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RESEARCH ARTICLE

Impacts of the unsaturation degree of long-chain fatty acids on the volatile fatty acid profiles of rumen microbial fermentation in goats *in vitro*

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Abstract
This study investigated the impacts of the degree of unsaturation (unsaturity) of long-chain fatty acids on volatile fatty acid (VFA) profiles of rumen fermentation *in vitro*. Six types of long-chain fatty acids, including stearic acid (C18:0, control group), oleic acid (C18:1, *n*-9), linoleic acid (C18:2, *n*-6), α-linolenic acid (C18:3, *n*-3), arachidonic acid (C20:4, *n*-6) and eicosapentaenoic acid (C20:5, *n*-3), were tested. Rumen fluid from three goats fitted with ruminal fistulae was used as inoculum and the inclusion rate of long-chain fatty acid was at 3% (w/w) of substrate. Samples were taken for VFA analysis at 0, 3, 6, 9, 12, 18 and 24 h of incubation, respectively. The analysis showed that there were significant differences in the total VFA among treatments, sampling time points, and treatment×time point interactions ($P<0.01$). α-Linolenic acid had the highest total VFA ($P<0.01$) among different long-chain fatty acids tested. The molar proportion of acetate in total VFA significantly differed among treatments ($P<0.01$) and sampling time points ($P<0.01$), but not treatment×time point interactions ($P>0.05$). In contrast, the molar proportion of propionate did not differ among treatments during the whole incubation ($P>0.05$). However, for butyrate molar proportions, significant differences were found not only among sampling time points but also among treatments and treatment×time point interactions ($P<0.01$), with eicosapentaenoic acid having the highest value ($P<0.01$). Additionally, no statistically significant differences were found in the acetate to propionate ratios among treatments groups ($P>0.05$), even the treatments stearic acid and α-linolenic acid were numerically higher than the others. The inclusion of 3% long-chain unsaturated fatty acids differing in the degree of unsaturation brought out a significant quadratic regression relation between the total VFA concentration and the double bond number of fatty acid. In conclusion, the α-linolenic acid with 3 double bonds appeared better for improving rumen microbial fermentation and the total VFA concentration.

Keywords: volatile fatty acid, unsaturation degree, long-chain fatty acid, *in vitro* fermentation

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1. Introduction

Volatile fatty acids (VFA) produced in the rumen have a great influence on ruminant metabolism as they provide 70–80% of digestible energy required by the host animal (Van 1996; Feng 2004; Bannink *et al.* 2008). Different types of VFA have different impacts on the physiological function of ruminants. The VFA can also help maintain an

ideal environment in the rumen, which is beneficial to rumen microbial growth (Bensadoun *et al.* 1962).

Rumen microorganisms could rapidly degrade fats in the diet to glycerine and free fatty acids, while free fatty acids with a free carboxyl, whether saturated or not, have some toxic actions on rumen microorganisms (Jenkins 1993). The unsaturated fatty acids in the rumen can inhibit the growth of protozoa (Dijkstra *et al.* 2000), some types of bacterial genera (Zhao *et al.* 2005; Liu *et al.* 2011) and the engulfment activity of protozoa on bacteria (Wang *et al.* 2010). The rumen microbes can only adapt to low levels of fats while high levels of fats in the rumen may affect their normal metabolism and growth, particularly for ruminal protozoa lacking cell walls. Therefore, we could manage ruminal protozoa family through adding oils or fatty acids and consequently manipulate rumen flora and their fermentation patterns (McGinn *et al.* 2004).

Previous studies suggest that unsaturated fatty acids in oils or fats may modify ruminal microbes and ecosystem, and rumen fermentation as well. Some kinds of plant oil richly containing unsaturated fatty acid are able to change rumen fermentation pattern by enhancing the molar percentage of propionate (Machmüller *et al.* 1998; Jalč and Čerešňáková 2002). Zhang *et al.* (2008) have even demonstrated that the acetate to propionate ratio decreased significantly by adding octadeca carbon fatty acids *in vitro*. Potu *et al.* (2011) have shown that the supplements of lipid with different long-chain fatty acid compositions have some discrepancies when acetic acid and propionic acid were produced in the rumen fermentation process. Acetate, butyrate and total VFA decrease with the increasing degree of unsaturation of C18 fatty acids (oleic acid, linoleic and linolenic acids) *in vitro* (Li *et al.* 2012). These reports suggest that the effects of long-chain fatty acids on rumen fermentation have differences and may be related to the unsaturation of long-chain fatty acids.

Our previous study found that the inclusion of 3% of long-chain unsaturated fatty acids increased bacterial protein content, whereas reduced protozoal protein content and enhanced dehydrogenase activity of rumen microorganism (Gao *et al.* 2016), which may result in the change of the pattern of rumen fermentation. Thus, this study was to examine the effects of six types of long-chain fatty acids varying in the degrees of unsaturation on the VFA profiles and their concentrations of rumen fermentation *in vitro*.

2. Materials and methods

2.1. Animals and management

Three fistulated Xuhuai White goats with similar age (1.5 years old) and live weight, (29.4±2.7) kg, from the Experimental Farm of Yangzhou University, China were used to provide rumen liquor as culture inoculum. These animals were fed at 07:00 and 19:00 in equal amounts of a diet containing 28% corn grains, 2% soybean and 70% *Leymus chinensis* hay. The daily feed allowance on the basis of dry matter was 2.5% of animal live weight. The animals had free access to clean drinking water at all the times. The use of animals and the experimental procedures were approved by the Animal Care and Use Committee of Yangzhou University, Jiangsu Province, China.

2.2. Experimental design, procedures and sampling

This work was part of a larger study. The experimental design and the *in vitro* substrates were the same as the work described by Gao *et al.* (2016). In brief, six kinds of long-chain fatty acids with different numbers of double bonds, including stearic acid (C18:0, control group), oleic acid (C18:1, *n*-9), linoleic acid (C18:2, *n*-6), α-linolenic acid (C18:3, *n*-3), arachidonic acid (C20:4, *n*-6), and eicosapentaenoic acid (C20:5, *n*-3), were added at 3% of substrate weight for *in vitro* incubation. Since stearic acid did not affect ruminal fermentation or slightly increased rumen microbes *in vitro* (Chalupa *et al.* 1984; Zhang *et al.* 2008), stearic acid containing nil double bond was used as a control in this experiment. Incubations were run in triplicate, and a set of appropriate blank (without substrates) was included.

Around 300 mL of rumen fluid per goat were obtained using a vacuum pump through the rumen fistula and mixed as inoculum before morning feeding. The rumen fluid was filtered through four layers of gauze into an aseptic saline bottle which was aerated with CO_2 and preheated at 39°C. Artificial saliva salt was made according to Menke and Steingass (1988) before the *in vitro* culture. The *in vitro* culture medium was prepared by mixing artificial saliva and rumen fluid in a ratio of 2:1 (v/v). The artificial saliva was used to neutralize fatty acids produced during fermentation in order to maintain pH within a normal range. A glass culture bottle used for incubation was added with 1.50 g of the substrate and 150 mL of the culture medium, flushed with CO_2 and then sealed. The bottles were placed in a water bath (39°C) and constantly shaken at 50 r min^{-1} for 24 h.

A sample of the fermentation fluid (2 mL each) was taken from each bottle at 0, 3, 6, 9, 12, 18 and 24 h of incubation and stored at –20°C for VFA analysis.

2.3. Laboratory analysis

The concentrations of VFAs in fermentation fluid were determined using a GC-14B gas chromatograph (Shimadzu Corp., Kyoto) using the method as described by Xiong *et al.* (1999). The testing condition was listed as follow, capillary

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