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Central Nervous Disorder of Calves Consuming Colostrum Containing 4 Methyl-Imidazole or Colostrum from Cows Fed Excess Ammoniated Hay

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Abstract

Holstein cows were fed fresh hay with a high sugar content and treated with 6% liquid ammonia as their main ration, for 3 weeks before and after calving. Their colostrum (test milk) was compared to normal colostrum to which 4 methyl-imidazole (4MI milk) had been added, and to normal colostrum lacking 4MI (control). Ten male Holstein calves (2 hours to 12 days of age) were used in this trial. Following the consumption of test milk, 4MI milk or normal colostrum, the symptoms of a central nervous system disorder were compared. Additional parameters monitored included heart function analyzed through electrocardiogram (ECG), auditory brainstem response (ABR), hematological and blood chemistry profiles. Two calves became febrile, hyperexcitable, and displayed an abnormal circling behavior within 2 days after the consumption of test milk. ABR decreased and disappeared intermittently. Four out of 6 calves given 4MI milk developed similar signs and showed a similar disappearance of ABR, while 2 older calves (6 and 12 days) did not develop fever or did not exhibit circling or abnormal ABR. Nearly all the experimental calves showed an elevation of blood pyruvate and lactate concentrations coinciding with the onset of illness. No remarkable changes in other blood components were detected. The autopsy did not reveal any pathological changes. These findings suggest that the age of the calf is related to the toxicity of a compound and in this experiment therefore affected the comparison of clinical signs induced by either 4MI milk or test milk.

Discipline: Animal health
Additional key words: auditory brainstem response (ABR), electrocardiogram, neurotoxicity, feed poisoning

Introduction

Ammoniation of low quality roughages, such as rice or wheat straw, allows them to be used more efficiently as ruminant feeds[2,14]. However, hay with a high sugar content is reported to yield toxins during the process of ammoniation[1,10].

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When hay is fed as a major component of the ration to growing cattle, resulting neurotoxicity has been designated as bovine bonkers or crazy cow syndrome[4,6,11,12]. Also, when ammoniated forage is fed to cows in late pregnancy or early lactation, toxins passing through their milk may cause similar symptoms in the suckling calves.

One potential toxin is considered to be 4 methyl-imidazole (4MI), which is produced by the chemical reaction between sugar and ammonia[1,8,9,12,17]. Concentrations of 4MI are low in ammoniated rice straw and wheat straw, but higher in ammoniated hay because of hay's relatively higher sugar content. It has not yet been determined with certainty, however, whether 4MI is the only causative agent of such poisoning in cattle[16].

In the present study, we investigated possible causes and attempted to characterize the disorder of this condition. Poisoning was induced experimentally in newborn calves which consumed colostrum (test milk) from cows that had been fed ammoniated roughage. Another group of calves consumed normal colostrum to which 4MI had intentionally been added (4MI milk). The experiment allowed a direct comparison of the symptoms which appeared in the 2 groups of calves.

Materials and methods

1) Animals and milk

Ten Holstein calves, ranging from 2 hours to 12 days of age and from 35 to 51 kg in weight, were used for the experiments. Two were fed test milk, 7 were fed 4MI milk, and a control calf was fed colostrum from a cow which had received standard (non-ammoniated) feed.

Test milk consisted of colostrum obtained from 2 cows 1 to 3 days following calving. For 3 weeks before and after delivery, these cows had been maintained on a sole ration of ammoniated grasses. This feed consisted mainly of grasses and weeds with a high sugar content, and had been treated with 6% liquid ammonia. After milking, test milk was stored for as long as 2 months by freezing at −20°C until use. Test milk was given to calves at 6 h and again at 11 h after birth, then 2 or 3 times daily until abnormal clinical signs were noted.

Colostrum from cows on standard ration was used to prepare 4MI milk by the addition of 4 methyl-imidazole. The dose ranged from 50 to 300 mg/kg body weight, or 8.6 to 16.3 mg/calf, respectively (Table 1). Colostrum containing these amounts of 4MI was fed either once or twice during the experiment to each of the 7 test calves. Calf No. 9217 received a 10 to 50 mg dose of 4MI 3 times daily over a period of 10 days (Table 1).

2) Auditory brainstem response (ABR) and electrocardiogram (EGC)

ABR was analyzed by measuring the auditory

Table 1. Experimental design

Calf No.	Age	Weight (kg)	Dose of 4MI (mg/kg)	Repeated administration of 4MI[a] (mg/kg)
4MI group				
9203	6 d	42	200	no
9204	12 d	50	300	150
9212	12 h	35	50	150
9213	8 d	44	200	no
9214	2 d	45	200	200
9215	2 h	41	200	no
9217	2 h	43	10–50/hd[b]	3 × 10 d[c]
Test milk group				
9216	6 h	44	8.6[d]	
9303	11 h	51	16.3	
Control calf				
9205	6 d	47	none	none

a): 4 methyl-imidazole. b): total 4MI dose per head. c): 3 times daily administration for 10 days. d): mg of 4MI contained in colostrum fed.

evoked potentials (Neuropak 4 mini MEB-53407. Nihonkoden Ind. Co. Ltd., Tokyo, Japan). Active (+) electrode was placed at the vertex reference (−) electrode at the root of the ear and a third electrode (ground) on the bridge, respectively. Earphones were positioned on both ears, and the reactions to clicks of 100 db in intensity, at either 100 Hz and 1 kHz were measured. ABR results were expressed as arithmetic means of the response to 1,000 clicks.

EGC was recorded by telemetry using the A-B lead method.

ABR and ECG readings were obtained both 1 h before and 1 h after feeding of test milk, as well as whenever abnormal clinical signs were observed in the experimental calves.

3) Determination of blood components and histopathological examination

Blood was collected by jugular vein puncture once each morning before feeding of test milk, and again at the time of onset of any abnormal clinical signs. The number of erythrocytes and leukocytes, hematocrit and blood glucose levels were determined in freshly collected samples. Then plasma or serum was separated and stored by freezing at −20°C until further analysis.

Total serum protein was determined by refractometry. Individual protein fractions and albumin: globulin ratio by electrophoresis; blood urea nitrogen (BUN) by the diacetylmonoxium method; lactic acid by the method of Barker and Summerson; ammonia, blood sugar, pyruvic acid, aspartate aminotransferase (AST), γ-GTP and CPK activities, total cholesterol, NEFA and TG each by enzymatic methods. Calcium (Ca) and magnesium (Mg) were analyzed using an atomic absorption apparatus. Numbers of erythrocytes and leukocytes were determined by automated cell counts. Hematocrit (Ht) was measured by the capillary tube method.

Finally, experimental calves were anesthetized, necropsied, and their tissues were examined histopathologically.

4) 4 Methyl-imidazole determination

After the test milk was homogenized at 1,000 rpm and 6 strokes, 20 μl of TCA was added to 1 ml of milk for deproteinization. Milk was then centrifuged at 12,000 rpm for 10 min. After the supernatant was filtered (Filter, Shodex MX-13T, Showadenko Co. Ltd. Tokyo, Japan), 4MI concentration was measured by ion pair high speed liquid chromotography[5].

Results

1) Clinical findings

Abnormal clinical signs were first noted 2 days after initial intake of test milk in calf No. 9216, and 3.5 days later, similar signs were observed in calf No. 9303.

In the 4MI group, clinical signs were observed in all the 6 calves except for No. 9217 between

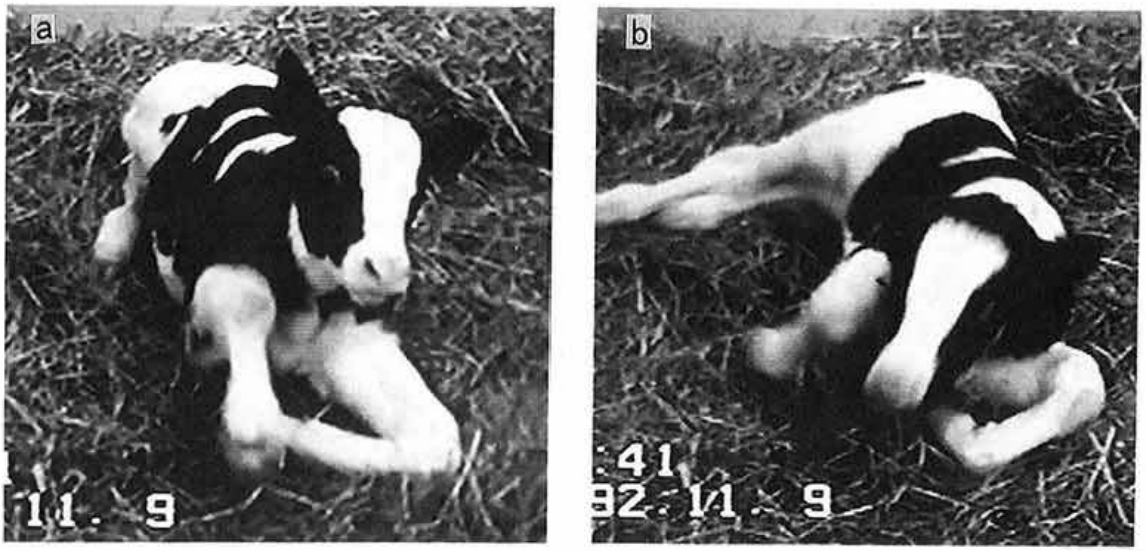

Plate 1. Abnormal clinical signs in the test milk group calf No. 9216
Calf began to circle around the barn for several minutes and fell to the floor (a).
Thereafter epileptoid seizures appeared (b).

1 h and 24 h after the initial administration (Table 1).

In the test milk group, calves first exhibited excessive foamy salivation and reacted to sounds with increased sensitivity. Then, muscular tremors of extremities were accompanied by occasional vocalization (bawling). Suddenly, the animals began to circle around the barn for a period of several minutes. At peak excitement stage, calves fell to the floor with epileptoid seizures lasting from 20 s to several minutes (Plate 1). After rising and resting quietly, there was a recurence of similar seizures. Body temperature was elevated at this time, occasionally reaching 43°C or a higher value.

In the 4MI group, 4 calves (Nos. 9212~9215) exhibited a similar sequential development of clinical signs. Some died after repeated seizures. In comparison, Nos. 9203 and 9204 calves in the 4MI group (treated at 6 and 12 days of age, respectively) displayed hyperexcitability to sounds, muscular tremors and disturbance in the coordination of movements. In contrast to the test milk group, these 2 calves did not develop fever, did not circle, nor develop epileptoid seizures. No. 9217 calf did not show any abnormal symptoms except for a mild, temporary sensitivity to sound stimulation (Table 2).

2) ABR and ECG findings

ABR of 2 calves in the test milk group showed a definite, stable and symmetrical wave pattern before drinking test milk, but during the seizures an abnormal wave pattern appeared. Normal wave component disappeared, reactivity was decreased and the wave pattern became asymmetrical. These changes were transitory. At rest, even in the absence of

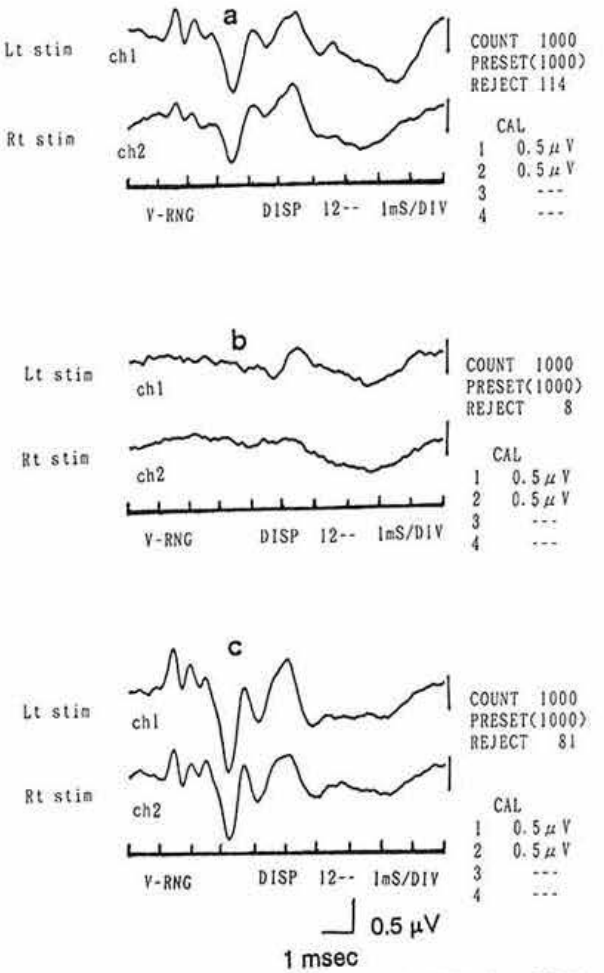

Fig. 1. Auditory brainstem response (ABR) in calf No. 9216 before and after drinking colostrum from cow fed ammoniated hay
a): Before onset abnormal clinical signs.
b): At onset of seizure.
c): After prostration occurred.

Table 2. Abnormal clinical signs

Calf No.	Foamy saliva	Sound reaction	Muscle tremor	Fever sweat	Seizure	Circling	Bawling	Time of onset (h)
4MI group								
9203	+	+	+	−	−	−	+	2
9204	+	+	+	−	−	−	−	2
9212	+	+	+	+	−	+	−	24
9213	+	+	+	+	+	+	+	1.5
9214	+	+	+	+	+	+	+	2
9215	+	+	+	+	+	+	+	1
9217*	−	±	−	−	−	−	−	−
Test milk group								
9216	+	+	+	+	+	+	+	48
9303	+	+	+	+	+	+	+	84
Control calf								
9205*	−	−	−	−	−	−	−	

* See text.

complete recovery, the normal wave pattern reappeared (Fig 1).

Four calves in the 4MI group (treated as early as 2 days after birth) showed similar abnormalities. On the other hand, abnormal ABR patterns were not observed in 2 calves (treated at an older age) which more closely resembled the control.

Tachycardia was evident on ECG after the onset of abnormal symptoms in all the animals in both groups. Tachycardia continued, not only during the seizures, but also at rest, except for calf No. 9214 in the 4MI group which showed temporary bradycardia only during the seizures (Fig 2).

3) Blood components

Few hematological or biochemical abnormalities were detected in comparison with base-line values prior to the ingestion of 4MI or test milk. However, calves in both groups showed higher concentrations of lactic acid and pyruvic acid at the onset of clinical abnormalities. Increase of the lactic acid concentration was especially conspicuous (Fig. 3).

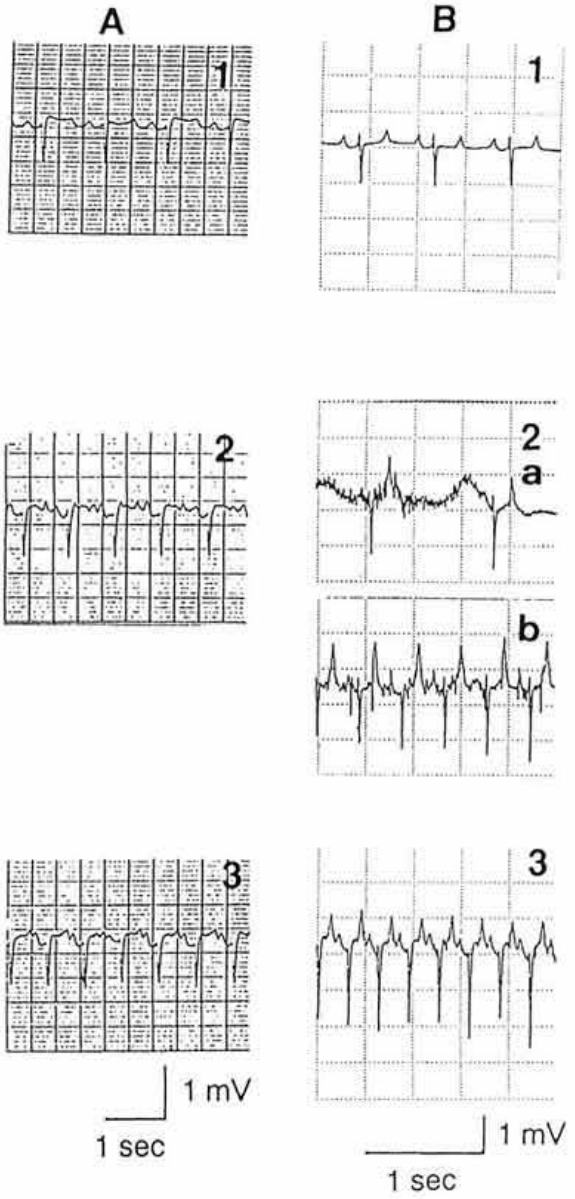

Fig. 2. Electrocardiogram (ECG) of poisoned calves
A: Calf No. 9216 after drinking colostrum from cow fed ammoniated hay.
B: Calf No. 9215 after drinking normal colostrum with 4 methyl-imidazole (4MI) additive.
1: Before onset of abnormal clinical signs.
2: At onset of seizure.
a: Bradycardia in calf No. 9214
b: Tachycardia in calf No. 9215
3: After prostration occurred.

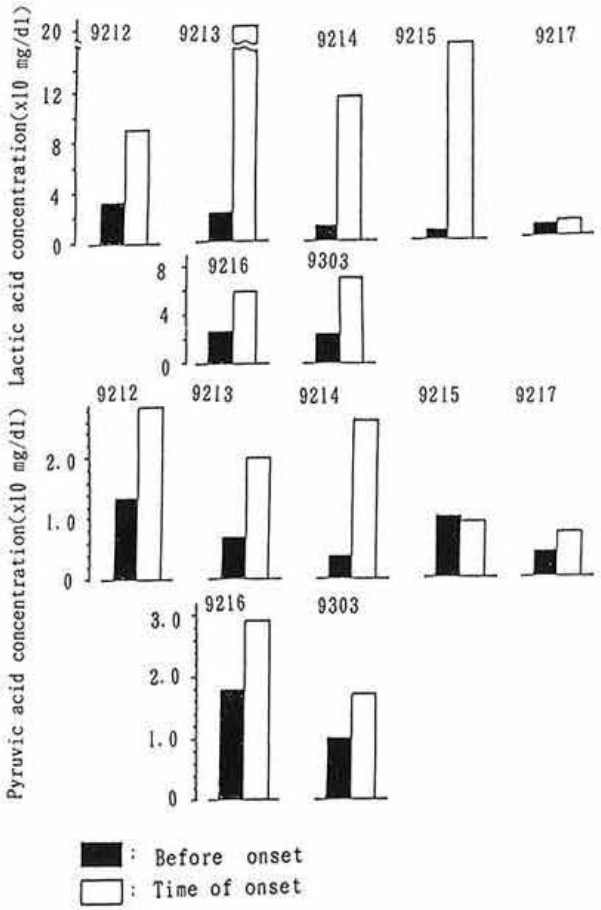

Fig. 3. Changes in serum lactic acid and pyruvic acid levels in calves after receiving normal colostrum with added 4MI (4MI group) or after receiving colostrum from cows fed ammoniated hay (test milk group)
4MI group: Calves 9212 through 9217.
Test milk group: Calves 9216 and 9303.

4) Pathological findings

No gross and microscopic lesions specifically associated with this disease were observed in the experimental calves.

Discussion

Since the introduction of ammoniated molasses in the 1950s, a hyper-excitability syndrome has frequently been observed in cattle consuming this feedstuff[8,9], presumably due to the formation of 4MI by the reaction of ammonia and sugar[3]. It has also been noted that high concentrations of 4MI resulted from ammoniation of roughages, especially hay with a high content of reducing sugars. As with ammoniated molasses, it was inferred that 4MI is a major toxic component resulting from the ammoniation of roughages[8].

In this study, a series of tests monitored the onset of the clinical symptoms in newborn calves consuming colostrum obtained from cows fed ammoniated hay. A comparison was made with calves consuming normal colostrum, to which known concentrations of 4MI had been added. A control calf received normal colostrum without the addition of 4MI.

Within 2 days after birth, calves fed colostrum with added 4MI developed abnormal clinical signs, abnormal ABR and ECG recordings, and blood chemistry profiles very similar to those of the calves fed colostrum from cows maintained on ammoniated hay. The major clinical signs in common were hyperexcitability, circling behavior and seizures, as previously reported. However, at this stage of illness, calves were able to perform coordinated movements without apparent lameness and without abnormal twisting of the head and neck. No pathological lesions for this disease were detected by gross and microscopic examinations.

It was therefore inferred that the ingestion of 4MI induced this functional abnormality in the brain stem at a level higher than the cerebellum. For this reason it seemed that testing of the central nervous system function by encephalometry techniques might be informative. In preliminary experiments, it was determined that ABR could be monitored without effect on the wave pattern, both in the presence or the absence of anesthesia in experimental calves. Therefore anesthesia was not required in this study. ABR of normal or diseased cattle had not been previously reported.

In calves in the test milk group and calves in the 4MI group, ABR disappeared during the seizures, for unknown reasons. In human beings, the disappearance of ABR is noted in meningitis and alcoholism, or can be caused by trauma, or by tumors of the brain stem or medulla oblongata[15].

It is generally recognized that the nervus vestibulocochlearis does not normally transfer sound signals to the central nervous system, but maintains a tonic state when loud sounds reach the external ear. At that time the wave pattern in the first half corresponding to the response of the nervus vestibulocochlearis disappeared[15]. In this study, during a seizure, not only the first half of the wave pattern reflecting the function of the middle ear, but the second half corresponding to the function of the brain stem also disappeared. Therefore, during the seizure, it is assumed that the central nervous system[16] shows widespread impairment.

In this study, calves treated with 4MI at 6 and 12 days of age did not develop any abnormality in ABR or in the blood components. This difference in age susceptibility may be due to the lack of absorption of toxin or simply due to the growth of the calf.

The cause of tachycardia in all the calves associated with the onset of abnormal clinical signs appeared to be due to the stimulation of the sympathetic nerve pathways. Bradycardia was likely due to change in respiration accompanying the seizures.

In both treatment groups in this study, a marked febrile response accompanied the onset of abnormal clinical signs. Fever was not noted, however, in older experimental calves. It is therefore suggested that 4MI or another toxin was absorbed into the ventricle of the newborn calf, which stimulated the thermoreceptor cells of the brain. Fever had not previously been reported as a symptom of this toxic syndrome.

In the colostrum obtained from cows fed ammoniated hay, a fluorescent alkaloid compound highly toxic for mice was detected, and was yielded under the same conditions which resulted in the formation of 4MI. However, this compound was very unstable, with a chemical structure hardly identifiable[16]. Toxicity of ammonia itself was also considered, but blood chemistry ruled out direct ammonia poisoning.

In this study an increase in the blood lactate and pyruvate concentrations was detected. In previous reports[16], these increases were attributed to thiamin deficiency. However, in this study, thiamin levels were within expected normal ranges. Since similar increases were noted in the 4MI group, they were probably caused by vigorous muscle movements and

associated with the depletion of muscular glycogen.

Since the 4MI concentration within the colostrum of cows fed ammoniated hay ranged from 0.2 to 104 ppm, the total dose of 4MI given to calves in the test milk group ranged from 8 to 16 mg. However in the 4MI group, symptoms were noted only after treatment with 4MI at 15 times this level. These results suggest that the causative substance is not exclusively 4MI[7,13]. Furthermore, since different clinical signs were noted in calves at 6 and 12 days of age, other toxins than 4MI may be involved.

Based on the results of this study, it was confirmed that when an excessive amount of ammoniated hay was fed to pre-parturient cows, a central nervous disorder developed in newborn calves through the milk, and that the clinical manifestations observed closely resembled those noted in newborn calves exposed to high doses of 4MI. Consequently, an excessive supply of ammoniated roughages with a high content of reserve carbohydrates to cow should be avoided.

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