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Evaluation of different types of calcined magnesites as feed supplement in small ruminant

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ABSTRACT

Magnesium (Mg) deficiency in ruminants can be avoided by the inclusion of magnesium oxide (MgO) supplements in their diets. However, the behaviour of MgO supplements in the rumen depends on various factors such as ore-origin, calcination conditions, particle size distribution etc. Moreover, in most cases the solubility of MgO supplements (both *in vitro* and *in vivo*) is not related with their apparent bioavailability, so their quality should be evaluated primarily by using both *in vitro* and *in vivo* tests before using them in practice. Thus, the objectives of this study were: to determine: (a) the particle size distribution of four MgO supplements (Δ_1, Δ_2, Δ_3 and Δ_4) with different chemical composition and their both *in vitro* and *in vivo* solubility and (b) their apparent bioavailability. The solubility of MgO supplements was tested in an aqueous NH_4NO_3 solution (*in vitro*) and in sheep (*in vivo*). Five fistulated wether sheep of Karaguniko breed were used in a Latin-square (5 × 5) design to determine the *in vivo* solubility. The sheep were kept in metabolism cages and were given a basal diet (800 g alfalfa hay and 600 g concentrates). The same diet plus 2 g of each MgO supplement was given to each sheep respectively during a 17-days period in order to determine their apparent bioavailability. The results showed that the solubility was depended on both the amounts of fine particles and the reactivity of the MgO supplements ($P < 0.05$). A decrease in the solubility of the MgO supplements was found by increasing Fe_2O_3 and Al_2O_3 content. Despite the fact that for the most MgO supplements (Δ_2, Δ_3 and Δ_4) a close relationship was found between solubility (both *in vitro* and *in vivo*) with apparent bioavailability this did not occur in the case of Δ_1 MgO supplement. Moreover, the Δ_4 MgO supplement was evaluated as the best candidate for feed supplement in ruminant's diets, not only due to its better particle size distribution, but also to its significantly high solubility (both *in vitro* and *in vivo*) and bioavailability which was due to its high purity (> 90%) and reactivity (49 s) and lower content in Fe_2O_3 and Al_2O_3 compounds compared to the others. In conclusion, the properties of MgO supplements should be tested both *in vitro* and *in vivo* before their use in practice since they are affected by various factors.

1. Introduction

Magnesium (Mg) is an essential macro element for all mammals. It is one of the main structural compounds of bones (Castiglioni et al., 2013). About 70% of the total Mg content is stored in bones (ARC, 1980; Hays and Swenson, 1993a,b) indicating its important role to skeletal mineralization. Intracellular Mg is vital for numerous physiological functions. More specifically, Mg is fundamental for ATP, the main source of energy in the cells. Moreover, Mg is a co-factor for more than 300 enzyme systems (Lehninger, 1950) involved in lipid, protein and nucleic acid synthesis, cell growth as well as in the stabilization of mitochondrial membranes (Aikawa, 1981; Ryan, 1991; Shills, 1997). Additionally, Mg also acts as a signal transducer (Li et al., 2011) and/or as neuromuscular transmitter (Hays and Swenson, 1993a,b).

Both clinical and subclinical Mg deficiency causes problems in ruminants such as excitability, muscles twitching of the face and ears, uncoordinated walking gait, convulsion, paralysis, and death (Berger, 2003; Brozos et al., 2011). Ruminant's diets, and especially those of lactated ones, should be supplemented with Mg.

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However, in order to have Mg absorption across the rumen wall into the blood-stream, via two different mechanisms (Martens and Schweigel, 2001), the dietary Mg must firstly be dissolved or solubilised in the rumen fluid. The amount of Mg absorbed is associated with high Mg apparent availability and retention, although the capacity of absorption is modulated by the physiological status of the animal. The amount of Mg absorbed in excess of requirement is excreted with urine to maintain Mg balance. Renal handling of Mg is a filtration-reabsorption process in which a part of the filtered Mg is reabsorbed and the remainder is excreted in urine (Averill and Heaton, 1966; Quamme and Cole, 2004). When plasma Mg falls below 0.2 mmol/L in sheep virtually all of the filtered Mg is reabsorbed and urinary Mg excretion declines to zero (Rook and Balch, 1958; Storry and Rook, 1963).

Magnesium oxide (MgO), magnesium sulphate ($MgSO_4$) and magnesium chloride ($MgCl_2$) are the most common sources of supplemental Mg in animal's diets. Even though $MgSO_4$ and $MgCl_2$ have slightly higher bioavailability at around 58%, compared to the MgO, the last one is the most commonly used Mg source (NRC, 2001). This is due to the fact that $MgSO_4$ and $MgCl_2$ are also sources of anionic salts that can acidify the blood pH and lower the dietary cation/anion difference. On the other hand, MgO can have a dual purpose in dairy ruminants and beef rations, as both Mg source and a rumen alkalizing agent that helps managing rumen pH when needed (Erdman, 1988). MgO produced from Magnesite, is an $MgCO_3$-rich ore, which has to be heated (calcined) to oxidize Mg-carbonate into Mg-oxide. The bioavailability of MgO varies from 28 to 50%, depending on particle size (finer is more available), source (seawater source may be more available), calcination temperature (Schonewille et al., 1992) and geographical (ore) origin (Van Ravenswaay et al., 1989). A good quality MgO, in order to be used as animal feed supplement, should have bioavailability close to 50%. Thus, it is recommended to assess its quality before use in practice.

Taking into consideration the aforementioned, the objectives of this study were to determine: (a) the particle size distribution of four MgO supplements (Δ_1, Δ_2, Δ_3 and Δ_4) with different chemical composition and their both *in vitro* and in *vivo* solubility and (b) their apparent bioavailability.

2. Materials and methods

2.1. Place of research execution

The experiment was conducted at the Agricultural University of Athens, Greece. Housing and care of the animals, conforming to the guidelines of the Ethical Committee of the Faculty of Animal Science and Aquaculture of the Agricultural University of Athens (025/10112015).

The first part of the present study concerned the determination of particles size distribution of four different calcined magnesite (MgO) supplements (Δ_1, Δ_2, Δ_3 and Δ_4) used in animal feeding. Furthermore, the solubility of the MgO supplements was also determined both *in vitro* and in *vivo*. In the second part of the present study the apparent bioavailability of the four MgO supplements was measured *in vivo* on sheep. The chemical composition of the four MgO supplements (Table 1) as well as for their reactivity were done according to Vogel's (1989) and BSI (2005) methodologies.

2.2. Information on the animals

Five, 3 years old, wether ruminal fistulated sheep of Karaguniko breed, with average body weight 56.0 ± 2 kg, were used for the *in vivo* determination of solubility of the four MgO supplements as well as for its apparent bioavailability. The sheep were kept in metal metabolism cages. In accordance with our routine health management protocol, 5 months before the experiment, the sheep had been vaccinated with a Clostridium VAC (Provet, Greece) which contains antibodies for the *Clostridium septicum*, *Clostridium perfringens* type A, C and D, *Clostridium sordellii* and *Clostridium novyi* type B and *Clostridium chauvoei*, and treated with ivermectin for internal (Vetermec, Veterin, PROVET, Greece) and cypermethrin for external (Ectopor, Premier Shukuroglon, Hellas, A.E) parasites. Throughout the experimental period each sheep was fed individually with 800 g of whole alfalfa hay and 600 g concentrates (Table 2) according to its daily individual energy and crude protein requirements (NRC, 2007). The quantities of feed offered to the sheep were the same throughout the experimental period since the animals where mature and castrated with no fluctuations in its requirements. No refusals were left after each feeding. The alfalfa hay was provided separately from the concentrates while both of them were offered to the animals twice a day (in two equal parts at about 07.30 h and 15.00 h each day). Throughout the experiment the sheep had access to tap water *at libitum* and the quantity drunk by the animals were recorded. The diet composition and the Mg concentrations in both diet and water are presented in Table 2.

Table 1
Chemical composition (%) and reactivity of the four MgO types (Δ_1, Δ_2, Δ_3 and Δ_4).

	Δ_1	Δ_2	Δ_3	Δ_4	Analar
MgO (%)	84.90	84.40	92.40	94.00	95.00
SiO_2 (%)	6.66	9.45	1.57	0.66	–
CaO (%)	3.47	2.37	2.97	2.59	–
Fe_2O_3 (%)	2.65	0.88	0.34	0.07	–
Al_2O_3 (%)	1.28	0.10	0.05	0.04	–
L.O.I (%)	0.65	2.57	2.42	2.47	–
Reactivity (sec)	>600	71	91	49	–

Table 2
Concentrate composition and Mg concentration of the feeds and water.

Diet	(g/kg)
Barley	820
Gluten meal	150
Calcium carbonate	10
Dibasic Calcium phosphate	10
Sodium chloride	5
Premix of vitamins	5
Mg Concentration (ppm)	
Alfalfa hay	4115
Concentrate	2829
Water	5.5

2.3. Methodology used in the research

2.3.1. Particles size distribution

For the particles size distribution determination six parallel samples of 100 g each, from each of the four MgO supplements (Δ_1, Δ_2, Δ_3 and Δ_4) were used which were distributed, with an appropriate sieving machine (Endecotts LTD, London, England), in eight different classes (> 1 mm, 500 μm–1 mm, 425–500 μm, 300–425 μm, 250–300 μm, 150–250 μm, 125–150 μm and <125 μm diameter).

2.3.2. In vitro *solubility*

The Durrant and Draycott (1976) method was used to determine the solubility of the MgO supplements *in vitro*. According to this method 1 g of each MgO supplement was added to 200 ml of a NH_4NO_3 solution (pKa = 4.1). More specifically, ammonium nitrate p.a. (MERC, Germany) was diluted with distilled water to a final concentration 1 M. For each MgO supplement six parallel samples were used and tested. Each series of samples was shaken for