Animal Feed Science and Technology 237 (2018) 8–18

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

Progressive adaptation of sheep to a microencapsulated blend of essential oils: Ruminal fermentation, methane emission, nutrient digestibility, and microbial protein synthesis

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ARTICLE INFO

Keywords:
Phenylpropanes
Terpene hydrocarbons
Rumen manipulation
Purine derivatives
Nitrogen retention

ABSTRACT

The combination of various active components in essential oils (EO) may be highly effective as a natural dietary supplementation option to manipulate ruminal fermentation and decrease methane (CH_4) emissions. Nine rumen cannulated Santa Inês sheep (55 ± 3.7 kg of body weight) were randomly divided into three groups to receive three experimental treatments: control (basal diet without additives); basal diet with 200 (EO200) mg of EO of a microencapsulated blend of essential oils (MBEO)/kg dietary DM offered; and basal diet with 400 (EO400) mg of EO of MBEO/kg dietary DM offered. The experiment was recorded in a triple 3 × 3 Latin Square design. The number of days necessary to observe the manipulation of rumen fermentation with MBEO was determined with an evaluation of nutrient digestibility, CH_4 emissions, and microbial protein synthesis. Ruminal samples were collected at day zero (the day before starting the dietary treatments), and on days 1, 2, 3, 7, and 15 after MBEO introduction, while the subsequent 7 days were assigned for the nutrient digestibility trial and CH_4 determination. On day 3, propionate concentrations began to increase ($P < 0.01$) for EO200 and EO400, which resulted in an associated decrease ($P = 0.03$) in the acetate: propionate ratio; this effect lasted until day 15. The enhancement ($P = 0.003$) in the overall mean of total short-chain fatty acids was consistent with the decrease ($P = 0.06$) in the overall mean of ruminal pH for both MBEO doses, compared to the control. A decline ($P < 0.01$) in the protozoal count was observed after a week in the EO400 treatment, whereas no differences were observed in the EO200 and control treatments. No significant differences among the three treatments were detected for ammonia concentration, dry matter intake, or total tract nutrient digestibility. A reduction ($P = 0.009$) in CH_4 emissions was observed in the EO200 and EO400 [24.5 and 27.6 l/kg digestible organic matter (DOM), respectively] treatments, compared to the control (38.2 l/kg DOM). Among the treatments, EO400 resulted in the highest body nitrogen (N) retention ($P = 0.03$) and microbial protein synthesis ($P < 0.01$). The results suggest that the addition of MBEO favorably modifies the ruminal

Abbreviations: ADFom, ash-free acid detergent fibre; AOAC, Association of Official Analytical Chemists; aNDFom, ash-free neutral detergent fibre treated with heat-stable amylase expressed exclusively of residual ash; BW, body weight; C2/C3, acetate to propionate ratio; CH_4, methane; CP, crude protein; DOM, digestible organic matter; EO, essential oils; DM, dry matter; DMI, dry matter intake; lignin(sa), lignin determined by solubilisation of cellulose with sulfuric acid; MBEO, microencapsulated blend of essential oils; MN_{PD}, daily duodenal flux of microbial N; N, nitrogen; OM, organic matter; PD_a, microbial purines absorbed from the small intestine; PD, urinary purine derivatives; SCFAs, short-chain fatty acids; SE, standard error

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https://doi.org/10.1016/j.anifeedsci.2018.01.004
Received 6 May 2017; Received in revised form 16 December 2017; Accepted 2 January 2018
0377-8401/

fermentation process, and 3 days of treatment is necessary before a stable and modified ruminal fermentation pattern can be observed. This pattern would last for a period of two weeks.

1. Introduction

The combination of various phenylpropanes and terpene hydrocarbons, active components in essential oils (EO), may be highly effective as a natural dietary supplementation option to manipulate ruminal fermentation and decrease methane (CH_4) emissions (Benchaar et al., 2006; Bassolé and Juliani, 2012; Benchaar and Greathead, 2011). This practice can lead to maximizing feed utilization, which is eventually reflected in overall animal productivity (Spanghero et al., 2009; Kissels et al., 2017). More importantly, unlike dietary antibiotics (*e.g.* ionophores), these EO combinations do not alter the activities of ruminal microbes towards a specific mode of action, and therefore present more potent mechanisms of action that are less likely to lose their effectiveness over time (Kissels et al., 2017).

The principal interest for producers in using EO in their animals might not be the reduction of CH_4 *per se*, but rather the benefits of redirecting the related energy that is conserved towards the production of milk or meat (Eckard et al., 2010; Soltan et al., 2018). Dietary EO additives are more likely to be used during critical periods in animal production (including transition, the onset of lactation, and steer finishing), which tend to span short periods of time, from a few days to a few weeks (Blanch et al., 2016). Thus, there is generally only a limited amount of time available in which to determine the power of EO blends in achieving the desired outcome. The progressive adaptation to EO blends, defined as the critical time during which EO begin to manipulate ruminal fermentation in a stable fashion, is typically incorporated into these critical periods of animal life, thus illustrating their importance. Research has suggested that rumen microbial communities can progressively adapt to dietary EO, but most *in vivo* studies have not taken dietary history into account. Observations are recorded at least two weeks after the first administration, and may present averages only (Castillejos et al., 2007; Spanghero et al., 2009; Geraci et al., 2012; Cobellis et al., 2016; Blanch et al., 2016). In addition, these studies have focused on the effects of EO on rumen microbial fermentation, while scarce data are available on the synthesis of microbial protein (Calsamiglia et al., 2007). However, it was reported that the action of EO not only occurred in the rumen but also extended to the entire gastrointestinal tracts (Cieslak et al., 2013). Thus, the action may affect the digestion of feed nutrients and absorption of microbial protein.

Recently, many *in vitro* studies have provided clear insight into the initial adaptation period (usually two weeks) for EO, but the studies did not account for the biological conditions of live animals, *e.g.* the capacity of rumen microbial populations, the *in vivo* effective dose, the dilution rate, or the stability of pH with buffering solutions (Benchaar and Greathead, 2011; Patra and Yu, 2015; Soltan et al., 2018). Moreover, the loss of some unstable or volatile active compounds from EO blends might affect the stability of their chemical composition along with the susceptibility of different microbes (Cobellis et al., 2016). Therefore, the objective of this study was to understand how progressive adaptation to a microencapsulated blend of EO (MBEO) would affect ruminal fermentation, CH_4 emissions, nutrient digestibility, and microbial protein synthesis.

2. Material and methods

The experiment was performed at the Centre for Nuclear Energy in Agriculture (CENA), University of São Paulo (USP), Piracicaba, State of São Paulo, Brazil. The protocol was approved by the Internal Commission for Ethics in Experimentation with Animals of CENA/USP (approval no. CIEEA/CENA 001/2011).

2.1. Experimental design, treatments, and basal diet

Nine rumen cannulated Santa Inês sheep (55 ± 3.7 kg of BW; mean ± SE) were individually allotted to indoor pens, divided into three groups, and randomly distributed in a triple 3 × 3 Latin square design for three consecutive periods of 37 days each. To remove MBEO residual effects, at the beginning of each experimental period all sheep were fed *ad libitum* a basal diet without additives for 15 days. After this time, the animals were fed their experimental diets for 22 days (the sampling period) in the following three treatments: control (basal diet without additives); basal diet with 200 (EO200) mg of EO (MBEO)/kg dietary DM added; and basal diet with 400 (EO400) mg of EO (MBEO)/kg dietary DM added. The amount of the offered diet, during the progressive adaptation period to MBEO, was calculated according to DMI of the first 15 days, during which the animals were fed the basal diet only, without additives. Adjustments were made if needed, and refused feed did not exceed 10% of daily intake. To determine DMI, orts were weighed daily and were not offered back to animals.

The first 15 days of the sampling period were dedicated to ruminal fluid collection. The next 7 days were dedicated to the digestibility trial, and the last 2 days of that trial focused on CH_4 determination. The animals were fed the experimental diets twice per day in equal portions, at 0900 and 1600 h, and had free access to fresh water.

The tested MBEO was a commercial product composed of a specific blend of natural essential oils in a standardized chemical formulation of cinnamaldehyde, eugenol, carvacrol, and capsicum oleoresin. Active components represent 15% of the product and are microencapsulated in a fat matrix (Activo Premium, GRASP Ind. e Com. LTDA, Curitiba, Paraná, Brazil).

Per kg of DM, the basal diet consisted of 500 g of hay prepared from Bermuda grass (*Cynodon dactylon* (L.) Pers.; hybrid "Tifton-