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Both monensin and plant extract alter ruminal fermentation in sheep but only monensin affects the expression of genes involved in acid-base transport of the ruminal epithelium

Hamidreza Mirzaei-Alamouti[a], Saeedeh Moradi[a], Zahra Shahalizadeh[a], Maryam Razavian[a], Hamid Amanlou[a], Taher Harkinezhad[a], Iraj Jafari-Anarkooli[b], Carolin Deiner[c], Jörg R. Aschenbach[c,*]

[a] Department of Animal Science, University of Zanjan, Zanjan, 45371-38791, Iran
[b] Department of Anatomy, Zanjan University of Medical Sciences, Zanjan, 45139-156111, Iran
[c] Institute of Veterinary Physiology, Free University of Berlin, Oertzenweg 19b, 14163 Berlin, Germany

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ABSTRACT

The effects of a plant extract (broadleaf plantain, peppermint) or monensin on dry matter intake (DMI), growth performance, ruminal fermentation products, as well as morphology and the expression of genes involved in acid-base regulation of the ruminal epithelium were determined. Thirty two male Afshari finishing lambs on a high-concentrate diet were subjected to four consecutive 14-d periods on one of four treatments with supplementation of (1) no additive (control, CON), (2) 2 g blend of plant extract/(d × lamb) (PE), or (3) 30 mg monensin/(d × lamb) (MON) over all four treatment periods. The fourth group received periodical inclusion of 30 mg monensin/(d × lamb) in periods 1 and 3 with no additive in periods 2 and 4 (MON-p). Despite no significant overall effects on DMI, ruminal pH, average daily gain (ADG), ADG:DMI ratio and plasma albumin, the interactions of treatment × period were significant for these measures ($P < 0.01$). Compared with CON, periods with decreased DMI occurred selectively in groups MON (period 1 and 2) and MON-p (period 2 and 3), a period with decreased ruminal pH occurred in PE (period 1), and periods with decreased plasma albumin occurred in groups MON (period 1) and MON-p (period 2) (all $P < 0.05$ or lower). In period 2, MON sheep had higher ADG than PE and MON-p ($P < 0.05$) and a higher ADG:DMI ratio than all other groups ($P < 0.05$). While total concentrations of ruminal short chain fatty acids were unchanged, increased propionate proportions and decreased acetate proportions were observed in PE ($P < 0.05$) and, as a trend, in MON, resulting in decreased acetate:propionate ratios in PE and MON compared to CON ($P < 0.05$). The proportion of butyrate was decreased by MON only ($P < 0.05$). Compared with CON, the relative mRNA abundance of Na^+/H^+ exchanger isoforms NHE1 and NHE3 were lower and that of monocarboxylate transporter isoform MCT1 was greater for MON ($P < 0.05$). In MON-p animals, the mRNA abundance of NHE1 was lower and that of MCT4 was greater compared to CON ($P < 0.05$). Morphometry results did not differ between treatments. It is concluded that

Abbreviations: CON, no additive control; PE, 2 g blend of plant extract/(d × lamb); MON, 30 mg monensin/(d × lamb) for 56 days; MON-p, 30 mg monensin/(d × lamb) for the first 14 days and for days 29–42; ADG, average daily gain; NHE, sodium/proton exchanger; MCT, monocarboxylate transporter; SARA, subacute ruminal acidosis; SCFA, short chain fatty acids; qPCR, quantitative real-time polymerase chain reaction; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; $SCFA^-$, SCFA anion; DRA, downregulated-in-adenoma (anion exchanger); PAT, putative anion transporter; AE, anion exchanger.

* Corresponding author.
E-mail address: joerg.aschenbach@fu-berlin.de (J.R. Aschenbach).

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monensin and the used plant extract were equally effective to increase ruminal propionate fermentation. Moreover, the study elucidated for the first time that the ruminal epithelium itself is affected by the ionophore properties of monensin and needs to adapt epithelial acid-base transporter expression.

1. Introduction

Ionophore antibiotics have been added to high-grain diets as feed additives for approximately four decades based on their potential to alter fermentation pattern, to promote growth, and to decrease the occurrence of subacute ruminal acidosis (SARA; Nagaraja et al., 1982; Bergen and Bates, 1984; Duffield et al., 2012a). In many countries, however, the use of antimicrobial agents as feed additives has been banned based on emerging antimicrobial multi-resistance in both animals and humans, posing a serious risk to the public health. Albeit monensin does not appear to specifically contribute to multi-resistance (Bonnet et al., 2009; Bush, 2012), there is yet a growing interest to replace its use by alternatives like essential oils or other bioactive compounds contained in or purified from plant extracts. Moreover, an often overlooked side effect of monensin is that it acts not only on bacteria and protozoa but also facilitates permeation of Na^+ and K^+ ions across cell membranes of birds and mammals (Novilla, 1992; Bush, 2012). From this perspective, it is surprising that no one has considered potential effects of monensin on the ruminal epithelium which is directly exposed to high concentrations of the ionophore during feed supplementation.

The most prominent effect of monensin is stimulation of ruminal propionate fermentation (Ellis et al., 2012; Yang et al., 2014), which likely also underlies its growth-promoting effects. A similar effect on ruminal propionate fermentation has been described to essential oils (Khiaosa-ard and Zebeli, 2013). Some purified essential oil preparations or plant extracts containing essential oil compounds are already commercially available and could thus be promising alternatives to monensin application in the growth promotion of sheep.

Proceeding from the above considerations, the present study had two main objectives. The first objective was to assess the growth-promoting and fermentation effects of a commercial plant extract as a replacement for monensin in sheep fed on an easily fermentable diet. The second objective was to assess the influence of plant extract and monensin on gene expression in the ruminal epithelium. We hypothesized that the ionophore properties of monensin would interfere with the regulation of intracellular pH and, consequently, affect the expression of key genes involved in intracellular pH regulation and/or the transport of short chain fatty acids (SCFA). Such expected impact on acid-base homestasis of the ruminal epithelium could further trigger morphological alterations of the ruminal epithelium, Therefore, a further objective was to characterize how the dietary feed additives affect rumen histology in lambs.

2. Animals, materials and methods

2.1. Animals and diets

Thirty two male Afshari lambs, with an initial body weight (BW) of 41.4 ± 2.0 kg and ~6 mo of age were randomly assigned to one of the four dietary treatments according to a completely randomized design. All procedures involving animal care and management were approved by the University of Zanjan Animal Care Committee (ID 1353). Lambs were dewormed with oral suspension of albendazol (5 mg/kg BW) using drencher before the study and confined to individual pens (171 × 83 cm) with concrete floors equipped with water and feed troughs.

The experiment lasted for 77 d, including a 21-day adaptation to the experimental diet and four consecutive 14-d treatment periods. In the adaptation period, Afshari lambs were gradually transferred to a dietary forage-to-concentrate ratio of 20:80. Such high-concentrate diets are commonly used in this dual purpose (meat and milk) large-size breed to achieve high growth rates in very intensive systems. The preceding diet was gradually replaced by an increasing fraction (plus 50 g/kg per day) of the high-concentrate diet. Ingredients and nutrient composition of this diet are shown in Table 1 (160 g/kg crude protein, CP; 11.5 MJ/kg metabolizable energy, ME). The diet was formulated using the software Cornell Net Carbohydrate and Protein System for Sheep (CNCPS_S, version 1.0.21; Cornell University, Ithaka, NY). Experimental treatments consisted of the high-concentrate diet supplemented with (1) no additive (control, CON), (2) 2 g commercial blend of plant extract/(d × lamb) (PE), (3) or 30 mg monensin/(d × lamb) (MON) during all four treatment periods. (4) A fourth group received periodical inclusion of 30 mg monensin/(d × lamb) in period 1 (days 1 through 14) and period 3 (days 29 through 42) but no feed additive in periods 2 and 4 (MON-p). The commercial blend of plant extract contained 400 mg/g plantain extract (*Plantago major*), 50 mg/g peppermint extract (*Mentha piperita*) enriched with essences modified from original ingredients, 50 mg/g tragacanth gum, and 500 mg/g sugar and pectin in prilled form; the granules are coated by lipids (commercial name, PLANTAGEL, Dineh Iran Pvt., Ltd.). Plantago major extract contained aucubin (200 mg/g), plantaginin (250 mg/g), succinic acid (120 mg/g), adenine (80 mg/g), choline (70 mg/g), and others (280 mg/g), and mentha piperita extract contained menthol (420 mg/g), menthone (150 mg/g), isomenthone (77 mg/g), pulegone (43 mg/g), menthofuran (85 mg/g), 1,8-cineole (84 mg/g), limonene (20 mg/g), b-pinene (15 mg/g), neomenthol (24 mg/g), carvacrol (12 mg/g), and others (70 mg/g). The respective herbs were