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ScienceDirect
Livestock Science 113 (2008) 307–310
LIVESTOCK SCIENCE
www.elsevier.com/locate/livsci

Short communication

Effects of natural zeolite clinoptilolite on passive immunity and diarrhea in newborn Holstein calves

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Received 18 May 2007; received in revised form 2 August 2007; accepted 7 August 2007

Abstract

In the present study, the effects of natural zeolite clinoptilolite on absorption of immunoglobulins from colostrum and incidence of enteric diseases were evaluated. In a completely randomised design, thirty Holstein calves were fed pooled colostrum and then milk containing zero (control), 0.5 (T1), 1.0 (T2), 1.5 (T3) and 2.0 (T4) g clinoptilolite per kg body weight per day through day 45. Blood was collected after birth and at 24 h of age and plasma IgG and IgM concentrations were determined. Fecal consistency score and severity of diarrhea were recorded for each calf twice daily. Calves receiving T3 and T4 had lower ($P<0.05$) plasma IgG concentration than control and other treatments. Calves on T2 had higher ($P<0.05$) plasma IgG concentration than T3 and T4, but not T1 and control. Inclusion of clinoptilolite to colostrum did not affect ($P>0.05$) IgM absorption from the intestine of newborn calves. Fecal consistency scores were lower ($P<0.05$) for calves on T1 and T2 and higher for calves on T3 and T4 than calves on control. Percent calf days with diarrhea followed the same trend. In overall, seven calves died, those being one each on control and T1, two on T3 and three on T4. Based upon these results, addition of 1.0 g clinoptilolite per kg body weight per day to colostrum and milk could reduce diarrhea, but its effect on passive immunity was negligible. Over 1.0 g/kg body weight per day, clinoptilolite had adverse effect on passive immunity and diarrhea.

Keywords: Calves; Clinoptilolite; Colostrum; Diarrhea; Immunoglobulin

1. Introduction

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, consisting of three dimensional frameworks of SiO_4^{-4} and AlO_4^{-5} tetrahedra linked through the shared oxygen atoms. Both natural and synthetic zeolites are porous materials, characterized by the ability to lose and gain water reversibly, to adsorb molecules of appropriate cross-sectional diameter (adsorption property, or acting as molecular sieves) and to exchange their constituent cations (ion-exchange property) without major change of their structure (Tomlinson, 1998). The exploitation of these properties underlies the use of clinoptilolite, a main compound of the natural zeolites, in a wide range of industrial and agricultural applications and particularly in animal nutrition since mid 1960s (Mumpton and Fishman, 1977; Sadeghi and Shawrang, 2006). In the medical practice, clinoptilolite is effective as anti-diarrheic drug (Rodriguez-Fluentes et al., 1997), antibacterial and antiviral properties (Grce and Pavelic, 2005). Recently, the European Commission has provisionally authorised the use of clinoptilolite of volcanic or sedimentary origin as additive in feedstuffs

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doi:10.1016/j.livsci.2007.08.010

for farm animals (European Commission Regulation, 2001).

The neonatal calves are born with no immunoglobulins in the blood stream and rely on them from colostrum through passive immunity transfer (Davis and Drackley, 1998). Passive immunity is critical to the survival and health of neonatal calves. Low blood immunoglobulin concentrations are directly related to calf morbidity and mortality (Besser and Gay, 1994), as well as long-term calf performance (Wittum and Perino, 1995). Unfortunately, transfer of passive immunity to neonatal calves is too often inadequate, resulting in excessive rates of morbidity and mortality.

In the literature, there were reported that the addition of zeolites to colostrum could enhance passive immunity and decrease susceptibility of neonates to infections (Mumpton and Fishman, 1977; Stojic et al., 1995; Nikkhah et al., 2002). Our experiment was designed to supply more accurate information on the effects of clinoptilolite to absorption of immunoglobulins and incidence of diarrhea in newborn calves.

2. Materials and methods

2.1. Clinoptilolite source and composition

Clinoptilolite (Anzymite®) was supplied by the Afrandtosca Company (Afrandtosca Co., Tehran, Iran) from Semnan Zeolite Mines located in the central region of Iran. Chemical composition of clinoptilolite used in this study based on X ray diffraction was: 695.3 g/kg SiO_2, 36.7 g/kg CaO, 27.7 g/kg K_2O, 77.5 g/kg Al_2O_3, 6.3 g/kg Na_2O, 14.1 g/kg Fe_2O_3, 1.9 g/kg TiO_2 and 129.4 g/kg LOI (loss of ignition).

2.2. Colostrum collection

Fresh colostrum was collected from donor cows as they calved. First and second milking colostrum was used to make the pools. When enough colostrum had been collected, it was thawed, pooled and refrozen (−20 °C) in 2 L portions in sealed plastic bags. Two 50 ml samples were collected for determining of IgG and IgM concentrations.

2.3. Calves and treatments

Thirty Holstein calves (16 male, 14 female) born between June 8, 2006, and September 6, 2006, at the Animal Research Center of Karaj (Iran) were used. All calvings were supervised to assure that calves had no opportunity to nurse the dam. Calves were removed from the dam within 10 min of birth, moved to the calf facility, weighed, and placed in an individual stall bedded with wheat straw; and their navels were dipped with iodine. Calves were fed, via nipple bottle, 2 L of colostrum containing zero (control), 0.5 (T1), 1.0 (T2), 1.5 (T3) and 2.0 (T4) g clinoptilolite powder per kg body weight per day as soon as possible after birth and 12, 24 and 36 h later. Any treatment volume refused was force-fed using an esophageal feeder. These treatments continued when calves fed milk until day 45.

2.4. Blood sampling

To ensure that calves had no initial circulating immunoglobulin, blood samples were collected from all calves as soon as possible after birth, prior to treatment administration and then 24 h of age. Blood samples were collected by jugular venipuncture into evacuated tubes containing EDTA. Plasma was separated by centrifugation (3000 ×*g* for 15 min) and frozen at −20 °C prior to analysis for IgG and IgM by single radial immunodiffusion (sRID kit; VMRD, inc. Pullman, WA, USA). Apparent efficiency of IgG absorption at 24 h of sampling was calculated as: [plasma IgG concentration (grams per liter) × plasma volume (liters) / IgG intake (grams)] × 100. Plasma volume was assumed to be 9.10% of body weight as previously reported in Holstein calves (Quigley et al., 1998).

2.5. Health status evaluation

The calves were daily observed for signs of disease throughout the monitoring period and any disease problems were recorded. Special attention was given to possible adverse effects noticed in the groups received clinoptilolite. Fecal consistency score and severity were assessed visually and characterized as: 1 = normal (soft without fluid), 2 = soft (semi-solid, mostly solid), 3 = runny (semi-solid, mostly fluid), and 4 = watery (all fluid) according to Larson et al. (1977) for each calf twice daily. The assessor was an experienced person, who was unaware of the treatment each calf was assigned to. Body temperatures were recorded daily for each calf with temperatures ≥ 37.8 °C considered elevated. All health status data were averaged in four periods (days 0–5, 6–12, 13–25 and 26–42).

2.6. Statistical analysis

Analysis of variance was performed using the general linear model procedures of SAS (1996) using a completely randomised design. Birth body weight was evaluated in the model as a covariate, but was not statistically significant ($P > 0.05$). Therefore, unadjusted means are presented. Sex of calf did not contribute significantly ($P > 0.05$) and was not included in the model. When differences were significant ($P < 0.05$), means were separated using Duncan's test (Steel and Torrie, 1980).

3. Results

3.1. Plasma immunoglobulin concentration

The average of plasma IgG and IgM concentrations are in Table 1. Plasma IgG and IgM before feeding were below detectable concentrations of the assay and did not

Table 1
The average plasma IgG and IgM concentrations of calves

Parameters	Control	Treatments				SEM[1]
		1	2	3	4	
Calf birth weight, kg	41.8	42.4	41.2	40.9	41.8	1.82
Plasma IgG, mg/ml						
0 h	<1	<1	<1	<1	<1	–
24 h	15.8[a]	16.2[a]	16.5[a]	12.5[b]	11.8[b]	1.60
Efficiency of IgG absorption, %	26.5[a]	27.6[a]	27.3[a]	20.5[b]	19.8[b]	1.63
Plasma IgM, mg/ml						
0 h	<0.1	<0.1	<0.1	<0.1	<0.1	–
24 h	1.9	2.1	2.0	2.0	1.8	0.18

[a, b] Means in the same row followed by different superscripts differ at $P<0.05$.
[1] SEM: standard error of means.

produce rings on the radial immunodiffusion plates. Therefore, they were assumed to be zero. At 24 h of age, plasma IgG concentration were different ($P<0.05$) among treatments. Calves on T2 had highest, but were not differ with calves on control and T1, and those on T3 and T4 had lowest plasma IgG concentration. Calves on T1 and T4 had highest and lowest ($P<0.05$) apparent efficiency absorption of IgG at 24 h of age, respectively. Addition of zeolite to colostrum had no effect ($P>0.05$) on plasma IgM concentration at 24 h of age. Numerically, calves on T1 had highest and those on T4 had lowest plasma IgM concentration.

3.2. Health status

The calf fecal score, diarrhea severity and mortality are presented in Table 2. Calf fecal consistency scores were lowest ($P<0.05$) for calves on T1 and T2 and highest for calves on T3 and T4. Calves on T4 scoured more ($P<0.05$) days than calves on control and other treatments.

Calf days with rectal temperature greater than 37.8 °C were greater ($P<0.01$) for T3 and T4 than control, T1 and T2 at days 13–25, and were greater numerically at days 26–45 (calves on T4 were statistically different compared to those treatments). Although these variables were statistically different among treatments, these small numerical differences do not suggest biological differences.

Four calves died within the first 5 days of life, those being one each on control, T3 (day 3) and two on T4 (days 3 and 4). In overall, seven calves died in this experiment with no mortality of calf on T2.

4. Discussion

The results of this study confirm the published data which indicate that the dietary use of clinoptilolite in appropriate amount reduces incidence, severity and duration of diarrhea in neonates (Mumpton and Fishman, 1977; Stojic et al., 1995; Rodriguez-Fluentes et al., 1997; Nikkhah et al., 2002), but not enhances passive immunity. In the above studies, beneficial effects of clinoptilolite on passive immunity have been reported, but investigations have not resolved the exact mechanism by which these benefits are achieved. Addition of 0.5 and 1.0 g clinoptilolite per kg body weight per day (i.e., for a 40 kg calf, 20 and 40 g per day or 10 and 20 g in morning and afternoon meals, respectively) to colostrum and milk reduced fecal score and its severity. Clinoptilolite retarding effect on intestinal passage rate and their water adsorption property leads to the appearance of drier and more compact feces (Mumpton and Fishman, 1977). The ameliorative effect of clinoptilolite on diarrhea of calves

Table 2
Calf fecal score, diarrhea severity and mortality of calves through day 45

Parameters	Control	Treatments				SEM[1]
		1	2	3	4	
Average fecal score[2]						
0 to 5 days	1.2[c]	1.0[c]	1.0[c]	1.5[b]	1.8[a]	0.21
6 to 12 days	2.1[c]	1.9[c]	1.8[c]	2.6[b]	3.1[a]	0.24
13 to 25 days	1.5[b]	1.4[b]	1.1[c]	1.7[b]	2.2[a]	0.24
26 to 45 days	1.3[bc]	1.1[cd]	1.0[d]	1.5[b]	1.8[a]	0.19
Calf days with diarrhea, %						
0 to 5 days	5[a]	3[b]	0[c]	3[b]	4[ab]	1.4
6 to 12 days	43[ab]	29[c]	25[c]	41[b]	48[a]	4.8
13 to 25 days	18[c]	17[c]	14[d]	21[b]	25[a]	2.2
26 to 45 days	10[b]	4[c]	3[c]	8[b]	15[a]	2.1
Calf days with temperature ≥37.8 °C, %						
0 to 5 days	10	9	9	10	11	2.5
6 to 12 days	13[bc]	11[cd]	10[d]	15[b]	18[a]	2.0
13 to 25 days	2[c]	2[c]	1[c]	5[b]	7[a]	1.5
26 to 45 days	2[b]	1[b]	1[b]	3[ab]	5[a]	1.7
Mortality status						
0 to 5 days	1	0	0	1	2	–
6 to 12 days	0	0	0	1	1	–
13 to 25 days	0	1	0	0	0	–
26 to 45 days	0	0	0	0	0	–
No. of calves died	1	1	0	2	3	–

[a, b, c, d] Means in the same row followed by different superscripts differ at $P<0.05$.
[1] SEM: standard error of means.
[2] Fecal score system: 1 = normal (soft without fluid), 2 = soft (semi-solid, mostly solid), 3 = runny (semi-solid, mostly fluid), and 4 = watery (all fluid).

maybe results from either the alteration of metabolic acidosis, through effects on osmotic pressure in the intestinal lumen, or the increased retention of the enterotoxigenic *Escherichia coli*. There is no evidence in the available literature for retention of *E. coli* on the outer surface of zeolite particles. However, clinoptilolite is capable to adsorb and partially inactivate enterotoxin of *Escherichia coli*, thus constricting its attachment to the intestinal cell membrane receptors (Ramu et al., 1997).

The adverse effect of T3 and T4 on passive immunity and diarrhea of calves maybe related to increase of the abomasal pH and osmotic pressure in the small intestine because of ion-exchange property of clinoptilolite. Inhibiting effect of clinoptilolite on clotting of colostrum and milk proteins (calves on T3 and T4 excreted fermented whole milk) resulted in increase of fecal score and diarrhea severity.

5. Conclusion

Based upon these results, addition of 1.0 g clinoptilolite per kg body weight per day to colostrum and milk is appropriate dose for reducing incidence and severity of diarrhea in newborn Holstein calves. Extending the clinoptilolite application over 1.0 g/kg body weight per day to colostrum and milk was considered excessive. Over 1.0 g/kg body weight per day, clinoptilolite had adverse effect on passive immunity and diarrhea.

Acknowledgments

The authors gratefully thank the Tehran Science and Research Branch, Islamic Azad University for financial support. They would also especially like to thank Prof. A. Nikkhah for his comments, Dr. M. Amin Afshar for statistical guidance and Mrs. Saeedeh Mousavy for technical assistance.

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