Effect of curd suppression in a milk replacer on physiological parameters in calves. I. Digestibility of nutrients and body mass-gain

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Two trials were conducted to determine the effect of coagulation suppression of milk and milk replacers on apparent digestibility coefficients and body mass-gain of calves. In Experiment 1, two groups of five Holstein bull calves, two to four days of age, were given a milk replacer in which casein coagulation either was normal (CM), or was prevented by the precipitation of Ca^{++} with an oxalic acid - sodium hydroxide buffer (NCM). Apparent digestibility of dry matter, organic matter, crude protein and fat was 87 and 91%, 88 and 92%, 75 and 84% and 83 and 87% for the NCM and CM treatments, respectively. Treatment means did not differ statistically, although there was a tendency towards lower crude protein digestibility for the NCM treatment. Owing to Ca^{++} precipitation in the one treatment, apparent ash and calcium digestibility (availability) coefficients were significantly lower for the NCM treatment than for the CM treatment, viz. 65 vs. 83% and 29 vs. 78%, respectively. In Experiment 2 (growth trial), four groups of seven Friesian bull calves, two to four days of age, were used. Whole milk (WM) and a commercial coagulable milk replacer (CM), were used either directly, or after treatment with oxalic acid (NWM and NCM). Body mass-gain (kg) and efficiency of feed conversion (kg dry matter intake/kg gain) over the 28-day experimental period were respectively 7,3 and 1,8 (WM); 7,3 and 1,8 (NWM); 4,3 and 3,9 (CM) and 4,9 and 2,9 (NCM). Both whole milk treatments were superior to milk replacer treatments, but prevention of coagulation *per se* had no significant effect on calf performance and on the occurrence of diarrhoea.

Twee proewe is uitgevoer om die invloed van voorkoming van koagulering van melk en melksurrogate op skynbare verteerbaarheidskoëffisiënte en massatoename van kalwers te bepaal. Twee groepe van vyf Holsteinbulkalwers, tussen twee- en vier-dae-ouderdom, is in Eksperiment 1 gebruik. Een melksurrogaat is geformuleer en kaseïenkoagulering is in die een behandeling (NCM) voorkom deur die presipitering van Ca^{++} met 'n oksaalsuur-natriumhidroksiedbuffer. Die ander groep kalwers (CM) het onbehandelde (koaguleerbare) melksurrogaat ontvang. Skynbare verteerbaarheid van droëmateriaal, organiese materiaal, ruproteïen en vet was 87 en 91%, 88 en 92%, 75 en 84% en 83 en 87% vir die NCM- en CM-behandelings, onderskeidelik. Verskille tussen behandelingsgemiddeldes was nie statisties betekenisvol nie, hoewel daar 'n neiging tot laer ruproteïenverteerbaarheid by die NCM-behandeling was. As gevolg van Ca^{++} presipitering in die een behandeling, was skynbare as- en kalsiumverteerbaarheid (-beskikbaarheid) betekenisvol laer vir die NCM-behandeling as vir die CM-behandeling, naamlik 65 vs. 83% en 29 vs. 78%, onderskeidelik. In Eksperiment 2 (groeistudie), is vier groepe van sewe Friesbulkalwers, tussen twee- en vier-dae-ouderdom, gebruik. Die behandeling was volmelk (WM), nie-koaguleerbare volmelk (NWM), 'n kommersiële koaguleerbare melksurrogaat (CM) en oksaalsuurbehandelde melksurrogaat (NCM). Massatoename (kg) oor die 28-dae-proefperiode en doeltreffendheid van voeromsetting (kg droëmateriaalinname/kg massatoename) was onderskeidelik 7,3 en 1,8 (WM); 7,3 en 1,8 (NWM); 4,3 en 3,9 (CM) en 4,9 en 2,9 (NCM). Hoewel albei volmelkbehandelings betekenisvol beter resultate gelewer het as die melksurrogaatbehandelings, het die voorkoming van koagulering *per se* geen invloed op kalfprestasie en op die voorkoms van diarree gehad nie.

Keywords: Body mass-gain, calves, casein curd formation, digestibility, milk replacers.

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It has been shown that abomasal curd development resulted in extended retention times of milk replacer components, and therefore had a delaying effect on abomasal emptying rate of these components (Cruywagen, Brisson & Meissner, 1990). Since an extended retention time of milk replacer components in the abomasum exposes the nutrients to prolonged gastric digestion, it is possible that coagulable milk replacers may result in higher digestibility than non-coagulable milk replacers. However, contradictory results have been reported in the literature.

According to Roy (1974), the inability of non-milk proteins to clot in the abomasum results in reduced protein digestibility, especially during the first three weeks of life. Evidence of the beneficial effect of curd development on the digestibility of protein and lipids was reported (Guilloteau, Toullec & Patureau-Mirand, 1979; Paruelle, Toullec, Frantzen & Mathieu, 1972) and it was suggested (Jenkins & Emmons, 1979) that at least part of the better calf performance obtained with low-pressure dispersion of fat in a milk replacer, compared to homogenization, might have resulted from the firmer curd development in the abomasum observed with the former technique. Bouchard, Brisson & Julien (1973) reported that large amounts of whey and bacterial sludge (rendering the milk replacer virtually incoagulable) did not have any effect on DM and CP digestibility. Although clot prevention in whole milk resulted in reduced apparent DM digestibility, Jenkins & Emmons (1982) reported that, for milk replacers based on skimmed milk powder, whey, fish protein and tallow, clot prevention had no effect on the digestibility of DM, nitrogen and lipids.

Age of the calf has a marked effect on the digestibility of nutrients. Lister & Emmons (1976) reported that calves under the age of three weeks require a diet based on skimmed milk that has not been severely heat-treated, and that curd formation is a suitable index for the quality of such milk replacer diets. According to Toullec, Frantzen & Mathieu (1974) and Toullec, Mathieu, Vassal & Pion (1969), suppression of coagulation had no effect on digestibility of nutrients in calves more than three weeks of age. However, digestibility was markedly reduced when curd development was inhibited in calves under three weeks of age.

In many documented studies on the effect of coagulation on digestibility of nutrients, milk protein was either substituted by non-coagulable proteins, or predigested to render the protein incoagulable. In the present study, coagulation was prevented by the precipitation of Ca^{++}. The protein source was therefore not denaturated.

Two trials were conducted in the present study to obtain:

1. apparent digestibility coefficients of coagulable and non-coagulable milk replacer components in calves under two weeks of age and
2. growth rate and efficiency of feed conversion in calves receiving coagulable and non-coagulable milk or milk replacer from birth to 30 days of age.

The second trial was planned after results of the first trial had been analysed.

Materials and Methods

Experiment 1

Ten male Holstein calves, two to four days of age, were purchased from dairy farmers in areas surrounding Québec City, Canada. After weighing, calves were allotted to two treatments in a randomized block design, stratified according to initial body weight. Animals were kept individually in 1,37 × 0,76 m elevated wooden pens with partially slatted wooden floors.

A coagulable milk replacer, containing 43,2% low-heat skimmed milk powder, 25,8% crude protein and 21,1% fat, was formulated (Cruywagen *et al.*, 1990). Curd formation was inhibited in one of the treatments by precipitation of Ca^{++} in the milk replacer (MR) with an oxalic acid (0,25 M) — NaOH (0,35 M) buffer solution. This buffer solution completely inhibited curd formation, and restored pH to 6,5 when added to the reconstituted milk replacer at a rate of 6,6 ml/10 g MR powder. Throughout the trial, each calf received 12 g MR powder/kg initial body mass on a daily basis. The milk replacer was reconstituted with water (45 °C) to 13% dry matter. The coagulating, untreated milk replacer was fed to one group (CM treatment), while the other group received the non-coagulating, oxalic acid-treated milk replacer (NCM treatment). In the latter case, a reaction time of 15 min was allowed before feeding to ensure sufficient precipitation of Ca^{++}. This treatment has previously been used to study the role of coagulation suppression in young calves (Petit, Ivan & Brisson, 1987; Cruywagen *et al.*, 1990). Animals were bucket-fed twice daily at 08h00 and 16h00.

Following an adaptation period of four days, faeces were quantitatively collected over a period of seven days in preweighed plastic bags which were attached to permanently fixed chute bags glued to the calves. Bags were changed daily and the wet faecal mass was accurately determined. The bags containing the wet excretion were stored at −25 °C up to the end of the collection period. After completion of the trial, the faeces were thawed, thoroughly mixed, and the samples pooled in order to obtain the total excretion of each calf. Representative samples of *ca.* 200 g were taken from the pooled sample of each calf, freeze-dried, and stored at 4 °C, until required for chemical analysis.

Faecal dry matter and nitrogen contents were determined following procedures described for abomasal contents by Cruywagen *et al.* (1990). Faecal fat determination was based on the petroleum ether extraction of fat, following HCl hydrolysis (Frankel, Reitman & Sonnenwirth, 1963). Ash was determined by incineration at 550 °C for 12 h. For calcium determination, the cooled ash was dissolved in a few drops of nitric acid, transferred to a 25-ml volumetric flask and brought to volume with distilled water. Appropriate dilutions were made with 1% lanthanum chloride and calcium was determined with the aid of an atomic absorption spectrophotometer at a wavelength of 211 nm.

In order to monitor blood calcium, a sample was drawn from the jugular vein of each calf prior to the morning (08h00) feeding on the second, as well as the last day of the trial. Blood was obtained by venipuncture into partially evacuated tubes containing potassium–EDTA as anticoagulant. The blood was immediately chilled, centrifuged, and the resulting plasma was stored in 10-ml vials at −25 °C until required for chemical analysis.

Total concentration of calcium in plasma was also determined by the atomic absorption technique. Plasma samples were prepared for analysis by adding 4 ml distilled water to 0,25 ml plasma, followed by the addition of 0,5 ml stock lanthanum solution (Perkin–Elmer, 1973). The solution was then accurately diluted to 5 ml. This method of dilution prevented the precipitation of protein in the plasma. Calcium in the solution was determined as described above.

Experiment 2

Twenty-eight male Friesian calves, two to four days of age, were obtained from the dairy herd of the Highveld Region (Potchefstroom) as well as from dairy producers in the Potchefstroom–Viljoenskroon Area. The animals were weighed and then allotted to four treatments in a randomized block design, stratified according to initial body mass. Animals were kept individually in 1,50 × 0,60 m elevated pens with expanded metal floors.

Calves received either a coagulable commercial milk replacer (CM), the same milk replacer treated with oxalic acid (NCM), whole milk (WM), or whole milk treated with oxalic acid (NWM). The milk replacer contained 40% skimmed milk powder, 23% crude protein, and 13% fat. The amount of oxalic acid added, and feeding schedule were the same as described above. No dry rations were offered, and the trial lasted for 28 days.

For both trials, the experimental design was a randomized block and an analysis of variance was performed on the data (Little & Hills, 1975). *F* tests were executed to determine significant differences.

Results and Discussion

Experiment 1

Apparent digestibility coefficients of all relevant nutrients are presented in Table 1.

No significant difference was found between treatments with regard to apparent dry matter (DM) and organic matter (OM) digestibilities. Variation in digestibility values was higher in the NCM treatment than in the CM treatment. Dry matter digestibilities of 77,0 and 82,3%, and OM digestibilities of 78,6 and 83,4% were reported by Toullec *et al.* (1969) for preruminant calves from 15—26 days of age, receiving a milk replacer which contained whey as the sole source of nitrogen. According to Toullec *et al.* (1974), DM and OM digestibilities in calves between 14 and 20 days of age were 94,7 and 94,2% for a coagulable milk replacer, and 86,5 and 87,6% for a non-coagulable milk replacer, respectively. In the latter treatment, coagulation was prevented by the addition of either sodium citrate or hydrochloric acid.

The abomasal curd consists predominantly of protein (casein) and fat. Since it had been shown that the CM treatment resulted in significantly extended retention of abomasal crude protein (CP) (Cruywagen *et al.*, 1990), improved CP digestibility was anticipated for this treatment as a result of more extensive gastric proteolysis and a relative slow passage rate through the duodenum. However, although the difference between treatment means was 8,7%, this difference did not prove to be statistically significant. It should be mentioned that variances for CP digestibility were not homogenous and two non-parametric tests, viz. Mann-Whitney (Snedecor & Cochran, 1980) and 'bootstrap' (Diaconis & Efron, 1983), were also used to analyse the data. The Mann-Whitney test indicated no significant differences ($P > 0{,}05$), whereas 'bootstrap' did indicate significant differences ($P < 0{,}05$). It is therefore concluded that, although curd suppression did not affect CP digestibility significantly, there was at least a tendency towards lower CP digestibility in the NCM treatment.

Bouchard *et al.* (1973) reported apparent CP digestibilities of 84,4% for a milk replacer based on skimmed milk (coagulable) and 87,9% for a milk replacer based on dried bacterial sludge and whey powder (non-coagulable). When alkane yeast meals and soybean meal provided 70—75% of the total protein of milk replacers (the rest being supplied by whey), CP digestibilities of 85 and 79%, respectively, were reported (Paruelle *et al.*, 1972) for calves older than one month. Digestibility was about 10 percentage points lower in both cases for calves between 15 and 24 days of age. They suggested that the lower CP digestibility at a younger age was due to insufficient adaptation of the digestive system of the very young calf to the change of the gastro-intestinal transit resulting from the absence of protein coagulation in the abomasum. According to Toullec *et al.* (1974), CP digestibility in calves between 14 and 20 days of age, was 91,9% for a coagulable milk replacer and 87,0% for a non-coagulable milk replacer. The difference was not significant due to the large variation observed in both treatments. Grongnet, Patureau–Mirand, Toullec & Prugnaud (1981) reported apparent CP digestibilities of 87,1% for a coagulable milk replacer and 88,8 and 85,9% for two milk replacers manifesting poor curd development. Crude protein digestibilities of 73,5% and 96,5% were reported by Cruywagen (1982) for milk replacers which contained 20% and 50% colostrum, respectively. The above documented results clearly show that important differences in crude protein digestibility can be observed between various protein sources incorporated in milk replacers for young calves. In most cases, however, it is impossible to distinguish between treatments solely on the basis of curd formation in the abomasum. In the present study, curd formation was prevented without protein denaturation in order to isolate the effect of coagulation suppression.

Prevention of abomasal curd development had no detrimental effect on apparent fat digestibility (Table 1). Contradictory results have previously been reported. According to Guilloteau *et al.* (1979) and Jenkins, Kramer & Emmons (1981), curd development resulted in improved fat digestibility, whereas Jenkins & Emmons (1982) suggested that clot prevention had no detrimental effect on the digestibility of lipids in milk replacers. Toullec *et al.*, (1974) reported that apparent fat digestibility in calves between 14 and 20 days of age was 89,8% for a milk replacer based on skimmed milk powder, and 76,5% and 69,0% when coagulation was prevented by the addition of citrate and hydrochloric acid, respectively.

Apparant total mineral (ash) and calcium digestibilities (availabilities) were significantly higher ($P < 0{,}01$) for the CM treatment than for the NCM treatment. A major part of the lower mineral availability observed for the NCM treatment may be ascribed to the precipitation of Ca^{++} by oxalic acid. This resulted in the formation of an insoluble calcium-oxalate complex. Although most of the calcium in the NCM treatment reacted with oxalic acid, it is of interest to note that this treatment did not result in complete inavailability of calcium.

Table 1 Apparent nutrient digestibility of coagulable and non-coagulable milk replacers (%) fed to calves ($x \pm SD$)

	Treatment[a]		
Nutrient	NCM	CM	Calculated *F* value
Dry matter	86,6 ± 4,7	91,0 ± 1,8	5,76
Organic matter	88,4 ± 4,4	91,8 ± 1,2	3,36
Crude protein	75,0 ± 10,6	83,7 ± 3,6	2,48
Fat	83,3 ± 8,5	86,5 ± 4,6	0,66
Ash	64,6 ± 5,2	82,8 ± 5,6	43,80**
Calcium	28,7 ± 4,6	78,1 ± 8,6	97,95**

[a] NCM = non-coagulable milk replacer; CM = coagulable milk replacer.

** $P < 0{,}01$.

Table 2 Overnight fasting blood calcium levels (mM/*l*) of calves which received coagulable and non-coagulable milk replacers (x ± *SD*)

Age (days)	Treatment[a] NCM	CM	Calculated *F* value
4	2,18 ± 0,13	2,29 ± 0,11	2,31
14	3,08 ± 0,09	2,92 ± 0,07	17,01*

[a] NCM = non-coagulable milk replacer; CM = coagulable milk replacer.
* $P < 0{,}05$.

Overnight fasting blood calcium levels of calves are presented in Table 2.

Calcium-binding substances such as oxalates and phytates are known to depress Ca availability in non-ruminant and preruminant animals (Braithwaite, 1976). In the present study, blood plasma Ca was near 2 mM/*l* at four days of age, and near 3 mM/*l* at 14 days of age (Table 2). There was no statistical difference in blood Ca between treatments at four days, but at 14 days, blood Ca in calves fed NCM was higher ($P < 0{,}05$) than in those fed CM. Fisher (1976) reported fasting blood Ca levels of 3,04 mM/*l* at nine days of age for calves which received whole milk. Results from the present study suggest that sufficient Ca was made available for maintaining normal blood levels in NCM-fed calves, even when part of the Ca was precipitated with oxalic acid. Although it appears that sufficient Ca may have been absorbed from NCM to maintain normal blood levels, a contribution from body reserves may have supplemented these levels.

Prevention of abomasal curd development did not affect the occurrence of diarrhoea. Mean diarrhoea days per calf during the 11-day digestibility trial were 1,6 for calves in the CM treatment and 1,8 for those in the NCM treatment. Therefore, diarrhoea could not explain tendencies in digestibility coefficients observed in the present work, nor could it explain greater individual variation observed among calves on the NCM treatment compared to the CM treatment.

Table 3 Body mass-gain (kg) and efficiency of feed conversion (kg dry matter intake/kg gain) of calves which received coagulable and non-coagulable milk or milk replacer up to one month of age (x ± *SD*)

Item	Treatment[1] WM	NWM	CM	NCM
Body mass-gain	7,3 ± 0,6[a]	7,3 ± 1,0[a]	4,3 ± 1,9[b]	4,9 ± 1,0[b]
EFC[2]	1,8 ± 0,2[a]	1,8 ± 0,3[a]	3,9 ± 1,6[b]	2,9 ± 0,7[b]

[1] WM = coagulable whole milk; NWM = non-coagulable whole milk; CM = coagulable milk replacer; NCM = non-coagulable milk replacer.
[2] Efficiency of feed conversion.
[a,b] Values with different superscripts differed highly significantly ($P < 0{,}01$).

Experiment 2

Experiment 2 was conducted to examine the effect of curd prevention of whole milk and milk replacer on body mass-gain and efficiency of feed conversion (EFC) of calves up to one month of age. Results are presented in Table 3.

Feeding whole milk to calves resulted in higher ($P<0{,}01$) body mass-gains and superior EFC ratios (see Table 3). Preventing abomasal curd development, however, did not impair growth or EFC when either whole milk or the milk replacer was fed. Treatments had no effect on the occurrence of diarrhoea.

These results contradict those reported by Toullec *et al.* (1974), where clotting was prevented by the addition of hydrochloric acid, as well as results obtained by Lister & Emmons (1976) who evaluated skimmed milk powder that was spray-dried at high and low temperatures. In the latter case, impaired gains observed for calves which received high-temperature treated milk powder were ascribed to a reduction in curd firmness. Jenkins & Emmons (1982) obtained only slightly lower (non-significant) weight gains up to four weeks of age for calves receiving non-coagulable milk or milk replacer compared to coagulable diets.

Conclusion

In the present study, the effect of curd suppression in the abomasum was studied with milk or milk replacers based on the same source of undenaturated protein. It is therefore believed that the ability of the milk replacer to coagulate in the abomasum was the only variable between treatments.

Milk is the natural food for young calves, and the casein curd-forming ability of milk, as well as milk replacers based on milk products, may be essential for the well-being of young calves. However, in the present work, the absence of curd development had no effect on body mass-gain, EFC ratio, or the occurrence of diarrhoea, and only tended (not significantly) to decrease OM, CP and fat digestibility. The actual advantage of abomasal curd development was not readily apparent under these circumstances. Under the conditions of the present study, the milk replacer was fed at a constant level of 12 g DM/kg initial body mass. It may be justifiable to examine the effect of curd development under conditions of higher, and increasing feeding levels.

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