Small Ruminant Research 125 (2015) 56–63

Contents lists available at ScienceDirect

Small Ruminant Research

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

The effect of cumin (*Cuminum cyminum*) seed extract on the inhibition of PUFA biohydrogenation in the rumen of lactating goats via changes in the activity of rumen bacteria and linoleate isomerase enzyme

Vahideh Heidarian Miri[a,*], Seyed Hadi Ebrahimi[b], Amrish Kumar Tyagi[a]

[a] Department of Dairy Cattle Nutrition, National Dairy Research Institute (NDRI), Karnal, Haryana 132001, India
[b] Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran

ARTICLE INFO

Article history:
Received 18 July 2014
Received in revised form 17 January 2015
Accepted 24 February 2015
Available online 1 March 2015

Keywords:
Cuminum cyminum
Conjugated linoleic acids
Goat
Linoleate isomerase

ABSTRACT

In this study, two experiments were conducted to evaluate the effect of cumin (*Cuminum cyminum*) seed extract (CSE) on PUFA biohydrogenation. The first experiment was arranged in a completely randomized design in which eighteen crossbred goats were assigned to three dietary treatments: one group received basal diet (control; C0) consisting of forage and concentrate at equal ratio while the other two groups received the same diet with supplemented CSE (at 1.27% and 2.53% of dry matter intake as C1 and C2, respectively). The rumen content was sampled on day 27 and 29 of the experiment and then subjected to volatile fatty acids (VFA) and long chain fatty acids analyses. The CSE had no effect on molar proportion of VFA ($P > 0.05$). However, the addition of the CSE to the diet of goats increased the ruminal concentration of rumenic acid (RA; C18:2(c9,t11)) by 34.8% ($P = 0.041$) and vaccenic acid (VA; C18:1(11)) by 11.4% ($P = 0.01$) in lower supplemented group in comparison to the CSE-free diet. In the CSE-fed goats, the concentration of linoleic acid (LA; C18:2(9,12)) and linolenic acid (LNA; 18:3(9,12,15)) was higher ($P < 0.05$) and stearic acid (SA; C18:0) was lower ($P = 0.036$) than that of the control group, suggesting the inhibition of biohydrogenation. There was no dose dependent effect of the CSE on PUFA content. In the second experiment, linoleate isomerase activity and the growth of five strains of *Butyrivibrio* sp. and ten predominant species of rumen bacteria was tested at presence of the CSE. The results revealed that CSE did not inhibit the growth of *Butyrivibrio* sp. but it decreased the growth of some main species of the rumen bacteria such as *Prevotella brevis*, *Streptococcus bovis*, *Peptostreptococcus anaerobius* and *Lachnospira multiparus* ($P < 0.05$), which are involved in ruminal biohydrogenation. Moreover, the linoleate isomerase activity of the only stearate forming bacterium, *Butyrivibrio proteoclasticus*, decreased by 53% in the presence of the CSE (4.25 μg CSE/ml; $P = 0.002$). Initially, the inhibition of LA metabolism by this species decreases the rate of RA and VA formation, but it gradually results in the accumulation of RA and VA. It was found that CSE altered the bacterial biohydrogenation activity, thus increasing RA and VA concentration in the rumen.

* Corresponding author at: Mashhad Feed Mill, Mashhad, Khorasan-e-Razavi, Iran. Tel.: +98 51 38765999; fax: +98 513 8801980.
E-mail addresses: vhheydarian@gmail.com, v.heidarian@mashhadfeedmill.com (V. Heidarian Miri).

http://dx.doi.org/10.1016/j.smallrumres.2015.02.017
0921-4488/

1. Introduction

Since polyunsaturated fatty acids (PUFA) content in ruminant feedstuffs undergo ruminal biohydrogenation, the meat and milk contain only a small amount of linoleic acid (LA; C18:2(9,12)) and linolenic acid (LNA; 18:3(9,12,15)). However, microbial biohydrogenation of LA and LNA, which is mostly performed by rumen bacteria, forms intermediates, conjugated linoleic acid (CLA) and vaccenic acid (VA; C18:1(11)) that have a healthy effect on humans. The VA is a precursor of the main form of CLA, i.e. rumenic acid (RA, C18:2(c9,t11)) in animal tissues (Harfoot and Hazlewood, 1997; Belury, 2002; Griinari et al., 2000).

The research focus on the ruminant product quality is to enhance health promoting PUFA, particularly CLA content (Palmquist et al., 2005; Li et al., 2010; Tyagi and Heidarian, 2010). Previous studies have shown that plants contain a wide variety of secondary metabolites with antimicrobial activity and the potential, in certain amounts, to modulate fatty acid biohydrogenation (Vasta et al., 2010; Brogna et al., 2011). These compounds, exerting antimicrobial effect on biohydrogenating species in the rumen (Min et al., 2005; Durmic et al., 2008) accumulate biohydrogenation intermediates and enhance PUFA and CLA content (Vasta et al., 2008; Khiaosa-Ard et al., 2009; Ramos-Morales et al., 2013).

In vitro experiments indicate that the inclusion of cumin (*Cuminum cyminum*) in ruminal fluid increased PUFA concentration, enhanced the digestibility of dry and organic matters and mitigated methane emission (Khan and Chaudhry, 2010; Kilic et al., 2011; Chaudhry and Khan, 2012; Heidarian et al., 2013b). Recently, Heidarian et al. (2013a) reported that the dietary inclusion of cumin extract enhanced milk RA concentration by 20% in lactating goats. However, the effect of cumin on biohydrogenation pathways and isolates of rumen bacteria has not been adequately investigated.

The present study aimed to evaluate the effect of cumin seed extract (CSE) on fatty acid biohydrogenation in the rumen. To this end, two in vitro experiments were conducted to assess the results in greater details: (i) the effect of the CSE on the growth of the bacteria species that produce biohydrogenation reaction and the predominant species of ruminal bacteria and (ii) the effect of the CSE on the enzymatic conversion of LA to RA, which is achieved through the direct impact of the CSE on linoleate isomerase (LA-I, EC = 5.2.1.5).

2. Materials and methods

2.1. Experimental design, animals and management

The experiment was carried out at the National Dairy Research Institute (NDRI) in Karnal, India. The animal care and the procedures used were approved by Institute Ethics Committee of Animal Experiments in NDRI. A total of eighteen crossbred (Alpine × Beetal) dairy goats, with an average body weight of 34 ± 1.95 kg were randomly assigned to three groups, each consisting of six animals. All goats were free from physiological, anatomical and infectious diseases, kept in singular pens and received the same ratio of basal diet consisting of fresh berseem and concentrate. The nutrient requirements of goats were estimated according to NRC sheep and goat (2007). Thus, the concentrate contained (as fed basis) the following materials: maize 57%; ground nut cake (GNC) 40%; mineral mixture 2%, and common salt 1%. Cumin was supplemented at 1.27% and 2.53% of DMI, (1 and 2 g/L of rumen volume or 12.7 and 25.3 g/kg of DMI) as methanolic extract of cumin seeds (purchased from AMSAR Pvt. Ltd., Indore, India). The CSE was used as aqueous–methanolic (1:1) extract of the cumin seed powder, which was introduced as a powder form and mixed with daily concentrate mixture to C1 and C2 groups. The rumen volume was calculated separately for each animal according to formula introduced by Owens and Goetsch (1986). The control group (C0) did not receive the CSE supplement. The refusal of concentrate and roughage was removed daily and weighed at 0700 h before the supplement of the fresh feed for the calculation of dry matter intake (DMI). Goats were adapted to the experimental diets over a period of 21 days. Animals were milked twice a day (at 0600 and 1600 h). The data referring to the milk fatty acid profile was presented in a companion paper by Heidarian et al. (2013a). The rumen samples were collected 3 h after the morning feeding using the stomach tube for two consecutive days in weeks 1 and 4. The rumen sample data were generated during the second 2 days in the first week (0 day) and the last 2 days in the last week for covariance analysis. Immediately after collection, samples were stored in ice and transferred to the lab. A subsample of 300 g of each rumen sample was freeze-dried for fatty acid analysis.

Table 1
Chemical composition of diet consumed by goats.

Attributes	Chemical composition		
	Hay	Concentrate mixture	Cumin seed extract
Dry matter (%)	11.90	89.00	94.6
Composition (%DM[a])			
Organic matter	83.62	92.84	95.1
Crude protein	18.84	20.44	6.2
Ether extract	3.47	4.22	20
Neutral detergent fiber	40.27	53.30	3.0
Acid detergent fiber	31.30	10.31	0.6
Ash	16.37	7.16	4.9
Total phenol	–	–	26.3
Total tannins	–	–	13.5
Saponins	–	–	5.04

[a] Dry matter.

2.2. Ruminal fatty acids analysis

Total lipids of rumen samples were extracted from 0.5 g of freeze-dried and homogenized sample using the method proposed by O'Fallon et al. (2007). In this method, nonadecanoic acid (C19:0; 0.5 mg of C19:0/ml of methanol) was added as internal standard (0.2 ml), fatty acid extraction and methylation were performed after adding 0.7 ml of 10 M KOH and 5.3 ml of methanol to water. Fatty acid methyl ester (FAME) was synthesized after adding 12 M H_2SO_4 to water. FAMEs were dissolved in hexane, placed into a GC vial, capped and then kept at −20 °C until GC analysis. Fatty acid analysis was performed on a gas chromatograph (Nucon 5700, Nucon Engineering CO, New Delhi, India) equipped with a capillary column (60 m, 0.25 mm, 0.25 μm; ID-BPX70) and FID detector as well as 0.5 μl methyl esters which was injected in hexane at 10:1 split ratio. The GC conditions were as follows: the injector temperature was maintained at 220 °C and detector temperature was set at 250 °C; the initial oven temperature was kept at 70 °C and increased at a heating rate of 2 °C/min with a final oven temperature of 250 °C. Helium was used as a carrier gas. Fatty acids were identified in accordance with standards purchased from Sigma–Aldrich (Supelco Inc., Bellefonte, PA, USA) and quantification was implemented using the internal standard. Trans C18:1 was analyzed as a mixture of isomers in which vaccenic acid (*trans*11 C18:1) was the predominant one (Cruz-Hernandez et al., 2004; Fritsche et al., 2000). C16:1 was measured as the sum of n-9 and n-7 isomers plus a co-eluting branched chain fatty acid (methyl 14-methylhexadecanoate), which was reported in one value. The results of rumen fatty acids were expressed as the percentage of total identified fatty acids.

VFA were analyzed using the method proposed by Makkar (2010). Individual VFA in the samples were determined using gas chromatograph (Nucon 5700, Nucon Engineers, New Delhi) equipped with flame ionization detector and stainless steel column, which was packed with chromosorb 101 mesh 80–100 (length: 1.5 m; o.d: 3.175 mm; i.d.: 2 mm). Analytical conditions for the fractionation of VFA were as follow: the injection port temperature was 210 °C; the column temperature was 180 °C and the detector temperature was 230 °C. The flow rate of carrier gas (nitrogen) was 30 ml/min.

2.3. Feedstuff analysis

Chemical composition (Table 1) and total lipid (Table 2) of the hay, concentrate and the CSE were extracted from 1 g of freeze-dried sample using the method of O'Fallon et al. (2007), as described earlier.

2.4. Bacteria and growth conditions

The growth study included fifteen strains of main bacteria species in the rumen (Table 3). *Butyrivibrio fibrisolvens* D1, *Butyrivibrio hungateii* A38 and *B. fibrisolvens* OB156 belonged to group A, biohydrogenating bacteria, *Butyrivibrio proteoclasticus* B316 and *B. proteoclasticus* UC142 belonged to group B of ruminal biohydrogenating bacteria. Other bacterial strains used in this study are listed in Table 3. All cultures were grown under O_2-free CO_2 at a temperature of 39 °C in DSM Medium 704 in 12.5 cm × 1.5 cm culture tubes, which was closed with screw caps and fitted with butyl