Production performance of dairy cows after the dietary addition of clinoptilolite

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ABSTRACT

Clays are frequently proposed for the addition to dairy cattle diets to reduce the transfer of mycotoxins in milk. This study examined the effect of the addition of Clinoptilolite to the diet of lactating dairy cows on milk production and milk composition. Thirty-two lactating Holstein cows (average lactation length: 142 d) were blocked according to milk production, parity, and days of lactation for assignment to one of two dietary treatments: control diet; control diet + Clinoptilolite, 200g/d. The control diet was based on corn and alfalfa silages, hay and concentrates, and did not contain aflatoxins above the safe level. The experimental period lasted 76 days. The Clinoptilolite supplementation did not affect milk yield and milk composition other than urea contents. The urea level in milk was negatively affected by Clinoptilolite addition (control group 29.7 mg/100 ml vs Clinoptilolite group 31.3 mg/100 ml). The dietary addition of Clinoptilolite did not change pH, ammonia content and VFA molar percentages in the rumen. No dietary effect on mineral contents of blood plasma (Na, K, Zn, and Ca) was observed. In case of clinoptilolite use in dairy cattle feeding, scarce negative effect on milk production and quality are expected.

Key words: Clinoptilolite, Dairy cow, Feeding, Milk, Milk composition.

RIASSUNTO

VALUTAZIONE DELL’INTEGRAZIONE DI CLINOPTILOLITE NELLA DIETA DI BOVINE IN LATTAZIONE SULLE PRODUZIONI DI LATTE.

Le zeoliti sono allumino-silicati naturali, alle quali viene riconosciuta un’importante azione adsorbente, in particolare nei confronti dello ione ammonio e delle micotossine. In natura ne esistono più di 50 tipi mentre oltre 200 sono quelle di sintesi. La clinoptilolite è una zeolite naturale tra le più impiegate nell’alimentazione dei bovini sia da latte che da carne, grazie alla sua ottima capacità di scambio cationico.

Trentadue bovine frisone con una durata media di lattazione di 142 giorni, sono state divise in due gruppi bilanciati per produzione di latte, ordine di parto e giorni di lattazione. Nel caso delle bovine del gruppo trattato sono stati aggiunti 200 g/capo/giorno di Clinoptilolite, alla dieta base. Tale dieta era costituita da insilato di mais (19%), fieno polilfito di prato stabile (17%), insilato di medica (10%), mangime concentrato (52%) e farina di pesce (1,5%). Durante la prova, nella dieta il contenuto di aflatoxine totali rilevato era inferiore alla soglia dei 10 ppb. Il periodo di osservazione ha avuto una durata di tre mesi, nei quali si sono raccolti complessivamente 544 campioni di latte, 32 campioni di succo ruminale e 32 campioni di sangue. L’aggiunta della clinoptilolite alla dieta non ha influenzato significativamente sia la produzione di latte
Introduction

Clinoptilolite is a clay mineral that belongs to the zeolites group. Chemically, it is a hydrated aluminosilicate - (Na, K)xAl2 Si3 O x12n+24H2O. In the structural unit of Clinoptilolite, some of the quadrivalent silicon is replaced by trivalent aluminium, giving rise to a deficiency of positive charge. (Mumpton and Fishman, 1977). This charge is balanced by the presence of mono- and divalent cations, such as Na⁺, Ca²⁺, and K⁺, elsewhere in the structure (Mumpton and Fishman, 1977).

By the nature of their chemical and physical properties, crystalline zeolites have a high attraction for water and large number of positively charged ions, such as K⁺, NH₄⁺, Ca²⁺, and Mg²⁺, which can be reversibly bound or released, depending upon the surrounding conditions.

The clinoptilolite is claimed to reduce the toxic effect of high NH₄⁺ content of ruminal fluids when non-protein nitrogen (NPN) compounds, such as urea and diuret, are added to diets of cattle (Mumpton and Fishman, 1977; Galindo et al., 1986). Hemken et al. (1984) introduced both natural and synthetic zeolites into the rumen of test animals. Ammonium ions formed by the enzyme degradation of NPN were immediately ion exchanged into the zeolite structure and held there for several hours until released by the regenerative action of Na⁺ entering the rumen in saliva during the after-feeding fermentation period. The gradual release of NH₄⁺ allowed rumen microorganisms to synthesize cellular proteins continuously available for the animal digestive system.

Johnson et al. (1988) reported no effect on the ammonium concentration in the rumen fluid and limited effect of the addition of 2% clinoptilolite on milk quality.

Vyaizenen and Budyjanu et al. (1995) reported the results of the studies of application of natural zeolites, which decreased the concentration of radionuclides in milk. On the other hand, Piva et al. (1987) reported limited effect of the addition 3% zeolite in the diet, with a decreased transfer of radionuclides in milk. Zeolites in the diet are also claimed to permit the adsorption of aflatoxins: in rats fed aflatoxin-contaminated diet, aluminosilicate improved haematological and biochemical parameters and histological picture of both liver and kidneys (Abdel-Wahhab et al., 2002). Ramos and Hernandez (1997) presented a review on the prevention of aflotoxicosis in farm animals by means of hydrated sodium calcium aluminosilicate addition to feedstuffs, while Huwig et al. (2001) discussed the mode of action of different adsorbents for the mycotoxin detoxication of animal feed. In addition, a clinoptilolite-rich zeolite in sheep diet could show some protective effect for rumen microbiota against the organophosphate poison (Nistiar et al., 2000).

The addition of zeolites, hydrated aluminosilicates of alkali, and alkaline earth cations to the diets of lactating dairy cows has not been studied extensively. However, under tropical conditions, the introduction of zeolite in a more rational feeding system of dairy cows, could contribute to improve production and health (Lopez et al., 2001).

Another reason to use zeolites in the feed is to reduce the release of ammonia in the manure. The longer time required for cow manure release in the soil is attributed to complex organic compounds and to the higher concentration of ammonia complex. Borja et al. (1993) observed that the digester with zeolite addition had the best behaviour during experiments, while inhibition of anaerobic
degradation was obtained in a control digester with suspended biomass.

The results on the efficacy of zeolites to reduce ammonia release in the environment and to contrast toxic dietary factors on health, production and milk composition of dairy cows are still scarce. However, before this increase in knowledge, it is important to determine if zeolites directly affect normal production parameters of dairy cows.

The objective of this study was to assess the effect of the dietary clinoptilolite addition on production and milk composition of dairy cows, on rumen fluid composition, and on the content of certain minerals in the blood.

Material and methods

In this trial, 32 lactating Holstein cows (average lactation length: 142 d) were blocked according to milk production, parity and days of lactation for the assignment to one of two dietary treatments: Control and Clinoptilolite. The Control group was fed a base diet consisting of corn silage, alfalfa silage, commercial feed compound (composition in Table 2), permanent meadow hay and minerals. Fish meal was included in the feed compound, as the trial was carried out before the ban of the product which was adopted within the measures for the prevention of the diffusion of BSE. The target intake was 20 kg of dry matter. The feeding was twice a day at 09.00 and 15.00 h in a TMR. The composition of the base diet is shown in Table 1. The clinoptilolite group was fed the same diet, supplemented with 10 g/kg d.m. clinoptilolite rock (Flint, 77%; Aluminum, 15%; Magnesium, 3%; Sodium, 2%; Calcium, 1%; Iron, 1%; Potassium, 1%). According to the company that provided the zeolite, the mineralogical composition the zeolite was: Clinoptilolite, 45%; Mordenite, 20%, Quartz, 12.5%; Kaolinite, 10.8%; Feldspar, 11.7%. The clinoptilolite was dispersed over the TMR in the feeding lane and accurately mixed with a pitchfork.

The experimental period lasted 76 days, from 26 July to 10 October. The cows were milked twice a day (06.00 and 16.00 h). Milk yield was recorded at 0 d and twice a week during the experimental period. Individual milk samples from morning and evening milking were collected, for a 272 total samples per group.

Rumen fluid and blood samples were collected twice on 45 and 180 days after the start of the trial, from 8 cows per group, about 1/2-1 hour after the morning milking. The rumen fluid was sam-

| Table 1. Composition of the base diet. |
|---------------------------------------|
| Mixed diet ingredients:               |
| Alfalfa silage %DM 9.74                |
| Corn silage % 18.98                    |
| Permanent meadow hay % 17.15          |
| Feed compound % 51.55                 |
| Fish Meal % 1.37                      |
| Monocalcium-phosphate % 1.21          |
| Chemical composition and nutritional facts: |
| Dry Matter % 54.53                     |
| Crude Protein %DM 18.70               |
| Fat % 4.35                            |
| Ash % 9.56                           |
| NDF % 30.90                          |
| Starch % 22.34                        |
| Milk F U % 0.92                       |

| Table 2. Ingredient composition of the feed compound. |
|-----------------------------------------------------|
| Ingredient                                          |
| %DM                                                 |
| Ground Corn                                         |
| Soybean meal 44 CP                                  |
| Whole extruded Soybean                              |
| Wheat Bran                                          |
| Wheat Middings                                      |
| Cane Molasses                                       |
| Minerals – Vitamins 1                                |
| Calcium Carbonate                                   |
| Sodium Bicarbonate                                  |
| Dicalcium Phosphate                                 |
| Salt                                                |
| Magnesium Oxide                                     |
| 41.97                                               |
| 21.57                                               |
| 10.20                                               |
| 7.89                                                |
| 7.98                                                |
| 3.34                                                |
| 1.58                                                |
| 1.72                                                |
| 1.66                                                |
| 1.07                                                |
| 0.55                                                |
| 0.44                                                |

1 Contained in kg of feed compound: Vitamin A 90.000 UI; Vitamin D 6000 UI; Vitamin E 60 mg; Vitamin PP 900 mg; Vitamin B1 7,50 mg; Vitamin B2 7,50 mg; Zinc 240 mg/kg; Iron 150 mg/kg; Selenium 1mg/kg; Iodium 3 mg/kg.
plied by rumenocentesis, inserting a 20 cm needle into the ventral rumen through the abdominal wall, and aspirating 20 ml of rumen fluid (Garrett et al., 1999). The ruminal samples were immediately kept in ice for 2 h, and frozen (-18°C). Blood samples (20 ml) were drawn by caudal venipuncture and collected in heparinized Vacutainers. Each tube was centrifuged at 2500 x g for 15 min to separate plasma, kept in ice for 2 h, and frozen (-18°C) for later analysis.

Dry matter was determined from samples of corn silage by drying at 80°C for 48 h. Determination of the nutrients was performed for ash by heating to 500°C for 5 h; for N by the Kjeldahl procedure; for ADF, NDF, and ADL according to the procedures recommended by Van Soest et al. (1991). The crude protein, ether extract, and crude cellulose were determined by methods A.O.A.C. (1995). The starch was determined by the polarimetric method (A.O.A.C.,1985) and Milk F U were estimated by I.N.R.A. (1988).

The total content of aflatoxins in the diet was measured by HPLC as reported by Tedeschi et al. (1984).

Fat, protein, somatic cell and urea contents of milk were determined by infrared analysis (Milkoscan; Foss Electric, Hillerod, Denmark).

In rumen fluid samples, pH, ammonia, and volatile fatty acids (VFA) were carried out. The ammonia in rumen fluid was measured by the method of A.O.A.C. (1995). The volatile fatty acids were determined using a gas chromatograph (Carlo Erba Instruments, Rodano-Milano, Italy), according to Hamada et al. (1968).

Plasma was analysed for calcium, sodium, potassium and zinc through atomic adsorption spectrophotometry (model 5000; Perkin-Elmer, Inc., Milano, Italy), according to the indications of the company (Perkin-Elmer, 1976).

After a preliminary analysis on separated values for morning and evening samplings, the average values of milk production and milk composition for each day of control were analysed, with the exception of those for milk urea. Statistical analysis of the results was performed by SAS using the Mixed procedure.

The effect of the day of control was defined as period. Consequently the model included diet, cow within diet, period, diet by period. For each parameter, the value observed at the day before the start of the trial was used as covariate. After a preliminary analysis on the variables, the mature equivalent milk yield (MEMY) at the starting month of
the trial and the day of calving were included as covariate only in the case of milk production. MEMY values were acquired from the official recording systems files held by Italian Breeder association (AIA, Rome).

The data of ruminal fluid composition and plasma mineral contents were analysed by a one-factor analysis of variance (diet).

Results and discussion

The total content of aflatoxin in the diet was below 10 ppb of diet, thus no relevant interference of Clinoptilolite with aflatoxin absorption was expected. Consequently, for this aspect, it can be stated that in the present trial the evaluation of the effects of the zeolite on milk production was performed under normal dietary conditions.

The results concerning milk production and milk composition are given in Table 3. Overall means of milk yield, and protein and fat percentage were 31 kg; 3.23%; 3.24%. No significant difference was found between the control group and the clinoptilolite group in milk yield, milk protein contents, fat contents, and somatic cells. The urea level in milk was higher in clinoptilolite group than in control group (+5.4% for morning samples, p<0.05, and +7.8% for evening samples, p<0.01). For the values of milk urea in morning samples, a statistically significant interaction between diet and day of sampling (p<0.01) was observed. The plot of morning urea for the two different groups against the day of sampling is shown in Figure 1. The values for the treated group were higher in 11 cases of 16 and in one case the control group showed higher values.

An effect of clinoptilolite addition on milk production was not observed. Bergero et al. (1995) obtained the same result by using the same clinoptilolite. In normal environment, supplementing the cows' diet with 2% zeolite A, Johnson et al. (1988) found decreased feed intake and milk production. On the contrary, under high temperature conditions, in dairy cows supplemented with sodium-zeolite, feed intake increased and milk production was not affected (Roussell et al., 1991). D'Yachenko and Lysenko (1988) observed that the addition of type A or B zeolite to a traditional Russian diet increased milk production.

In the present trial, fat and protein content were not improved by the clinoptilolite addition. Hornig et al. (1999) observed a significant increase
in fat, protein and lactose content, feeding 240 cows a diet containing 2% of clinoptilolite in a period of 13 weeks, while Garcia Lopez et al., (1988) found only an improvement in milk fat, with the same dietary addition. The absence of effect in our experiment could be related to the lower dose of supplementation.

The results of rumen fluid composition are presented in Table 4. The ammonia level in rumen fluid was the same for the two diets (15.4 mmol l\(^{-1}\)). The molar ratios of VFA and the pH were not affected by the diet.

In our trial the urea level was higher in the Clinoptilolite group than in the Control group; this result is difficult to interpret. Hornig et al. (1999) did not see significant differences of urea concentration on milk, with a 2% addition of clinoptilolite in the diet. Bergero (1995), using the same product, observed a slight increase in urea level of milk in the Clinoptilolite group, but it was not statistically significant. Urea levels in milk are highly variable within each subject, and urea is affected by many factors (temperature, diet, quality of feeds, water, season, stage of lactation etc.) (Castagnetti et al., 1995; Jonker et al., 1998).

It is possible that, after the release of ammonia consequent to each meal, clinoptilolite absorbs high levels of ammonia concentration of rumen, then clinoptilolite releases ammonia when its concentration is reduced. This could cause a problem between energy and nitrogen use in the rumen. Mumpston and Fishman (1977) reported that the zeolite’s ability to act as a reservoir can result in the protection of the animal against ammonia overload in the rumen. They calculated that the action of clinoptilolite using the equivalent of 500 g of additive for 50 liters of rumen fluid is selective enough for the ion ammonia.

It is interesting to compare the milk urea values with the results obtained after the rumen biopsy. Our rumen samplings at 45 and 180 days did not show any effect of the diet on ammonia concentration in rumen fluid. This apparently contrasts with the observation on milk urea. However, the sampling of rumen was done only twice, at a fixed time and in a single point. So it can be related only to a particular moment (i.e. 1/2-1 h after morning milking).

The results from other reports can help in the interpretation of our data. In rumen-fistulated ewes administered various amounts of urea, the supplementation of crushed clinoptilolite rock could not prevent the urea toxicity, as indicated by free ammonia concentrations in the rumen (Stephenson et al., 1992). Hornig et al. (1999) did not observe a decrease in ammonia concentration in rumen fluid of dairy cows. Thus the inclusion of clinoptilolite in dairy cow diets should not positively affect the concentration of ammonia in the rumen and, consequently, the urea level in milk.

| Table 4. Effect of clinoptilolite supplementation on pH, ammonia and VFA rumen fluid. |
|-----------------|-------|-------|-------|
| Sample          | Control | Clinoptilolite | SEM |
| pH              | 6.81 | 7.01 | 0.12 |
| Ammonia         | 15.4 | 15.4 | 0.94 |
| VFA (molar %)   |       |       |       |
| - Acetate       | 59.8 | 61.2 | 1.3  |
| - Propionate    | 25.8 | 26.7 | 1.5  |
| - Butyrate      | 14.3 | 12.0 | 1.2  |

No statistical significant difference
This contrasts with the early experiments reviewed by Mumpton and Fishman (1977) and with their calculation. In our trial the dietary levels of soluble proteins were quite high due to the presence of alfalfa silage, so we can state that when the diet is high in soluble proteins there are some risks of a rise in the urea level in milk. In conclusion, with clinoptilolite we should not in any case expect an improvement in urea values in milk.

No differences for rumen pH between the Control group and Clinoptilolite group were found. Both pH's are high, with respect to the 6.3 pH value, physiological for this stage of lactation. This can be again related to the higher presence of soluble protein in the diet. Johnson et al. (1988) reported non significant changes of rumen pH when natural zeolite was included in the cattle diet, whereas Galindo et al. (1986) observed a reduction of the same rumen parameter.

The inclusion of clinoptilolite in the diet had no effect on rumen concentration of volatile fatty acids (VFA). Molar proportion of acetate, butyrate and minor acids in rumen fluid was not different among treatments. This could mean that clinoptilolite did not adsorb these volatile fatty acids. Johnson et al. (1988) using 2.0% clinoptilolite in the cow diet reported a decrease in propionate concentration with an increase in acetate to propionate ratio. On the contrary, in steers supplemented with clinoptilolite, propionate increased with the 2.5% doses, but not with the 1.25 and 5.0% dose (McCollum and Galyen, 1983).

In Table 5 the data for mineral blood contents are shown. No statistically significant difference was found between the control group and the Clinoptilolite group for Calcium, Potassium, Zinc, and Sodium contents.

The overall mean values of the Na and K in plasma were within the range reported for dairy cows by Bertoni and Piccoli Cappelli (2000). The value for calcium and zinc is over the standard range proposed by the same Authors. We sampled the blood after the morning milking and when the new total mixed ratio was already available; consequently it is possible that the short time span from the meal could have affected the average values, in general very buffered (Bertoni and Piccoli Cappelli, 2000). The same consideration can apply for high zinc values (Bertoni and Piccoli Cappelli, 2000).

The Clinoptilolite in this experiment did not produce any effect on mineral concentration in blood. Dry cows treated with 1 kg of zeolite/d had greater serum Ca concentration in zeolite-treated cows than the control and did not contract milk fever or experience subclinical hypocalcaemia (Thilsing Hansen and Jorgensen, 2001). Sweeney et al. (1980) observed a decrease in potassium concentration in plasma blood of dairy cattle fed zeolite. It’s probable that there is an exchange between potassium ion and sodium ion, depending on the starting composition of the zeolite.

**Conclusions**

The object of this trial was the evaluation of the addition of clinoptilolite to the diet of dairy cows, due to the possible applications of the prod-

| Table 5. Effect of clinoptilolite supplementation on plasma mineral contents. |
|--------------------------|--------------------------|--------------------------|
| Sample                  | Control | Clinoptilolite | SEM |
| Sodium (mmol/l)         | 162.5  | 132.8  | 4.0  |
| Potassium ("           | 3.7   | 3.8   | 0.18 |
| Calcium ("              | 5.1   | 5.0   | 0.17 |
| Zinc ("                 | 20.6  | 21.8  | 1.7  |

*No statistical significant difference*
uct as ion exchanger. The clinoptilolite does not affect milk production, milk fat, milk protein and somatic cell count. The milk urea level can be increased by clinoptilolite inclusion in the diet. This experiment confirms that milk urea is not a parameter of simple interpretation. The result of analysis of rumen fluid indicates the short selectivity of clinoptilolite on ammonia ion.

Finally, in case of use of clinoptilolite in dairy cattle feeding, scarce negative effect on milk production and quality are expected.

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