Animal Feed Science and Technology 165 (2011) 125–130

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Short communication

Determination of γ-glutamyl-S-ethenyl-cysteine in narbon vetch (*Vicia narbonensis* L.) seeds by high performance liquid chromatography

R. Sánchez-Vioque[a,*], J. Girón-Calle[b], M.F. Rodriguez-Conde[a], J. Vioque[b], M. De-los-Mozos-Pascual[a], O. Santana-Méridas[a], M.E. Izquierdo-Melero[a], M. Alaiz[b]

[a] *Departamento de Cultivos Herbáceos, Centro Investigación Agraria Albaladejito, Junta de Comunidades de Castilla – La Mancha, Ctra. Toledo – Cuenca, Km 174, 16194 Cuenca, Spain*

[b] *Departamento de Fisiología y Tecnología de Productos Vegetales, Instituto de la Grasa, Consejo Superior de Investigaciones Científicas. Avda. Padre Garcia Tejero, 4. 41012 Sevilla, Spain*

ARTICLE INFO

Article history:
Received 8 June 2010
Received in revised form 25 January 2011
Accepted 4 February 2011

Keywords:
Antinutritional factor
Feed
Legume
Livestock
Peptide
Toxicity

ABSTRACT

A new method for fast and easy determination of γ-glutamyl-S-ethenyl-cysteine (GEC) in narbon vetch (*Vicia narbonensis* L.) seeds was developed as a tool for the selection of low GEC varieties. Samples ($n = 20$) were analyzed by reverse-phase HPLC, using isocratic elution and monitoring at 215 nm. Calibration curve showed very good linearity of the response between 2.5 and 20 μg GEC. The limits of detection and quantification were 0.013 and 0.049 mg/mL, respectively. The repeatability (average coefficient of variation (CV) = 0.023) and reproducibility (average CV = 0.016) of the method were within the recommendations for the validation of HPLC methods. The method yielded similar results in comparison with a previously established method.

1. Introduction

In addition to being a protein rich animal feed, narbon vetch (*Vicia narbonensis* L.) has been proposed in recent decades as a rotation crop in sustainable agricultural systems. Crop rotation has numerous advantages as compared to cereal monoculture, including an improved maintenance of organic matter and nitrogen status in the soil, and a better disease and pest control, which results in higher production yields. Narbon vetch exhibits an erect growth habit and a reduced pod shattering that facilitates the mechanical harvesting, is well adapted to marginal lands, shows a good tolerance to drought and cold, and requires low chemical inputs like fertilizers or herbicides. Because of these favourable agronomic characteristics, this species is very well suited for the production of straw and grain for livestock in dry areas like Central and West Asia, North Africa and Southern Australia (ICARDA, 2006; Jones and Singh, 2000; Saxena et al., 1993; Siddique et al., 1999). This crop has also shown a good adaptability and yield in some regions of Mexico and China (Flores and Sánchez, 2009; Nan et al., 2006). In

Abbreviations: CV, coefficient of variation; GEC, γ-glutamyl-S-ethenyl-cysteine; LOD, limit of detection; LOQ, limit of quantification; S/N, signal/noise ratio; S/Q, signal/concentration ratio.

* Corresponding author. Tel.: +34 969 21 37 63; fax: +34 969 23 21 51.
E-mail address: rsanchezv@jccm.es (R. Sánchez-Vioque).

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doi:10.1016/j.anifeedsci.2011.02.006

Spain the crop has recently gained significance as a valuable alternative for the diversification of the agriculture and to offset the home production deficit of plant protein for livestock (García et al., 2006).

The chemical composition of narbon vetch is similar to that of other grain legumes. Protein concentration in the grain and straw of some cultivars is higher than 300 and 100 g/kg, respectively (Abd El Moneim, 1992; Aletor et al., 1994). Narbon vetch is more appropriate for feeding ruminants than monogastric animals because some antinutritional factors can be destroyed in the rumen, although the grain has an unpleasant taste resulting in a reduced intake by both ruminants and monogastrics. In sheep, this results in the animals eating less grain and at a slower pace, although there is some controversy about the consequences on growth. Thus, while Jacques et al. (1994) found that a more even consumption over time leads to a better utilization of the grain, Hadjipanayiotou (2000) observed a reduction in growth when compared with other cereal and legume based diets. More severe effects have been found when feeding narbon vetch to monogastrics, including nephritis and damage to red blood cells in pigs (Davies, 1987). Wali et al. (2005) found acute tubular nephrosis in broiler chickens, whereas Eason et al. (1990) reported no significant effects of a diet containing 100 g/kg narbon vetch as compared to other protein sources. The discrepancies on the negative effects of feeding narbon vetch are probably due to differences in the genetic background and age of the animals and to different experimental feeding designs. In addition, and maybe most importantly, different varieties of narbon vetch were used in the studies cited above (Enneking, 1994a).

Most of the detrimental effects of narbon vetch on animals have been attributed to the presence in the seeds of γ-glutamyl-S-ethenyl-cysteine (GEC), an antinutritional dipeptide that was first isolated and crystallised by Enneking et al. (1998). GEC, as other antinutritional factors in legumes, is important in the physiology of the seed as a storage compound. It is also present as a defensive mechanism against insects, predators, microorganisms, and other plants. Its content in narbon vetch seeds is between 10 and 30 g/kg (Francis et al., 1999).

Because of the antinutritional effects of GEC on livestock, the development of cultivars with a low GEC content is a major goal of narbon vetch breeding programs, and therein the importance of methods for the determination of this compound. Nevertheless, the only method currently available for analysis of GEC is based on capillary electrophoresis, which is not always readily available in laboratories (Arias et al., 2005; Berger et al., 1999; Enneking, 1994b). The objective of this work is to develop a HPLC method that facilitates a rapid and easy determination of GEC as an alternative to capillary electrophoresis.

2. Materials and methods

2.1. Plant material

The commercial cultivar 'Altair' was donated by Agrosa Semillas Selectas S.A. (Guadalajara, Spain). All other narbon vetch accessions were donated by the Centro de Recursos Fitogenéticos, CRF (Madrid, Spain). Seeds were multiplied during the 2001–2002 season in the trial fields of the Centro de Investigación Agraria Albaladejito placed in Cuenca (Spain) and preserved in the the Banco de Germoplasma Vegetal de Cuenca (BGV-CU). Around 30 g of seeds of each accession were ground to pass a 0.08-mm screen in a Ultra Centrifugal ZM 1000 mill (Retsch, Haan, Germany) and kept at 4 °C in plastic bags until analysis.

2.2. Reagents

HPLC grade solvents were used for HPLC chromatography. Purified GEC was a gift from Dr. M.E. Tate (University of Adelaide, Australia). The use of this material as a standard for capillary electrophoresis has been previously reported (Arias et al., 2005). All other chemicals were of analytical grade

2.3. Extraction of GEC from narbon vetch seeds

Ground seeds (500 mg) were extracted twice by stirring in 5 mL of ethanol/water (70/30, v/v) for 1 h. Supernatants resulting from centrifugation of the extracts at 5000 rpm for 30 min were taken to a volume of 10 mL (Arias et al., 2005).

2.4. HPLC analysis

The HPLC system (Beckman-Coulter, Brea, CA, USA) consisted of a 126 solvent module, 166 detector and IBM personal computer. Data acquisition and processing were effected with a 32 Karat 7.0 version software (Beckman-Coulter, Brea, CA, USA). Extracs (20 μL) were directly injected in a Discovery BIO Wide Pore C18 (25 cm × 4.6 mm, 5 μm) column (Supelco, Bellefonte, PA, USA). Elution was performed at 1 mL/min and at room temperature using trifluoroacetic acid in water (1 mL/L)/trifluoroacetic acid in acetonitrile (1 mL/L) 90/10 (v/v). Elution was monitored by UV absorption at 215 nm. Calibration was carried out by injecting 2.5–20 μg GEC standard.

2.5. Evaluation of the HPLC method

Evaluation was carried out by determination of linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability, and reproducibility. Linearity of a method is defined by its ability to produce results that are directly, or by a well-defined