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Bioactive compounds, aucubin and acteoside, in plantain (*Plantago lanceolata* L.) and their effect on *in vitro* rumen fermentation

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ABSTRACT

Plantain (*Plantago lanceolata* L.) contains bioactive compounds with antimicrobial activity that can potentially influence ruminal fermentation. This study aimed to identify the concentration of the bioactive compounds catalpol, aucubin, and acteoside in plantain cv. 'Ceres Tonic' through two consecutive growing seasons (2011–2012 and 2012–2013). Then the herbage with highest levels of bioactive compounds was used to evaluate their effect on rumen *in vitro* fermentation. Plantain cv. 'Ceres Tonic' had almost nil concentration of catalpol. Both aucubin and acteoside concentrations increased ($P < 0.05$) through the growing season. Aucubin increased from 1.78 to 3.80 mg/g DM in the first and from 0.44 to 6.87 mg/g DM in the second growing season; while, acteoside increased from 23.6 to 35.4 mg/g DM and from 0.5 to 41.7 mg/g DM, respectively. The *in vitro* experiment evaluated the effect of aucubin and acteoside on ammonia (NH_3), volatile fatty acids (VFA) and gas production (GP) parameters. Aucubin and acteoside were added to chicory (*Cichorium intybus* L.) as negative and to plantain as positive controls. The treatments were (i) chicory (CH); (ii) chicory + 10 mg aucubin/g DM (C+au); (iii) chicory + 20 mg aucubin/g DM (C+2au); (iv) chicory + 40 mg acteoside/g DM (C+ac); (v) plantain naturally containing 7 mg/g DM of aucubin and 36 mg/g DM of acteoside (PL); (vi) plantain + extra 10 mg aucubin/g DM (P+au); and (vii) plantain + extra 36 mg acteoside/g DM (P+ac). Plantain with natural concentrations of bioactives produced 40% less NH_3 than chicory over 24 h. The exogenous addition of both bioactive compounds reduced net NH_3 production by CH and PL. The increase in potential GP from acteoside fermentation suggested its use as an energy source. Whereas, the addition of aucubin reduced the rate of GP at dose level, potentially due to its bactericide activity. Therefore, acteoside would have a greater positive effect than aucubin on ruminant animals.

Abbreviations: A, potential gas production; A:P, acetate propionate ratio; ac, acteoside; ADF, acid detergent fibre; au, aucubin; BCVFA, branched chain volatile fatty acids; CH, Chicory; C+au, Chicory plus 10 mg aucubin/g DM; C+2au, chicory plus 20 mg aucubin/g DM; C+ac, chicory plus 40 mg acteoside/g DM; CO_2, carbon dioxide; CP, crude protein; DM, dry matter; GP, gas production; HPLC, high performance liquid chromatography; HWSC, hot water soluble carbohydrates; ME, metabolisable energy; MeOH, methanol; N, nitrogen; N_2O, nitrous oxide; NDF, neutral detergent fibre; NH_3, ammonia; OM, organic matter; OMD, organic matter digestibility; PL, plantain; P+au, plantain plus 10 mg aucubin/g DM; P+ac, plantain plus 36 mg acteoside/g DM; $R^{1/2A}$, fermentation rate at $T^{1/2A}$; RFC, readily fermentable carbohydrates; SC, structural carbohydrate; $T^{1/2A}$, half time when the potential GP was reached; V24 h, volume of gas produced after 24 h incubation; VFA, volatile fatty acids.

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1. Introduction

Plantain (*Plantago lanceolata* L.) is a herb containing secondary, bioactive, compounds that may influence N cycling in pastoral livestock systems (Pacheco and Waghorn, 2008). The most well-known bioactive compounds in plantain are the iridoid glycosides: aucubin and catalpol; and the phenylpropanoid glycoside, acteoside (Syn. verbascoside) (Stewart, 1996; Tamura and Nishibe, 2002). These compounds have been reported to have antimicrobial and antifungal effects (Andary et al., 1982; Davini et al., 1986; Kim et al., 2000), and it has been suggested that these compounds may influence the rumen micro flora of grazing ruminants and ultimately their nutrient utilisation (Burke et al., 2000; Swainson and Hoskin, 2006); however, their impact on rumen fermentation is unclear.

The presence of bioactive compounds in plantain with the potential to affect rumen fermentation is likely to have important implications for rumen N efficiency (Stewart, 1996). A reduction in the NH_3 concentration in the rumen is desirable because it could decrease N losses to the environment, including via urine (Attwood et al., 1998). Dairy cows grazing, at the same N intake, either diverse pasture containing plantain or perennial ryegrass *Lolium perenne* L. had a substantially lower N concentration in their urine (Totty et al., 2013). Totty et al. (2013) did not determine if this lower N concentration in the urine was by improvement of N efficiency or N dilution in the urine. However, greater urine flows have been recorded in animals grazing plantain (Wilman and Derrick, 1994), consistent with the diuretic effect of iridoid glycosides (Tamura and Nishibe, 2002). Urine N is immediately available for leaching down the soil profile and volatilisation resulting in significant nitrous oxide (N_2O) emissions. The antimicrobial effect of aucubin, but not for acteoside (Andary et al., 1982), has been well documented (Davini et al., 1986; Bartholomaeus and Ahokas, 1995; Kim et al., 2000), and the effect of these bioactive compounds on rumen fermentation appears to not have been assessed. Our hypothesis is that the bioactive compounds in plantain will decrease NH_3 production and affect volatile fatty acids (VFA) production in the rumen. This study examined the seasonal concentration of these bioactive compounds in plantain cv. 'Ceres Tonic' and evaluated the *in vitro* fermentation of plantain and compared it to chicory (*Cichorium intybus* L.), a perennial herb known not to contain these bioactive compounds, by the exogenous addition of aucubin and acteoside.

2. Materials and methods

2.1. Experimental site of plant material

The plantain evaluated in this study was obtained from a grazing trial at Dairy 1, Massey University, Palmerston North, New Zealand (40°22′S, 175°36′E) from October, 2011 until May, 2013. The grazing trial evaluated plantain (cv. 'Ceres Tonic') and chicory (cv. 'Grassland Choice') pastures grazed every two and four weeks throughout two growing seasons (December, 2011–May, 2012 and August, 2012–May, 2013). The pastures of plantain and chicory were established in October, 2011 in plots (300 m^2) arranged in a randomised block factorial design with five replicates. Plantain and chicory were grazed exactly every two and every four weeks with dairy cows immediately after the morning milking (0600 h) and until the swards achieved a residual height of 70–100 mm (approximately 5 h).

2.2. Plant material sampling

The herbage from the plantain and chicory pastures was collected by taking a hand plucked sample from multiple sites in both the plantain and chicory plots at different dates through both growing seasons. During the first growing season (2011–2012), the herbage from all plots was collected at two dates: (i) summer (December, 2011) and (ii) autumn (May, 2012). During the second growing season herbage samples were taken on three dates: (i) spring (October, 2012), (ii) summer (January, 2013), and (iii) autumn (May, 2013). Samples were stored at −20 °C until later analysis.

2.3. Laboratory analysis of bioactive compounds

Catalpol, aucubin and acteoside in plantain and chicory were determined by high-performance liquid chromatography (HPLC). Plantain and chicory samples were stored at −20 °C, then freeze-dried and ground to pass through a 1 mm diameter sieve. A 100 mg aliquot from each of the ground samples was taken for extraction of aucubin, catalpol, and acteoside, with 10 mL of methanol (MeOH) in 15 mL tubes and shaken for 2 h at room temperature. The solid plant material was filtered out using grade 41 quantitative filter papers (Whatman Co., Ltd., England). Then, 2 mL of the filtrate was diluted in 8 mL of ultra-pure water, and further filtered using 0.2 μm syringe filters (Whatman Co., Ltd., England) and a 20 μL aliquot used for HPLC analysis for the simultaneous determination of catalpol and aucubin. Whereas for acteoside, 2 mL of the filtrate undiluted was filtered using 0.2 μm syringe filter (Whatman Co., Ltd., England) and a 20 μL of aliquot used for HPLC analysis.

Commercially available catalpol, aucubin, and acteoside (99% pure; Extrasynthese S.A, France) were used as standards. The standard solution contained 2 mg each of catalpol and aucubin in 50 mL of 20% MeOH and 1 mg of acteoside in 5 mL of pure MeOH. High-performance liquid chromatographywas performed at 40 °C using a 100 mm × 6.0 YMC pack ODS-A column protected by a YMC guard pack (YMC America, Inc). The mobile phase was 1% acetronitrile in water for catalpol and aucubin and 29% MeOH in water (containing 5% acetic acid) for acteoside. The flow rate was 1 mL/min. For catalpol and aucubin, wavelength detection was performed at 240 nm and for acteoside at 330 nm. The HPLC system consisted of a Dionex