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Rumen fermentation pattern, lipid metabolism and the microbial community of sheep fed a high-concentrate diet supplemented with a mix of medicinal plants

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ABSTRACT

The aim of this study was to determine the effects of a medicinal plant mixture (MP), sunflower oil (SO) and a combination of the medicinal plant mixture and sunflower oil (MPSO) on fermentation end products, the fatty acid composition of the rumen fluid, the ruminal microbial population as well as antioxidant status in the blood of sheep fed a high-concentrate diet. Four rumen-fistulated rams were randomly assigned according to a 4 × 4 Latin square design and fed a basal diet consisting of 720 g DM/day meadow hay and 540 g DM/day barley grain. The basal diet was supplemented with no additive (control), a medicinal plant mixture (MP, 10% replacement of meadow hay; 72 g DM/day), sunflower oil (SO, 36 g/day) and the combination (MPSO). Before the in vivo experiment, 24 h ruminal incubations in vitro were performed (meadow hay/barley grain; 400/600, w/w; MP; 10% replacement of meadow hay; SO; 3.5% DM; MPSO). The in vitro experiment with MP and MPSO increased the in vitro dry matter digestibility ($P < 0.001$) and decreased methane production ($P = 0.021$), while the MPSO treatment increased the concentration of polyunsaturated fatty acids (PUFA; $P < 0.001$). In sheep, the rumen characteristics were not affected by the treatments. No treatment effect on the rumen eubacterial population was detected by the 16S-polymerase chain reaction-denaturizing gradient gel electrophoresis method. The beneficial effects of MP and MPSO on PUFA concentration observed in vitro were not fully confirmed in vivo. However, these results could point to the promising effects of using a medicinal plant mixture in high-concentrate diets of ruminants without adverse effects on the fermentation characteristics and microbial ecosystem in sheep rumen.

1. Introduction

The maintaining of high productivity in ruminants and increasing the quality of their products is associated with the use of high-concentrate diets that have negative effects on the rumen ecosystem. Recently, natural products, such as probiotics, prebiotics, organic acids or secondary plant compounds, have been tested to mitigate these negative effects and to enhance the efficiency of feed utilization. However, little information is available on the potential of traditional medicinal herbs in the form of whole plants to modify rumen fermentation characteristics (García-Gonzáles et al., 2008). It is well known that animals grazing on grassland sometimes seek out plants with certain medicinal, antioxidant or immunological effects (Hosoda et al., 2006; Fraisse et al., 2007). However, intensification of animal production prevents such animal self-medication; therefore, feeding plant materials to ruminants represents a new option in ruminant nutrition. The major active compounds of medicinal plants (essential oils, saponins, flavonoids, tannins and polyphenols)

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have been tested as extracts with effects on the microbial population (Patra and Saxena, 2009), methanogenesis (Bodas et al., 2012) or lipolysis in the rumen (Khiaosa-Ard et al., 2009; Jayanegara et al., 2011a). Kälber et al. (2011) reported that the α-linolenic acid concentration in milk fat could be enhanced by feeding flowering dicots as a source of phenolic compounds. However, the addition of dietary fats together with a medicinal plant mixture could influence the microbial ecosystem in the rumen toward the enhancing of essential polyunsaturated fatty acids (PUFA) in ruminant products and could also mitigate the negative effects of high-concentrate diets. Therefore, the objective of this study was to investigate the effect of a medicinal plant mixture (as a component of a diet ration) and sunflower oil (as a source of PUFA) on rumen fermentation pattern, lipid metabolism and the microbial population of sheep fed high-concentrate diets.

2. Materials and methods

Before the in vivo experiment, the in vitro ruminal incubations were performed in order to assess the effect of the mixture of medicinal plants with sunflower oil on rumen fermentation pattern and lipid metabolism in high-concentrate diets. On the basis of the results obtained in vitro, an in vivo experiment was conducted on sheep to verify the in vitro results.

2.1. Animals, diets and experimental design

Four rumen fistulated rams (Lacaune versus Suffolk; 1.5 years of age; 45.0 ± 2.5 kg weight) were used in a 4 × 4 Latin square design. The experimental protocol was approved by the Ethical Committee of the Institute of Animal Physiology SAS and State Veterinary and Food Office (Ro-2061/13-221). The animals were housed separately and randomly assigned to one of the four experimental diets. The basal diet consisted of 720 g DM/day meadow hay and 540 g DM/day barley grain. The chemical composition of the meadow hay and the barley grain, respectively, was (mean values, g/kg DM): DM: 916 (868); N: 17 (8.5); CP: 103 (53); ADF–ADF: 365 (84); NDF–NDF: 498 (aNDF 331); ash: 70 (34); fat: 21 (8.7). The basal diet was supplemented with no additive (control), the medicinal plant mixture (MP, 10% replacement of meadow hay; 72 g DM/day), sunflower oil (SO, 44 g/day) or with a combination of the medicinal plant mixture and sunflower oil (MPSO). The chemical composition of the MP mixture was (mean values, g/kg DM) DM: 896; N: 11; CP: 67; ADF–ADF: 376; NDF–NDF: 409; ash: 90.7; fat: 21; total phenols: 25.4. The MP mixture contained the following seven plants from commercial sources: roots of dandelion (*Taraxacum officinale* L.) and calamus (*Acorus calamus* L.), flowers of marigold (*Calendula officinalis* L.) and whole overground herbs of St. John's-wort (*Hypericum perforatum* L.), yarrow (*Achillea millefolium* L.), nettle (*Urtica dioica* L.) and chicory (*Cichorium intybus* L.). The dry medicinal plant materials were mixed in equal proportions and the mixture of medicinal plants (MPM) was standard throughout the in vitro experiment. The selection of medicinal plants was based on information available about their beneficial effect in traditional human medicine: their carminative and anti-inflammatory effects (Kresánek and Kresánek, 2008). Sunflower oil, a material rich in linoleic acid (533 g/kg of fatty acids, FA), was obtained from a commercial source. To obtain a uniform rumen eukaryotic and prokaryotic population of experimental animals, a one-time infusion of rumen fluid from all four rams was performed, with subsequent inoculation via ruminal fistula during the adaptation period (i.e., prior to the experiments). The rams were weighed at the beginning and at the end of the experiment. To allow the sheep to adapt to the oil-supplemented diets, all sheep were fed a diet supplemented with a mixture made up of sunflower oil (18 g DM/day) plus MP (20 g DM/day) for 4 weeks prior to the first experimental period. The supplemental oil and MP were homogenously mixed with barley grain. The experiment was conducted for four periods. Each of the four periods consisted of a 7 day washout phase, a 12 day adaptation phase, and 2 day collection phase. The washout phase was used to reduce carry over effect from previous experimental diets. During this phase the rams had access to basal diet. Samples of rumen fluid and blood were collected at 07:00 h on the 13th and 14th day of each experimental period. Blood samples were collected into heparinized tubes and immediately centrifuged at 1200 × *g* for 15 min at 4 °C. Ruminal fluids were separated from the feed particles by filtering through four layers of gauze and centrifuging at 15,000 × *g* for 15 min at 4 °C. Samples of plasma and supernatant of rumen fluid were stored at −80 °C until analysis of total antioxidant capacity as the ferric-reducing antioxidant power (FRAP) value. A pellet of total rumen microorganisms was stored at −80 °C and used for total DNA analysis. The mixed rumen contents were fixed with an 8% formaldehyde solution in equal volumes for the estimation of the biomass of ciliates and total bacteria.

2.2. Twenty-four-hour in vitro batch culture experiment

The ruminal inoculum used in this in vitro experiment was obtained from 4 rumen cannulated rams (Lacaune versus Suffolk; 1.5 years of age; 45.0 ± 2.5 kg weight) before the morning feeding. The rumen fluid was squeezed through four layers of gauze, purged with CO_2 and mixed at a 1:1 ratio with McDougall's buffer (McDougall, 1948). The inoculum was dispensed in volumes of 35 ml into fermentation bottles (120 ml) containing 0.25 g of substrate. The meadow hay and barley grain (400:600, w/w) were used as the components of a high-concentrate substrate for in vitro experiment. Four diet substrates were used as follows: (1) meadow hay (0.1 g) and barley grain (0.15 g) (control), (2) meadow hay (0.09 g) with medicinal plant mixture (0.01 g) and barley grain (0.15 g) (MP), (3) meadow hay (0.1 g) and barley grain (0.15 g) with 3.5% of DM sunflower oil (SO), and (4) meadow hay (0.09 g) with medicinal plant mixture (0.01 g), barley grain (0.15 g) and 3.5% of DM sunflower oil (MPSO). Meadow hay in MP and MPSO was substituted for 10% of medicinal plant mixture. Incubations were performed for 24 h at 39 ± 0.5 °C. For each treatment, nine replicates (9 incubation bottles) were prepared and three replicate bottles were also used for the blank (no substrate). The experiment was repeated three times within three consecutive days ($n = 3 \times 9$).

2.3. Measurements and chemical analysis

Samples of diet substrates were analyzed in triplicate for DM (No. 967.03), ash (No. 942.05), nitrogen (No. 978.02), fat (No. 9836.23) and crude protein (No. 990.03) using standard methods (AOAC, 1990). The amounts of acid-detergent (ADF) and neutral-detergent fiber (NDF) were determined according to Van Soest et al. (1991) using a Fibertec System 2021 FiberCap (Foss Analytical AB, Höganäs, Sweden). In forages, NDF–NDF was assayed without a heat-stable amylase and expressed inclusive of residual ash. In concentrates, aNDF–NDF was assayed with a heat-stable amylase and expressed inclusive of residual ash. ADF–ADF was expressed inclusive of residual ash. The amount of total phenols was determined according to Makkar et al. (1993), using the Folin–Ciocalteu method, and the result was expressed as the tannic acid equivalent. Qualitative chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants were carried out in extracts using standard procedures (Yadav and Agarwala, 2011). The in vitro dry matter digestibility (IVDMD) was estimated from the difference in the substrate weight before and after incubation (Mellenberger et al., 1970). The gas production during in vitro incubation was measured using the pressure transducer technique (Váradyová et al., 2005). The pH of the rumen fluid was measured using a pH meter (InoLab pH Level 1, Weilheim, Germany). The concentrations of short-chain fatty acids (SCFA) and methane were determined according to Cottyn and Boucque (1968), using a PerkinElmer Clarus 500 gas chromatograph (PerkinElmer, Inc., Shelton, CT, USA), with crotonic acid as the internal standard. The concentration of ammonia nitrogen was determined in the supernatant fluid by the phenol–hypochlorite method (Broderick and Kang, 1980). Fatty acids (FA) were extracted and analyzed from lyophilized samples according to Váradyová et al. (2008). Fatty acid methyl esters were determined by gas chromatography (PerkinElmer, Inc., Shelton, CT, USA) using a commercial C4–C24 FA methyl ester mixture (Supelco, Bellefonte, PA, USA). Ciliated protozoa were counted microscopically according to Williams and Coleman (1992). The biomass of total bacteria was estimated by direct bacterial count through image analysis of pictures taken under bright field illumination of smears of formaldehyde-fixed samples stained with methylene blue. Two smears of known dimensions per sample of known volume were prepared according to the Breed method (Horáková, 1988). Twenty randomly selected pictures per smear were taken and the images processed and analyzed using ImageJ software (ImageJ software documentation).

2.4. Qualitative analysis of eubacterial population by PCR-DGGE

Total DNA from the frozen samples was isolated using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The quality of the isolated DNA was assessed by 0.8% agarose gel electrophoresis. Isolated DNA was subsequently used