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Fortification of dried distillers grains plus solubles with grape seed meal in the diet modulates methane mitigation and rumen microbiota in Rusitec

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ABSTRACT

The role of dried distillers grains plus solubles (DDGS) and associative effects of different levels of grape seed meal (GSM) fortified in DDGS, used as both protein and energy sources in the diet, on ruminal fermentation and microbiota were investigated using rumen-simulation technique. All diets consisted of hay and concentrate mixture with a ratio of 48:52 [dry matter (DM) basis], but were different in the concentrate composition. The control diet contained soybean meal (13.5% of diet DM) and barley grain (37%), whereas DDGS treatments, unfortified DDGS (19.5% of diet DM), or DDGS fortified with GSM, either at 1, 5, 10, or 20% were used entirely in place of soybean meal and part of barley grain at a 19.5 to 25% inclusion level. All diets had similar DM, organic matter, and crude protein contents, but consisted of increasing neutral detergent fiber and decreasing nonfiber carbohydrates levels with DDGS-GSM inclusion. Compared with the soy-based control diet, the unfortified DDGS treatment elevated ammonia concentration (19.1%) of rumen fluid associated with greater crude protein degradation (~19.5%). Methane formation decreased with increasing GSM fortification levels (≥5%) in DDGS by which the methane concentration significantly decreased by 18.9 to 23.4 and 12.8 to 17.6% compared with control and unfortified DDGS, respectively. Compared with control, unfortified DDGS decreased butyrate proportion, and GSM fortification in the diet further decreased this variable. The proportions of genus *Prevotella* and *Clostridium* cluster XIVa were enhanced by the presence of DDGS without any associative effect of GSM fortification. The abundance of methanogenic archaea was similar, but their composition differed among treatments; whereas *Methanosphaera* spp. remained unchanged, proportion of *Methanobrevibacter* spp. decreased in DDGS-based diets, being the lowest with 20% GSM inclusion. The abundance of *Ruminococcus flavefaciens*, anaerobic fungi, and protozoa were decreased by the GSM inclusion. As revealed by principal component analysis, these variables were the microorganisms associated with the methane formation. Grape seed meal fortification level in the diet decreased DM and organic matter degradation, but this effect was more related to a depression of nonfiber carbohydrates degradation. It can be concluded that DDGS fortified with GSM can favorably modulate ruminal fermentation.
Key words: dried distillers grains plus solubles, grape seed meal fortification, ruminal fermentation, ruminal microbiota, methane mitigation

INTRODUCTION

Incorporation of industrial by-products in animal diets is an economically and environmentally viable practice for livestock production, especially for ruminants that can take advantages of fiber-rich and low-quality feedstuffs. Dried distillers grains plus solubles (**DDGS**) are a by-product of ethanol production by yeast fermentation of grain starch. The fermentation process removes starch of grains and, in turn, enriches the content of other nutrients, making DDGS an excellent source of protein, energy, and nonforage fiber in cattle diets (Abdelqader et al., 2009). However, DDGS can alter the characteristics of the diet because DDGS is low in physically effective fiber (small particle size of high specific density) and the NDF of DDGS is highly digestible (Zhang et al., 2010a). Such dietary characteristics may negatively alter ruminal fermentation and rumen health (Li et al., 2012b; Zebeli et al., 2012). Supporting this notion, some studies report that DDGS resulted in undesired changes in ruminal fermentation characteristics (Loy et al., 2007; Li et al., 2012b). In dairy cows, DDGS replacing barley silage decreased

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ruminal pH, rumination, chewing activity, and milk fat percentage (Penner et al., 2009; Zhang et al., 2010a,b). Environmentally, DDGS can lead to increased ruminal ammonia concentration and total N excretion in cattle (Hünerberg et al., 2013).

Due to the antimicrobial properties of plant secondary compounds, previous research attempted to use plant secondary compounds to lower ruminal protein degradation as well as to decrease greenhouse gas emissions in cattle fed DDGS; some success in those studies has been reported (Hao et al., 2011; Li et al., 2012b). In ruminant nutrition, tannins are of interest when supplied in small amounts. For example, in addition to their effects on microbes and mitigating methane emissions (Jayanegara et al., 2010), tannins are also able to build indigestible complexes with certain protein and carbohydrate fractions under the rumen condition, thus decreasing the rate of ruminal degradation and subsequently increasing the flow of these nutrients for the lower gut digestion (Patra and Saxena, 2011). Grape seed meal (**GSM**), the residue from grape seeds after oil extraction, is a rich source of tannin phenols (Shi et al., 2003), making GSM an interesting by-product to be used in diets containing high energy and protein ingredients such as DDGS. We hypothesized that incorporating GSM in the DDGS component of the diet may favorably modulate ruminal fermentation in a way that GSM phenols affect the activity of ruminal microbiota by interacting with various protein and carbohydrate fractions of the DDGS or other components of the diet. Hence, this could shift substrate availability and potentially shape substrate preferences of rumen microbiota. In the present study, we evaluated effects of DDGS with or without graded levels of GSM, used entirely in place of soybean meal and part of barley grain, on the abundance of rumen microbiota and ruminal fermentation characteristics in vitro using the rumen-simulation technique (**Rusitec**).

MATERIALS AND METHODS

Treatments and Experimental Diets

Six dietary treatments were used; all containing 48% second-cut meadow hay and 52% concentrate mixture (DM basis; Table 1). The concentrate mixture differed in its composition among diets. The control diet contained 37% barley grain, 13.5% soybean meal, and 1.5% mineral-vitamin mix in diet DM. For the second diet, the entire portion of soybean meal and part of barley were replaced with DDGS (ActiProt, AGRANA Stärke GmbH, Tulln, Austria) at a level of 19.5% in total diet DM. For the other 4 diets the same substitution manner was performed but DDGS fortified with GSM, either at 1, 5, 10, or 20% GSM, were used instead of pure DDGS, respectively. The GSM-fortified DDGS products were provided by E. Taufratzhofer (Vinolis Traubenkernöl, Gumpoldskirchen, Austria). To keep all 6 diets at similar OM (~92%) and CP (~16.6%) levels, amount of the 4 DDGS-fortified products was 19.5, 20.5, 22.0, and 25% in diet DM, respectively. Before use, hay was chopped to about 1-cm in length, whereas the concentrate ingredients were ground through a 4-mm sieve. Grape seed meals used were a mixture of red and white grapes (40 and 60% wt/wt, respectively). As analyzed, both GSM contained total phenols 38 to 39 mg/g of DM, which was in a similar range as a previous report (Shi et al., 2003). More details regarding chemical composition of the diet ingredients are illustrated in Table 1. The analyzed chemical composition of the individual ingredients was used to formulate the diets.

Experimental Design, Rusitec Procedure, and Sample Collection

Two Rusitec systems, each consisting of 6 incubation units, thus the experimental units, with an effective volume of 800 mL, were used in this experiment. The experiment was a completely randomized design, whereby 6 diets were tested in 3 experimental runs with 2 replicates in each run, resulting in 6 independent measurements per each treatment. The procedure of Rusitec is explained in details in a previous study (Klevenhusen et al., 2014). In brief, each experimental run lasted 10 d, whereby the first 5 d were used for equilibration of the system and the last 5 d were used for samplings. Equilibration of the system was monitored by the redox potential. On the first day of each run, ruminal fluid and solid digesta were obtained from 2 out of the 3 nonlactating rumen-cannulated Brown Swiss cows kept at the Clinic for Ruminants at the University of Veterinary Medicine (Vienna, Austria) at about 3 h after morning feeding. Only 1 donor cow was available for the second experimental run. The donor cows had been fed with hay ad libitum and a daily allowance of 0.5 kg of commercial concentrates (KuhKorn PLUS Energie, Garant-Tiernahrung GmbH, Pölchlarn, Austria). The cows were kept according to Austrian guidelines for animal welfare. Before use, ruminal fluid of the cows was mixed and filtered through 4 layers of medical gauze (1 mm pore size). Each fermenter was inoculated with 600 mL of strained ruminal fluid and 100 mL of artificial saliva. Subsequently, a pair of nylon bags (120 × 65 mm, 150 μm pore size, Fa. Linker Industrie-Technik GmbH, Kassel, Germany) was added to each fermenter, one filled with the experimental diet and another bag filled with solid ruminal digesta. On the second day, for each fermenter the digesta bag was