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Effects of dietary non-ionic surfactant and forage to concentrate ratio on bacterial population and fatty acid composition of rumen bacteria and plasma of goats

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ABSTRACT

This study was conducted to investigate effects of alkyl polyglycoside (APG) in diets that varied in forage to concentrate ratio (F:C) on ruminal microbial and plasma fatty acid (FA) composition and bacterial population in goats. A 2 × 2 factorial experiment was arranged within a 4 × 4 Latin square design using four wether goats fitted with permanent ruminal fistulae. Treatments included two levels of dietary F:C (40:60 *vs.* 60:40, on an air dry basis) and APG (no APG *vs.* 6.5 g APG per day per animal). Dietary APG inclusion increased the proportion of *iso*-14:0 (P=0.03), and decreased the proportions of *anteiso*-17:0 (P=0.04) and monounsaturated fatty acids (MUFA) (P=0.04) in solid associated bacteria (SAB), but had no effect on FA in liquid associated bacteria (LAB) or in plasma. In addition, APG decreased (P<0.05) the *Ruminococcus albus* population. The major effects of APG were independent of F:C ratio. High dietary F:C decreased the proportions of 17:0 (P=0.01) and 9c12c18:2 (P=0.05) in LAB, decreased *iso*-13:0 (P=0.04) in SAB and 9c12c18:2 (P=0.05) in plasma, increased the proportions of *anteiso*-13:0 (P=0.04) in LAB, and 17:0 (P=0.0003) and 9c18:1 (P=0.03) in plasma, but had no effect on the ruminal bacterial population. These results indicate that, in both low and high F:C diets, dietary APG alter the FA composition of ruminal bacteria, which further imply a change in function and survival of rumen bacteria.

1. Introduction

Ruminal microbial cells are an important source of fatty acids (FA) for ruminants. Microbial FA originates from: *de novo* synthesis, direct incorporation of preformed precursor molecules from dietary sources (Harfoot and Hazlewood, 1997), hydrogenation, desaturation of long-chain FA (Yanez-Ruiz et al., 2006; Varadyova et al., 2008), and physical adsorption of dietary FA onto bacterial extracellular structure (Kucuk et al., 2008). Numerous factors can alter ruminal bacterial FA

Abbreviations: APG, alkyl polyglycoside; AA, amino acids; ADF, acid detergent fiber; CP, crude protein; DM, dry matter; EE, ether extract; F:C, forage to concentration ratio; FA, fatty acid; LAB, liquid associated bacteria; MUFA, monounsaturated fatty acids; NDF, neutral detergent fiber; NIS, non-ionic surfactants; OBCFA, odd-and branched-chain fatty acid; OM, organic matter; PUFA, polyunsaturated fatty acids; SAB, solid associated bacteria; SFA, saturated fatty acids; VFA, volatile fatty acids.

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composition, such as dietary fat supplements (Or-Rashid et al., 2007; Varadyova et al., 2008), dietary forage to concentrate ratio (F:C), forage source (Hussein et al., 1995; Bas et al., 2003; Vlaeminck et al., 2006), and dietary additives (*e.g.*, plant extracts; Vasta et al., 2009; Tan et al., 2011).

Alkyl polyglycosides (APG) are a type of mild non-ionic surfactant (NIS) derived from a reaction of corn starch glucose and a natural fatty alcohol (decyl polyglucose). These surfactants are used in cosmetic lotions (Weuthen et al., 1995) and cleaning agents (Nickel et al., 1996), and are commercially available as yellowish colored aqueous paste. Alkyl polyglycosides are non-toxic, biodegradable (Nickel et al., 1992; Hill and Rhode, 1999) and GRAS (generally regarded as safe) (GRAS notice 000237). In addition, APG are characterised in terms of their physicochemical properties of emulsification and dispersion (Nickel et al., 1992, 1996). The glycoside of APG is an efficient nonionic emulsifier and the fatty alcohol is a co-emulsifier (Weuthen et al., 1995).

Cong et al. (2009) reported that APG increased *in vitro* ruminal dry matter (DM) and organic matter (OM) digestibility of cereal straw, and volatile fatty acids (VFA) concentrations in a dose dependent manner. Dietary inclusion of APG increased total tract digestibility of OM and neutral detergent fiber (NDF), concentrations of ruminal ammonia-N and VFA, and the ratio of acetate to propionate in goats (Yuan et al., 2009, 2010). In addition, we also found that dietary inclusion of APG affected amino acid (AA) composition of ruminal bacteria under conditions of different dietary F:C (Zeng et al., 2011). Lee et al. (2003) and Hwang et al. (2008) reported that Tween 80 (Sigma–Aldrich, Kyunggi-do, Korea), another type of NIS with similar properties as APG, stimulated the growth rate of anaerobic microorganisms, but decreased adhesion of rumen cellulolytic bacteria to forage. The increase in nutrient digestibility and changes to ruminal metabolism by APG inclusion might be due to a change in microbial populations and bacterial FA composition, but there is no information available about these effects in the literature. Furthermore, a change in bacterial FA composition could result in a change in proportions of FA in plasma of sheep (Zhang et al., 2006). However, there is no literature on the effects of APG on plasma FA profile. Therefore, this study was designed to investigate the effects of dietary APG on rumen bacterial populations, as well as bacterial and plasma FA composition, in goats fed diets that varied in F:C.

2. Materials and methods

2.1. Animal, experimental design and treatments

The experiment was approved by the Animal Care Committee, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China.

Four ruminally fistulated Liuyang Black wether goats (a local breed in southern China) with initial body weight of 19.2 ± 1.1 kg were randomly assigned to a 4×4 Latin square design with four dietary treatments. Treatments included two factors: dietary F:C (40:60 *vs.* 60:40, on an air dry basis) and APG (no APG, None; 6.5 g APG per animal per day, APG). The APG (D-Glucopyranoside, C9-11 alkyl, oligomeric, degree of polymerization 16) was in liquid form (Hunan Diyuan Co., Ltd., Changsha, Hunan, China; 0.5 g/mL). Maize stover (OM 850 g/kg DM, neutral detergent fiber [NDF] 723 g/kg DM, acid detergent fiber [ADF] 475 g/kg DM, crude protein [CP] 68 g/kg DM and ether extract 13.4 g/kg DM) was supplied as forage. Finely ground corn, soybean meal and wheat bran were also used in the diets in different proportions, and diets were formulated to provide the same (100 g/kg DM) concentration of CP (Table 1). The supplemented APG was manually mixed into the concentrate before starting the experiment.

Each of the 4 periods consisted of 22 days, with the first 18 days for adaptation and the last 4 days for sample collection. All goats were kept in individual pens and had free access to fresh water. Feed intake was restricted to 540 g DM/d to provide 1.1 times maintenance metabolic energy requirements of the goats (Zhang and Zhang, 1998). Dietary forage and concentrate were offered simultaneously as a total mixed ration in two equal portions at 08:00 and 19:00. No refusals occurred after the adaptation period.

2.2. Sample collection

Before the morning feeding on day 19 of each period, blood samples were taken from the jugular vein and collected into heparinized tubes. These were centrifuged for 15 min at $1500 \times g$ at 4 °C, and the plasma was collected and stored at −20 °C. In addition, about 30 mL of rumen contents were taken from each goat at 0 h (before the morning feeding, 08:00), 1, 2, 3, 6 and 9 h after the morning feeding through the rumen cannula. The pH was measured immediately using a pH meter (model 2000, Beckman Instruments Inc., Fullerton, CA, USA).

On day 20, about 30 mL of rumen contents were sampled at 2 h before the morning feeding, and tightly squeezed through four layers of cheesecloth. The filtrate and particles were immediately frozen at −70 °C prior to DNA extraction and real-time polymerase chain reaction (qPCR) analysis.

On day 20–22, about 80 mL of rumen contents were sampled at 2 h before and after each feeding (four times per day, 3 days). The microbial fractions were separated by differential centrifugation according to the method of Legay-carmier and Bauchart (1989). Briefly, rumen contents were squeezed through eight layers of cheesecloth. Total particles were washed in saline solution (0.009 cold NaCl, 100 mL/100 g particles) and squeezed again to remove free-floating bacteria of the liquid phase loosely associated with particles. The filtrates were pooled and centrifuged at $500 \times g$ for 10 min at 4 °C to remove feed particles and protozoa. The supernatant obtained was centrifuged at $27{,}000 \times g$ for