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Manipulating rumen fermentation and methanogenesis using an essential oil and monensin in beef cattle fed a tropical grass hay

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ABSTRACT

The objective of this study was to determine if a specific blend of essential oils (CRINA® Ruminants) compared to monensin could reduce enteric methane production in beef cattle fed medium to low quality Rhodes grass (*Chloris gayana*) hay. Five Brahman steers [mean live weight (LW); 226 kg] were allocated to one of five groups: control (no additive), CRINA1 (CRINA 1 g/d), CRINA2 (CRINA 2 g/d), Mon1 (monensin 60 mg/d) and Mon2 (monensin 250 mg/d) as a 5 × 5 Latin square. Individual LW, dry matter (DM, kg/d) intake, rumen pH, fermentation patterns, and ruminal fungal colonisation was measured. Methyl coenzyme-M reductase (*mcrA*) clone libraries (methanogen diversity) were generated from microbial DNA extracted from the rumen. Total methane production (g/d) was measured over 24 h using open circuit respiration chambers. The DM intake for animals given CRINA at either dose rate was not different ($P>0.05$) to the control (5.4 kg/d). However, Mon2 ($P<0.05$) reduced DM intake by 18%, compared with the control with no effect on rumen pH or total VFA production. CRINA significantly increased butyrate and iso-valerate concentrations compared with the control. Mon2 also reduced acetate:propionate compared with CRINA and the control. Based on sporangia counts from rumen fluid collected throughout the experimental period a reduction in fungal colonisation was observed for both monensin and CRINA treatments.

Abbreviations: CRINA, CRINA® Ruminants (DSM Nutritional Products Ltd.); ADFom, acid detergent fibre (ADFom) exclusive of residual ash; ADG, average daily gain; aNDFom, neutral detergent fibre with the addition of heat stable α-amylase and sodium sulphite; ANOVA, analysis of variance; CP, crude protein; CRINA1, Crina1 (1.0 g/d); CRINA2, Crina2 (2.0 g/d); CSIRO, Commonwealth Scientific and Industrial Research Organisation; d, day; DM, dry matter; DNA, deoxyribonucleic acid; EO, essential oil; EU, European Union; g, gram; GE, gross energy; GHG, greenhouse gas; h, hour; kg, kilogram; kPa, kilopascal; LSD, least significant difference; LW, live weight; mg, milligramme; min, minute; MJ, mega Joule; mM, micro molar; mm, millimetre; mm^2, square millimetre; mL, millilitre; Mon1, Monensin1 (60 mg/d); Mon2, Monensin2 (250 mg/d); N, nitrogen; OM, organic matter; RCC, rumen cluster C; sem, standard error of the mean; VFA, volatile fatty acid.

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[1] It is with regret that this paper could not be published prior to Dr Elliott's death on 18/11/2012. Bob was a great advocate of applied research and will be missed by many.

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The use of Mon1 or CRINA did not affect methane production. Mean methane production was reduced to 10.2 g/kg DM intake for Mon2 treated animals, compared with the control group (14.6 g/d DM intake), but this was also associated with lower DM intakes. A shift in methanogen diversity for monensin treated animals was due to a decrease in *Methanomicrobium* genus and concurrent increase in *Methanobrevibacter* genus. The specific blend of essential oils used in this study had no direct effect on methane emissions; however the potential to manipulate rumen fungi with CRINA and/or monensin and the relationship with methanogens may be a novel strategy to indirectly reduce enteric methanogenesis.

1. Introduction

In Australia methane from ruminant production systems contribute significantly to the national agricultural greenhouse gas (GHG) emission profile. Enteric methanogenesis represents an energy loss to the ruminant and can be affected by a number of factors, including level of feed intake, diet characteristics, addition of lipids or ionophores to the diet, changes in rumen micro-flora and level of animal productivity (Johnson and Johnson, 1995; McAllister et al., 1996). Ionophores are established inhibitors of enteric methanogenesis, but since the feeding of ionophores was banned by the EU in 2006 there has been growing interest in essential oils (EO) as viable alternatives in manipulating rumen fermentation (Calsamiglia et al., 2007; Benchaar et al., 2008; Hart et al., 2008).

The inclusion of CRINA® Ruminants (DSM Nutritional Products Ltd., Basel, Switzerland) in diets fed to cattle and sheep has been reported to increase DM intake and milk production in dairy cows (Schmidt et al., 2004), reduce amino acid deamination (Newbold et al., 2004), inhibit hyper-ammonia producing bacteria (McIntosh et al., 2003) and increase ruminal pH (Varga et al., 2004). The product is a defined patented mix of natural and synthetic essential oils (EO); namely thymol, eugenol, vanillin, limonene and guaiacol on an organic carrier (Rossi, 1995). The antimicrobial role of EO against bacteria, fungi, protozoa and viruses is attributed to an effect on cell membranes, although alternative modes of action have been proposed (Grainger et al., 2008; Greathead, 2003; Calsamiglia et al., 2007). Previous studies with growing beef cattle fed a 75% barley silage diet supplemented with CRINA at a rate of 1 g/d reported no effects on ruminal fermentation or methane emissions (Beauchemin and McGinn, 2006) although the lack of response may have been influenced by the concentration within the rumen.

Monensin (Rumensin®; Elanco Animal Health Pty Ltd., Indianapolis, IN) is approved for use in several countries including Australia and has been shown to improve feed utilisation, reduce feed intake variation in beef cattle (Goodrich et al., 1984; Stock et al., 1995), and promote higher ruminal propionate concentrations and reduced acetate:propionate ratio (Russell and Strobel, 1989; Chow et al., 1994). Ionophores are lipophilic compounds toxic to many bacteria, protozoa and fungi (Russell, 1996), and the extent of methane inhibition has been shown to be related to dose rate and type of ration. An indirect effect on the symbiotic relationship between ruminal protozoa and methanogens has been shown to be responsible for decreases in enteric methanogenesis (Ushida and Jouany, 1996). Methanogenic rumen archaea can now be described using the sequence divergence within the methyl coenzyme-M reductase subunit A (mcrA) gene (Denman et al., 2007) and this has become a useful tool to identify changes associated with the inclusion of antimethanogenic compounds in the diet of ruminant animals.

The purpose of this study was to determine the effect of two feed additives; CRINA® Ruminants (1 and 2 g/d) and monensin (60 and 250 mg/d) on rumen fermentation, enteric methane production and rumen methanogen population diversity for steers fed a Rhodes grass (*Chloris gayana*) hay diet.

2. Materials and methods

The experimental protocol complied with the Australian Code of Practice for the care and use of Animals for Scientific Purposes (Aust. Gov. National Health and Medical Research Council, 2004) and was approved by the local Animal Experimentation and Ethics Committee (RH247/08).

2.1. Animal trial

Five rumen fistulated Brahman (*BBos. indicus*) steers [mean ± sem. live weight (LW); 226 ± 8.1 kg], approximately 1 year old, were used in a 5 × 5 Latin square design. Steers were allocated to one of five treatments involving the supplementation of CRINA, at a rate of 1.0 g/d (CRINA1) or 2.0 g/d (CRINA2), and monensin (monensin as Rumensin™ 10%; Elanco Animal Health Pty Ltd., Greenfield, IN) at a rate of 60 mg/d (Mon1) or 250 mg/d (Mon2) for 40 d periods with a control group (no supplement) as a contrast.

Steers were initially fed medium quality Rhodes grass (*C. gayana*) hay *ad libitum* [hay chemical composition (g/kg DM) of ash, 87; crude protein (CP) 126; neutral detergent fibre (aNDFom) 688; acid detergent fibre (ADFom) 403; and gross energy (GE, MJ/kg), 18.6] without CRINA or monensin for 14 d before being transferred to individual pens under controlled animal