

## A Simple Test for the Demonstration of Abomasal Juice in Rumen Samples of Young Calves

Digestive disorders were shown to constitute the most important group of diagnoses among dairy calf mortalities in Denmark (Lauridsen 1987). Although infectious agents undoubtedly play an important role in such fatalities, Dirksen & Garry (1987) have drawn the attention to the importance of non-infectious diseases in the forestomachs of young artificially reared calves.

Abnormal ruminal content with a pH  $\leq 5$ , may be due to ruminal drinking (van Weeren *et al.* 1988) or abomaso-ruminal reflux (Dirksen & Garry 1987). In farm investigations, differentiation between these 2 conditions is of importance when efforts are made to associate digestive disorders with nutrition and management. Dirksen & Garry (1987) describe the clinical symptoms associated with chronic ruminal drinking and they suggest chloride determination of rumen liquor samples as a means of diagnosing abomaso-ruminal reflux. However, Dirr (1988) found that chloride titration was of limited value due to difficulties of defining a normal range.

The present investigation introduces a new principle for the diagnosis of abomaso-ruminal reflux in calves. It is based on the presence of the specific milk coagulation factor chymosin (rennin) in the abomasal juice of calves and it thus utilizes the ability of rumen samples from reflux calves to coagulate milk. Four healthy 2 to 3 week old male Jersey calves with a bodyweight of 26-28 kg were used. The calves had been bucket-fed twice

daily with 2 plus 2 liters of a skim milk based milk replacer. In addition, they had access to a calf starter concentrate and hay and water.

Rumen liquor samples were obtained with the apparatus described by Geishhauser (1990) fitted with a calf stomach tube (article 480010, Eichemeyer, D-7200 Tutthingen). Samples were taken before and at intervals after the experimental meal was given.

Calves no. 1 and 2 were given 500 ml milk replacer milk by stomach tube. Calves 3 and 4 were also given 500 ml milk but, in addition, they received 100 ml of abomasal content obtained from a calf slaughtered 90 min after it had been fed a milk replacer meal of 2 l. The pH of the rumen samples was determined by a standard laboratory pH-meter.

The rumen samples were tested as follows. Freshly obtained milk from a healthy cow was mixed with the rumen samples in amounts indicated below in black California Mastitis Test (CMT) plates used for semi-quantitative cell counts in milk (Dittman Moore, Langenfelde, D-3006 Burgwedel 1). Milk was added to the 4 compartments of the CMT plates and milk in excess of 2 ml was poured off. The following amounts of rumen liquor were added to each compartment by means of a disposable syringe: a: 0.25 ml, b: 0.50 ml, c: 1.0 ml and d: 2.0 ml. The plates were agitated in horizontal circular movements and left at room temperature for 15 min. Reading was performed at intervals indicated in Table 2. When no coagulation

Table 1. pH of the rumen at various time before and after tube feeding.

Calf No.	30 min. before	30 min. after	60 min. after	90 min. after	240 min. after
1	6.8	5.7	5.4	n.d.	n.d.
2	6.4	4.9	4.8	4.8	6.1
3	6.4	5.2	5.3	5.5	6.2
4	6.8	5.4	5.2	5.2	n.d.

n.d. = not done.

was observed, the plates were incubated for another 15 min at 38°C. When the mixture of milk and rumen sample appeared as junket the test was recorded as having coagulated.

Table 1 shows a drop in pH in all 4 calves following tube feeding with 500 ml of milk replacer. It also shows that the pH remained

lower than before drenching, for more than 90 min. The addition of 100 ml of abomasal juice appeared not to influence pH of the ruminal fluid.

The results of the milk coagulation test presented in Table 2 clearly reflect the experimental conditions. No spontaneous coagula-

Table 2. Results of the milk coagulation test. The following amounts of rumen liquor were added to the 4 compartments of the CMT plates: a: 0,25 ml, b: 0,50 ml, c: 1,0 ml, d: 2,0 ml. Coagulation is indicated with a »C« whereas »-« indicate a negative test (absence of coagulation).

Time (min)	Test temp (°C)	Reading time (min)	Calf No.:															
			1				2				3				4			
			a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
-30	20	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	38	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+30	20	5	-	-	-	-	-	-	-	-	-	-	C	C	-	C	C	C
	20	10	-	-	-	-	-	-	-	-	-	C	C	C	-	C	C	C
	20	15	-	-	-	-	-	-	-	-	-	C	C	C	-	C	C	C
	38	15	-	-	-	-	-	-	-	-	C	C	C	C	C	C	C	C
+60	20	5	-	-	-	-	-	-	-	-	-	-	C	C	-	C	C	C
	20	10	-	-	-	-	-	-	-	-	-	C	C	C	-	C	C	C
	20	15	-	-	-	-	-	-	-	-	-	C	C	C	-	C	C	C
	38	15	-	-	-	-	-	-	-	-	C	C	C	C	C	C	C	C
+90	20	5	*	*	*	*	-	-	-	-	-	-	C	C	-	-	C	C
	20	10	*	*	*	*	-	-	-	-	-	-	C	C	-	C	C	C
	20	15	*	*	*	*	-	-	-	-	-	-	C	C	-	C	C	C
	38	15	*	*	*	*	-	-	-	-	C	C	C	C	C	C	C	C

\* no sample obtained due to blocking.

tion occurred before drenching or in the 2 calves (calf no 1 and 2) after drenching with milk replacer alone. In contrast, the addition of 100 ml of abomasal juice could be traced consistently up to 90 min after drenching when 1 or 2 ml of rumen liquor was added to 2 ml of milk. Less than 1 ml gave consistent results at 38°C but not at room temperature (20°C).

With the equipment used, samples of rumen liquor were easily obtained. However, at 1 occasion of the 16 samplings planned, blocking of the tube had occurred.

The recorded drop in ruminal pH after drenching with milk replacer was to be expected parallel to the finding by others who have made observations on spontaneous or experimentally induced ruminal drinkers (*van Weeren et al.* 1988, *Dirr* 1988, *Dirksen & Garry* 1987). On the other hand, reflux of abomasal juice, of the magnitude induced in the present experiment, i.e. approximately 20% of the expected rumen volume (*Nickel et al.* 1982), did not appear to lower rumen pH in itself. However, spontaneous reflux may behave differently when abomasal juice plus milk is refluxed.

The coagulation test was simple to perform since it only required fresh milk and a CMT plate. The results indicate that the admixture of 1 ml of rumen liquor with 2 ml of fresh milk in a CMT plate at room temperature will reveal within 15 min whether or not abomaso-ruminal reflux has occurred in the calf during the preceding 90 min.

The evaluation of the milk coagulation test under field conditions remains to be made. Hopefully the test will prove useful in clarify-

ing the significance of reflux to production and health in young dairy calves.

#### Acknowledgement

This investigation was supported by a grant from Leo Pharmaceutical Research Foundation.

*R. J. Jørgensen, A. Agerholm, S. Jessen and T. Thorsen*

Department of Clinical Studies, Division of Internal Medicine, Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

#### References

- Dirksen G, Garry F*: Diseases of the forestomachs in calves. *Comp. cont. Educ. Vet. Pract.*, 1987, 9, 140-147, 173-179.
- Dirr L*: Untersuchungen über die Dysfunktionen des Schlundrinnenreflex beim jungen Kalb (Investigations on the oesophageal groove reflex in young calves). Veterinary Faculty, Ludwig-Maximilians University, Munich, 1988. Thesis.
- Geishauser T*: Entwicklung und Prüfung eines Gerätes zur Pansensaftentnahme und -übertragung sowie zur eingabe flüssiger Arzneimittel beim erwachsenen Rind (Development and test of an apparatus for rumen liquor sampling and -transfer as well as for drenching in adult cattle). *Proc. XVI World Buiatrics Congr. Salvador, Brazil, 13-17 August 1990*, p.685-690.
- Lauridsen B H*: Obduktionsfund i kalvemateriale tilført destruktionsanstalt (Post mortem diagnosis of calves at a rendering plant). *Dansk Vet. Tidsskrift*, 1987, 70, 425-433.
- Nickel R, Schummer A, Seiferle E*: Lehrbuch der Anatomie der Haustiere (Textbook of the anatomy of domestic animals). Vol. II. 5. Ed, Paul Parey Publ. Berlin/Hamburg 1982, p. 161-167.
- van Weeren A, Breukink H J, Mouwen J M V M, Wensing T*: Clinical and pathological consequences of ruminal drinking in a calf model. *Proc. XV World Buiatric Congr., Palma de Mallorca, Spain 1988*, 445-448.

(Received December 16, 1991; Accepted February 3, 1992).

Reprints may be requested from: Rolf Jess Jørgensen, Department of Clinical Studies, Division of Internal Medicine, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark.