Small Ruminant Research 144 (2016) 126–134

Contents lists available at ScienceDirect

Small Ruminant Research

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

Effect of dietary oil sources on fatty acid composition of ruminal digesta and populations of specific bacteria involved in hydrogenation of 18-carbon unsaturated fatty acid in finishing lambs

Tianzhang Zhao[a,b], Yong Ma[a], Yanghua Qu[a], Hailing Luo[a,*], Kun Liu[a], Zhaoyun Zuo[a], Xiaonan Lu[a]

[a] State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, 100193 Beijing, PR China
[b] Department of Biology, Jining Normal University, 012000 Wulanchabu, Inner Mongolia, PR China

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 19 June 2016
Accepted 30 June 2016
Available online 1 July 2016

Keywords:
Oil source
Fatty acid
Bio-hydrogenation
Bacteria
Rumen
Lamb

ABSTRACT

This study aimed to investigate the relationship of fatty acid composition with specific bacteria involved in hydrogenation of 18-carbon unsaturated fatty acid in response to dietary oil sources. 28 finishing Bamei lambs were randomly allocated to 4 diets composed of dehydrated hay and concentrate mixture containing no additional lipid (CONT), supplemented with 40 g/kg dry matter (DM) of fish oil (FO), 40 g/kg DM of sunflower oil (SFO), or 10 g/kg DM of fish oil plus 30 g/kg DM of sunflower oil (FOSFO). After 120 days of feeding, ruminal fluid and digesta were harvested from each lamb for FA composition and fermentation characteristic analysis, respectively. QPCR was undertaken for determining the relative contents of *Butyrivibrio fibrisolvens* and *Butyrivibrio proteoclasticus* in ruminal fluid. Compared with CONT, both SFO and FOSFO did not influence the major ruminal fermentation parameters except for total volatile FA (TVFA), however FO fed alone shifted rumen fermentation toward propionate at the expense of acetate with no change in molar proportions of other individual volatile FA (VFA). Dietary oil supplements resulted in the accumulation of *trans*-11 C18:1 in ruminal digesta, and FO inclusion simultaneously induced a marked decrease in C18:0 concentrations. Sunflower oil inclusion resulted in a small decrease in the relative proportion of *B. fibrisolvens*, however, the relative proportion of *B. proteoclasticus* was substantially less for lambs fed fish oil supplements compared with CONT. QPCR analysis indicated that a weak relationship between the number of *B. proteoclasticus* and C18:0 concentrations ($P < 0.01$, $R^2 = 0.3293$). In general, unprotected FO, SFO, or FOSFO affected ruminal fermentation and produced series of bio-hydrogenation intermediates. Alterations in ruminal bio-hydrogenation were associated with changes in the abundance of *B. proteoclasticus*, but *B. proteoclasticus* was not the dominant bacterium in producing C18:0.

1. Introduction

Conjugated linoleic acid (CLA) is a group of positional and geometric (*cis* or *trans*) isomers of linoleic acid with a conjugated double bond. The most representative CLA isomers are *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. CLA has been shown to exert various potent physiological functions such as anticancerogenic, antidiabetic, and antiatherogenic effects, as well as effects on the immune system, bone metabolism, and body composition (Schmid et al., 2006). Also, reports suggest that physiological effects of CLA are different between the isomers, for example the *trans*-10, *cis*-12 CLA isomer is anticarcinogenic, antiobese and antidiabetic, whereas the *cis*-9, *trans*-11 CLA isomer is mainly anticarcinogenic (Koba and Yanagita, 2014). It has been demonstrated that CLA in ruminant products are mainly formed by endogenous synthesis, which involves the action of Δ9-desaturase on *trans*-11 18:1 (vaccenic acid, TVA) for *cis*-9, *trans*-11CLA and *trans*-7 18:1 for *trans*-7, *cis*-9 CLA in mammary gland or adipose tissues (Griinari et al., 2000; Corl et al., 2001). *trans*-11 18:1 is an important 18:1 intermediate due to the biohydrogenation of dietary 18-carbon polyunsaturated FA (PUFA) to saturated FA (SFA) in the rumen. The bacteria involved in the different steps of the biohydrogenation pathway have been categorized as Group A and B (Harfoot and Hazelwood, 1997): group A bacteria, which belong to the *Butyrivibrio fibrisolvens* group, hydro-

* Corresponding author at: China Agricultural University, College of Animal Science and Technology, 2 Yuan Mingyuan Xilu, Haidian District, Beijing, PR China.
E-mail address: luohailing@cau.edu.cn (H. Luo).

http://dx.doi.org/10.1016/j.smallrumres.2016.06.012
0921-4488/

genate 18:2n-6 and 18:3n-3 to *trans*-11 18:1; in contrast, group B bacteria biohydrogenate the same FA to 18:0. The only Group B bacteria isolated from the rumen capable of converting PUFA to SFA are closely related to *Butyrivibrio proteoclasticus* (*B. proteoclasticus*), which are also part of the *Butyrivibrio fibrisolvens* group (Paillard et al., 2007a; Boeckaert et al., 2008; Moon et al., 2008; Huws et al., 2010). Because group B bacteria (18:0 producers) form a tight grouping in which strains cluster together close to *B. proteoclasticus* (van de Vossenberg and Joblin, 2003; Wallace et al., 2006), the 18:0 producers are described here as *B. proteoclasticus*.

Several factors (seasonal variations, animal genetics, and production practices) have been found to influence the CLA content in ruminant products. Diet composition is the major factor influencing the fatty acid (FA) composition of meat and milk from ruminants because the FA which reach the duodenum are, at least in part, of dietary origin as well as the result of rumen microbial bio-hydrogenation of dietary lipids (Buccioni et al., 2012). In the past decade, numerous studies demonstrated that marine oil supplementation could affect rumen lipid metabolism by altering the activity of specific ruminal bacteria involved in bio-hydrogenation and isomerization of dietary PUFA (Huws et al., 2010). Fish oil supplementation can modify the FA profile of ruminal contents, resulting in accumulation of CLA and *trans*-11 C18:1 in ruminant dairy products (Chilliard et al., 2003; Chow et al., 2004; Wasowska et al., 2006; Shingfield and Griinari, 2007). Kim et al. (2008) and Shingfield et al. (2012) reported that fish oil supplementation significantly increased the ruminal outflow of *trans* C18:1 and resulted in a linear decrease in numbers of *B. proteoclasticus*. However, recent studies indicated that *B. proteoclasticus* was not dominant in the ruminal C18:0-producing process of lactating or nonlactating sheep fed diets containing fish oil, marine algae, or/and sunflower oil (Toral et al., 2010; Belenguer et al., 2010; Toral et al., 2012). Furthermore, ruminant meat products are another important human food source for *cis*-9, *trans*-11 CLA and long-chain n-3 FA. Previous studies have mostly focused on the effect of dietary plant oil and marine lipid supplementation on ruminal FA metabolism and concomitant changes in the rumen bacterial community in dairy cows and adult sheep. Moreover, based on the suggested interspecies differences in lipid metabolism (Shingfield et al., 2009), there might be different bacteria existing for bio-hydrogenation profiles.

The objective of this study was to investigate the effects of different dietary oil sources on FA composition of ruminal digesta and populations of bacteria involved in bio-hydrogenation of dietary PUFA to produce CLA and *trans*-11 18:1, and try to identify the true role of *B. proteoclasticus* on bio-hydrogenation of 18-carbon unsaturated FA (UFA) in the rumen of finishing lambs.

2. Materials and methods

The work described in this study was conducted in accordance with the requirements of the Animal Care and Use Committee of China Agricultural University. Lambs were bought from China Bamei Lamb Breeding Center in Linhe, Inner Mongolia. The feedlot experiment was conducted from May to October.

2.1. Animals, diets and experimental design

According to the weight and age of the lambs, twenty-eight 60-day-old male Bamei lambs were randomly assigned to one of four dietary treatments on the basis of body weight (18.89 ± 3.29 kg). Four diets composed of concentrate mixture and forage (60:40) were formulated to meet the nutrient requirements for finishing lambs (NRC, 2007). As shown in Table 1, the 40 g/kg oil addition level in grain supplements was chosen based upon the study of Awawdeh et al. (2009). In addition, Moore et al. (1986) and Ngidi et al. (1990) reported that a negative effect was found for higher inclusion of oils. FOSFO diet was mixed with a proportion of 1:3 for fish oil (FO) and sunflower oil (FSO), according to the study of AbuGhazaleh (2008), who indicated that high level of FO supplementation might induce milk fat depression in dairy cows. All diets were formulated to be isonitrogenous by adding expanded urea, and isocaloric by adding extra wheat bran. The forage consisted of 750 g/kg Chinese *chinensis* and 250 g/kg lucerne hay. The commercial concentrate mixture was produced in an industrial unit of animal feeds and oils were included by partially replacing cracked corn in the concentrate mixture supplement. Menhaden fish oil (Omega Protein Inc., Hammond, LA) and Luhua sunflower oil (Inner Mongolia Luhua Group Co., Ltd., Linhe, China) were used in this study. Concentrate diets and lipid supplements were finely mixed, well bagged and stored at room temperature. Ingredients and chemical composition of the diets are shown in Table 1, and the FA composition of grain supplements, forage and oil supplements are shown in Table 2. During the 120-day-feedlot period, all lambs were free access to (ad libitum) feed and clean water.

Table 1
Ingredients and chemical composition of grain supplements.

Ingredients, g/kg DM	Dietary treatments[a]			
	CONT	FO	SFO	FOSFO
Cracked corn	567	392	392	392
Soybean meal, 48% CP	148	120	120	120
Wheat bran	92	350	350	350
DDGS[b]	150	38	38	38
Fish oil	0	40	0	10
Sunflower oil	0	0	40	30
Expanded urea[c]	0	17	17	17
Salt	8	8	8	8
Limestone	5	5	5	5
Dicalcium phosphate	8	8	8	8
Sodium bicarbonate	5	5	5	5
Vitamin/mineral premix[d]	17	17	17	17
Chemical composition,[e] g/kg DM				
ME, MJ/kg DM	10.77	10.62	10.72	10.62
CP	175.8	174.9	175.8	174.8
EE	28.9	55.8	54.4	53.8
aNDF	227.3	232.1	233.8	242.8
ADF	68.9	70.7	72	76
TFA	38.21	75.27	73.07	71.63

[a] CONT, FO, SFO, and FOSFO refer to dietary treatments containing no additional fat, 40 g/kg fish oil, 40 g/kg sunflower oil, and 10 g/kg fish oil + 30 g/kg sunflower oil on DM basis, respectively.
[b] DDGS = distillers dried grains with solubles.
[c] Expanded urea is produced by gelatinization of maize, urea and coagulant to make urea with a sustained release in rumen.
[d] Contained per kilogram of premix (Zhengzhou Ruipu Biological Engineering Co., Ltd., Zhengzhou, China): vitamin A, 3,700,000 IU; vitamin D_3, 680,000 IU; vitamin E, 6000 mg; Mn, 15.26 g; Zn, 15.18 g; Fe, 6.60 g; Cu, 3.75 g; I, 270 mg; Co, 97 mg; Se, 179 mg plus antioxidant.
[e] All values are analyzed values except metabolizable energy; ME = metabolizable energy; CP = crude protein; EE = ether extract; aNDF = neutral detergent fiber; ADF = acid detergent fiber; TFA = total fatty acids.

2.2. Slaughtering and rumen sampling

At the end of feedlot period, all lambs were transported to a commercial abattoir, and slaughtered after being fasted for 12 h as reported previously (Ponnampalam et al., 2001). Digestive organs were pulled out, and an incision was made in the reticulo-rumen with a knife. The whole rumen contents, representing both liquids and solids, were collected from at least 4 different locations of each rumen and pooled for each animal (Chaudhry and Mohamed, 2012). Digesta samples were filtered, vacuum packaged and stored at −20 °C until analysis for FA composition. Ruminal fluid samples were obtained by filtering the remaining rumen content through