



Short communication

The effect of digestive activity of pig gastro-intestinal tract on zeolite-rich rocks: An *in vitro* study

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ABSTRACT

Two zeolite-rich rock samples extracted from Campanian Ignimbrite tuff quarries, southern Italy, usually used as binding and anticaking agents in animal feeding, were characterized and tested in terms of their resistance in stomach, small and large intestine of pig, using a three step *in vitro* digestibility trial. The mineralogical composition and major and trace-elements analysis were performed after each digestion step using XRPD and ICP-OES techniques. The main zeolite phases identified are phillipsite and chabazite. Results showed that *in vitro* physical-chemical conditions (pH 2–3 and T 39 °C) the release of Pb and other elements from the zeolite structure is extremely low and due to the limited breakdown of the zeolite framework and glass/amorphous matter hydrolysis, by the gastric liquids. This aspect also suggests that zeolites, after the total permanence in the animal body digestive tract, can further perform their important function of cation exchanger, with also relevant relapses on the growth and health of the animal species, as well as on the environment. Moreover, the contact of zeolite material with liquids of small and large intestine leads to negligible release of undesirable elements, which are far less than those envisaged by the European Regulations.

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1. Introduction

Zeolitic materials have been far back proposed as additives in animal diets as, further than their function as anticaking and binding agents, they also act as cation exchangers, thus immobilizing the ammonium ion during the digestive process [1–4]. The positive effect is a lower dispersion of ammonia in the environment, derived from the animal manure, and an improvement of weight gain in finishing pigs [5]. Such advantages are particularly evident in those areal contexts where a high concentration of livestock holdings occurs. The use of these materials in the European Union (EU) area as additives in animal feed is currently a well-established practice as witnessed by the plentiful literature of the last decades [6] and references therein]. In particular, further

deepening deserves the aspect concerning the formulation of animal feeding that, since indirectly entering the human food chain, requires following well defined European Commission Regulations. These Regulations assess the maximum allowable limits of noxious elements and compounds, in particular for those commonly defined as undesirable elements: Pb, Cd, As, and Hg. Natural zeolites admitted in the formulation of animal feeding are natrolite-phonolite (E566) [7] and clinoptilolite of volcanic origin (E567) [8]. Phillipsite-rich rocks (PHIL 75) were later equalized to E566. For all these materials, the European Commission Guidelines [9] establish the allowable limits (mg/kg) for As, Pb and Hg, further than other organic compounds.

Since the zeolite additives investigated in the present study are volcanic tuffs, they may carry variable amounts of elements such as Pb, As, Sb, Hg, Cd, Ni, Cr, elements that can be bioavailable as a function of the medium (gastric and, intestine liquids) with which they interact. UNI [10] accounts for a particularly aggressive

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methodology to evaluate the release of undesired metals; it was in fact demonstrated [6] that it is definitely not comparable with the real medium occurring in the digestive apparatus of the animals. However, systematic checks carried out in qualified laboratories operating in conformity to UNI [11] by Italiana Zeoliti S.r.l. on a PHIL 75 batch, a volcanic tuff linked to the activity of Campi Flegrei (southern Italy), demonstrated that the release of Pb, As, Cd and Hg is always lower than that prescribed by law although the treatment determines the total breakdown of zeolites.

Considering the large use of zeolites in the swine feeding and the potentially dangerous for the animal and human health, a multidisciplinary investigation is required. To achieve this goal, an *in vitro* trial was carried out to evaluate the modifications occurring in zeolite-rich rock in the swine gastro-intestinal tracts (stomach, small and large intestine).

2. Experimental

The present research took advantage of geological resources widely occurring on the Campania region (southern Italy) and commonly used in the animal feed production. Investigated samples belong to industrial batches produced by the Italiana Zeoliti (S.r.l.) which uses materials usually mined in quarries from southern Italy (Naples and Benevento provinces, Campania Region). Two bulk rock samples of some hundred tons, extracted from quarries of homogeneous zeolite tuff in 2012 and 2013, were characterized and tested as animal feeding. They both belong to the yellow facies of the Campanian Ignimbrite formation [12], extracted from Comiziano, Naples (APO) (Apostolico & Tanagro ITZ company) and Dugenta, Benevento (DUG) (E.T.E.P. company).

The mineralogy of all samples was evaluated by X-ray powder diffraction (XRPD) using a PANalytical X'Pert Pro modular diffractometer (CuK α radiation, 40 kV, 40 mA, 4–50° 2 θ scanning interval, 0.017° equivalent step size, 60 s per step equivalent counting time, RTMS X'Celerator detector). Powders with grain size <10 μ m were obtained using a McCrone micronising mill (agate cylinders and wet grinding time 15 min). An α -Al $_2$ O $_3$ internal standard (1 μ m, Buehler Micropolish) was added to each sample in an amount of 20 wt. %. Quantitative mineralogical analyses were performed using combined Rietveld [13] and RIR methods [14,15] by means of TOPAS 4.2 software (BRUKER AXS). Atomic starting coordinates for identified crystalline phases were taken from literature data [16,17].

A three-steps *in vitro* digestibility trial was made to simulate the passage of the zeolitic samples throughout the whole digestive tract of the pigs.

First step: gastric digestion. Fifty-four replicates (twenty-seven APO and twenty-seven DUG) of about 1 g were weighed to an accuracy of ± 0.1 mg into 100 mL glass bottles. The bottles were filled with 75 mL of 37% HCl in ultrapure water to reach pH values ranging between 1 and 2; to the mixture were then added 2 mL of a freshly prepared solution containing 25 mg pepsin (porcine, 2000 FIP-U/g, Merck No. 7190). The flasks were closed with a rubber stopper and placed on multipoint stirrers (Variomag multipoint HP 15) in a thermostatically-controlled heating chamber at 39 °C for 6 h to simulate the gastric digestion according to Huang et al. [18]. At the end of incubation, liquid and solid were separated by filtering on Whatman 42 filter papers and collected. Nine replicates for each material were stored at 4 °C and then analyzed. Mineralogical composition of solids were obtained by XRPD, whereas all the solutions were analyzed by ICP-OES, using the methodology described by Mercurio et al. [6], to determine the following elements: Ca, Na, K, Mg, Al, Si, Fe (radial setting), Mn, Pb, Cd (axial setting), Sb, As and Hg (cold vapors and hydride generation).

Second step: small-intestine digestion. For each material the remaining 18 gastric digested zeolitic samples were used to simulate the passage in the further tracts of the pig digestive system. Samples were placed in 100 mL glass bottles. The whole digestive tract of three Italian Large White \times Italian Landrace pigs (live weight 143.2 ± 4.3 kg) was collected in a specialized slaughter house, opportunely sealed and transported as soon as possible (~30 min) in the laboratories of the Department of Veterinary Medicine and Animal Science. The content of the small intestine was diluted 2:1 with a phosphate buffer (0.2 M, pH 6.8) + 5 mL of a 0.6 M NaOH solution. The pH was then adjusted to 6.8 with a 1 M HCl or a 1 M NaOH solution; 75 mL of this buffer were then added to the 18 glass bottles with the stomach-digested zeolitic samples. The bottles were incubated at 39 °C for 18 h. At the end of the incubation, nine replicates (solid and liquid) for each material were stored at 4 °C and then analyzed as reported in the previous step.

Third step: large-intestine digestion. For each material, 9 gastric-small intestine digested zeolites were placed in glass bottles filled (75 mL) with the content of the large intestine diluted 1:4 with the buffered solution (pH 6.8) and incubated at 39 °C for 24 h. At the end of incubation, a similar procedure as reported for stomach and small intestine digestion was followed.

3. Results

Table 1 reports the mineralogical and chemical composition of the source materials (APO and DUG). APO is characterized by a 51 wt. % total zeolite content, with phillipsite (35 wt. %) prevailing over chabazite (15 wt. %); limited amounts of analcime (1 wt. %) also occur. By contrast, DUG shows a total zeolite content of 37 wt. % with chabazite (23 wt. %) prevailing on phillipsite (13 wt. %) and analcime (1 wt. %). The chemical composition of these two zeolites, although quite similar, as both belonging to the same trachytic pyroclastic formation, is characterized by different alkaline and alkaline earths content. APO shows K + Na prevailing over Ca,

Table 1
Representative XRPD mineralogical and chemical composition (by ICP-OES) of the source materials.

	APO	DUG
<i>Mineralogical composition, wt. %</i>		
Sme	Tr.	1 \pm 1
Cbz	15 \pm 1	23 \pm 1
Phi	35 \pm 2	13 \pm 2
Anl	1 \pm 1	1 \pm 1
Cpx	4 \pm 1	2 \pm 1
Cal	1 \pm 1	1 \pm 1
Ab	8 \pm 2	6 \pm 2
Sa	19 \pm 3	20 \pm 3
Bt	2 \pm 1	1 \pm 1
Am	15 \pm 3	32 \pm 4
<i>Zeolite^a</i>	51	37
<i>Chemical composition, wt. %</i>		
SiO $_2$	55.63	53.96
TiO $_2$	0.47	0.44
Al $_2$ O $_3$	16.36	15.88
Fe $_2$ O $_3$	4.21	3.99
MnO	0.16	0.15
MgO	0.69	0.95
CaO	3.58	4.32
Na $_2$ O	1.32	0.85
K $_2$ O	7.33	5.97
P $_2$ O $_5$	0.09	0.09
LOI	10.18	13.49

Sme = smectite; Cbz = chabazite; Phi = phillipsite; Anl = analcime; Cpx = clinopyroxene; Cal = calcite; Ab = albite; Sa = sanidine; Bt = biotite; Am = amorphous. Tr. = traces.

^a Sum of Cbz + Phi + Anl.

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