The effect of heat treatment on the nutritive value of milk for the young calf

8.* The effect of the pre-heating treatment of spray-dried skim milk on the pH and the contents of total, protein and non-protein nitrogen of the pyloric outflow

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1. Four Ayrshire bull calves between 8 and 34 days of age and fitted with duodenal and ileal re-entrant cannulas were used to study the effect of heat treatment of the milk they received on the pH and nitrogen composition of the pyloric outflow and ileal contents.

2. Milk A contained a spray-dried skim-milk powder pre-heated during the drying process at 74° for 30 min and milk B a similar powder pre-heated at 77° for 15 sec. In milk A about 50 % of the non-casein protein N had been denatured.

3. Milk B resulted in a lower pH than milk A in the pyloric outflow throughout the sampling period of 6·5 h after feeding. It resulted also in an increased volume of outflow during the 1st h after feeding, a reduced output of undigested protein, an increased output of non-protein nitrogen (NPN) and a different pattern of flow of NPN during the first 4 h after feeding.

4. These differences between milk A and milk B were associated largely with different clotting characteristics, which were demonstrated in vitro at two levels of addition of rennet with or without the addition of calcium. The buffering capacity of the two milks was similar.

5. Variation between calves in their response to these two milks was attributed to the age of the calves and to differences in inherent clotting or proteolytic activity.

6. In the ileal outflow, bacterial activity, as measured by dehydrogenase activity, was positively related to N concentration, but the N concentration when milk A was given did not appear to differ from that when milk B was given.

7. One calf had diarrhoea when given milk A at a young age. This was associated with an increased pyloric outflow, an increased outflow of undigested protein but little difference in the rate of proteolysis, and a high pH. In the ileal outflow the volume and amount of N was much increased although the N concentration was reduced.

8. It is concluded that the detrimental effect of milk A, found in earlier experiments, was largely associated with high pH and poor digestibility of protein in the abomasum, conditions which allow multiplication of coliform organisms in the intestine.

Earlier experiments on the effect of heat treatment of milk showed that milk diets, which had been heated sufficiently to denature a large proportion of the whey proteins, had a detrimental effect on the calf (Shillam & Roy, 1961). When such diets were given after the colostrum-feeding period, they tended to predispose the calves to an *Escherichia coli* localized intestinal infection (Wood, 1955). Associated with the denaturation of the whey proteins is a reduction in ionizable calcium (Hostettler & Stein, 1958), the release of SH groups (Zweig & Block, 1953), poor clotting ability by rennet (Shillam & Roy, 1963*b*), and reduced digestibility, but no effect on the biological value, of the protein (Shillam & Roy, 1963*a*).

The detrimental effects of these heat-treated milks were manifest in three ways. First, under low 'infection' conditions, weight gains of calves were reduced by as much as 30% in the first 3 weeks of life (Shillam & Roy, 1963a); secondly, the rate of build-up of 'infection', when large numbers of susceptible calves were passed through a calf-house, was increased (Roy, Palmer, Shillam, Ingram & Wood, 1955); and thirdly, the incidences of diarrhoea and mortality were increased once the 'infection' had been built up, as evidenced by the dominance of one or two strains of E. coli (Wood, 1955; Shillam, Roy & Ingram, 1962a). It is known that E. coli become established in the alimentary tract very soon after birth and reach peak viable counts in the duodenum at 1–4 days of age. In normal calves the E. coli population declines to a low level, 10–10^2/ml, by 10 days of age. In contrast, calves whose deaths were associated with an E. coli localized intestinal infection had viable counts of 10^7–10^8/ml in the duodenum at 10 days of age (Ingram, 1962).

Although it was thought that the depression in weight gain induced by a 'severely' heat-treated milk, under conditions where there was little diarrhoea, might be due to a subclinical infection, chlortetracycline supplementation was without effect (Shillam & Roy, 1963a). Addition of calcium improved the clotting ability of such milks in vitro, but supplementation of the milk with Ca had only a very slight beneficial effect on the calf (Shillam & Roy, 1963b). As might be expected from the finding that there was no change in the biological value of the protein, supplementation with methionine was also ineffective (Roy & Shillam, 1961, 1962).

The addition of undenatured whey proteins appeared to be the only treatment that would improve the performance of calves given a 'severely' heat-treated milk (Shillam, Roy & Ingram, 1962b). This was in keeping with the finding that the presence of denatured whey proteins increases the time required for casein to be coagulated by rennet (Kannan & Jenness, 1961),

Since putrefactive diarrhoea in infants has been associated with the presence of abnormal amounts of protein and peptides in the chyme, and with the increased production of amines from the decarboxylation of amino acids by the saccharo-proteolytic components of the flora, including E. coli (reviewed by Weijers & van de Kamer, 1965), a study of the composition of the chyme resulting from the feeding of milks given different heat treatments might reveal the causes of the detrimental effect of a 'severely' heat-treated milk.

An experiment was made, therefore, to study the flow of chyme and the degradation of N compounds in the alimentary tract of the calf, by means of re-entrant cannulas, after the calves had been given 'severely' and 'mildly' heat-treated milks.

EXPERIMENTAL

Calves

Four Ayrshire bull calves (calves 1–4) that had not suckled their dams were used. The calves were brought into the calf house within 8 h of birth and were kept in individual pens on straw bedding at an ambient temperature of 20°.

Diets

Colostrum. Each calf was given, within 36 h of birth, 3·5 kg whole colostrum obtained from the first two milkings after parturition. Thereafter, the calves were given milk B (see below) until cannulation.

Milk substitute. Two diets were used in the experiment; they were of similar composition except for the spray-dried skim-milk powder used. During the drying process, skim-milk powder 'A' had received a 'severe' pre-heating treatment (74° for 30 min) and milk powder 'B' a 'mild' pre-heating treatment (77° for 15 sec). These powders were prepared by the same processes as powders A and B, used in the experiments of Shillam *et al.* (1962*a, b*) and Shillam & Roy (1963*a, b*). The composition of the diets is given in Table 1. The values for N partition were those determined by the method of Aschaffenburg & Drewry (1959) on batches of powder A and B, used in the earlier experiments. The calves were fed twice daily at 09.00 and 16.00 h, the milk, warmed to 37°, being given in equal portions. Details of the calves, together with the quantity and type of milk given, for the various collection periods are given in Table 2.

Table 1. *Composition of the diets*

	Milk A	Milk B
Non-vitaminized margarine (%)	2·0	2·0
Spray-dried skim milk (%)	—	—
Powder A	9·8	—
Powder B	—	9·8
Water (%)	88·2	88·2
Vitamin A (i.u./calf daily)	3500	3500
Vitamin D (i.u./calf daily)	700	700

Nitrogen partition (mg/100 g milk)

	Milk A	Milk B
Total N	573	559
Casein N*	487	436
Non-casein N	86	123
Non-casein protein N	48	86
Total albumin N	28	62
β-lactoglobulin N	20	39
Residual albumin N†	8	23
Proteose-peptone + globulin N	20	24
Non-protein N	38	37

* Includes denatured non-casein protein N.

† Sum of α-lactalbumin, 'blood' serum albumin and two minor components (see Aschaffenburg & Drewry, 1959).

Insertion of re-entrant cannulas and recovery of the calves

Between 2 and 6 days of age, each calf was fitted with a Perspex re-entrant cannula either in the duodenum or in both the duodenum and the ileum. The proximal duodenal cannula was fitted immediately posterior to the pyloric sphincter and the distal ileal cannula was sited as close as possible to the caecum. A few hours after the operation, 200–400 ml 3% glucose solution were offered to the calf in several portions and on the following day 800–1200 ml diluted milk (equal parts milk B and 3% glucose solution) in four to six portions were given. As soon as the calf consumed the

required amount of milk regularly, it was transferred to a metabolism crate and the experiments were begun.

Table 2. *Details of the Ayrshire calves and their diets*

Calf no.	Period	Birth wt (kg)	Age (days)	Diet Milk	Vol. given (l./feed)	Remarks
1	—	34·0	27–28	A	3·5	
			29–30	B	3·5	
2	—	38·8	14–15	B	3·5	
			16–17	A	3·5	
3	a	33·8	20–21	A	2·5	Developed diarrhoea
			24–25	B	2·5	Diarrhoea continued
	b		31–34	B	3·5	
			35–38	A	3·5	
4	a	36·0	8–9	B	2·5	
			10–11	A	2·5	
	b		14–15	B	3·5	
			16–17	A	3·5	

Sampling from the alimentary tract

Pyloric chyme. After the morning feed on the days in the experimental periods shown in Table 2, samples of pyloric chyme were taken every 5–25 min (in amounts of 50–150 ml abomasal outflow) until 6·5 h after feeding, the intervals being shorter during the first 2–3 h after feeding and longer thereafter. After pH determination, the chyme was homogenized and a sample of 5–10 % taken, the remainder being maintained at 37° until it was returned into the distal end of the re-entrant cannula. This was done at intervals just before the next sample was taken. Proteolytic activity in the sample was stopped by raising the pH to 7·5 with N-NaOH, and the sample was then cooled to −25°.

Ileal contents. Ileal contents were collected over 24 h periods into an empty rubber balloon, connected to the proximal cannula, to avoid the presence of air. Volume was measured less frequently than for pyloric chyme. A 10 % sample was taken for N determination and the remainder returned to the distal cannula. Dehydrogenase activity was determined on two or three occasions at regular intervals during each sampling period. For this, fresh ileal contents were collected shortly before the determination was carried out, and additional N determinations were made on these samples.

Chemical analyses

Total nitrogen (TN). Total N was determined by the Kjeldahl method using 5 ml pyloric chyme or 1–3 ml ileal contents.

Undigested protein N (PN). This fraction was precipitated from the pyloric chyme by adding trichloroacetic acid (TCA) up to a final concentration of 2 %. The denatured N was then separated by centrifugation. The precipitate was then rinsed with 3 % TCA and recentrifuged. The N content of the precipitate was determined by the biuret method as modified by Robinson & Hogden (1940). The coloured mixture was

centrifuged and the colour intensity was measured on a Unicam spectrophotometer at a wavelength of 560 nm.

Non-protein N (NPN). This was calculated as the difference between TN and undigested PN.

Dehydrogenase activity in ileal contents. Triphenyltetrazolium chloride (TTC) was used for estimating the dehydrogenase activity of the micro-organisms in the ileal contents. This method had been originally used for estimating the activity of soil bacteria (Stevenson, 1959) and has since been applied to study the activity of rumen micro-organisms (Tagari, Dror, Ascarelli & Bondi, 1964). Preliminary experiments showed that the maximum release of H^+ was obtained when the ileal contents were incubated for 5 min at a final concentration of 0·15 % TTC.

A 15 ml test tube was filled with CO_2, and 2 ml of recently collected and homogenized ileal contents were introduced. After addition of 0·4 ml 0·9 % TTC, the mixture was shaken once every minute whilst being incubated at 37° for 5 min. The reaction was then stopped by addition of 2·4 ml isopropanol. The mixture was allowed to stand for a further 10 min at 37° to obtain better extraction of the triphenylformazan and was centrifuged at not less than 2400 *g* for 20 min. The clear solution was decanted and diluted appropriately with 50 % isopropanol. The extinction was read at 485 nm in a Unicam spectrophotometer against a blank, which was prepared in the same way, but with isopropanol added before TTC.

pH. To express pH as an average value for the sampling period, the following calculation was made: the curves relating pH to time were integrated by measuring the areas below them and the values thus obtained were divided by the length of the sampling period of 6·5 h.

Determination of whey drainage in vitro

Reconstituted skim milk A or B (200 ml), containing 12·5 % of the appropriate skim-milk powder, was clotted at 37° by the addition of 0·19 or 0·38 ml commercial rennet (1:6000). The whey drainage at these two levels of addition of rennet was examined with and without the addition of 500 mg Ca/l. as $CaCl_2.2H_2O$. This concentration of Ca was used, since at a lower concentration milk A did not clot in a reasonable time; it was the same concentration as was used in the feeding experiments of Shillam & Roy (1963*b*). After 20 min for milk B or 50 min for milk A, the curd was cut and the whey allowed to drain through a cheese cloth, all collections of whey being made at 37°. Buffering capacity was determined on the whey of the two milks and on the homogenized clotted milks by titrating with 0·1 N-HCl.

RESULTS

Drainage of whey in vitro and buffering capacity of reconstituted spray-dried skim-milk powders A and B

Drainage of whey. The results for the volume of whey drained from the two reconstituted skim milks A and B after addition of 0·19 ml or 0·38 ml commercial rennet/200 ml milk with or without the addition of 500 mg Ca/l. are given in Fig. 1.

In the absence of Ca and at the lower concentration of rennet, the curd of milk B was firm and elastic, and the whey was clear and of greenish-yellow colour. Milk A produced only a flocculent clot at the lower concentration of rennet, and the clot was not much improved at the higher concentration. Great care had to be taken, when transferring milk A to the cheese cloth, as otherwise considerable amounts of the clot passed through the cloth. The whey from milk A drained very slowly, and was opaque and milky in appearance.
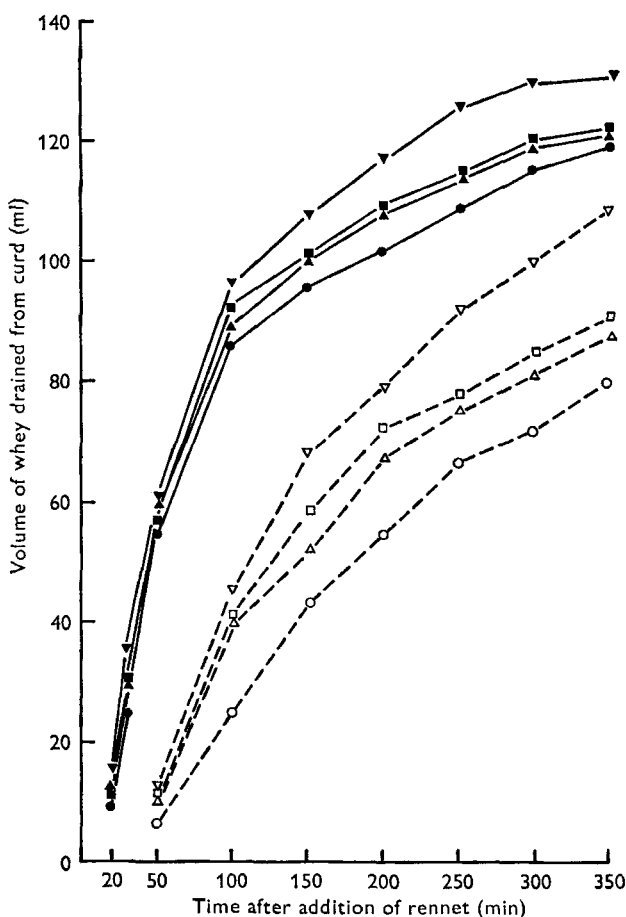

Fig. 1. Volume of whey drained from the curd in relation to time after addition of rennet, or rennet and calcium, to skim milk 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B). Open symbols, broken lines, milk A; solid symbols, solid lines, milk B; ○, ●, 0·19 ml rennet/200 ml milk; △, ▲, 0·38 ml rennet/200 ml milk; □, ■, 500 mg Ca/l.+0·19 ml rennet/200 ml milk; ▽, ▼, 500 mg Ca/l.+0·38 ml rennet/200 ml milk.

As can be seen from Fig. 1, the volume of whey that drained from milk B, irrespective of rennin concentration or the addition of Ca, was always greater than that from milk A. Furthermore, whey drainage from milk B began much earlier and was several times faster immediately after its transfer on to the cheese cloth; in the 100 min

period after addition of rennet 60–70% of the total whey output was already drained from milk B.

Doubling the concentration of rennet had less effect than the addition of Ca, but was greater with milk A than with milk B, especially during the first 100 min after addition of the rennet. Addition of Ca always increased the volume of whey produced from both milks. The combined effect of increased rennet concentration and Ca addition was greater than the effect of these two factors separately. The combined effect on milk A was such that the amount of whey drained, 350 min after the addition of rennet, approached that from milk B treated with the lower concentration of rennet but no Ca. Any improvement in the clot and drainage of whey from milk A resulted in a much clearer whey. It should be emphasized, however, that the combined effect on milk A did not result in outputs of whey similar to those from milk B during the early part of the experiment.

Buffering capacity. The results for the buffering capacity of 40 ml whey or 40 g homogenized clotted skim milk are given in Table 3. No difference in buffering capacity between milks A and B was found, although the buffering capacity was twice as high for the clotted skim milk as for the whey.

Table 3. *Volume of 0·1 N-HCl (ml) required to change the pH of the whey and clotted homogenized skim milks A and B*

pH	Milk A		Milk B	
	Whey (40 ml)	Clotted skim milk (40 g)	Whey (40 ml)	Clotted skim milk (40 g)
6·75	0·0	0·0	0·0	0·0
5·00	4·0	11·0	4·0	9·0
4·00	8·0	21·0	8·0	21·0
3·00	12·5	29·3	12·0	27·0
2·00	19·7	43·0	19·2	43·0

Nature of the pyloric chyme from calves given diets A and B

The nature of the pyloric chyme varied with time after feeding and differed according to the milk that had been given. Before a calf was fed the pyloric chyme was of thick consistency, opaque and usually white, but occasionally pale yellow. This latter colour probably resulted from staining with bile pigments. At this time, little difference was apparent as a result of different milks having been given at the previous feed.

After a calf was given milk B a clear, watery greenish-yellow fluid flowed into the duodenum containing, on occasions, small firm elastic pieces of clot. This type of outflow continued for 1–3 h after feeding and thereafter changed to a watery but white opaque milk-like liquid; 4–5 h after feeding, the outflow became more viscous and contained many small pieces of clot, a phenomenon probably associated with the extensive disintegration of the curd in the abomasum.

Although the chyme from milk A was similar to that from milk B during the period before feeding and from 4–5 h after feeding, the clots from milk A were much softer and when homogenized showed a slower rate of sedimentation. However, the chyme

from milk A differed markedly from that from milk B during the period from feeding until up to about 4 h after feeding, having the appearance of watery milk containing many small pieces of clot. The more viscous clot-containing liquid, which was thought to be the result of disintegration of the curd, always appeared in the outflow much earlier for milk A than for milk B.

Volume of the chyme

The absolute and cumulative volumes of chyme flowing from the abomasum into the duodenum in relation to time after feeding for all the periods when 3·5 l. milk/feed were given are shown in Table 4. Table 5 gives a comparison of the chyme outflow from calves 3 and 4 when given 2·5 l. or 3·5 l. milk/feed.

The volume of chyme outflow collected during the 1st h after feeding was greater for calves given milk B than for those given milk A. For the periods in which 3·5 l./feed were given, the increase in volume in the 1st h averaged 27%. Since the total volume produced in the complete sampling period tended to be greater for milk B, the percentage of the total volume that was produced in the 1st h averaged 25% for milk B compared with 21% for milk A. The exception to this finding was calf 3 which, when given 2·5 l. milk A/feed in period a, developed diarrhoea that continued when milk B was given. This calf produced an abnormal amount of chyme when given milk A. For calves given 3·5 l. milk/feed (Table 4), the difference in chyme output in the 1st h was maintained up to 4 h after feeding and then diverged slightly until the end of the sampling period, but relative to total output the difference had largely disappeared by 3–4 h after feeding.

In the comparison of the outflows which occurred after 3·5 l. or 2·5 l. milk/feed had been given (Table 5) the same differences between milks were evident except for the above-mentioned calf 3 given milk A in period a. In general, the outflow was greater at the higher level of intake, except for calf 3, whose outflow in period a, when it was given 2·5 l. milk A and when it had diarrhoea, was similar to the outflow in period b, when it was given 3·5 l. milk A and was apparently healthy.

When 3·5 l. milk were given the accumulated total volumes of outflow in 6·5 h were in general greater than the total milk intake, with a slight tendency for milk B to cause a greater increase than milk A. With 2·5 l. milk, the accumulated volumes of outflow were similar or less than the intake with, once again, the exception of calf 3 in period a, when milk A was given. This calf had the highest chyme output in relation to intake of all the trials, but although still having diarrhoea when given milk B, its outflow had returned to a normal volume, thus suggesting that milk B was having an ameliorating effect. This is borne out by the values for NPN expressed as a percentage of TN given later (see Table 6). The increase in total outflow in relation to intake in this experiment was considerably lower than that found 6·5 h after feeding 2·0 l. whole milk to Guernsey calves, namely about 138% (Mylrea, 1966).

pH of chyme

The changes in the acidity of the outflow are shown in Fig. 2. The trend of the changes in pH values during the sampling period were qualitatively similar for

Table 4. *Volume of chyme (ml), absolute and cumulative, passing into the duodenum of calves at periods of time from 0–1 h to 6–6·5 h after they had received 3·5 l. of a milk substitute containing 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) spray-dried skim milk*

(Values are the mean values obtained from collections made over 2 or 3 days)

Calf no.	Age (days)	Chyme from milk A							Total	Total as % of milk intake	Chyme from milk B							Total	Total as % of milk intake
		0–1 h	1–2 h	2–3 h	3–4 h	4–5 h	5–6 h	6–6·5 h			0–1 h	1–2 h	2–3 h	3–4 h	4–5 h	5–6 h	6–6·5 h		
1	27–30	986	800	950	850	437	343	56	4422	126	1162	652	672	715	602	495	140	4438	127
2	14–17	964	1115	642	602	550	515	205	4593	131	1107	960	947	642	437	320	105	4518	129
3	31–38	721	665	556	574	383	345	185	3429	98	1058	531	699	533	537	457	168	3983	114
4	16–17	735	527	720	682	594	432	206	3896	111	995	702	695	750	645	459	231	4477	128
Mean	14–17	851	777	717	677	491	408	163	4085	117	1080	711	753	660	555	433	161	4354	125
Mean cumulative volume		851	1628	2345	3022	3513	3922	4085	—	—	1080	1791	2545	3205	3790	4193	4354	—	—
Mean % of total volume		21	39	57	74	86	96	100	—	—	25	41	58	74	87	97	100	—	—

Table 5. *Volume of chyme, absolute and cumulative, passing into the duodenum of calves at periods of time from 0–1 h to 6–6·5 h after they had received a milk substitute containing 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) spray-dried skim milk at either 2·5 or 3·5 l./feed*

(Values are the mean values obtained from collections made over 2 or 3 days)

Calf no.	Period	Age (days)	Vol. of milk (l.)	Chyme from milk A							Total	Total as % of milk intake	Chyme from milk B							Total	Total as % of milk intake
				0–1 h	1–2 h	2–3 h	3–4 h	4–5 h	5–6 h	6–6·5 h			0–1 h	1–2 h	2–3 h	3–4 h	4–5 h	5–6 h	6–6·5 h		
				Absolute volume (ml)																	
3	a*	20–25	2·5	874	561	541	572	401	407	174	3530	141	802	518	453	323	190	236	73	2595	104
	b	31–38	3·5	721	665	556	574	383	345	185	3429	98	1058	531	699	533	537	457	168	3983	114
4	a	10–11	2·5	418	296	316	507	233	435	145	2350	94	662	356	269	209	245	345	190	2276	91
	b	16–17	3·5	735	527	720	682	594	432	206	3896	111	995	702	695	750	645	459	231	4477	128
				Cumulative volume† (ml)																	
3	a*	20–25	2·5	874 (25)	1435 (41)	1976 (56)	2548 (72)	2949 (84)	3356 (95)	3530 (100)			802 (31)	1320 (51)	1773 (68)	2096 (80)	2286 (88)	2522 (97)	2595 (100)		
	b	31–38	3·5	721 (21)	1386 (40)	1942 (57)	2516 (73)	2899 (85)	3244 (95)	3429 (100)			1058 (27)	1589 (40)	2288 (57)	2821 (71)	3358 (84)	3815 (95)	3983 (100)		
4	a	10–11	2·5	418 (17)	714 (30)	1030 (44)	1537 (65)	1770 (75)	2205 (94)	2350 (100)			662 (29)	1018 (45)	1287 (56)	1496 (66)	1741 (76)	2086 (92)	2276 (100)		
	b	16–17	3·5	735 (19)	1262 (32)	1982 (51)	2664 (68)	3258 (84)	3690 (95)	3896 (100)			995 (22)	1697 (38)	2392 (53)	3142 (70)	3787 (84)	4246 (95)	4477 (100)		

* At the end of the period on milk A this calf developed diarrhoea, which continued during the period on milk B.

† Figures in parentheses are % of total volume.

milks A and B. pH values obtained before the feed were between 1·8 and 2·9, increasing within a few minutes of feeding to 6·0–6·5 (range 4·5–6·8). During successive hours, the pH values decreased until at 4–5 h after the feed they were practically at their initial values; thereafter, there was little change.

In contrast to the similarity of the general changes in pH, there were marked quantitative differences in the pH values of the outflow resulting from the two milks.
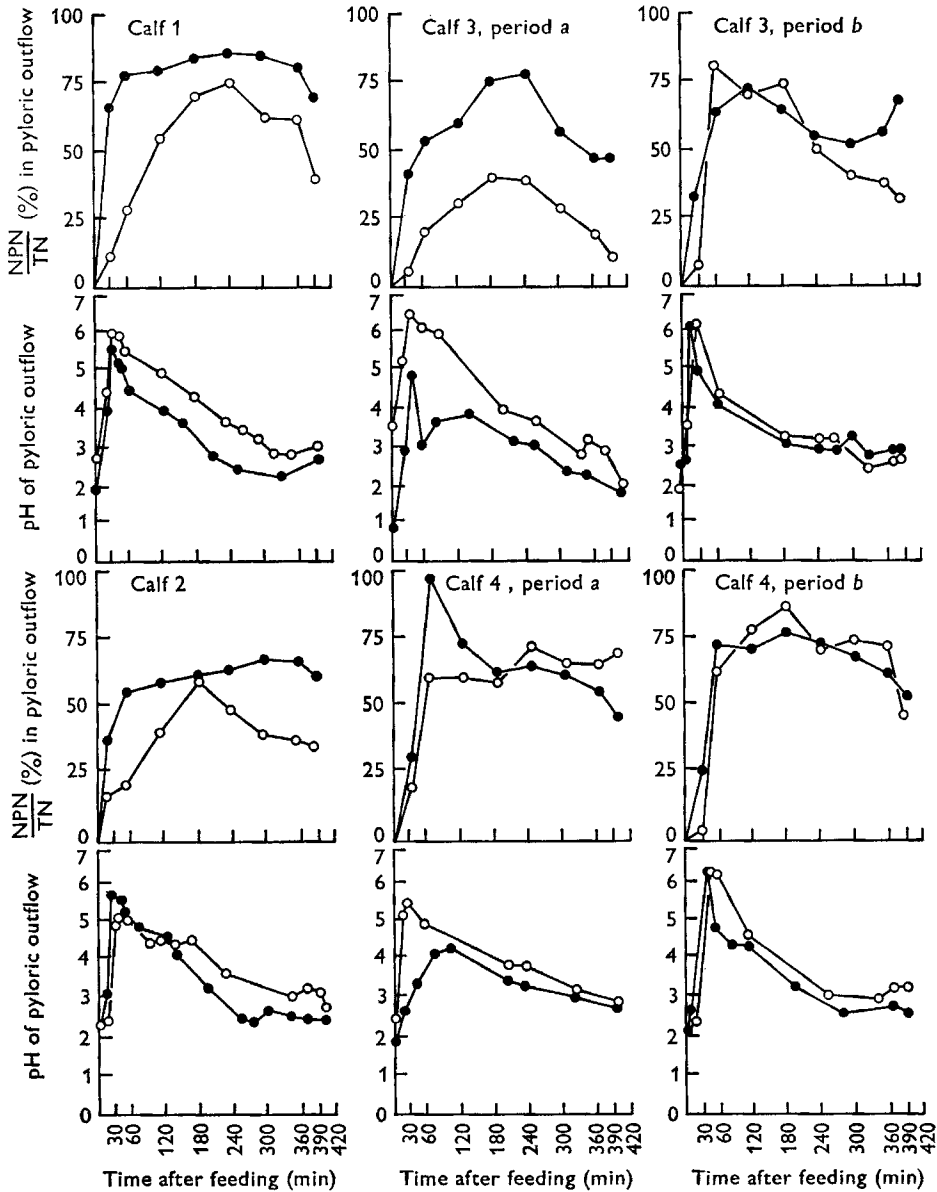

Fig. 2. Change in protein degradation, measured as non-protein nitrogen (NPN) in relation to total N (TN) of pyloric outflow of calves, and change in pH of pyloric outflow in relation to time after receiving a diet containing 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) skim-milk powder. ○, milk A; ●, milk B.

The pH of the outflow resulting from milk B was lower throughout the sampling period than that of the chyme from milk A. The greatest differences in pH after feeding occurred in the calves studied at a younger age and given 2·5 l. milk/feed (calves 3 and 4, period a). When these calves were older and were given 3·5 l./feed, the curves of daily changes in pH became more like those determined for calves 1 and 2.
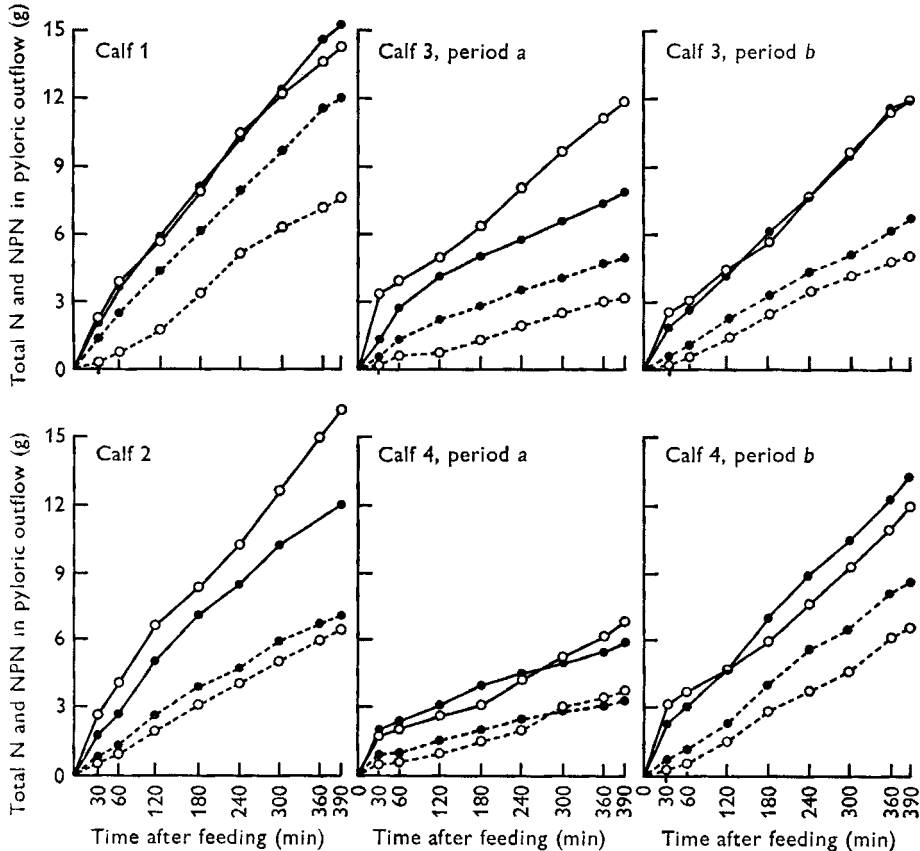

Fig. 3. Cumulative amounts of total nitrogen (TN) (solid lines) and non-protein N (NPN) (broken lines) in pyloric outflow of calves in relation to time after receiving a milk substitute containing 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) skim-milk powder. ○, milk A; ●, milk B.

N fractions in the chyme

Cumulative amounts of hourly outflow of TN and NPN for each of the collection periods (mean of either 2- or 3-day samples) are given in Fig. 3, whilst in Fig. 4 the mean values for protein N (PN) are shown. In most instances, TN output in the 1st h after feeding was higher for milk A than for milk B, but throughout the remainder of the sampling period there were considerable differences between calves and no consistent differences associated with the two milks were apparent. However, throughout the sampling period there was always a higher output of undigested PN and thus a lower amount of NPN when calves were given milk A. It is of particular interest to

note that, for both milks, the rate of output of protein was much higher in the first 30–60 min after feeding than during the rest of the sampling period; after that time PN output in relation to time appeared to be approximately linear.
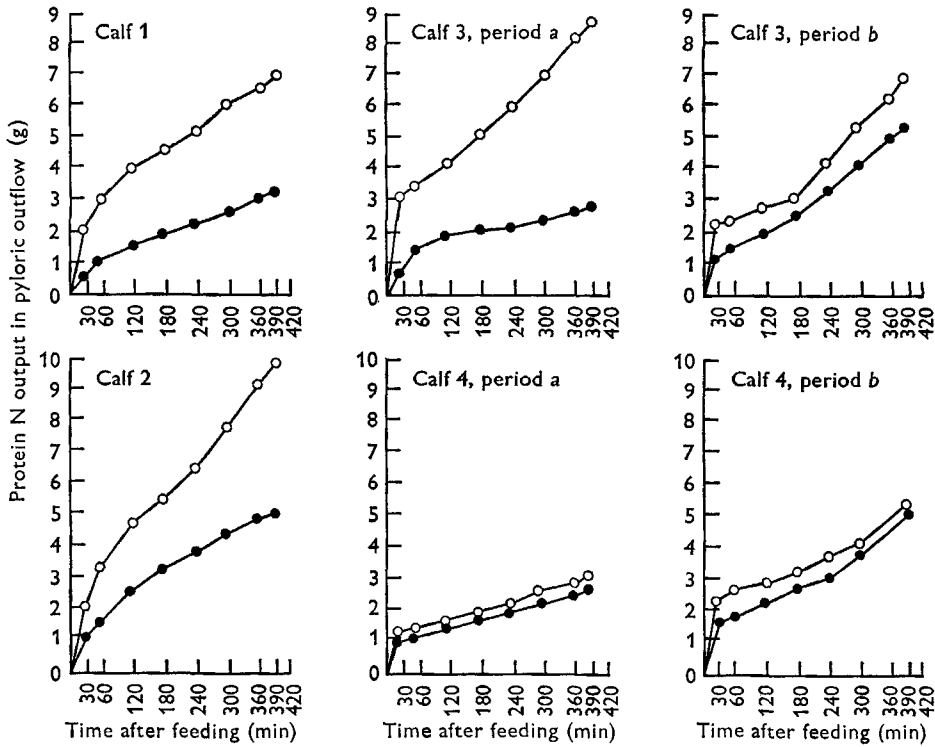

Fig. 4. Cumulative amounts of protein nitrogen in pyloric outflow of calves in relation to time after receiving a milk substitute containing 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) skim-milk powder. ○, milk A; ●, milk B.
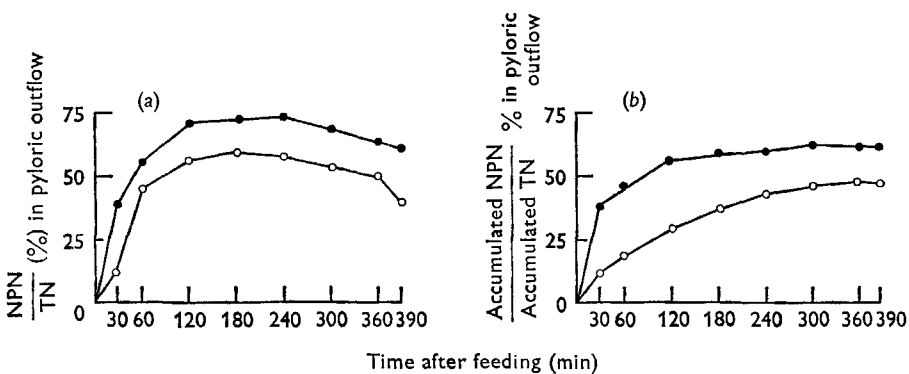

Fig. 5. Mean changes in protein degradation, measured as non-protein nitrogen (NPN) in relation to total N (TN) of pyloric outflow of calves, in relation to time after feeding for all collection periods when calves were given a diet containing a 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) skim-milk powder. (a) Absolute amounts in periodical pyloric outflow. (b) Cumulative amounts (by summation of (a) from beginning up to the time noted). ○, milk A; ●, milk B.

The ratio NPN:TN, i.e. the coefficient of protein degradation in the abomasum, is shown in Fig. 2 and summarized in Table 6 and in Fig. 5. The ratio increased faster for milk B than for milk A during the first 30–60 min after feeding. In most of the sample periods the coefficient for milk B after reaching its highest level did not change very much, whereas for milk A the peak was reached later and thereafter the coefficient immediately began to decrease. The results for calf 4, periods a and b, were exceptions to this finding. In most of the periods, irrespective of the milk given, a decrease in digestibility occurred towards the end of the sampling period.

The mean and individual values for total weights of N fractions produced in the sampling period, given in Table 6, show that PN output was always higher and NPN output lower for milk A than for milk B, except for calf 4, period a, when NPN output was slightly lower for milk B.

Effect of age and the variation between individual calves on clotting and distribution of N fractions in the chyme

The results for calves 3 and 4, which each had two successive experimental periods, a and b, allowed the effect of age to be studied within each individual calf; however, the effect was confounded by the increase in volume of diet from 2·5 l./feed in the first period to 3·5 l./feed in the second.

The main finding was that the shape of the pH curves and the average pH values resulting from milk A and milk B differed more when the calves were younger. The differences in the pH curves soon after sampling began were particularly noticeable in the first periods, whereas in the second the curves tended to be parallel. The average pH values were always lower with milk B than with milk A.

In calf 3, which developed diarrhoea in period a (Table 6), the outflow of chyme was rapid during the 1st h after feeding, and protein degradation was reduced. This calf, although still having diarrhoea when given milk B, appeared to digest the protein from this milk satisfactorily. The average pH value for this calf in period a was much higher than for calf 4 in period a, particularly when it received milk A. In contrast, in period b, there was little difference between calves 3 and 4 after either milk had been given. The difference in total PN outflow between these calves, when given milk A in period a, was particularly noticeable, the outflow being 8·8 g and 3·1 g in period a and 7·0 g and 5·5 g in period b for calves 3 and 4 respectively. The differences between these two calves were possibly the result of impaired clotting ability in calf 3, which improved as the calf grew older, and may have been responsible also for the diarrhoea. Since there were little differences in the output of digested protein (NPN) for these two calves given milk A in period a, it is probable that the proteolytic activities, in the abomasum, in contrast to clotting, were similar. In period b, the proteolytic activity improved, but more so for calf 4 than for calf 3. In any comparison of these two calves, it must be remembered that for equivalent sampling periods calf 3 was 1–2 weeks older than calf 4.

In contrast to these findings with milk A, the total PN outflows from milk B for calves 3 and 4 were similar in each of the experimental periods, the good clotting properties of milk B being sufficient to overcome the impaired clotting ability of calf 3.

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Another comparison between calves in their response to the different milks may be made between calf 2 and calf 4, period b; both calves were given 3·5 l./feed of the same milk at the same age. For milk A, protein degradation, as indicated by total

Table 6. *Total N, protein N, non-protein N and average pH values in chyme passing into the duodenum of calves during a period of 6·5 h after receiving a milk substitute containing 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) spray-dried skim milk*

| Calf no.	Milk	Period	Amount of diet (l.)	Total N (g/390 min)		Protein N (g/390 min)		Non-protein N (g/390 min)		NPN as % of total N		Average pH	
				A	B	A	B	A	B	A	B	A	B
1	A	—	3·5	14·4	—	6·9	—	7·4	—	51·8	—	3·86	—
	B	—	3·5	—	15·3	—	3·3	—	12·1	—	78·7	—	3·11
2	A	—	3·5	16·1	—	9·8	—	6·3	—	39·3	—	3·83	—
	B	—	3·5	—	12·1	—	5·0	—	7·0	—	58·4	—	3·43
3	A*	a	2·5	11·9	—	8·8	—	3·1	—	26·3	—	5·05	—
	B*	a	2·5	—	7·9	—	2·9	—	4·9	—	62·7	—	3·95
	A	b	3·5	12·0	—	7·0	—	5·0	—	41·9	—	3·76	—
	B	b	3·5	—	12·0	—	5·3	—	6·7	—	55·7	—	3·55
4	A	a	2·5	6·8	—	3·1	—	3·7	—	54·0	—	4·11	—
	B	a	2·5	—	5·9	—	2·7	—	3·2	—	54·8	—	3·54
	A	b	3·5	12·0	—	5·5	—	6·5	—	54·5	—	3·97	—
	B	b	3·5	—	13·4	—	5·3	—	8·1	—	60·2	—	3·58
Mean	A	—	—	12·2	—	6·8	—	5·3	—	44·6	—	4·09	—
	B	—	—	—	11·1	—	4·1	—	7·0	—	61·7	—	3·52

* Calf had diarrhoea.
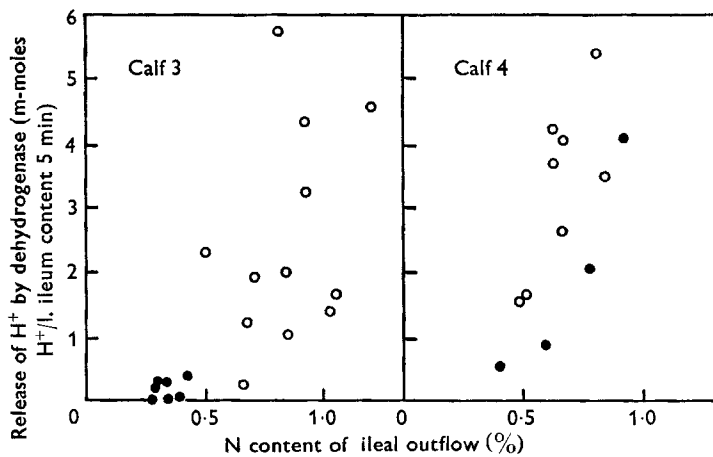
Fig. 6. Relationship of bacterial activity, as determined by release of H$^+$ by dehydrogenase, to protein content of ileal outflow of calves given 2·5 l./feed of a diet containing 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) skim-milk powder. Results are expressed as m-moles H$^+$ released by 1 l. ileum content in 5 min incubation. ○, milk A; ●, milk B.

NPN output, was similar for the two calves, namely 6·3 and 6·5 g/6·5 h for calves 2 and 4, period b respectively, but PN output of calf 2 was nearly twice that of calf 4, the values being 9·8 and 5·5 g PN respectively in the 6·5 h collection period. For milk B, with its improved clotting properties, PN output was similar for the two calves,

being 5·0 and 5·3 g PN/6·5 h for calves 2 and 4, period b respectively, whilst NPN output, indicative of protein degradation, was slightly better for calf 4 (7·0 and 8·1 g NPN/6·5 h for calves 2 and 4, period b respectively). Thus, calf 4 would appear to have had a considerably greater clotting ability and a slightly higher abomasal proteolytic activity than calf 2.

Table 7. *Volume of daily ileal outflow and its total nitrogen content in calves given 2·5 l./ feed of a milk substitute containing 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) spray-dried skim milk*

Day of experi- ment		Calf no. 3				Calf no. 4		
	Milk	Volume of outflow (ml)	N content (%)	Daily N outflow (g)	Milk	Volume of outflow (ml)	N content (%)	Daily N outflow (g)
1	A	211	0·97	2·05	B	317	0·65	2·06
2	A	293	0·96	2·81	B	394	0·74	2·91
3	A	496	1·01	5·00	A	473	0·75	3·54
4	A*	1167	0·67	7·82	A	515	0·68	3·50
5	B*	1991	0·39	7·76	A	519	0·72	3·73
6	B*	2035	0·36	7·32	A†	573	0·52	2·97

* On these days the calf had diarrhoea.
† The calf was kept on this diet for a further period but did not show any signs of diarrhoea.

Properties of ileal outflow, its N content and dehydrogenase activity

The consistency of ileal outflow differed during the sampling period. In the early part of the period, the outflow was very thick, viscous and dark brown or sometimes even greenish in colour. Within 2–3 h after feeding, the outflow became less viscous and its colour changed from brown to yellow. In the calf which had diarrhoea, it was watery and of a pale yellow colour. The TN content was always higher in the thick viscous outflow during the first 2–3 h after feeding than in the watery outflow later in the sampling period.

The results in period a for calves 3 and 4 given 2·5 l. milk A or B/feed are given in Table 7. The TN outflow through the ileum in calf 4, which was healthy throughout, was 2·1–3·7 g/24 h. In calf 3 similar amounts were found only on the first 2 days of observation when the calf's faeces were of normal consistency. As diarrhoea developed in this calf, the volume of ileal outflow increased markedly and the TN concentration in the outflow showed a pronounced decrease. Nevertheless, the total daily outflow of N was greatly increased.

The results given in Fig. 6 for dehydrogenase activity showed that there was a clear positive correlation between the concentration of H^+ released by bacteria and the N concentration in the outflow. The values obtained for each milk separately showed the same trend, but there tended to be a lower concentration of N in the ileal outflow and a lower dehydrogenase activity when the calves were given milk B. It should be remembered, however, that the extremely low N concentration and low dehydrogenase activity that occurred in calf 3 (period a), when given milk B, was accompanied by,

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and might be the result of, diarrhoea which began when the calf was receiving milk A and continued when it was receiving milk B. The increased volume of ileal outflow during diarrhoea was accompanied by a lower N concentration, possibly as a result of dilution of the outflow, and also of a reduced rate of bacterial activity.

DISCUSSION

There is clear evidence from the results of this experiment that, although there is individual variation in the response between calves, a diet containing a 'severely' pre-heated spray-dried skim-milk powder (milk A) results in marked differences in the pyloric outflow compared with a diet containing a 'mildly' pre-heated spray-dried skim-milk powder (milk B). One of the important findings was the low pH values in the outflow when milk B was given. As no difference in buffering capacity could be shown, it seems that lower pH values for milk B might result from greater amounts of HCl secreted in the abomasum. However, no measurement of the buffering capacity of the pyloric outflow was made. Jančařík (1962) showed that pasteurized milk caused a reduced secretion of HCl compared with fresh milk, although, when treated with *Lactobacillus acidophilus*, pasteurized milk did not have this effect.

Since the pre-heating treatment given to milk A (74° for 30 min) caused greater denaturation of the whey proteins than was caused by Holder pasteurization (63° for 30 min) (Shillam, Dawson & Roy, 1960), a reduction in the secretion of HCl after milk A was given may well have occurred. As the total pyloric outflow in 6·5 h in relation to intake tended to be greater for milk B than for milk A, it would seem that milk B probably stimulated a greater output of gastric secretion or saliva. The possibility of increased HCl production on the one hand, and a faster whey drainage from the curd of milk B on the other, would allow a higher ratio of HCl:curd for milk B. This may have caused the differences in the pH values of abomasal outflow from the two milks and may have led to differences of proteolytic activity. Abomasal secretion has been shown to be continuous but reaches a peak 2–3 h after feeding, whilst pepsin secretion increases to a peak 1 h after feeding (Marchenko, Budnaya, Khimina & Riyashko, 1964).

The dependence of N degradation upon average daily pH values in pyloric outflow was examined both by relative and absolute criteria. In relative terms the ratio NPN output to TN output, and in absolute terms the amount of NPN output were compared with pH. The results are summarized in Fig. 7. The regression coefficient of NPN:TN on pH and NPN on pH did not differ between milks, the over-all values being −21·5 ($P < 0.01$) and −3·51 ($P < 0.05$) respectively.

To explain the variation between calves in their response to the two milks, it is necessary to consider the optimum pH values for certain functions. For clotting by rennin the optimal pH is 6·5, whereas the optimal pH for proteolytic activity was found by J. W. G. Porter (1964, personal communication) to be 4 for rennin and 2 for pepsin. Moreover, the proteolytic activity of pepsin is much higher than that of rennin at their optimal pH values. Further, Porter found that at an early age some calves secrete mostly rennin, whereas others secrete more pepsin than rennin, but

pepsin secretion increases in calves as they grow older. These findings could explain some of the variation found between the calves in the present experiment.

To summarize the over-all results for proteolytic activity, the NPN outputs in relation to time of sampling for the period when 3·5 l. milk were given and also for all the periods are shown in Fig. 8. Besides the quantitative changes in the products of proteolysis with time, there were qualitative differences in the rate of activity. The
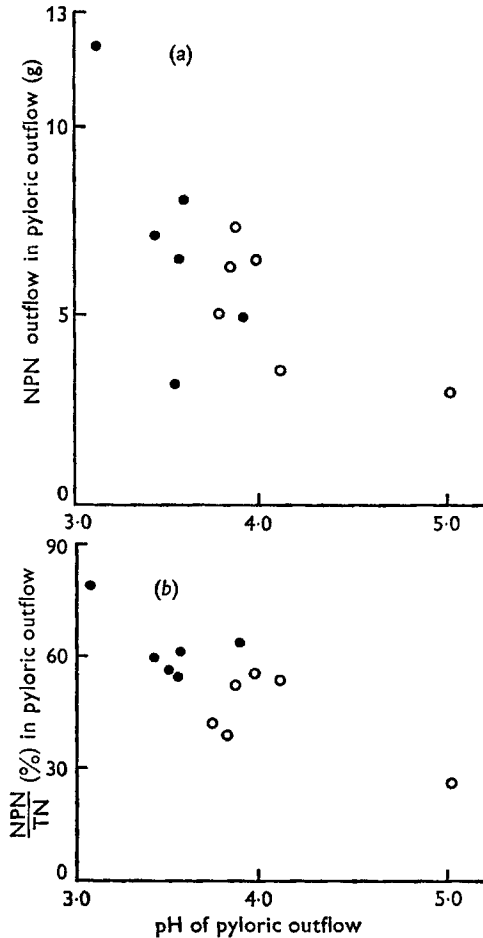

Fig. 7. Relationship of protein degradation in the abomasum to average pH of pyloric outflow of calves given a diet containing 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) skim-milk powder. (*a*) Relationship of total non-protein nitrogen (NPN) output in 6·5 h to pH; (*b*) relationship of the ratio of total NPN output to total N (TN) output in 6·5 h (as %) to pH. ○, milk A; ●, milk B.

amount of NPN outflow per min in the chyme from milk A was lowest immediately after feeding, and increased steadily with the acidification of the abomasal contents until it reached its peak 3–4 h after feeding and declined thereafter. In contrast to this, and rather unexpectedly, the outflow of NPN per min after milk B was given was highest immediately after feeding and decreased throughout the sampling period,

although acidification of the abomasal contents took place at a faster rate than with milk A.

Analysis of the composition of milk A showed that 50 % of the β-lactoglobulin N, 65 % of the residual albumin N (sum of the N of α-lactalbumin, blood serum albumin and two minor components) and 34 % of the immune globulin N had been denatured. The difference between the two diets in undenatured whey proteins was about 38 mg/100 g, or 1·3 g in 3·5 l. milk. The undenatured whey proteins, which are water-soluble, may be more readily available for rapid proteolysis, and may release NPN as peptides before clotting has occurred. Moreover, Alais (1963 a, b) has shown that
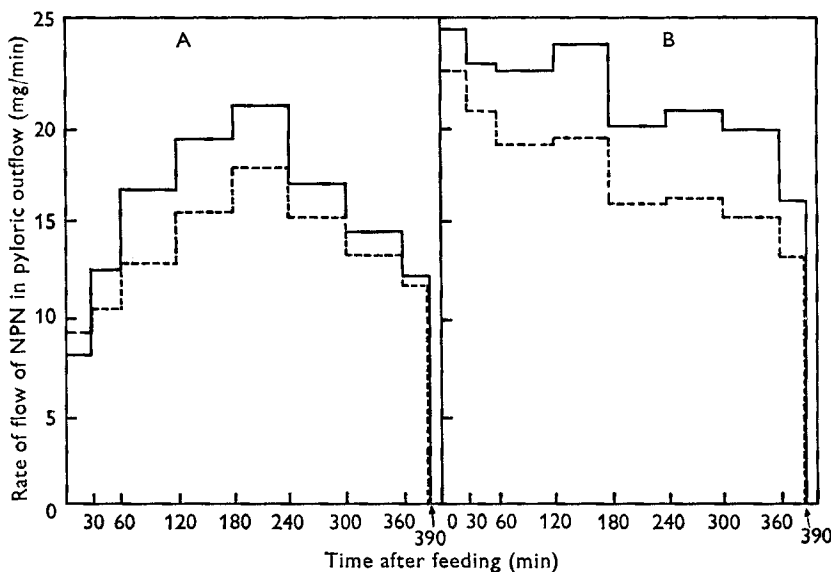

Fig. 8. Rate of flow of non-protein nitrogen (NPN) (mg/min) in pyloric outflow of calves during 6·5 h after receiving a diet containing 'severely' pre-heated (milk A) or 'mildly' pre-heated (milk B) skim-milk powder. —, mean results for calves given 3·5 l./feed; - - -, mean results for all the calves.

there is a release of glycopeptide from κ-casein by the action of rennin and this occurs before coagulation takes place. In addition, the retention of the casein in the abomasum as a result of improved clotting may account for the extended release of NPN later in the sampling period. Both these metabolic sources of NPN in addition to the original milk NPN and proteose-peptone N, which are unaffected by heat, are passed into the duodenum during the first 2 h after feeding at a faster rate and in a greater volume of chyme if the milk has had a 'mild' pre-heating treatment (milk B) than they do if the heat treatment has been more severe (milk A).

Proteolysis of milk A appeared to increase steadily up to 3 h after feeding and thereafter decreased. This decline also happened with milk B in spite of the increasing acidification of the abomasal contents. This decline could possibly be explained by the finding of Ash (1964) that HCl production reached a peak 30–90 min after feeding and then declined, whereas proteolytic activity increased within 15 min of feeding and thereafter rapidly decreased. However, the calves used by Ash obtained the milk by

sucking a nipple rather than by drinking from an open bucket. Thus, although proteolysis is no doubt increased by the level of acidity in the abomasal contents, it may be overruled by a decrease in the rate of enzyme secretion and the passage of enzyme with the chyme into the duodenum. The over-all effect therefore may have resulted in a decrease in proteolytic activity for both milks in the latter half of the sampling period.

The coefficient of protein degradation (NPN/TN) is affected not only by the proteolytic activity but also by the escape of protein from the abomasum as a result of poor clotting. The mean values given in Table 6 show that the lower coefficient of protein degradation when milk A was given was not only a result of the small amounts of NPN found in the abomasal outflow. The differences between the two diets in the amounts of undigested protein N flowing out of the abomasum were much greater than the differences between the amounts of NPN output. This can be seen from a study of the individual periods, in which the amounts of TN outflow were similar for the two milks; in all instances, the PN output was greater with milk A than with milk B.

It is concluded, therefore, that milk of a good clotting potential may assist in better digestion of the protein, but other factors such as the presence of undenatured whey proteins may be of considerable importance. This is in keeping with the finding that addition of Ca to milk A gave good clotting in vitro, but had only a marginal effect on the performance of the calves (Shillam & Roy, 1963 b).

One calf (calf 3, period a) developed severe diarrhoea when given milk A. The diarrhoea was preceded and accompanied by a relatively large volume of protein outflow from the abomasum, as well as high pH values and poor protein digestion. These three factors have been shown by Weijers & van de Kamer (1965) to be important in the aetiology of putrefactive diarrhoea, associated with the saccharo-proteolytic flora, in infants. Mylrea (1968) also found a large increase in volume of pyloric outflow in relation to intake with calves affected by non-specific diarrhoea, although the concentration of N in the outflow was not affected. Whether the high output of chyme after feeding resulted from its high pH or as a response to changes further down the intestine is not known. However, it was noticed in a preliminary trial that the return of large amounts of chyme to the distal duodenal cannula caused cessation of pyloric outflow until such time as the chyme became stained with bile pigments. Since Mylrea (1966) showed that duodenal contents of normal calves had a pH ranging from 4·5 to 5·5, and the average pH of the daily outflow from calf 3 on milk A was 5·05, the small alteration that was necessary to the pH of this chyme may have allowed accelerated passage. On the other hand, a delay in returning chyme to the distal cannula resulted in an increased rate of pyloric outflow. Thus, it is possible that the interaction of pH of the pyloric outflow and rapid passage from the duodenum could have influenced the pyloric output and that it was not a reflection of inadequate abomasal digestion alone.

The finding that higher bacterial activity was associated with higher concentration of N in the ileal outflow, and the high outflow of N in calf 3 when it had diarrhoea, are in keeping with the findings of Mylrea (1968) that ileal outflow of calves with enteritis contained six times as much N as that of normal calves. However, any increase in

bacterial activity as measured by dehydrogenase activity cannot be used as an indication of pathogenic numbers of bacteria without further knowledge of the levels of activity to be expected in normal calves.

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