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Small Ruminant Research 61 (2006) 63–71
Small Ruminant Research
www.elsevier.com/locate/smallrumres

Influence of natural magnesium sources on the in vitro fermentation and protozoan population in the rumen fluid collected from sheep

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Received 25 June 2004; received in revised form 8 December 2004; accepted 4 January 2005
Available online 3 March 2005

Abstract

The objective of this experiment was to determine the effect of two types of caustic calcinated magnesite (caustic magnesite (CM) and Agromag (AG)) upon the end products of in vitro fermentation (total gas, methane, total and individual fatty acids, and VFA) and protozoan population in the rumen fluid collected from sheep. Both magnesium additives (CM and AG) as natural products in the dose of 0.01 g were added to the fermentation bottles containing rumen inoculum from sheep and different substrates. Meadow hay (MH), wheat straw (WS), amorphous cellulose (AC) and barley grain (BG) were used as substrates and incubated with the buffered rumen fluid using an in vitro gas measuring technique during 72 h of incubation. The rumen protozoa, *Entodinium* spp., *Trichostomatids* and large *Entodiniomorphids* and the total protozoan concentration were counted after 24 h of incubation. The methane production was significantly decreased with CM or AG, respectively, by 58 or 62% (MH), by 65% (WS), by 52% (AC) and by 58% (BG). The total VFA concentration was significantly lower compared to control for CM plus MH, WS, AC, BG and AG plus WS. The total VFA concentration was significantly higher compared to control for AG plus AC. The effect of the both additives on ciliate population was not uniform and depended on the substrates used and protozoan type. Ciliate population was significantly increased in *Entodinium* spp. (AG plus BG) and *Diploplastron affinae* (CM or AG plus BG) compared to control. Tested additives significantly decreased population of *Entodinium* spp. (AG plus MH or AC), *Dasytricha ruminantium* (AG plus AC), *Ophryoscolex c. tricoronatus, Eremoplastron dilobum* and *Polyplastron multivesiculatum* (CM or AG plus BG). It can be concluded that both natural magnesium sources influenced rumen fermentation patterns and protozoan population in vitro depending on the type of the substrate used; therefore, the relative efficacy of individual tested additive cannot be determined from these experiments. In vivo experiments are required in future.

Keywords: Magnesium; Caustic magnesite; Agromag; In vitro; Fermentation; Protozoa; Sheep

1. Introduction

Current world trend in the production of ecologically clean foods has increased the demand for natural

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doi:10.1016/j.smallrumres.2005.01.004

mineral additives for animal nutrition with a positive influence on the human organisms. Most naturally occurring mineral deficiencies in herbivores are associated with specific regions and are directly related to the soil characteristics (McDowell, 1996). Grass in some areas is rich in potassium and inhibits magnesium absorption in cows. Absolute amount of magnesium absorption (g per day) depends on dietary magnesium intake (Jittakhot et al., 2004). To prevent hypomagnesaemia, it is common to supplement commercial concentrates with MgO. Some natural mineral additives, e.g. dolomite, silicate minerals (Váradyová et al., 2003a,b) and caustic calcinated magnesite (CM) are used in the agricultural practice. Caustic calcinated magnesite serves as natural sources of magnesium in the form of MgO. However, no investigation of the impact of caustic calcinated magnesite on the rumen fermentation pattern and rumen protozoa has been carried out. Finally, the production of volatile fatty acids and gases by rumen microbes is generally influenced by macro-minerals (Durand and Komisarczuk, 1988; Komisarczuk-Bony and Durand, 1991). The aim of this study was to examine the influence of two types of caustic calcinated magnesite (caustic magnesite CCM 86 and Agromag 78, Slovak Magnesite Works, Jelšava Inc.) sources on the rumen fermentation patterns with different substrates (meadow hay, wheat straw, cellulose amorphous and barley) and protozoan population in sheep in vitro.

2. Materials and methods

2.1. Inocula and method of incubation

To obtain high rumen ciliate population (Ankrah et al., 1990; Leng et al., 1986; Michałowski, 1975, 1977; Michałowski and Muszyński, 1978) and high fermentation activity (Hillman et al., 1995; Itabashi and Kandatsu, 1975) rumen fluid was collected 3 h after the morning feeding from three rumen fistulated Slovak Merino sheep. Sheep fed meadow hay ad libitum with free access to water 1 week before the collection of rumen fluid. The rumen fluid was transferred to the laboratory into two prewarmed thermos flasks, preheated to $39 \pm 0.5\,^{\circ}C$, squeezed through four layers of cheesecloth and purged with CO_2. The rumen fluid was mixed (1:2) with McDougall's buffer containing (g/l): $NaHCO_3$ 9.24, $Na_2HPO_4{\cdot}12H_2O$ 7.12, NaCl 0.47, $MgCl_2$ 0.47, KCl 0.45 and $CaCl_2$ 0.055 (McDougall, 1948); under continuous flushing with CO_2. After mixing, 35 ml of inoculum was pumped by an automatic pump into the preheated fermentation bottles (120 ml serum bottles) containing one kind of substrate. The incubations were performed in the incubator for 72 h at $39 \pm 0.5\,^{\circ}C$. The concentration of ciliate protozoa was counted (after 24 h) microscopically according to the procedure described by Coleman (1978). Protozoa were identified according to Dogiel (1927) and Ogimoto and Imai (1981).

2.2. Substrates and additives

The following four substrates (0.25 g/bottle) were used: meadow hay (MH), wheat straw (WS), amorphous cellulose (AC) and barley grain (BG). Meadow hay, wheat straw and barley grain were ground, sieved (particle size of 0.15–0.4 mm) bulked and stored in sealed plastic containers until required. The substrates were of intermediate quality. Content of K and Mg was as follow (g/kg): MH 15.6 and 22.5; WS 5.5 and 1.1; BG 5.0 and 1.1. Two magnesium additives caustic magnesite (CM) and Agromag (AG) (both from the Slovak Magnesite Works, Jelšava Inc.) in the amounts of 0.01 g were added into the culture bottles containing substrates. Chemical composition of the tested caustic calcinated magnesite is presented in Table 1. Substrates were incubated in eight replications. Eight replications were used for all the experimental groups (rumen inoculum plus substrate plus additive). Eight replicate bottles were used as controls (rumen inoculum plus substrate, no additive). 'Blank' fermentations (rumen

Table 1
Chemical composition of the tested caustic calcinated magnesite

	MgO (%)	CaO (%)	Fe_2O_3 (%)	SiO_2 (%)	Al_2O_3 (%)
Caustic magnesite CCM 86 (0–0.125 mm)	86.0	2.5	7.5	0.5	0.2
Agromag 78 (0–0.5 mm)	78.0	2.8	6.8	0.8	0.3