Biol Trace Elem Res (2011) 142:159–166
DOI 10.1007/s12011-010-8765-3

Effects of In-Feed Inclusion of Clinoptilolite on Blood Serum Concentrations of Aluminium and Inorganic Phosphorus and on Ruminal Ph and Volatile Fatty Acid Concentrations in Dairy Cows

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Received: 10 March 2010 / Accepted: 29 June 2010 /
Published online: 24 July 2010
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Abstract The experiment investigated the effects of the dietary inclusion of 200 g of the natural zeolite, clinoptilolite on the blood serum concentrations of aluminium (Al) and inorganic phosphorus (P) as well as on the ruminal pH and the ruminal concentrations of Al and P and of certain volatile fatty acids. Sixteen Holstein cows with a rumen fistula were randomly assigned to one of two groups. Group A cows (n=8) were fed the basal ration supplemented with 200 g of clinoptilolite per day, and group B cows (n=8) were fed the basal ration and served as controls. Blood and rumen fluid samples were collected at the same day of each week and at the same time (5 h after morning feeding) for 12 weeks. Clinoptilolite supplementation had no significant effect on ruminal and blood serum concentrations of Al and P. However, clinoptilolite significantly increased ruminal pH and acetate, and decreased ruminal propionate and valerate.

Keywords Clinoptilolite · Dairy cows · Aluminium · Volatile fatty acids · Ruminal pH · Phosphorus

Introduction

Over the last decades, the use of both natural and synthetic zeolites in animal nutrition has increased mainly to improve their performance and to protect against mycotoxins intoxication [1, 2]. These hydrated aluminosilicates of alkali and alkaline earth cations have unique properties such as the ability to lose and gain water reversibly to absorb molecules of appropriate diameter (absorption property) or acting as molecular sieves and

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to exchange their constituent cations without major change of their structure (ion-exchange property) [3, 4]. These properties make zeolites useful in animal nutrition.

Research results in dairy cattle suggest that the long-term dietary administration of a natural zeolite, clinoptilolite, has beneficial effects on their health status and performance [5–7]. One of the major concerns that arise from the use of clinoptilolite as feed additive in dairy cattle is whether it remains stable or is hydrolyzed through its passage from the rumino-intestinal tract. If a considerable amount of clinoptilolite is hydrolyzed, the released aluminium (Al) may interfere with the utilization of several minerals, with phosphorus absorption and metabolism being the most affected [8]. Although this hypothesis has not been tested yet for natural zeolites, some researchers recently found increased levels of Al in the rumen of cows that were receiving synthetic zeolite A, indicating that synthetic zeolites are hydrolyzed even in rumen [9]. Moreover, they observed reduced bioavailability of dietary phosphorus and significantly lower blood serum phosphorus in cows that were fed zeolite A [10].

Due to their ion-exchange property, natural zeolites, when added to acidic or basic aqueous solutions, act as regulatory factors [4]. Although many researchers have attempted to use this property in order to regulate the pH of rumen content for changing the fermentation patterns and for the prevention of subacute ruminal acidosis, their results have been contradictory.

Using different levels of dietary supplementation, some researchers observed that clinoptilolite does not affect [11–13], whereas others increase [14] or even reduce [13–15], ruminal pH. Despite the contradictory results about ruminal pH, it is well documented that both natural and synthetic zeolites cause changes in rumen fermentation patterns which affect the molar proportions of volatile fatty acids. However, the changes on the proportion of volatile fatty acids are not constant. In some studies it is concluded that zeolites increase the proportion of propionate [15, 16], and in others, decrease the proportion of propionate [17, 18] and valerate [9], and increase that of acetate [9, 17].

The aim of this study was to investigate whether the dietary inclusion of 200 g of the natural zeolite, clinoptilolite, has any effect on the blood serum concentrations of Al and inorganic phosphorus (P) as well as on the ruminal pH and the concentrations of Al and of certain volatile fatty acids.

Materials and Methods

Animals and Experimental Design

Sixteen clinically healthy non-pregnant Holstein dairy cows at the third and fourth lactation period were used in the study. Before the onset of the experiment, a rubber fistula (inner diameter, 5 cm) was surgically positioned in the dorsal sac of the rumen of each cow. They were kept in the clinic of farm animals of the Veterinary School of Aristotle University of Thessaloniki in a tethered stall with neck straps and were milked twice daily. Their average milk yield was 12.7 (SD, 1.4) kg per day.

The experiment started 3 weeks after the placing of rubber fistulas and lasted for 12 weeks. At the commencement of the experiment (day 0), the cows were randomly assigned to one of two groups that were similar in parity and milk yield. Group A consisted of eight cows, which were fed the basal ration supplemented with 200 g of clinoptilolite daily. Group B consisted of eight cows, which were fed the same basal ration without clinoptilolite supplementation and served as controls.

The basal ration that was offered to the animals throughout the experimental period consisted of 25 kg of corn silage, 2 kg of molasses and 4 kg of concentrates per day. The concentrate portion was comprised of 12.6% soybean meal, 16.0% maize grains, 22.0% wheat bran, 18.0% sunflower meal, 8.0% rape cake, 21.0% carob fruits, 0.2% vitamins and trace minerals premix, 1.3% salt, 0.5% calcium carbonate and 0.4% dicalcium phosphate. The ration was offered in an individual trough for each animal. Maize silage and molasses were offered twice daily at 0700 and 1900 hours. The amount of concentrate fed was divided into equal portions and offered twice a day during morning and afternoon milking, at 0800 and 2000 hours, respectively. In group A, clinoptilolite was offered with concentrate.

Prior to the onset of the experiment (day 0), a rumen fluid sample and a blood sample were obtained from each cow. The rumen fluid and blood samplings were repeated at the next day (day 1) and then at weekly intervals at the same day of each week (Tuesday) at 1300 hours. The rumen fluid was collected from the cannula into a sterile plastic tube and the ruminal pH was immediately determined by a portable pH-metre (CRISON® microp H 2002). Thereafter, the rumen fluid was frozen at −19°C until analysis. The blood sample was obtained by jugular vein puncture, in vacuum glass tubes with an 18-gauge needle. After clotting, the serum was separated by low-speed centrifugation (1.600×g for 15 min), transferred in plastic vials and frozen at −19°C until it was analysed.

Zeolitic Material

The zeolitic material used in the experiment had a particle size of <0.80 mm and contained approximately 92% clinoptilolite and the admixture was 8% opal ($SiO_2 \times nH_2O$) as determined by X-ray powder diffraction. The material's cation exchange capacity was 220 meq 100 g^{-1} and its chemical composition is SiO_2, 69.9%; Al_2O_3, 11.27%; CaO, 3.02%; MgO, 0.6%; Na_2O, 0.75%; K_2O, 2.23%; Fe_2O_3, 0.11%; and loss on ignition, 13.05%.

Chemical Analysis

Soluble P and Al in rumen fluid were analysed after a high-speed centrifugation (15.000 g for 30 min). The supernatant medium was measured by inductively coupled plasma optical emission spectometry. The blood serum concentration of inorganic phosphorus was determined by using the heteropoly acid-blue method [19], whereas blood serum Al concentrations were determined by inductively coupled plasma mass spectrometry according to the directive DIN 17294-2. Rumen fluid was analysed for short chain fatty acids using gas chromatography.

Statistical Analysis

The data were analysed with SPSS® 15.0. Before analysis, they were tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variances with the Levene test. The effect of clinoptilolite supplementation on the parameters evaluated was tested with repeated measures ANOVA with the treatment group used as between-subject factor and the values of each parameter on day 0 as covariates. At each sampling day, the data for each variable evaluated were analysed with independent-samples *t*-test in order to determine the significance of the differences among the experimental groups. In all cases, a significance level of $P \leq 0.05$ was used.

Results

The dietary administration of clinoptilolite had no significant effect on the average ruminal (mean ± SE, 6.26±0.14 and 6.27±0.14 mmol/L for groups A and B, respectively) and blood serum concentration of Al (mean ± SE, 28.97±1.80 and 29.23±1.80 mmol/L for groups A and B, respectively) throughout the experimental period and the differences among the experimental groups were not significantly different at any collection time ($P>0.05$). Similarly, the average concentration of soluble phosphorus in the rumen fluid (mean ± SE, 9.81±1.67 and 9.85±1.67 mmol/L for groups A and B, respectively) and the average blood serum inorganic phosphorus (mean ± SE, 2.13±0.01 and 2.13±0.01 mmol/L for groups A and B, respectively) were not significantly affected by clinoptilolite supplementation throughout the experiment and no significant difference was recorded in any sampling point ($P>0.05$).

Ruminal pH was affected by the addition of clinoptilolite to the concentrate and was significantly higher in group A compared to group B (mean ± SE, 6.58±0.06 and 6.11±0.06 mmol/L for groups A and B, respectively, $P<0.05$). As it is shown in Fig. 1, ruminal pH was significantly higher in group A than the other group at all collection times after the second week ($P<0.05$).

The ruminal concentrations of the total volatile fatty acids and the percentages of the molar proportions of ruminal acetate, propionate and valerate are presented in Fig. 2. The total volatile fatty acids concentration was not significantly affected by clinoptilolite supplementation (mean ± SE, 101.70±1.68 and 103.65±1.68 mmol/L for groups A and B, respectively; $P>0.05$) and no difference was recorded among groups at any sampling point ($P>0.05$). However, the molar proportion of acetate was significantly higher (mean ± SE, 71.17±0.26% and 64.50±0.26% for groups A and B, respectively, $P<0.05$) in group A than group B throughout the experiment as well as at each rumen fluid collection time ($P<0.05$). On the other hand, the molar proportions of propionate (mean ± SE, 15.22±2.12% and 17.98±2.12% for groups A and B, respectively) and valerate (mean ± SE, 1.55±0.10% and 2.21±0.10% for groups A and B, respectively) were significantly lower throughout the experiment and at each sampling point in group A compared to control group B ($P<0.05$). The molar proportions of butyrate (mean ± SE: 12.55±0.28% and 13.20±0.28% for groups

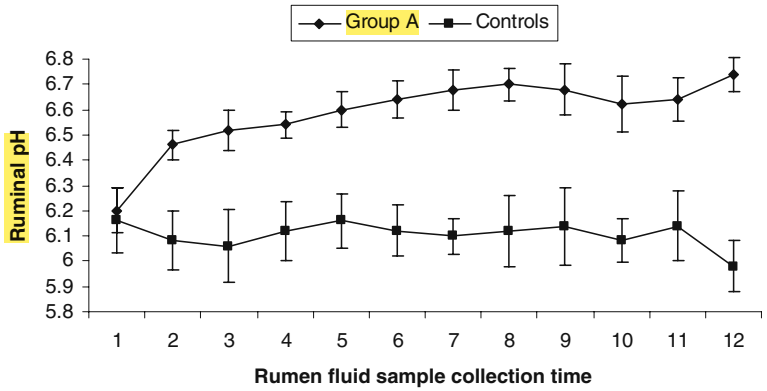

Fig. 1 Average (mean ± SE) ruminal pH values in rumen fluid samples that were obtained at weekly intervals for 12 weeks (rumen fluid collection time, 1–12), from the eight cows of group A that received a ration supplemented with 200 g of clinoptilolite per day and the eight cows of group B that were left untreated as controls

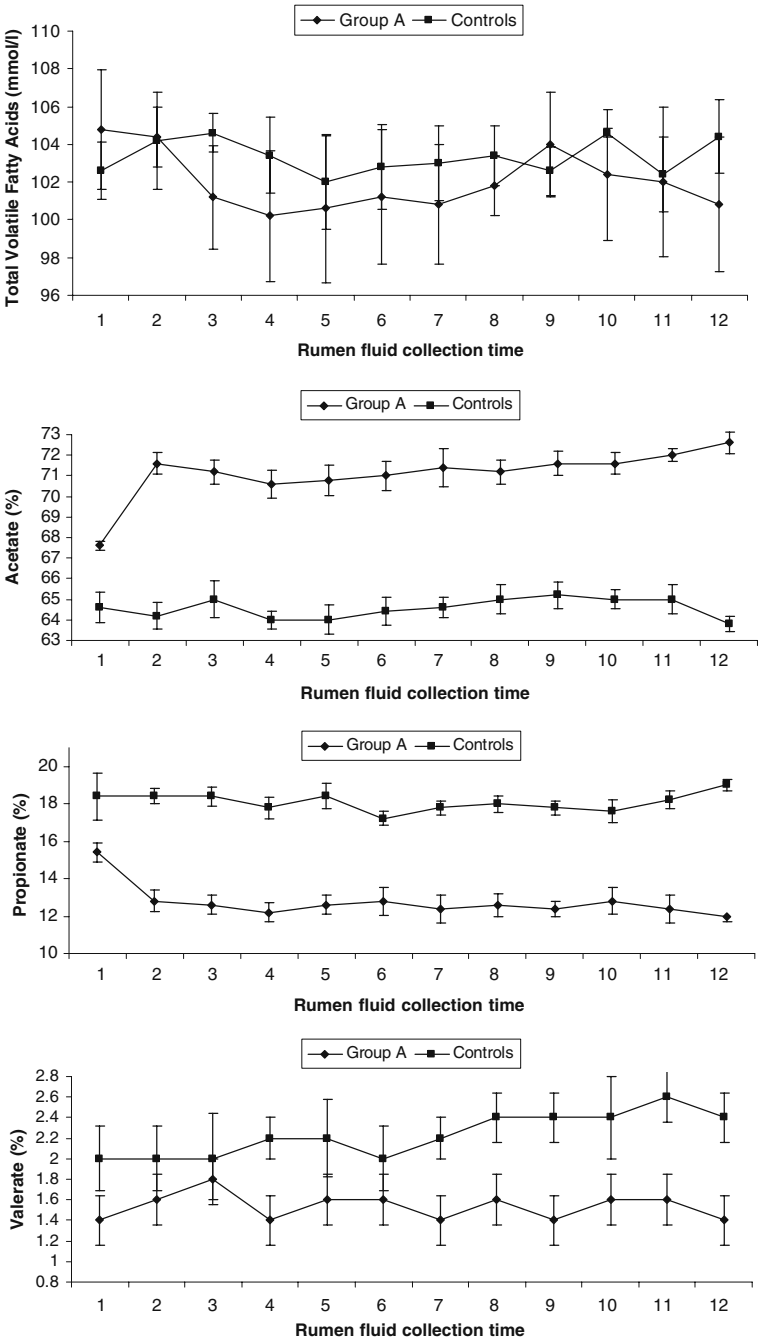

Fig. 2 Average (mean ± SE) total volatile fatty acid concentrations (mmol/L) and molar proportions of acetate, propionate and valerate (%) in rumen fluid samples that were obtained at weekly intervals for 12 weeks (rumen fluid collection time, 1–12), from the eight cows of group A that received a ration supplemented with 200 g of clinoptilolite per day and the eight cows of group B that were left untreated as controls

A and B, respectively) and iso-fatty acids (mean ± SE, 2.05±0.10% and 1.93±0,10% for groups A and B, respectively) were not influenced by clinoptilolite ($P>0.05$).

Discussion

The aim of this study was to investigate whether the dietary inclusion of 200 g of the natural zeolite, clinoptilolite, has any effect on the blood serum concentrations of Al and inorganic phosphorus as well as on the ruminal pH and the ruminal concentrations of Al and of certain volatile fatty acids.

The Al concentrations in rumen fluid and blood serum were not affected by clinoptilolite supplementation throughout the experimental period. This indicates that clinoptilolite appears to be stable at the acidic pH of the gastrointestinal tract. This point of view is confirmed by the results of previous studies in other animal species. It has been proven that clinoptilolite feeding has no significant effect on the urine concentration of silicon (Si), the other main component of zeolites, in lambs [20] and does not alter the concentration of Al in the kidney and the liver of pigs [21]. In contrast to the observations at the present study, other researchers found that the ruminal concentration of Al was significantly higher in dairy cows that were consuming synthetic zeolite A compared to the controls [10], proving that zeolite A was partially unstable at the ruminal pH. It is well-known that the stability of zeolites on the acidic pH is depended on the Si–Al ratio and they are more stable when this ratio is higher [22]. Clinoptilolite has a Si–Al-ratio of 5:1 while zeolite A has a 1:1 ratio and this is the reason why clinoptilolite is more stable in the acidic pH than synthetic zeolite A.

The concentration of soluble P in the rumen fluid was not significantly affected by clinoptilolite supplementation. This was rather expected since clinoptilolite was stable at the acidic ruminal pH. However, other researchers observed that the soluble phosphorus in the rumen fluid is negatively correlated with the intake of synthetic zeolite A and it was attributed to the formation of aluminium phosphate complexes that were insoluble on a pH range between six and seven [10]. Apart from rumen fluid P concentration, blood serum P was unaffected by clinoptilolite as well, suggesting that the bioavailability of inorganic phosphorus was not impaired by the dietary supplementation of clinoptilolite. This is confirmed by the results of previous studies in ruminants where no significant effect of clinoptilolite on blood serum P was recorded [5, 20, 23, 24]. On the contrary, the supplementation of synthetic zeolite A seems to have a negative effect on phosphorus metabolism, which is reflected in the low-serum P concentration [9, 25–27].

The dietary administration of clinoptilolite significantly increased the pH of the rumen fluid. This increase is attributed to the buffer effects of clinoptilolite when added to acidic or basic aqueous solutions [4]. However, at the first rumen fluid collection time the difference of the pH values was not significant among the groups, indicating that it is required more than 5 h from the first administration of clinoptilolite in order to increase the ruminal pH. The results of previous similar studies provide contradictory conclusions. In accordance to the present experiment, Eng et al. [14] observed that clinoptilolite increases the ruminal pH. Other researchers found that clinoptilolite either decreases [13, 15] or does not affect ruminal pH [11–13], but it has to be noted that the zeolitic material used at these studies had lower content of clinoptilolite in comparison with the material that was used here.

The total volatile fatty acid concentration was not significantly affected by clinoptilolite supplementation, which is in agreement with the observations in previous studies [10, 18]. However, the molar proportions of the volatile fatty acids evaluated were significantly

affected by clinoptilolite. Acetate was increased while propionate and valerate were decreased. The exact mechanism by which clinoptilolite might affect the molar proportion of volatile fatty acids in rumen is currently unknown and needs further investigation. Similar alterations of the molar proportions of the volatile fatty acids have been recorded in previous experiments as well [9, 17, 18], but other researchers observed that clinoptilolite increased the proportion of propionate [15, 16]. However, in the latter experiments clinoptilolite was added to high-concentrate diets and not at high-roughage diets as at the present study.

The main conclusions of the present study is that clinoptilolite remains practically stable in the rumen of dairy cattle without causing alterations on the blood serum Al levels and without impairing the bioavailability of dietary P. Furthermore, it was proven that when clinoptilolite is used as feed additive in dairy cattle, it increases the levels of ruminal pH and the molar proportion of acetate and reduces the proportions of propionate and valerate.

References

1. Trckova M, Matlova L, Drorska L, Palvic I (2004) Kaolin, bentonite and zeolites as feed supplements for animals: health advantages and risks. Vet Med Czech 49:389–399
2. Papaioannou D, Katsoulos PD, Panousis N et al (2005) The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: a review. Microporous Mesoporous Mater 84:161–170
3. Mumpton FA, Fishman PH (1977) The application of natural zeolites in animal science and aquaculture. J Anim Sci 45:1188–1203
4. Filippidis A, Godelitsas A, Charistos D, Misaelides P, Kassoli-Fournaraki A (1996) The chemical behaviour of natural zeolites in aqueous environments: interactions between low-silica zeolites and 1 M NaCl solutions of different initial pH-values. Appl Clay Sci 11:199–209
5. Katsoulos PD, Roubies N, Panousis N et al (2005) Effects of long-term dietary supplementation with clinoptilolite on incidence of parturient paresis and serum concentrations of total calcium, phosphate, magnesium, potassium and sodium in dairy cows. Am J Vet Res 12:2081–2085
6. Katsoulos PD, Panousis N, Roubies N et al (2006) Effects of long-term feeding of a diet supplemented with clinoptilolite to dairy cows on the incidence of ketosis, milk yield and liver function. Vet Rec 159:415–418
7. Karatzia MA (2010) Effect of dietary inclusion of clinoptilolite on antibody production by dairy cows vaccinated against *Escherichia coli*. Livest Sci 128:149–153
8. Allen VG (1984) Influence of dietary aluminium on nutrient utilization in ruminants. J Anim Sci 59:836–844
9. Grabherr H, Spolders M, Lebzien P, Huther L, Flachowsky G, Furll M, Grun M (2009) Effect of zeolite A on rumen fermentation and phosphorus metabolism in dairy cows. Arch Anim Nutr 63:321–336
10. Grabherr H, Spolders M, Furll M, Flachowsky G (2009) The effect of several doses of zeolite A on feed intake, energy metabolism and on mineral metabolism in dairy cows around calving. J Anim Physiol Anim Nutr 93:221–236
11. Bergero D, Rumello G, Sara C, D'Angelo A (1997) Effect of natural clinoptilolite or phillipsite in the feeding of lactating cows. In: Kirov G, Filizova L, Petrov O (eds) Natural zeolites-Sofia '95. Pensoft Publiushers, Sofia-Moscow, pp 67–72
12. Bosi P, Creston D, Casini L (2002) Prodction performance of dairy cows after the dietary addition of clinoptilolite. Ital J Anim Sci 1:187–195
13. Moghaddam G, Taghizadeh A (2001) Effect of zeolite nutrition on rumen ecosystem in dairy cow. In: Proceedings of the British society of animal science, Scarborough, March 2001, pp 155
14. Eng KS, Bectel R, Hutcheson DP (2003) The buffering activity of a potassium clinoptilolite zeolite in steers fed a high concentrate steam flaked grain-corn silage diet. J Dairy Sci 86:167, Supplement 1
15. McCollum MI, Galyean MI (1983) Effects of clinoptilolite on rumen fermentation, digestion and feedlot performance in beef steers fed high concentrate diets. J Anim Sci 56:517–524
16. Galyean MI, Chabot RC (1981) Effect of sodium bentonite, buffer salts, cement kiln dust and clinoptilolite on rumen characteristics of beef steers fed a high roughage diet. J Anim Sci 52:1197–1204

17. Sweeney TE, Cervantes A, Bull LS, Hemken RW (1984) Effect of dietary clinoptilolite on digestion and rumen fermentation in steers. In: Pond WG, Mumpton FA (eds) Zeo-agriculture. Use of natural zeolites in agriculture and aquaculture. Westview Press, Bowlder, pp 183–193
18. Johnson MA, Sweeney TF, Muller LD (1988) Effects of feeding synthetic zeolite A and sodium bicarbonate on milk production, nutrient digestion and rate of digesta passage in dairy cows. J Dairy Sci 71:946–953
19. Boltz DF, Lueck CH (1958) Phosphorus. In: Boltz DF (ed) Colorimetric determination of nonmetals. Interscience Publishers Inc., New York
20. Pond WG (1984) Response of growing lambs to clinoptilolite or zeolite Na-A added to corn, corn-fish meal and corn-soybean meal diets. J Anim Sci 59:1320–1328
21. Pond WG, Yen JT, Crouse JD (1989) Tissue mineral content in swine fed clinoptilolite. Bull Environ Contam Toxicol 42:735–742
22. Flaningen EM (1984) Adsorption properties of molecular sieve zeolites. In: Pond WG, Mumpton FA (eds) Zeo-Agriculture. Use of natural zeolites in agriculture and aquaculture. Westview Press, Bowlder, pp 55–68
23. Pond WG, Laurent SM, Orloff HD (1984) Effect of dietary clinoptilolite or zeolite Na-A on body weight gain and feed utilization of growing lambs fed urea or intract protein as a nitrogen supplement. Zeolites 4:127–132
24. Hutcheson DP (1984) Addition of clinoptilolite ores to the diets of feeder cattle. In: Pond WG, Mumpton FA (eds) Zeo-Agriculture. Use of natural zeolites in agriculture and aquaculture. Westview Press, Bowlder, pp 195–199
25. Thilsing-Hansen T, Jorgensen RJ, Enemark JMD, Larsen T (2002) The effect of zeolite A supplementation in the dry period on periparturient calcium, phosphorus and magnesium homeostasis. J Dairy Sci 85:1855–1862
26. Thilsing-Hansen T, Jorgensen RJ, Enemark JMD, Zelvyte R, Sederevicius A (2003) The effect of zeolite A supplementation in the dry period on blood mineral status around calving. Acta Vet Scand 97:87–93
27. Thilsing-Hansen T, Jorgensen RJ, Poulsen HD (2006) In vitro binding capacity of zeolite A to calcium, phosphorus and magnesium in rumen fluid as influenced by changes in pH. J Vet Med A 53:57–64