Effect of curd suppression in a milk replacer on physiological parameters in calves. II. Selected blood profiles

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Fourteen Holstein calves, two to four days of age, were randomly divided into two groups to determine the effect of abomasal curd suppression on selected blood profiles. Calves received 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). Jugular blood samples were taken before the morning feeding (0 h), as well as at 1, 2, 4 and 6 h post-feeding. Fasting (0 h) plasma free essential amino acid (EAA) concentration tended to be higher for the CM treatment than for the NCM treatment, while the contrary was observed for postprandial values up to 6 h post-feeding. Plasma EAA concentration increased significantly during the first hour post-feeding for the NCM treatment, whereas values remained fairly constant for the CM treatment. Plasma triglyceride concentration was significantly higher for the CM treatment at 0, 4 and 6 h post-feeding, while it was higher for the NCM treatment at 1 h post-feeding. The fasting plasma glucose concentration was similar for both treatments. Plasma glucose was significantly higher for the CM treatment at 2 h post-feeding, but the contrary was observed thereafter. For the CM treatment, the plasma glucose profile almost mirrored the triglyceride profile. It was concluded that profiles of plasma free EAA and triglycerides may be reliable indicators of *in vivo* curd-forming ability of a given milk replacer, and that abomasal curd suppression may have a detrimental effect on amino acid and caloric homeostasis.

Veertien Holsteinkalwers, tussen twee- en vier-dae-ouderdom, is ewekansig in twee groepe verdeel om die invloed van voorkoming van stolselvorming in die abomasum op geselekteerde bloedprofiele te bepaal. Een melksurrogaat is geformuleer en kaseïenkoagulering is in die een behandeling (NCM) voorkom deur presipitering van Ca^{++} met 'n oksaalsuur-natriumhidroksiedbuffer. Die ander groep kalwers (CM) het onbehandelde (koaguleerbare) melksurrogaat ontvang. Bloedmonsters is voor die oggendvoeding (0 h), asook 1, 2, 4 en 6 h na voeding geneem. Vastende (0 h) plasmavrye essensiële-aminosuur(EAA)-konsentrasie het geneig om hoër te wees vir die CM-behandeling as vir die NCM-behandeling, terwyl die teenoorgestelde waargeneen is vir die periode tot 6 h na voeding. Met die NCM-behandeling het die plasma-EAA-konsentrasie betekenisvol toegeneem gedurende die eerste uur na voeding, terwyl dit redelik konstant gebly het met die CM-behandeling. Plasmatrigliseriedkonsentrasie was betekenisvol hoër vir die CM-behandeling by 0, 4 en 6 h na voeding as vir die NCM-behandeling, terwyl die teenoorgestelde 1 h na voeding waargeneem is. Vastende plasmaglukosekonsentrasie was dieselfde vir albei behandelings. Een uur na voeding het die plasmaglukosewaardes geneig om hoër te wees vir die CM-behandeling en 2 h na voeding was dit betekenisvol hoër as vir die NCM-behandeling. Die teenoorgestelde is 4 en 6 h na voeding waargeneem. Vir die CM-behandeling was die plasmaglukoseprofiel feitlik die omgekeerde van die trigliseriedprofiel. Die gevolgtrekking is gemaak dat plasmavrye EAA- en trigliseriedprofiele moontlik betroubare indikators van *in vivo* stollingsvermoë van 'n gegewe melkvervanger kan wees, en dat voorkoming van abomasale stolselvorming moontlik 'n nadelige invloed op die handhawing van aminosuur- en kaloriese homeostase kan hê.

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It is well established that alterations in either the dietary crude protein intake, or the amino acid profile of dietary protein, will ultimately be reflected to some degree in blood profiles of non-ruminant animals (Munro, 1970; Fisher, 1976; Dove, 1978; Beynen & van Gils, 1983a). A number of investigators have exploited this fact to estimate amino acid requirements of pre-ruminant calves (Williams & Smith, 1974; Williams & Hewitt, 1979; Tzeng & Davis, 1980). Relationships between nutrients in the diet and blood profiles of calves have also been observed for lipids (Barrows, Heeg, McGilliard, Richard & Jacobson, 1980; Beynen, van Gils & den Engelsman, 1983), and for glucose (Anonymous, 1983; Beynen & van Gils, 1983b).

Curd suppression of a milk replacer had a marked effect on abomasal retention time of nutrients, and consequently on their rate of passage (Cruywagen, Brisson & Meissner, 1990). It could be expected then that the blood profiles of these nutrients would be influenced accordingly. Although the effect of various types of milk replacers on postprandial changes in concentrations of blood components is well documented, the effect of curd development *per se* on blood profiles of calves is less well-known. Guilloteau, Toullec, Patureau-Mirand & Prugnaud (1981) concluded that the metabolic utilization of amino acids, as reflected in blood free amino acid concentrations, were highly influenced by the duration of digesta retention in the abomasum.

According to Jenkins & Emmons (1982a), treatment for clot prevention in whole milk resulted in an increase in plasma essential amino acids and urea nitrogen, 2 and 6 h after feeding. When calves were fed liquid diets containing skimmed-milk powder or soybean protein concentrate, it was found (Beynen & van Gils, 1983a) that the dietary protein source had no significant effect on the concentration of glucose, urea, non-protein nitrogen, triglycerides, cholesterol and phospholipids in serum from fasting animals. However, postprandial increases in serum non-protein nitrogen, phospholipids and cholesterol were higher in the soybean protein group than in the skimmed-milk group, while postprandial glucose concentration tended to be higher in animals fed the skimmed-milk diet. It has been suggested that at least part of the difference in postprandial values of several serum components between calves receiving different milk replacers is related to the fact that soya protein, in contrast to casein, does not coagulate in the abomasum of the calf.

The present trial was designed to determine the effect of the presence or absence of curd development in the abomasum on plasma concentrations of free amino acids, triglycerides and glucose at different times post-feeding, as well as to relate the concentrations of these components to the abomasal retention time of certain milk replacer nutrients.

Materials and Methods

Fourteen male Holstein calves were randomly assigned to two dietary treatments. The diets were a coagulable milk replacer, containing 43,2% low-heat skimmed-milk powder, 25,8% crude protein and 21,1% fat (CM treatment) and the same milk replacer treated with oxalic acid (Cruywagen, Brisson & Meissner, 1990) to prevent curd forming in the abomasum (NCM treatment). Feeding schedule, housing and care of animals were described earlier (Cruywagen, Brisson, Tremblay & Meissner, 1990).

Blood samples were collected when the calves were nine days of age, at 0, 1, 2, 4 and 6 h post-feeding. The 0-h sample represents a fasting period of 16 h, i.e. taken before the morning feeding. Blood sampling was performed as

described previously (Cruywagen, Brisson, Tremblay & Meissner, 1990).

For the determination of plasma free amino acids, frozen plasma samples were allowed to thaw in a refrigerator at 4 °C. Each sample was deproteinized by adding 50 mg of solid sulfosalicylic acid to a conical centrifuge tube, to which 1 ml of plasma was added. The contents were thoroughly mixed and left for 1 h at 4 °C. After centrifugation at 3 000 × g for 15 min at 4 °C, the supernatant liquid was transferred to a separate tube and the pH was adjusted to 2,2 (using 0,3 M-lithium hydroxide). The liquid was then filtered through 0,22 µ. Plasma free amino acid concentrations were determined with a LKB 4400 Amino Acid Analyser (LKB Biochrom, London, UK), according to the method described in the instruction manual.

Plasma triglycerides were determined using a Peridochrom Triglycerides kit supplied by Boehringer Mannheim (Boehringer Mannheim Canada Ltd., Dorval, Québec). The method is based on the enzymatic hydrolysis of triglycerides with subsequent enzymatic determination of the liberated glycerol by colorimetry. For this purpose, a Bausch & Lomb Spectronic 2000 Colorimeter (Bausch & Lomb, Rochester, New York) was used with the wavelength set at 578 nm.

Plasma glucose concentrations were determined using the Glucose GOD–PAP (Cat. No. 166391) kit supplied by Boehringer Mannheim. The method is based on the enzymatic oxidation of glucose and the colorimetric determination of subsequently formed peroxide, at a wavelength of 510 nm.

The data were examined using a two-way statistical analysis with cell replicates (Snedecor & Cochran, 1980). The main effect and interaction were pooled and the method of orthogonal contrasts was applied. In other words, an alternative separation of the seven degrees of freedom, subdivided in single degrees of freedom with the following denominations: difference at times 1, 2, 3 and 4, and three remaining mean tendencies over time were used. Least significant differences were also calculated to
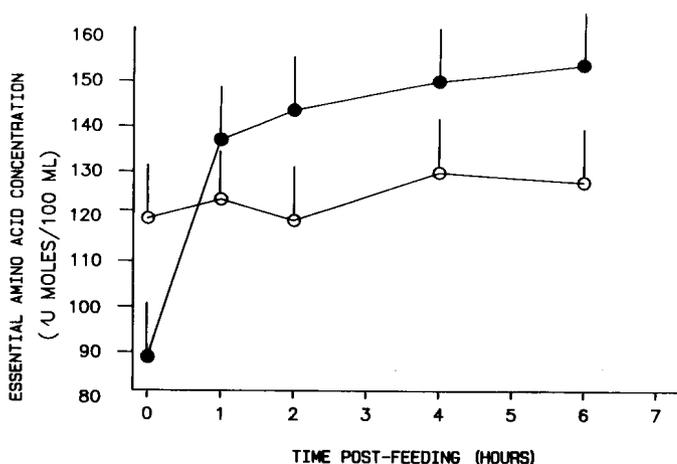

Figure 1 Postprandial plasma concentrations of total free essential amino acids in calves receiving milk replacer diets. O = Coagulable milk replacer; ● = non-coagulable milk replacer; vertical bars indicate standard errors.
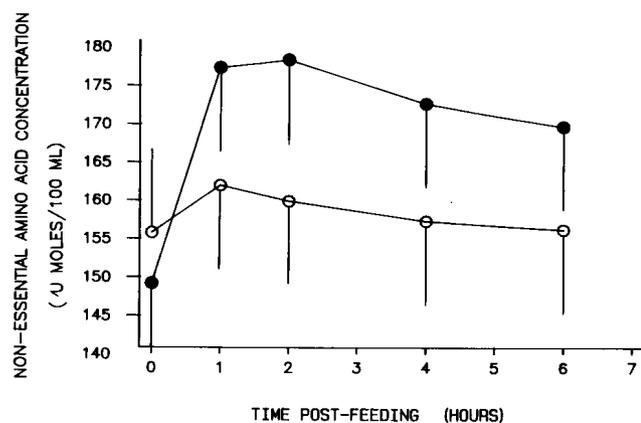

Figure 2 Postprandial plasma concentrations of total free non-essential amino acids in calves receiving milk replacer diets. O = Coagulable milk replacer; ● = non-coagulable milk replacer; vertical bars indicate standard errors.

compare plasma free amino acid concentrations within treatments, between different times post-feeding.

Results and Discussion

Plasma concentrations of total free essential amino acids (EAA) at different times post-feeding are presented in Figure 1.

Although total EAA values did not differ statistically between treatments at any given time post-feeding (referred to as a critical reference time, or CRT), it appears as if abomasal curd suppression tended to result in lower fasting EAA concentrations, but higher postprandial concentrations, compared to the CM treatment. Amino acid concentrations apparently varied more in the NCM treatment than in the CM treatment from 0 to 6 h CRT. This is in accordance with findings by Jenkins & Emmons (1982a) and Petit, Ivan & Brisson (1988a).

This observation suggests that abomasal curd suppression may alter the mechanism of maintaining essential amino acid homeostasis. Although the practical significance of this hypothesis is not obvious, Jenkins & Emmons (1982a), in the context of their publication, leave the impression that a faster absorption rate of EAA (as in the case of our NCM treatment) is not conducive to their utilization. These authors also reported that postprandial plasma urea levels showed a tendency to increase when non-coagulable milk was fed. Petit et al. (1988a) used a milk replacer similar to the one used in this study and showed that prevention of curd development induced higher blood urea levels than those found in the control. According to Jenkins & Emmons (1982a), it appears that the prevention of clot formation in milk may result in an increased deamination of amino acids by the liver and consequent wastage of dietary EAA. This might be ascribed to an overly rapid absorption of EAA from digested, unclotted milk protein, relative to their utilization for protein synthesis by the liver. The fact that duodenal infusion of glucose lowered blood urea in calves fed a non-coagulable milk replacer (Petit et al., 1988b) supports this hypothesis.

For the NCM treatment, postprandial EAA values were significantly ($P < 0,05$ at 1 h CRT and $P < 0,01$ at 6 h CRT) higher than fasting EAA values, while for the CM treatment, there was no difference between postprandial and fasting EAA values. Previous investigation (Cruywagen, Brisson & Meissner, 1990) showed that some casein clots remained in the abomasum for up to 16 h post-feeding. This may explain the higher plasma EAA concentrations at 0 h for a coagulable milk replacer.

Plasma concentrations of total free non-essential amino acids (NAA) at different times post-feeding are presented in Figure 2.

As for EAA, no significant differences in NAA concentration occurred between treatments at any CRT. Contrary to EAA, however, NAA values did not differ significantly from pre- to postprandial periods in either treatment. It appears that, as for EAA, NAA concentrations varied more in the NCM treatment than in the CM treatment from 0 to 6 h   CRT.

These results suggest that the *in vivo* curd-forming ability of milk replacers may be reflected by the profile of plasma free amino acids. A marked increase in amino acid concentration (especially EAA) during the first hour post-feeding would be indicative of poor, or total absence of, abomasal curd development.

Plasma triglyceride concentrations at different times post-feeding are presented in Figure 3.

The mean plasma triglyceride concentration at 0 h CRT was significantly ($P < 0,01$) higher for calves in the CM treatment than for those in the NCM treatment. It has been shown (Cruywagen, Brisson & Meissner, 1990) that some curd material is still present in the abomasa of calves fed the coagulable milk replacer (CM treatment) at 16 h post-feeding, i.e. immediately before the morning feeding, and that the fat retention value at 0 h CRT was 6,5% of ingested fat for the CM treatment as opposed to 2,4% for the NCM treatment. The slower passage rate of fat for the CM treatment may have resulted in a more uniform and continuous (though decreased) release of lipids into the duodenum,
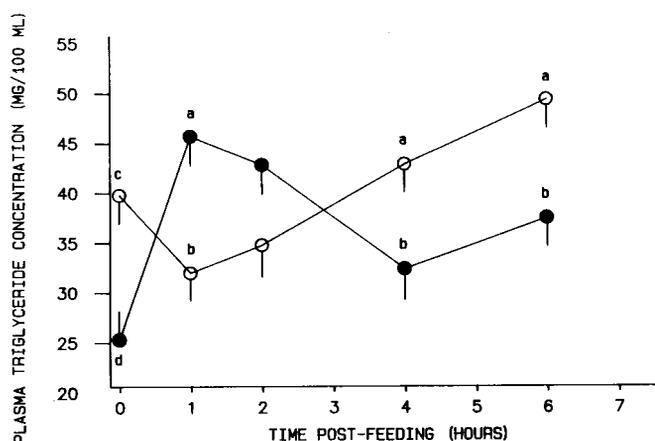

Figure 3 Postprandial concentrations of plasma triglycerides in calves receiving milk replacer diets. ○ = Coagulable milk replacer; ● = non-coagulable milk replacer. For each time point, different letters are different: a,b ($P < 0,05$); c,d ($P < 0,01$). Vertical bars indicate standard errors.
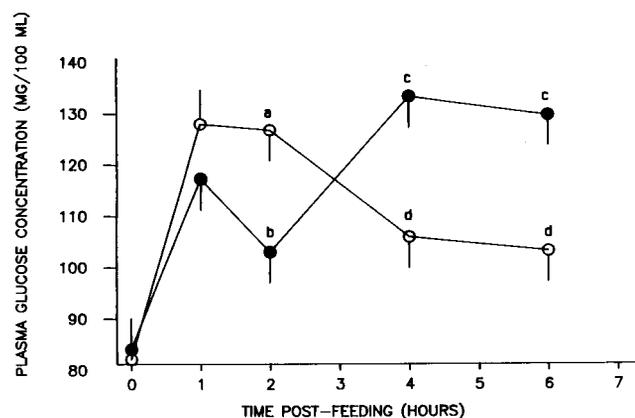
Figure 4 Postprandial concentrations of plasma glucose in calves receiving milk replacer diets. ○ = Coagulable milk replacer; ● = non-coagulable milk replacer. For each time point, different letters are different: a,b ($P < 0,05$); c,d ($P < 0,01$). Vertical bars indicate standard errors.

even at 16 h post-feeding. This would explain the higher plasma triglyceride value for the CM treatment at 0 h CRT.

At 1 h CRT, plasma triglyceride concentration was significantly ($P < 0,05$) higher for the NCM treatment than for the CM treatment. The rapid increase in plasma triglycerides of calves in the NCM treatment reflected the fast release of fat during the first hour post-feeding as observed previously for this treatment (Cruywagen, Brisson & Meissner, 1990). Furthermore, as noted by Bazin & Brisson (1976), it is possible that a fraction of the dietary lipids leaving the abomasum was rapidly absorbed due to the action of salivary lipase (Grosskopf, 1965), which can hydrolyse triglycerides containing long- or short-chain fatty acids (Otterby, Ramsey & Wise, 1964).

The significant decrease in plasma triglycerides observed for the CM treatment at 1 h CRT, may be partly explained by the probable inclusion of clot material from a previous meal within the new clot (Hill, Noakes & Lowe, 1970). Owing to initial whey syneresis, the release of lipids from the abomasum before 1 h CRT would be virtually negligible. However, since blood samples were not taken between 0 and 1 h post-feeding, the pattern of plasma triglyceride concentration for this period is unknown. Bazin & Brisson (1976) observed a sharp increase in triglyceride concentration 30 min after feeding a high-fat (coagulable) milk replacer, followed by a sharp decrease during the next 30 min. The initial rapid increase observed by these authors could have been the result of readily hydrolysed lipids passing from the abomasum before curd development had been completed, while the influx of lipids into the duodenum ceased temporarily following curd formation. In the present work, the difference between treatments was not statistically significant at 2 h CRT, but higher plasma triglyceride concentrations were obtained in the CM treatment at 4 h and at 6 h CRT. Although the peak value observed at 1 h CRT for the NCM treatment may be attributed to the rapid flow of fat from the abomasum during the first hour post-feeding (Cruywagen, Brisson & Meissner, 1990), the gradual increase in triglyceride concentration for the CM treatment from 1 to ·6 h post-feeding was presumably the result of enzymatic curd lysis. The plasma patterns, and especially the values at 0 and 1 h CRT, may be a valuable indicator of *in vivo* curd-forming ability of milk replacers.

Plasma glucose concentrations at different times post-feeding are presented in Figure 4.

A sharp increase in glucose concentration from 0 h to 1 h CRT was observed for both treatments. However, at 2 h CRT, plasma glucose concentrations in calves fed the coagulable (CM) milk replacer were significantly ($P < 0,05$) higher than in calves fed the non-coagulable (NCM) milk replacer. On the other hand, at 4 h and 6 h CRT, calves fed NCM displayed significantly ($P < 0,05$) higher plasma glucose concentrations than calves fed CM.

Bazin & Brisson (1976) reported a fasting plasma glucose concentration of 78 mg% for calves receiving a high-fat milk replacer (25% fat), which agrees with the value in the present study. The rapid increase in plasma glucose concentration during the first postprandial hour observed for the CM treatment may be attributed to the initial passage of whey into the duodenum. According to Coombe & Smith

(1974), peak glycemia seems related to a rapid hydrolysis and absorption of carbohydrates in the diets. On a dry matter basis, whey contains 70—75% lactose (Morrill, Melton, Dayton, Guy & Pallansch, 1971) which is hydrolysed to glucose and galactose in the gut (Coombe & Smith, 1973), where glucose is rapidly absorbed.

It is of interest to note that the plasma triglyceride profile for the CM treatment (Figure 3) was almost opposite to the plasma glucose profile (Figure 4): an increase in plasma glucose concentration was accompanied by a decrease in plasma triglyceride concentration, and *vice versa*. A similar effect may be observed for the NCM treatment, but only from 2—6 h post-feeding. Since curd development did not occur in the latter treatment, the negative starting index resulted in an initial increase in both plasma triglyceride and glucose concentrations. By examining Figures 3 and 4 simultaneously, it appears that the pattern of release of glucose and lipids from the abomasum and subsequent absorption from the gut, complement the homeostasis concept in the case of the CM treatment. It is therefore postulated that abomasal curd development may provide a natural mechanism to aid metabolic systems in maintaining caloric homeostasis.

Conclusions

The different blood profiles observed in the present investigation contributed to the hypothesis that curd suppression of a milk replacer may alter a natural mechanism to aid metabolic systems in maintaining essential amino acid homeostasis, as well as caloric homeostasis. However, the fact that prevention of curd development has had no effect on previously discussed (Cruywagen, Brisson, Tremblay & Meissner, 1990) digestibility parameters, body mass gain or EFC ratios, brings into question the validity of this proposition. It should be kept in mind though that no significant differences in total EAA concentrations could be discerned between treatments at any CRT, and that it would therefore be presumptuous to assume that a tendency towards a steadier plasma amino acid concentration would result in improved animal performance. Gastric emptying rates of different milk replacer components were reflected in blood profiles. Plasma free essential amino acid- and plasma triglyceride profiles appear to be especially reliable indicators of *in vivo* curd development. This observation may have practical application, since it was suggested (Cruywagen, Brisson & Meissner, 1990; Jenkins & Emmons, 1982b) that *in vitro* tests for coagulation may not support *in vivo* observations under all circumstances.

Acknowledgements

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