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A Chaso, R Pascual, Ja Madrid, Gm Salido. Bioavailability of fluoride from dietary sepiolite in the lamb. Annales de Recherches Vétérinaires, 1991, 22 (1), pp.71-75. hal-00902011

## HAL Id: hal-00902011 https://hal.science/hal-00902011

Submitted on 11 May 2020

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#### Ann Rech Vet (1991) 22, 71-75 © Elsecvier/INRA.

## Bioavailability of fluoride from dietary sepiolite in the lamb

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(Received 27 April 1990; accepted 21 September 1990).

**Summary** – Nine weeks after weaning, 12 lambs were randomised to 2 groups, each consisting of 6 animals. One group received a diet containing 213.9 mg/kg of fluor (F) in the form of sodium fluoride (NaF) and the other group received a diet containing 212.3 mg/kg of fluor in the form of sepiolite. The 24 h time courses of plasma fluoride concentrations showed that after feeding the average peak plasma concentration of the NaF-fed group was 0.75  $\mu$ g F/ml; that of the sepiolite-fed group was 0.35  $\mu$ g F/ml. The 12-h area under the plasma concentration curve (AUC) values in the NaF-fed group were higher with statistical significance (P < 0.001) at each time point. Compared with fluoride from NaF, the relative bioavailability of fluoride from sepiolite was found to be very weak.

#### lamb / bioavailability / fluoride / sepiolite

**Résumé** – **Biodisponibilité du fluor apporté par la sépiolite chez l'agneau.** Douze agneaux âgés de 9 semaines ont été répartis par tirage au sort en 2 groupes expérimentaux de 6 agneaux chacun. Un groupe recevait un aliment qui contenait 213,9 mg/kg de fluor sous la forme de fluorure de sodium. Le deuxième groupe était nourri avec un aliment contenant 212,3 mg/kg de fluor (F) sous la forme de sépiolite. L'évolution au cours de 24 h des concentrations plasmatiques d'ion fluor a montré qu'après la prise alimentaire, la concentration moyenne au moment du pic, dans le groupe ayant reçu du fluorure de sodium, était 0,75 µg F/ml et, dans le groupe ayant reçu de la sépiolite, elle était 0,35 µg F/ml. L'aire sous la courbe des concentrations plasmatiques d'ion fluor au cours de 12 h après le repas était plus élevée (P < 0,001) dans le groupe ayant reçu du fluorure de sodium que dans le groupe ayant reçu de la sépiolite. Par rapport au groupe ayant reçu du fluorure de sodium, la biodisponibilité du fluor provenant de la sépiolite était très faible.

#### agneau / biodisponibilité / fluor / sépiolite

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#### INTRODUCTION

In addition to its established cariostatic effect and its possible preventive or therapeutic roles in other major diseases, fluoride is a hazardous substance when large doses are taken acutely or when lower doses are taken chronically (Whitford, 1989). Various sources may contribute to the total fluoride intake by animals. The most commonly encountered sources of excessive fluoride intake are forages subjected to air-born contamination in areas near certain industrial plants, drinking water high in fluoride content, feed supplements and mineral mixtures high in fluoride content and vegetation growing on soils high in fluoride content (Shupe et al, 1963). To avoid the risk of fluorosis. the Conseil des Communautés Européennes (1974) established at 50 mg/kg the maximal concentration of fluoride in lamb feedstuff. Sepiolite, a clay of the sepiolite-palygorskite group, has been used as binder, carrier for supplements or growth promotor in chicken and pig feeding (Tortuero, 1982; Tortuero and Rioperez, 1989). Sepiolite from Vallecas (Spain) has an average fluorine content of 12 500 mg/kg (Alvarez *et al.*, 1987); then the sepiolite content in lamb feed must be < 0.4 %. This experiment was designed to determine in lambs the relative bioavailability of fluoride from sepiolite in comparison with the availability of sodium fluoride (NaF), a readily soluble compound added to the diet in comparable amounts.

#### MATERIALS AND METHODS

#### Animals

Twelve male lambs were purchased at weaning and fed a pelleted diet (control diet). After 9 wk (average weight 29.1 kg) they were divided into 2 groups of 6 animals, which respectively received a fluorine diet and sepiolite diet. Fluorine in the form of either sodium fluoride or sepiolite was added to the control diet for specific treatments (table I). Animals were prepared for study by placing chronic cannulae into the left jugular vein and fasted for 24 h prior to the experiment.

#### Experimental procedure

All experiments began at 10 h and no food or drink was allowed before 12 h. Each animal was individually fed *ad libitum* during a 2-h feeding period twice

Table I. Composition of experimental diets (% of diet).

Ingredients	Diets		
	Control	Sepiolite	Fluoride
Corn flour	23.0	23.0	23.0
Barley flour	52.2	50.2	52.3
Bran wheat	7.0	7.0	7.0
Soja flour (44 %)	15.2	15.2	15.2
Sodium chloride	0.4	0.4	0.4
Calcium carbonate	1.5	1.5	1.5
Calcium phosphate	0.5	0.5	0.5
Vitamin corrector	0.2	0.2	0.2
Sepiolite	-	2.0	-
Sodium fluoride (46 % fluor)	_	-	0.44
Fluor concentration (mg/kg)	29.0	212.3	213.9

a day. Twelve jugular blood samples were taken from each animal during a 24-h period for fluor (F) analysis. Fluoride analyses of the plasma (Fuchs *et al*, 1975) and diets (Melton *et al*, 1974) were carried out on duplicate samples with a fluoride combination electrode (Orion model 94-09) connected to a digital pH/mv meter ion analyzer (Crison model 517).

#### Calculations

F levels were calculated as net F concentrations by subtraction of the background F levels of the control samples from the F data obtained after intake of the diets. The area under the curve (AUC) of F plasma concentration *versus* time from 0 to 12 h was calculated by the trapezoidal rule and the bio-availability of F from sepiolite according to Trautner and Sie-



bert (1986). All data are expressed as mean  $\pm$  standard error of mean. Significance of differences was assessed by Student's *t*-test. Complete bioavailability of F administered as NaF was assumed (Ekstrand *et al*, 1978).

#### RESULTS

For NaF at different doses between 15 and 78 mg F, linear correlation was found with the area under the plasma concentration *versus* time curve (fig 1). The time courses of plasma fluoride concentrations of lambs after feeding are shown in figure 2. Plasma F profiles de-

Fig 1. Correlation between the quantity of fluor ingested as sodium fluoride and the area under the 12 h plasma fluor concentration curve (AUC) in lambs.

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Fluor concentration (µg/ml plasma)



	Sepiolite	Fluoride	Statistical significance
Diet consumed (g/feeding) Fluoride concentration (mg/kg) Fluoride consumed (mg/feeding) Area under curve (ng· h/ml plasma) Bioavailability (%)	$\begin{array}{c} 566.08\pm 33.89^{a}\\ 212.30\pm 2.15\\ 120.18\pm 7.19\\ 14.41\pm 0.81\\ 3.08 \end{array}$	$\begin{array}{c} 223.92 \pm 22.60^{a} \\ 213.90 \pm \ 2.73 \\ 45.98 \pm \ 4.89 \\ 178.83 \pm 24.42 \\ 100 \end{array}$	<i>P</i> < 0.001 NS <i>P</i> < 0.001 <i>P</i> < 0.001 <i>P</i> < 0.001

**Table II.** Fluor content, amounts ingested per feeding and net 12 h area under the plasma concentration curve (AUC) from different diets.

a: Mean  $\pm$  standard error of mean; n = 6 animals.

pended greatly on the type of diet ingested and similar curves were obtained with all subjects. The plasma fluoride concentration curve of the sepiolite fed group was relatively flat during the whole experimental period. In contrast, the peak concentrations of the NaF group occurred during feedings. The average peak plasma concentration of the NaF fed group was 0.75 µg F/ml, and that of the sepiolite fed group was 0.35 µg F/ml.

The average amount of fluoride ingested by the animals during the entire experiment was 45.98 mg for the NaF fed group and 120.18 mg for the sepiolite fed group. Feed and fluoride consumption values and AUC values have been summarized (table II). The NaF significantly affects the feed consumption and fluoride from sepiolite is poorly available in comparison with NaF.

#### DISCUSSION

In view of the adverse effects that fluoride can produce in animals (Shupe and Alther, 1966) it is essential to know how much fluoride can be ingested over a period without disrupting normal animal perform-

ance. F levels in plasma are good indicators of uptake in bioavailability studies involving lambs (Milhaud et al, 1985). In general, the reactive physiological and anatomical process of animals ingesting fluoride is governed by a number of factors, including level or amount of fluoride inaested, duration of indestion time. type and solubility of fluoride ingested and species of animal involved (Shupe et al, 1963). The recommendation of the Conseil Des Communautés Européennes (1974) assumes that the dietary availability of F is 50 mg/kg for lambs, but no differentiation is made between feedstuffs and mineral supplements. Most experimental studies of F toxicity in domestic animals have utilized NaF as the F source, and suggested tolerances are usually based on these data. In general, most investigators have found that bioavailability of F in mineral supplements is less than that of equivalent F in the form of NaF (Singer and Ophaug, 1979; Taves, 1983; Rao, 1984; Clay and Suttie, 1985). Our bioavailability data obtained in lambs clearly demonstrate that F from sepiolite is poorly available and indicates that sepiolite may contribute little to dietary F intake. The poor availability of F from sepiolite could be due to the fact that the F sepiolite bond is extremely strong,

it being very difficult to leach more than 20 % of the overall F content in this clay, even after drastic treatment (Alvarez *et al*, 1987).

Our results indicate that sepiolite does not significantly increase F levels in lamb plasma, when concentrations of up to 2.% clay are included in the diet. However, several authors have indicated the positive effect of clays in the feed efficiency of diverse animal species (Tortuero, 1982; Tortuero and Rioperez, 1989). Thus further investigations should be carried out on the effect of sepiolite on growth and feed conversion in lambs of different ages.

#### ACKNOWLEDGMENTS

The authors thank F Mogena and JA Andrada for their assistance in providing animal care for this study.

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