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Australian plants with potential to inhibit bacteria and processes involved in ruminal biohydrogenation of fatty acids☆

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Abstract

Conjugated linoleic acids (CLA) are health-promoting fatty acids found in foods derived from ruminant products that are formed in the rumen during bacterial biohydrogenation of linoleic acid (LA). Although selective antimicrobials might increase CLA production by manipulation of ruminal microflora, feeding of antibiotic growth promoters to livestock is declining due to fears of development of antibiotic resistance in human pathogens. This has initiated a search for alternatives, including plants containing bioactive compounds, which can have similar positive effects on ruminal microflora, but without negative impact on animal and human health. In this study we investigated effects of natural compounds in Australian plants on the bacteria and processes involved in ruminal biohydrogenation. Ethanolic extracts and essential oils were obtained from 91 Australian plants collected during 2004/2005 in Western Australia. Minimal Inhibitory Concentrations for the main bacterial

Abbreviations: CLA, conjugated linoleic acids; AGP, antibiotic growth promoters; LA, linoleic acid (*cis*-9,*cis*-12-18:2); SA, stearic acid (18:0); VA, vaccenic acid (*trans*-11-18:1); CALM, Department of Conservation and land management; CRC, Cooperative Research Centre; MIC, minimal inhibitory concentration; FA, fatty acids; FAME, fatty acid methyl esters; GC–MS, gas chromatography mass spectrometry

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species involved in rumen biohydrogenation were established using an agar dilution method, and effects of selected candidates on LA biohydrogenation was examined using an *in vitro* system with a mixed rumen bacterial population. A wide range of the plant extracts had selective inhibitory effect towards *Clostridium proteoclasticum* (which forms stearate from LA) without affecting *Butyrivibrio fibrisolvens* (which forms CLA and vaccenic acid, but not stearate). However, only a few plants, including *Acacia iteaphylla* and *Kennedia eximia*, inhibited LA metabolism or stearate formation in the mixed bacterial community *in vitro*. Further experiments are needed to investigate effects of selected candidates on CLA production *in vivo*.

Keywords: Conjugated linoleic acids; Rumen bacteria; Rumen hydrogenation; Plant extract

1. Introduction

Consumers are demanding animal products that are safe and are health-promoting. Such properties have been assigned to the group of unsaturated fatty acids (FA) called conjugated linoleic acids (CLA; Ha et al., 1987; Bessa et al., 2000; Bhattacharya et al., 2006), that are formed in the rumen by bacterial isomerization of dietary linoleic (LA), the first reaction in a multi-step process that ultimately produces the saturated FA stearic acid (SA; Harfoot and Hazlewood, 1997). Many intermediates are formed, but the two that are of importance to final levels of CLA in animal products are *cis*-9, *trans*-11-18:2 CLA isomer (i.e. rumenic acid) and vaccenic acid (VA). After absorption from the small intestine, CLA are incorporated into animal tissues and milk, while VA can be converted back to CLA by a desaturase in mammalian tissue (Harfoot and Hazlewood, 1997; Griinari and Bauman, 1999). Griinari and Bauman (1999) indicated that the flow from the rumen of VA plays a more important role than CLA in determining CLA concentration in animal tissues.

Many ruminal bacteria species have been implicated in ruminal biohydrogenation, including species of the genera *Butyrivibrio, Ruminococcus, Treponema – Borrelia, Micrococcus, Megasphaera, Eubacterium, Fusocillus* and *Clostridium* (Polan et al., 1964; Harfoot and Hazlewood, 1997; Maia et al., 2007). The most active species are in the *Butyrivibrio* group, where all bacteria form CLA from LA, while only *Clostridium proteoclasticum* (as previously isolated '*Fusocillus*' spp.) is found to convert VA to SA (Polan et al., 1964; Kemp et al., 1975; Maia et al., 2007; Paillard et al., 2007). Selective suppression of *Cl. proteoclasticum*, without affecting *B. fibrisolvens*, would provide more unsaturated acids, including VA and CLA, escaping the rumen to be absorbed and incorporated into animal tissues.

Increased CLA levels in ruminant-derived food products can be achieved by nutritional and management practices that facilitate higher forestomach output of CLA and VA. Feeding ruminants diets containing vegetable oils can boost forestomach CLA and VA outflow, but this can be impractical, and/or have negative effects on animal production (Griinari and Bauman, 1999), and some unsaturated FA are also toxic to rumen microbes (Harfoot and Hazlewood, 1997; Kim et al., 2000; Maia et al., 2007). Some antibiotics can decrease rate of biohydrogenation and preserve ruminal CLA *in vitro* (Fellner et al., 1997), via selective inhibition of some ruminal bacteria (Nagaraja and Taylor, 1987). Antibiotic growth promoters (AGP) are fed to livestock to improve growth and productivity and, although the