Animal Feed Science and Technology 196 (2014) 50–59

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

Effects of essential oils supplementation on *in vitro* and *in situ* feed digestion in beef cattle

A. Nanon [a,*], W. Suksombat [a], W.Z. Yang [b]

[a] *School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*

[b] *Agriculture and Agri-Food Canada, Lethbridge Research Centre, Alberta, Canada T1J 4B1*

ARTICLE INFO

Article history:
Received 23 September 2013
Received in revised form 16 July 2014
Accepted 21 July 2014

Keywords:
Essential oil
Feed digestibility
Gas production
Batch culture
In situ rumen method

ABSTRACT

The objective of this study was to investigate the effect of lemongrass oil (LMO) and a mixture of garlic and ginger oil (CEO) on gas production (GP) and feed digestibility using the batch culture and *in situ* ruminal technique. Four feeds: wheat distillers dried grains with solubles (DDGS), barley grain, grass hay, and a total mixed ration (TMR) were tested with varying essential oil (EO) dosages. The TMR consisted of 350 g/kg grass hay, 150 g/kg alfalfa hay, 200 g/kg barley grain, 100 g/kg corn DDGS, 100 g/kg wheat DDGS, 50 g/kg canola meal, and 50 g/kg vitamin and mineral supplement. The *in vitro* study was a complete randomized design with 2 × 4 factorial arrangement of two EO (LMO and CEO) combined with four dosages of EO (*i.e.*, 0, 100, 200, and 300 mg/kg substrate DM). Digestibilities of DM (DMD) and neutral detergent fibre (NDFD) were measured at 24 h and 48 h post incubation, while GP was read at 3, 6, 12, 24, 36, and 48 h post incubation. In situ ruminal degradability was measured using three ruminally fistulated beef heifers with incubation time of 4, 12, 24 or 48 h. There was no interaction on *in vitro* DMD and NDFD between EO source and its dose. The DMD and NDFD were greater with CEO compared to LMO for wheat DDGS ($P < 0.01$; 48 h) and barley grain ($P < 0.01$; 24 h), but lower for TMR ($P < 0.05$; 24 or 48 h). Increasing the dosage of EO linearly ($P < 0.01$) increased the DMD of wheat DDGS and barley grain at 24 h post incubation, and linearly ($P < 0.01$) and quadratically ($P < 0.05$) improved *in vitro* DMD and NDFD of grass hay and TMR with addition of LMO and CEO at 24 or 48 h post incubation. The cumulative GP was overall affected ($P < 0.01$) by both LMO and CEO in quadratic manner after 24, 36 or 48 h of incubation. *In situ* ruminal DMD of wheat DDGS and barley grain were higher ($P < 0.05$) at 4 or 24 h of incubation with CEO than with control or LMO which had no differences in DMD. However, the *in situ* DMD of grass hay and TMR were improved by both LMO and CEO supplementation after 24 h ($P < 0.01$) or 48 h ($P < 0.05$) post incubation. The dose of 200 mg/kg DM was likely cost-effective to improve DMD for both LMO and CEO. The LMO and CEO appeared to be more effective to improve the DMD of fibrous feeds since the *in vitro* DMD and NDFD of grass hay and TMR were consistently improved at 24 h or 48 h post incubation. These results suggested that the LMO and CEO used in the present study could be potentially developed as rumen modifier to improve feed digestibility in the rumen.

Abbreviations: EO, essential oils; LMO, lemongrass oil; CEO, equally blend of garlic oil and ginger oil, GP, gas production; OM, organic matter; DM, dry matter; DMD, dry matter disappearance; aNDF, neutral detergent fibre inclusive of residual ash; NDFD, neutral detergent fibre disappearance; ADF, acid detergent fibre; CP, crude protein; CPD, crude protein disappearance; TMR, total mixed ration.

* Corresponding author. Tel.: +66 44224378; fax: +66 44224150.
E-mail address: aiis_nanont@hotmail.com (A. Nanon).

http://dx.doi.org/10.1016/j.anifeedsci.2014.07.006
0377-8401/

1. Introduction

Nutritionists have been searching for alternative additives for reducing the need for in-feed antibiotics such as ionophores. Using antibiotics in animal feed is facing reduced social acceptance due to the appearance of residues and resistant strain bacteria. Plant essential oil (EO) from variety of sources have been intensively studied during the last decades by ruminant scientists aiming to develop rumen modifiers for manipulating rumen fermentation as documented by several review papers (Calsamiglia et al., 2007; Hart et al., 2008; Benchaar and Greathead, 2011). Many studies focused on cinnamon oil (cinnamaldehyde) or clove oil (eugenol) to evaluate the effects on rumen fermentation characteristics in particular (Busquet et al., 2006; Cardozo et al., 2005, 2006; Fraser et al., 2007). The researches demonstrated the ability of using EO to alter rumen fermentation and nutrient utilization in ruminants. Garlic, ginger, and lemongrass are plant extracts and herb of interest. These herbs are widely used in tropical countries as for one of human food composition. Lemongrass has been shown antibacterial (Valero and Salmeroìn, 2003), antioxidant (Cheel et al., 2005), and antihyper-NH_3-producing ruminal bacterial (McIntosh et al., 2003) activities as well as the effects on changes of blood metabolites and rumen fermentation in Holstein steers (Hosoda et al., 2006). However, lemongrass EO (LMO) was little evaluated on its effects on ruminal fermentation. In addition, garlic oils and garlic oil compounds have been explored as an alternative to antibiotics to manipulate rumen fermentation due to their well-known antimicrobial effects (Ramos-Morales et al., 2013). Garlic oil and garlic derived compounds have been demonstrated to have antimethanogenic property with mixed effects on rumen fermentation (Busquet et al., 2005a; Chaves et al., 2008). Ginger oil has also been detected to have inhibitory effects for 10 different micro-organisms (Hammer et al., 1999) but limited studies showed no effect on rumen VFA concentration in a continuous culture (Busquet et al., 2005b). Additive, antagonistic, and synergistic effects have occurred between components of EO (Burt, 2004), suggesting that combinations of EO of different composition, or specific combinations of EO secondary metabolites, may result in additive and/or synergetic effects which may enhance efficiency of rumen microbial fermentation. Finally, the responses of rumen fermentation and feed digestion to EO supplementation depended on type of substrates or the composition of diets fed to animals (Hart et al., 2008).

The objective of this study was to determine the effect of EO supplementation on gas production (GP) and rumen digestion of individual feed ingredients including wheat dried distillers grain with solubles (DDGS), barley grain, grass hay, and total mixed rations (TMR) using batch culture and *in situ* technique. Wheat DDGS is a by-product of ethanol plant and is commonly fed to livestock animals in western Canada and elsewhere in the world due to rapidly increased availability.

2. Material and methods

2.1. Experimental design, feeds, and treatments

Experiment 1 (Exp. 1) was a complete randomized design with 2×4 factorial arrangement of treatment. The EO were LMO and a combination of garlic oil and ginger oil at ratio of 1:1 (CEO), and were purchased commercially (purity >990 g/kg; Phodé S.A., Albi, France). The dosages of EO were 0, 100, 200, and 300 mg/kg substrate DM. The substrates included wheat DDGS, barley grain, grass hay, and TMR which consisted of 350 g/kg grass hay, 150 g/kg alfalfa hay, 200 g/kg barley grain, 100 g/kg corn DDGS, 100 g/kg wheat DDGS, 50 g/kg canola meal, and 5 g/kg vitamin and mineral supplement. The substrates were ground through 1 mm screen (standard model 4 Wiley Mill; Arthur Thomas Co., Philadelphia, PA, USA), and mixed with EO before weighing into a test bag. A 0.5 g (DM basis) of substrate was weighed into a ANKOM F57 filter bag (pore size of 50 μm, Ankom Technology Corp., Macedon, NY, USA), and sealed for *in vitro* incubation.

Inoculum for the batch culture was obtained from three ruminally fistulated beef heifers (Spayed beef heifer) fed a diet consisting of 640 g/kg barley silage, 60 g/kg grass hay, 270 g/kg dry-rolled barley grain, and 30 g/kg vitamin and mineral supplement. Rumen fluid was collected from different sites within the rumen, pooled, and squeezed through PeCAP® polyester screen (pore size 355 μm; B & S Thompson, Ville Mont-Royal, QC, Canada) into an insulated thermos, and transported immediately to the laboratory. All animal procedures were in accordance with the guidelines of the Canadian Council on Animal Care (1993).

In vitro incubations were in 100 ml glass bottles (with 3 replicates/run) fitted with rubber stoppers to prevent escape of fermentation gases. Sufficient anaerobic media was prepared the day before the incubation according to the method of Hall et al. (1998) except that cysteine HCl was not substituted for Na_2S. Forty-five millilitres of prewarmed media and 15 ml of inoculum were added anaerobically to the 100 ml bottles by flushing with oxygen free CO_2. Bottles were sealed immediately with a 14 mm butyl rubber stopper plus aluminium crimp cap and incubated at 39 °C for 24 or 48 h. The incubation was repeated with two runs.

At pre-determined time points, headspace gas production (GP) was measured at 3, 6, 12, 24, 36, and 48 h post incubation by inserting a 23 gauge (0.6 mm) needle attached to a pressure transducer (model PX4200-015GI, Omega Engineering, Inc., Laval, QC., Canada) connected to a visual display (Data Track, Christchurch, UK). A volume of 15 ml gas was sampled using a syringe and transferred into 6.8 ml Exetainer vials (Labco Ltd., Wycombe, Bucks, UK) for immediate measurement of CH_4. Methane concentration was determined using a gas chromatography (Varian 4900 GC; Agilent Technologies Canada Inc.,