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Effects of *Suaeda glauca* crushed seed on rumen microbial populations, ruminal fermentation, methane emission, and growth performance in Ujumqin lambs

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ABSTRACT

This study was conducted to evaluate the effects of *Suaeda glauca* crushed seed on rumen microbial populations, ruminal fermentation, methane emission, and growth performance in lambs. Thirty male Ujumqin lambs (a local breed, average body weight 25.5 ± 1.28 kg) were equally divided into three treatments for a 56 d feeding period. The treatments were control diet (CTR), 80 g of SG seed/kg of diet (SGS80, dry matter (DM) basis), and 160 g of SG seed/kg of diet (SGS160, DM basis). Ruminal samples were collected at 0, 3, 6 and 9 h after the morning feeding on d 28 and 56 to investigate treatment, sampling day, sampling hour, and their interactions effects on ruminal parameters and microorganism population. After the feeding trial, all lambs were moved to respiratory chambers to measure methane emission. Addition of SG seed decreased methanogen ($P < 0.05$), protozoa ($P < 0.01$), and *Fibrobacter succinogenes* populations ($P < 0.05$). Methanogen and protozoa populations were also affected by an interaction between treatment and sampling day ($P < 0.01$) and the effect of SG seed on methanogen and protozoa populations decreased with feeding time. Addition of SG seed did not change populations of fungi, *Ruminococcus flavefaciens*, and *Ruminococcus albus*. Lambs fed SG160 diet had lower rumen ammonia nitrogen concentration ($P < 0.01$) compared with lambs fed CTR diet. Ammonia nitrogen concentration was affected by an interaction between treatment and sampling day ($P < 0.01$). No SG seed effects were found in ruminal pH, total volatile fatty acid concentration and molar proportion of volatile fatty acid. Addition of SG seed increased average daily gain ($P < 0.05$) and dry matter intake ($P < 0.05$) of lambs but reduced methane emissions ($P < 0.05$). No SG seed effects were found in feed efficiency. In conclusion, addition of SG seed seemed to be a feasible means of decreasing methane emissions from lambs by reducing methanogen and protozoa populations.

Abbreviations: ADFom, acid detergent fiber expressed exclusive of residual ash; ADG, average daily gain; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; BW, body weight; CH_4, methane; CP, crude protein; DM, dry matter; DMI, dry matter intake; FA, fatty acid; FE, feed efficiency; qRT-PCR, quantitative real time polymerase chain reaction; SG, *Suaeda glauca*; UFA, unsaturated fatty acid; VFA, volatile fatty acid.

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1. Introduction

Methane (CH_4) contributes to the global warming phenomenon and plays a role in the destruction of the stratospheric ozone layer (IPCC, 2007). Methane is produced in the process of anaerobic enteric fermentation of feeds in animals, especially ruminants. It is estimated that ruminants are responsible for about 150–200 g/kg of the annual total anthropogenic CH_4 emissions (Moss et al., 2000). Moreover, enteric CH_4 from ruminants is 20–120 kJ/MJ of ingested gross energy loss to the animal (Johnson and Johnson, 1995). Therefore, the development of feeding strategies to mitigate CH_4 emissions from ruminants may bring not only environmental benefits for the planet but also nutritional benefits for the animal. Several studies have indicated that dietary manipulation could reduce CH_4 production in the rumen by inhibiting methanogenic bacteria or by diverting hydrogen away from CH_4 formation (McGinn et al., 2004; Mao et al., 2010).

The *Suaeda glauca* (SG), derived from an annual halophyte of *Chenopodiaceae*, can survive in saline and alkaline soils and form a large scale community around the saline-alkali lake and in the saline-alkali grassland. In the grassland of northeastern China, the SG hay yield per ha is approximately 6 t, of which 200 g/kg is seeds (Li and Yang, 2004). Several studies have reported that some vegetable oil or oilseeds rich in unsaturated fatty acid (UFA) decreased CH_4 production and altered fermentation patterns due to biohydrogenation and toxic effects of medium-chain or polyunsaturated fatty acids on methanogens and protozoa (Beauchemin et al., 2009; Mao et al., 2010). According to Yu et al. (2005), the SG seed contained approximately 244 g crude fat/kg DM and 887 g UFA/kg total fatty acids. Thus SG seed seems to be an effective hydrogen acceptor and inhibitor of microorganisms involved in methanogenesis. Previous studies found that SG seed improved growth performance and meat quality in sheep (Sun and Zhou, 2010; Sun et al., 2015). However, no information on the potential role of SG seed on reducing CH_4 emission from lambs was found in the literature reviewed. Thus, we hypothesized that SG seed could exhibit inhibitory properties on methanogens or protozoa and we designed this study to investigate the effects of dietary addition of SG seed on growth performance, CH_4 emission, ruminal fermentation, and microbial populations in lambs.

2. Materials and methods

2.1. Animals and diets

All procedures were approved by the Administration Office of Laboratory Animals, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences.

Thirty male Ujumqin lambs (a local breed) with average age of 112 ± 10.5 d, initial body weight (BW) of 25.5 ± 1.28 kg, and similar genetic background were equally divided into three treatments in a completely randomized design. The treatments were control diet (CTR), 80 g of SG seed/kg of diet (SGS80, dry matter (DM) basis), and 160 g of SG seed/kg of diet (SGS160, DM basis). The control diet was formulated according to feeding standards for meat-producing sheep and goats (NY/T 816-2004, Ministry of Agriculture, China; Table 1). Three diets had similar gross energy and crude protein contents. The ether extract contents were 24, 34, and 45 g/kg DM in the CTR, SGS80, and SGS160 diets, respectively. The diet was fed as a total mixed ration.

The SG seed (purchased from Yancheng Green Garden Saline Soil Agriculture Technology Co., Ltd, Jiangsu, China) was crushed using a roller mill with the roller setting adjusted to ensure the hulls were cracked. Chemical and fatty acid composition of the batch used in this experiment are shown in Table 1.

2.2. Chemical analysis

The DM was determined by oven drying at 105 °C overnight (AOAC method. 930.15, 1995). Crude protein (CP) was measured by a Kjeldahl nitrogen analysis (AOAC method. 954.01, 1995). Ether extract was determined using a Soxhlet apparatus (AOAC method. 945.16, 1995). Neutral detergent fiber (aNDFom) and acid detergent fiber (ADFom) contents were determined as explained by Van Soest et al. (1991) using heat stable amylase (A3306, Sigma) and sodium sulfite, and expressed without residual ash. Sodium content was determined using the standard operating methods for the flame spectrophotometry (AOAC method. 963.13, 1995). Gross energy content was determined using the values for heat of combustion by an adiabatic bomb calorimeter (Parr Instrument Co., 1970). The fatty acid compositions of SG seed and treatment diets were measured using gas chromatograph (GC-8A, Shimadzu Corp., Kyoto, Japan) as described by Kagan et al. (2011).

2.3. Growth performance

From day 1 to 56 of the trial, the lambs were housed individually in 30 uniform pens equipped with a feeder and free access to water. Lambs were fed diets for *ad libitum* intake. All lambs were individually fed twice daily at 0700 and 1700 h. Feed offered and refused was recorded daily, and daily samples were collected to determine dry matter intake (DMI). Body weight was individually recorded once weekly to calculate the average daily gain (ADG) and feed efficiency (FE, FE = DMI/ADG).