble carbohydrates decrease; therefore, digestibility is reduced (Yu et al., 2004; McDonald et al., 2011). Moreover, DM yield increases, but anti-nutrients (*i.e.,* nitrate and oxalate) decrease with increasing plant age (McDonald et al., 2011; Abbasi et al., 2012). The nitrogen (N) status of plant leaves is a main determinant of their photosynthetic capability and therefore their efficiency (Onyango, 2010). Soil N is the most important nutrient for plant growth and development, which has positive effect on DM yield, CP concentration and digestibility (Peyraud and Astigarraga, 1998; Almodares et al., 2009). To our knowledge, there is scarce information on the effects of growth stage and N fertilizer level on the phenolic compounds, *in situ* degradability and *in vitro* ruminal fermentation variables in amaranth forage. Hence, the aim of this study was to assess the influences of two growth stages (early flowering or milk stage of kernels) and three levels of N fertilizer (120, 180 or 240 kg of N/ha) on the chemical composition, phenolic compounds, *in situ* DM and CP degradability, and *in vitro* ruminal fermentation variables in amaranth (*A. hypochondriacus*) forage.

2. Materials and methods

2.1. Forage preparation and sampling method

A. hypochondriacus was sowed in the experimental field located in Tarbiat Modares University (Tehran, Iran). The area is at an altitude of 1215 m, with a mean annual rainfall and temperature of 306 mm and 15 °C, respectively. The soil at the experimental site is soft loam-sandy. The treatments were two growth stages (early flowering or milk stage of kernels) and three levels of N (as urea) fertilizer (120, 180 or 240 kg of N/ha). Six treatments were assessed as split plot arrangement based on randomized complete block design with four replicates (four plots per treatment) of two samples each. The N fertilizer level and growth stage were considered as main- and sub- plots, respectively. There were 24 plots of 9 m^2 each. Weeds were controlled through row cultivation during the first weeks after plant sowing to reduce competition. Twelve plots were harvested at the early flowering (40 days after sowing) and 12 at the milk stage of kernels (60 days after sowing) by cutting the whole-plants with knife to a 5-cm stubble height. From each harvested plot (each replicate), two samples of approximately 12 kg each were taken for chemical analyses, and *in vitro* and *in situ* studies. Each sample was enough to provide sufficient sub-samples necessary for later experiments.

2.2. Chemical analyses

The samples were analyzed for DM (oven-dried at 60 °C to a constant weight), ash (No. 924.05), CP (No. 988.05) and ether extract (EE) (No. 920.39) using procedures of AOAC International (1998). The concentration of ash-free NDF (NDFom) was